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Contents

Chapter 25
Molecular Markers for Genetic Diversity and Bayoud Disease Resistance in Date Palm

MyH. Sedra

Abstract The date palm (*Phoenix dactylifera L*) is a monocotyledoneus woody perennial and dioecious plant with a long generation life time. Traditional and modern genetic improvement in date palm need long time and considerable funds. The molecular markers can assist the selection and give better and efficient research strategies. Several researches cited in this overview paper showed the use molecular markers as tools to evaluate genetic diversity and genotyping of date palm cultivars. Based on statistical analysis, some informative molecular markers which are associated to some phenological characters in date palm. Previous study of the date-palm mitochondrial DNA had evidenced two plasmid-like DNAs that seem to be linked to resistance to Bayoud disease but these markers cannot distinguish both studied cultivars. The research using several hundred RAPD and ISSR primers allowed identifying several markers candidates which can distinguish partially or totally between resistant and susceptible cultivars of date palm. The difficulty and relatively weak efficiency were probably due to the nature of genetic status of resistance. These preliminary researches open new doors to explore in the use of molecular technologies in the development of programme breeding of date palm in order to select rapidly new varieties desired by farmers and more demanded by different markets. They also may give area in research and construction programme of date palm genetic map.

Keywords Date palm • Bayoud • Molecular techniques • Selection • Resistance • Fingerprinting

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25.1 Introduction

The date palm (*Phoenix dactylifera L*) is a monocotyledonous woody perennial belonging to the Arecaceae family, which comprises 183 genera and more than 2,300 species (Moore 1973). Date palm is a dioecious plant with a long generation life time (a period of 4–5 years is necessary to reach the first flowering). One of the earliest cultivated tree crops it is believed to be native to the Arabian Gulf region, possibly southern Iraq. In very early times, the date palm was introduced by man into northern India, North Africa and southern Spain, where it plays a major role in arid zones. The date palm has traditionally been propagated vegetatively from offshoots produced by elite individual trees and more recently by plants derived from tissue culture. Although the number of cultivars in the world is about 5,000, the offshoot mode of distribution has probably limited the genetic diversity. In Morocco, the date palm is one of the most important traditional crops of the oases. In addition to its important ecological and social roles, this tree plays a significant role in human nutrition and animal feed, and is used to produce a wide range of end-products. In Morocco, around 4.8 million date palms are cultivated over an area of approximately 48,000 ha. To the present, more than 453 known cultivars have been identified. These are represented by some 2.1 million trees; the remaining 2.7 million trees originate from natural seed propagation and are commonly known as *khalts* (Sedra et al. 1996). The major constraints of date palm production are drought, low productivity and post-harvest techniques on traditional farms, as well as pests and diseases. Bayoud is the most serious fungal disease of the date palm caused by *Fusarium oxysporum* f. sp. *albedinis* which occurs in the major date palm-growing areas of Morocco, in a large portion of western and southern Algeria and in some areas of Mauritania (Sedra 2003a, c, 2007a, b). It is a serious disease in these North African countries and represents a serious threat to those countries which are still bayoud-free. Since 1963, the Moroccan National Agricultural Research Institute (INRA) has carried out, in collaboration with national and international partners, several scientific and applied investigations in order to serve date palm farmers and preserve the ecosystem of oases.

At present, the most promising means to control bayoud disease is the genetic approach using resistant cultivars. Several clones resistant to bayoud have been selected (Sedra 1990a, 1995, 1997, 2001, 2003a,b, 2005a), but they are each represented by only one to a few trees. In order to produce sufficient numbers of nursery plants for the reconstitution of date palm groves destroyed by bayoud, mass micropropagation of selected resistant date palms clones is essential. Among Moroccan cultivars, seven are resistant to bayoud and the behavior towards this disease of several hundred other cultivars among the majority of *khalt* trees is still unknown. Field observations alone of this behavior are not sufficient and the evaluation of this material by artificial inoculation using pathogens needs considerable funding and relatively lengthy time periods (Sedra 1993). Phenological and agro-morphological characters as quantitative or qualitative descriptors have been determined (Sedra 2001), but they cannot distinguish between resistant and susceptible cultivars, except for

the importance of a black color at the leaf bases**,** which is indicative (Sedra 1990b). This approach, based on phenological characters, does not easily allow early detection of resistant lines for young plants. To the contrary, molecular markers may identify the change in behavior of palm trees, which is expressed from certain regions of the DNA, or the total composition of DNA.

Pathologists and breeders cannot ignore the progress made in plant biotechnology, including the application of DNA-based markers for quality assurance. DNA-based tests for date palm identification and detection of some characters include techniques such as: RAPD (Random Amplified Polymorphic DNA); RFLP (Restriction Fragment Length Polymorphism) (Botstein et al. 1980); random-amplified polymorphism DNA markers (RAPD) (Williams et al. 1990), AFLP (Amplification Fragment Length Polymorphism) (Blears et al. 1998; Vos et al. 1995); RDA (Representational Difference Analysis) (Cullis et al. 1999; Powell et al. 1996) simple sequence repeat polymorphism or microsatellites (SSR) (Akkaya et al. 1992; Gupta et al. 1994; Morgante and Oliviery 1993) and inter simple sequence repeat (ISSR) (Ajibade et al. 2000; Fang and Roose 1999; Fang et al. 1997; Stepansky et al. 1999).

25.2 Genetic Diversity and Characterization of Cultivars

Sedra (2001) enumerated 342 date-palm descriptors of which 105 are descriptive characters (tree, inflorescence, fruit); 132 agronomic characters (maturity, pollination, resistance, etc.), 62 chemical characters (chemical structure, etc.); 6 biochemical scorers (enzymes, etc.) and 37 RAPD molecular markers. The statistical analyses using the Static and Systat programs on a sample of more than 90 date palm cultivars grown in North Africa permitted an appreciation of the interrelationships observed between all quantitative and qualitative characters and to determine those that proved to be discriminative (Sedra 2001, 2003c; Sedra et al. 1996). Therefore, 25 quantitative characters have been recognized as discriminative, of which 9 are very highly discriminative (0–1%), 16 highly discriminative (1–5%) and 11 correlated to highly discriminative quantitative characters (Sedra 2001).

RAPD technology appears very effective for identifying accessions of date palm, although the overall polymorphism exhibited is rather low (Sedra et al. 1998) in comparison with results reported for other cultivated species (Hu and Quiros 1991; Koller et al. 1993; Mossler et al. 1992; Wolff and Van Run 1993; Yang and Quiros 1993). Previous molecular markers studies (Aît Chitt et al. 1995; Corniquel and Mercier 1994) involved a restricted set of date palm cultivars and were less rewarding. The relatively low polymorphism and lack of evident organization observed among the date palm cultivars grown in Morocco could be related to the mode of introduction and maintenance of germplasm (Sedra et al. 1998). The foundation of the germplasm is somewhat limited. The fact that the cultivars from Tunisia and Iraq did not markedly diverge from the genetic diversity present in Morocco suggests a narrow genetic diversity of populations from which the present cultivars have been derived and maintained over several centuries (Sedra et al. 1998). Exchange of cultivars

between plantations and periodic development of new recombinant cultivars through sexual reproduction and seedling selection also may have played an important role. In addition, selection by farmers concerns mainly end-use quality-related genes which may represent only a small fraction of the date palm genome.

Cultivars showing resistance to bayoud disease in three different genetic groups may indicate the presence of several genetic resistance sources (Sedra et al. 1998). A combination of such potentially different sources of resistance could therefore be of interest in the framework of breeding programs. Date palm breeding and genetic improvements have relied, and continue to rely, on traditional methods. Advances in selection for agronomically-important traits, such as fruit quality or disease resistance, are difficult due to the species' long generation time. The Moroccan experience in date palm genetic improvement by crosses and mass selection reveals this reality (Djerbi et al. 1986; Louvet and Toutain 1973; Saaidi et al. 1981; Sedra 1995, 1997, 2003a, 2005a). The resistance character to bayoud disease seems the least complicated characters for date palm tree. Relatively fast methods were developed for the assessment of resistance to bayoud in different stages of plant growth (seedlings, young plants and adult trees) in the field and in laboratory (*in vitro* screening and in the greenhouse) (Sedra 1994a,b; Sedra and Besri 1994; Sedra et al. 1993). These methods of conventional breeding and hybridization programs are limited practically and financially when it is necessary to evaluate populations with a very large number of individuals.

Genetic engineering and molecular markers have not thus far been used for the improvement of date palm tree, but they are likely to play an important role in the future development of this crop. Until recently, there was little study of crops at the genome level; however, the next few years should bring molecular breeding technology to this plant group. Molecular breeding can be defined as the application of DNA-based analysis of genome polymorphism to breeding programs. Molecular markers can be linked to genes of interest like disease resistance, allowing indirect selection of the desired genotypes. Technologies for genome fingerprinting (molecular marker detection) include RFLP, RAPD, AFLP, SSR and ISSR. The use of amplified fragment length polymorphism (AFLP) has some advantages in terms of use in the identification of diagnostic or specific markers. Although these markers are generally dominants, the AFLP technique does not require previous knowledge of the DNA sequence, generates reproducible fingerprinting profiles and allows the amplification of a high number of DNA fragments per reaction, enabling the detection of specific amplified fragments (Vos et al. 1995). All of the molecular techniques (RAPD, AFLP, SSR and ISSR) have been applied to evaluate the genetic diversity and identification of date palm cultivars (Adawy et al. 2002, 2005; Cao and Chao 2002; Lacaze and Brackpool 2000; Saker and Moursy 1999; Sedra 2007c; Sedra et al. 1998) and for genetic comparison and the identification of vitroplants obtained by tissue-culture techniques from mother adult palms (Diaz et al. 2003; Saker et al. 2006; Sedra 2005b). Detection of somaclonal variations in tissue culture-derived date palm plants of some Egyptian cultivars has been accomplished using isozyme analysis and RAPD fingerprints (Saker et al. 2000). The genetic variations occurred in approximately 4% of the

analyzed plants representing 70 regenerated plants. On the other hand, Rival et al. (1998) identified a molecular marker linked to somaclonal variations in African oil palm.

Molecular markers associated with bayoud resistance would be suitable for rapid and efficient screening of field grown palms produced by mass selection, hybrid plants from crosses in breeding programs, as well as plantlets from *in vitro* tissue culture.

25.3 Resistance in Date Palm

25.3.1 Biochemical and Plasmid Mitochondrial DNA Markers

In this approach, many markers that are correlated to resistant palms have been reported, such as isozymes (Baaziz 1990; Bendiab et al. 1993; Bennaceur et al. 1991), polyphenolics (El Hadrami et al. 1996; El Idrissi-Tourane et al. 1996), and mitochondrial plasmid-like DNAs (Benslimane et al. 1994). However, the correlation between the date-palm phenotype and the described marker has not been clearly established. In fact, previous studies of date-palm mitochondrial DNA provided evidence of two plasmid-like DNAs, called the S and R plasmids that are of 1,454 and 1,345 bp respectively (Benslimane 1995). These plasmids are of about 99% sequence similarity. A 109 bp sequence is only present in the S plasmid (Benslimane et al. 1996). The S and R plasmids were found in the mitochondria of two Moroccan cultivars: the first one is bayoud susceptible and contained the S plasmid, and the second containing the R plasmid is bayoud resistant. This suggested that S and R DNAs could be correlated to date-palm susceptibility to and resistance against bayoud. In Tunisia, Trifi (2001) has extended a similar study to nine Tunisian date-palm cultivars in order to obtain a deeper insight of the relationship that exists between these plasmids and the bayoud-tree phenotype (susceptibility/resistance). The analysis of results based on the detection of the mitochondrial plasmids did not agree with the date-palm phenotype against the bayoud, because seven of the nine cultivars tested showed the S plasmid. Surprisingly, the S plasmid and both S and R plasmids have been detected respectively in cvs. Deglet Noor and Horra. However, both of the tested cultivars have been shown susceptible to bayoud according to Saaidi (1992) and Sedra (1992). This finding showed that the correlation between the date-palm phenotype and these markers has not been clearly established. Trifi (2001) suggested that this feature could be justified by interrelations involving nuclear and mitochondrial genomes. This is supported by Flamand et al. (1993) who reported that the mitochondrial plasmids that arise by recombination events are controlled by the nuclear genome and by Sedra et al. (1998) who suggested a multi-gene control of date-palm bayoud-resistance/susceptibility. Otherwise, the genetic resistance can be controlled by dominant genes and additive genes (Djerbi and Sedra 1986; Sedra 2003a). This has been shown when the percentage of resistant individuals of progenies from S (susceptible) × R (resistant) or R × S parents and S × S parents reaches, respectively, more than 50% and 5%.

The abovementioned situation encouraged researchers to develop other efficient and potential molecular markers associated with bayoud resistance in date palm and how the polymerase chain reaction (PCR) technology allowed a powerful approach suitable in the rapid screening of selected bayoud-resistant individuals.

25.3.2 RAPD and ISSR Markers for Characterization and Relationship with Phenological and Agronomic Traits Including Resistance to Bayoud Disease

Data based on molecular markers such RAPDs, have been developed to molecularly characterize date-palm genotypes of cultivars and to examine their phylogenetic relationships (Sedra 2000; Sedra et al. 1998; Soliman et al. 2003; Trifi et al. 2000). Therefore, the search of many other markers is required to obtain a deeper understanding of the genetic organization in date-palm cultivars. Among the markers that can be investigated, microsatellites may be the more efficient. In fact, microsatellites are interspersed in the genomes (Gupta et al. 1994; Sanchez et al. 1996) and they constitute discrete markers suitable in DNA fingerprinting. In addition, microsatellites are also informative about many loci and are suitable to discriminate closely-related genotype variants (Fang and Roose 1997). Microsatellites are small arrays (typically <100 bp) of simple di- and tri-nucleotide repeats (Scribner and Pearce 2000). The inter simple sequence repeat (ISSR) technique permits the detection of polymorphism in microsatellites and inter-microsatellites loci without previous knowledge of the DNA sequence. The sequences of repeats and anchored nucleotides are randomly selected (Fang et al. 1997). ISSR strategy was therefore performed to access the DNA diversity among crop genotypes. The ISSR markers were tested to examine the genetic variability and molecular fingerprinting of Egyptian date palm cultivars (Adawy et al. 2002, 2005; Ben Saleh and El-Helaly 2003). A similar strategy has been employed to distinguish ecotypes in closely-related groups such as vigna bean (Ajibade et al. 2000), citrus (Fang and Roose 1999; Fang et al. 1997) and melon (Stepansky et al. 1999).

The ISSRs and RAPDs markers were tested as informative markers to identify some other markers eventually related to date palm cultivar phenotype responses to bayoud disease. For this molecular approach, at least seven resistant and seven susceptible cultivars of date palm tree (Table 25.1) and numerous susceptible and resistant young plant-hybrids derived from controlled crosses Black Bousthammi (resistant female) × INRA-A18 (susceptible male) and Jihel (susceptible female) x male INRA-NP4-Boufegopus (resistant male) were used. The DNA extraction method was based on CTAB protocol. Yield of genomic DNA varied from 250 to 450 μg from 4 g of non-dried samples or from 0.2 to 0.3 g of dried-lyophilized leaflets samples.

The RAPD analysis and PCR were achieved according to the protocol used by Sedra et al. (1998) with some slight modifications. Oligonucleotide primers (10 mers)

Table 25.1 Moroccan date palm cultivars tested in a study concerning the research on molecular markers associated with resistance to bayoud disease

Cultivars	Main geographical area	Fruit quality	Phenotype to bayoud disease
Boufeggous ou Moussa	Bani	Fair	Resistant
Black Bousthammi	Bani, Draa valley,Tafilalet, Saghro, Anti-Atlas	Fair	Resistant
Boukhanni	Draa valley	Moderate	Resistant
Iklane	Anti-Atlas, Bani, Draa valley, Saghro	Fair	Resistant
Najda	Draa valley	Good	Resistant
Sairlayalate	Bani	Moderate	Resistant
Tademainte	Anti-Atlas, Bani, Draa valley, Oriental, Saghro	Fair	Resistant
White Bousthammi	Anti-Atlas, Bani	Fair	Resistant
Ahardane	Draa valley, Anti-Atlas, Saghro, Bani, Oriental		Susceptible
Boufeggous	Draa valley, Tafilalet, Ziz, Anti-Atlas, Saghro, Bani, Ferkala, Gheris, Guir, Todra, Oriental, between Saghro and High-Atlas	Good	Susceptible
Bourar	Draa valley, Bani, Saghro, Tafilalet	Good	Susceptible
Bouskri	Bani, Draa valley, Saghro,Todra, Oriental, Tafilalet, between Saghro and High-Atlas, Anti-Atlas	Moderate	Susceptible
Deglet Noor	Oriental	Good	Susceptible
Jihel	Draa valley, Bani, Anti-Atlas, Tafilalet, Saghro, between Saghro and High-Atlas	Good	Susceptible
Medjool	Tafilalet, Ziz valley, Oriental, Draa valley, Saghro, between Saghro and High-Atlas	Excellent	Susceptible

were purchased from Operon Technologies Inc., Alameda CA (OP) and The University of British Columbia (UBC) Vancouver. A total of 550 primers were used; 13 (OP) were already selected by Sedra et al. (1998) and 100 (UBC) interesting primers which were selected among more than 400 primers (Sedra 2007c) as primers generating polymorphism. Afterward, other RAPD primers were selected. For ISSR analysis, PCR was realized using 46 oligonucleotides composed of defined, short tandem repeat sequences representing different ISSR microsatellites used as genetic primers in PCR amplifications in Moroccan date palm cultivars. PCR was performed and adapted according to the protocol by Sedra and Zhar (2010). Only distinct reproducible, well-resolved fragments were scored as present (1) or absent (0) for both date cultivars. PCR generated band profiles obtained in seven resistant and seven susceptible cultivars cited in the Table 25.1 were analyzed to produce a genetic distance matrix using the formula of Nei and Li (1979). The genetic distance matrix was then computed using the UPGMA cluster analysis. A dendrogram was constructed using the average linkage between groups in order to evaluate the relation between resistant cultivars.

Table 25.2 Examples of selected RAPD primers that revealed interesting molecular markers which allowed good genetic diversity of date palm (bases of nucleotides between 3′ and 5′)

Primer sequences			
CACAGACACC	CCTGGGCTTA	AAGCTGCGAG	GATCCATTGC
CCGCTACCGA	CCTGGGTGGA	GGTCTCTCCC	GCTGGGCCGA
GAGGGAAGAG	TCCGGGTTTG	GCTTCCCCTT	AGGGAGTTCC
GGGAACGTGT	CCGGCCCCAA	TGTCGGTTGC	TCCACGGACG
GTCCACTGTG	GCGGCTGGAG	ATGTGTTGCG	CTGAGGCAAA
CCGAACACGG	GGGCAATGAT	CTGGGGATTT	CTGTCCAGCA
CTCCATGGGG	CTCGGGTGGG	GAGCACTTAC	CTGAAGGGGA
CCACACTACC	CGTCTGCCCG	CACGGCGAGT	ATGTTCCAGG
CACCCGGATG	AGTAGACGGG	CGGTTTGGAA	GCCCGACGCG
TGGTCGCAGA	ATCCCAAGAG	TGCACTGGAG	GGGTGAACCG
GGACACCACT	TGACCGAGAC	GAAGCGCGAT	TACCGACGGA
CTGGGGACTT	TTAGCGGTCT	CAGCGAACTA	GAGTAAGCGG
GTCGCCGTCA	ATTGGGCGAT	CATGTGCTTG	GCATCTACCG
TCTGGTGAGG	CGGTTACTAG	TCACACGTGC	CGACAGTCCC
GGTCTACACC	GTAGACGAGC	ACAGGTAGAC	CGGTGGCGAA
CACCGTATCC	GCGGTTGAGG	CCCGTCAATA	CGGCCCACGT
CATCCGTGCT	ATCTGGCAGC	AAGCCTCCCC	GCTGGTACCC
AGGGCGTAAG	GAGCCAGAAG	CTAGGGGCTG	ATCTAGGGAC
CTCACGTTGG	ACAGGGAACG	GTCGCCGTCA	TCTGGTGAGG
CTCCATGGGG	CTGGGGACTT	TCGTCTAGCT	GGTCTACACC
CCACACTACC	CCGAACACGG	AGGGCGTAAG	CACCGTATCC
CTCACGTTGG	GGGAACGTGT	GGACACCACT	CACCCGGATG
CACAGACACC	ACAGGCAGAC	GAGGGAAGAG	TGGTCGCAGA
ATACAGGGAG	CATCCGTGCT	GTCCACTGTG	CCGCTACCGA

25.3.2.1 Selection of RAPD and ISSR Molecular Markers Allowing Genetic Diversity and Cultivar Genotyping

In order to select interesting primers, a total of 550 RAPD primers 10-Decamer were tested on date palm DNA and 170 of them were selected permitting identification of more than 300 polymorphic markers which are able to detect polymorphism and genetic diversity and to identify date-palm cultivars. The percentage of polymorphism may reach 70% and 1–5 polymorphic bands per primer were generally generated. Tables 25.2 and 25.3 and Fig. 25.1 present examples of selected primers (Sedra 2007c). For ISSR primers, the PCR analysis applied to 46 primers (short sequences) tested, allowed the selection of 21 primers that showed a high rate of polymorphism among 45 date palm cultivars studied. These primers have revealed more than 80 polymorphic markers. The percentage of polymorphism varies from 43% to 100% (average 80%) according to primers and the average of 6.1 polymorphic bands per primer was generated. Table 25.4 and Fig. 25.2 present some examples of selected ISSR primers. For both RAPD and ISSR markers, the results were reproducible.

Table 25.3 Examples of selected RAPD molecular markers and their sizes that revealed good genetic diversity of date palm and cultivar identification

RAPD primers	Primer sequences	Molecular marker weight (kb)
OP-D3	5′-GTCGCCGTCA-3′	0.15
OP-D4	5′-TCTGGTGAGG-3′	0.7
OP-D10	5′-GGTCTACACC-3′	0.1
OP-D12	5′-CACCGTATCC-3′	1.1
OP-D12	5′-CACCGTATCC-3′	1.9
OP-D15	5′-CATCCGTGCT-3′	1
OP-D15	5′-CATCCGTGCT-3′	1.06
OP-D16	5′-AGGGCGTAAG-3′	0.83
OP-D16	5′-AGGGCGTAAG-3′	1.06
OP-J4	5′-CCGAACACGG-3′	2.5
OP-J5	5′-CTCCATGGGG-3′	1.12
OP-J13	5′-CCACACTACC-3′	0.9
OP-J14	5′-CACCCGGATG-3′	1.1
OP-J18	5′-TGGTCGCAGA-3′	1.47
OP-M5	5′-GGGAACGTGT-3′	1.16
OP-M11	5′- GTCCACTGTG-3′	1.16
OP-M11	5′- GTCCACTGTG-3′	0.94
OP-M11	5′- GTCCACTGTG-3′	0.67
OP-N1	5′-CTCACGTTGG-3′	0.5
OP-L6	5′-GAGGGAAGAG-3′	0.86
OP-X4	5′-CCGCTACCGA-3′	2.29
UBC-3	5′-CCTGGGCTTA-3′	0.38
UBC-3	5′-CCTGGGCTTA-3′	0.46
UBC-3	5′-CCTGGGCTTA-3′	0.7
UBC-13	5′-CCTGGGTGGA-3′	0.76
UBC-173	5′-CAGGCGGCGT-3′	0.75
UBC-201	5′-CTGGGGATTT-3′	1.47
UBC-209	5′-TGCACTGGAG-3′	1.4
UBC-213	5′-CAGCGAACTA-3′	0.63
UBC-256	5′-TGCAGTCGAA-3′	1.42
UBC-276	5′-AGGATCAAGC-3′	0.35
UBC-302	5′-CGGCCCACGT-3′	2.52
UBC-304	5′-AGTCCTCGCC-3′	0.75
UBC-310	5′-GAGCCAGAAG-3′	1.02

25.3.2.2 Molecular Markers and Quantitative and Qualitative Descriptors of Date Palm

Preliminary studies based on statistical analysis revealed the relationship between 31 quantitative and qualitative descriptors of date palm tree and 34 selected RAPD molecular markers (from m1 to m34) (Sedra 2007c). As cited above, phenological and agro-morphological characters cannot distinguish between resistant and susceptible

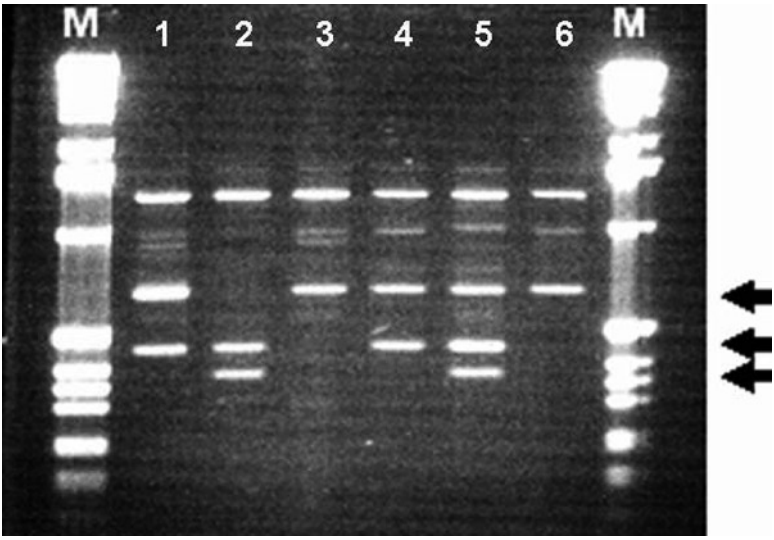

Fig. 25.1 Examples of date palm DNA fragments amplified using RAPD primer UBC-3. Ethidium bromide-stained agarose gel of amplification fragments. From lane *1* to lane *6*: Sampled Moroccan cvs. respectively, Boukhani, Bouzeggar, Boufegous ou Moussa, Outokdime, Iklane and Ahardane. M: Standard molecular weight size (λ/EcoR1/Hind III.BAP). The *arrows* indicate the discriminative markers

Table 25.4 Examples of selected ISSR primers that revealed interesting molecular markers which allowed good genetic diversity of date palm (bases of nucleotides between 3′ and 5′)

ISSR primers	Primer sequences	ISSR primers	Primer sequences
Mic 3	$(AC)_{10}$	Mic 15	$(TC)_{10}$
Mic 4	$(AGT)_5$	Mic 16	$(AG)_{12}$
Mic 5	$(ATC)_5$	Mic 17	$(GA)_{13}$
Mic 6	$(GATA)_4$	Mic 18	$(GGGA)_4$
Mic 7	$(GACA)_4$	Mic 19	$(TA)_{14}$
Mic 9	$(TGTC)_4$	Mic 21	$(CTCACA)_4$
Mic 10	$(AAC)_8$	Mic 50	$(AGG)_8$ TC
Mic 11	$(TATG)_4$	Mic 51	$(TCC)_5$ AG
Mic 12	$(AAG)_8$	Mic 52	$(ATG)_5$ AG
Mic 13	$(TTC)_8$	Mic 54	$(TCC)_5$ G
Mic 14	$(CT)_8$		

cultivars, except the importance of the black color in palm leaf bases which showed an indication of a resistant cultivar (Sedra 1990b). The results based on molecular analysis permitted the identification of some informative molecular markers that have a relationship with some descriptors of date palm; for example, width of the stalk carrying the fruits; width of the spathe; length of the thorns (spines) situated in the middle of the leaf; number of inflorescences (tiny flowers) on the spike situated in the

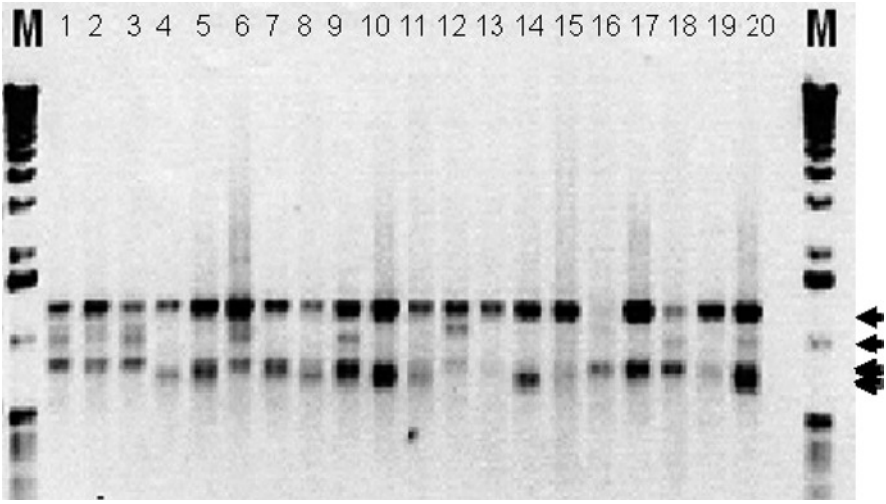

Fig. 25.2 Examples of DNA fragments amplified using ISSR primer $(CTCACA)_4$ in 20 Moroccan date palm cultivars. Ethidium bromide-stained agarose gel of amplification fragments. Lanes *1* and *20* contain fragments of sampled cultivars. M: Standard molecular weight size (λ/EcoR1/Hind III. BAP). The *arrows* indicate the discriminative markers

middle; angle made between the apical leaflets of the palm leave; date-fruit shape; color of the bases of the palm leaves (Table 25.5) (Sedra 2007c). This last case is interesting for our present studied topic. The informative molecular markers grouped with this character are m11, m21, m33 and m25 of which primer name and marker size are cited in Table 25.5.

25.3.2.3 Molecular Markers and Resistance to Bayoud Disease in Date Palm

Table 25.6 indicates the examples of molecular markers revealed by RAPD and ISSR techniques which are candidates to be associated with resistance to bayoud disease in the date palm. It appears that the markers cited have been detected in different resistant cultivars. The RAPD marker UBC-145-1.22 is present in five resistant cultivars among seven studied (Table 25.7 and Fig. 25.3). The RAPD-UBC-578-1.50 is present in 5/6 resistant cultivars. The ISSR marker Mic19-1.37 is detected in both six resistant cultivars studied (Table 25.7 and Fig. 25.4). Other markers are only detected in one or a few resistant cultivars. In results not presented here, these markers have been revealed in the majority of resistant hybrids (young plantlets) derived from crossing a resistant parent with the other susceptible parent. These markers therefore can be transmitted to the progeny. These results suggest that the resistance could be encoded by different genes.

Table 25.5 Relationship between certain informative molecular markers and certain quantitative and qualitative descriptors of date palm

Descriptors	Informative molecular markers
Quantitative descriptors	
Percentage of length of the part of the thorns (spines)	m1
Width of the spathe	m10, m25, m30
Width of the stalk carrying the dates	m35
Length of the thorn at the middle	m14, m2, m4, m14
Total number of the inflorescences or tiny flowers on middle spike	m30
Total number of the thorns	m10, m30, m25
Angle made between the apical leaflets of the palm leaf	m30, m20, m25, m10
Angle made between the thorn at the middle and the rachis	m27, m3
Qualitative descriptors	
Date shape	m24, m9, m22, m5, m12, m27, m32
Density of leave grouping	m22, m1, m15, m11, m9, m17, m21
Consistency of the leaflets of the palm	m14, m17, m21
Consistency of thorns	m11
Color of the bases of the palm leaves	m11, m21, m33, m25
The arching of the palm leaf in relation to the trunk of tree	m13, m31, m34, m11, m30, m37

Table 25.6 Examples of the sizes of some informative RAPD molecular markers

Informative molecular markers	Name and size (kb)
m11	OP-J14-0.12
m19	OP-D12-1.9
m21	OP-D12-1.58
m22	OP-J4-2.5
m24	OP-D16-0.83
m25	OP-N1-0.5
m32	OP-L6-0.86
m33	OP-M5-1.16
m34	OP-M11-1.16

The dendrogram of 14 Moroccan date-palm cultivars (seven susceptible and seven resistant cited in Table 25.1) constructed by genetic distance using 79 ISSR markers showed two groups which each contains two sub-groups (Fig. 25.5). Each sub-group comprises at least one or two resistant cultivars. This supposes that the resistance may have several sources localized in different regions. These data agree with those describing the application of RAPD molecular tools in date-palm variability analysis and previously reported (Sedra et al. 1998).

Table 25.7 Examples of molecular markers revealed by RAPD and ISSR techniques and that are candidates to be associated with resistance to bayoud disease in the date palm

Primer	Total number of revealed bands	Size of markers candidates (Kb)	Number and origin (cultivars)	
			No. markers detected/studied resistant cultivars	Name of cultivars
RAPD-OP-D16	6	1.06	3/7	Black Bousthammi, Iklane, Tadmainte
RAPD-OP-D19	6	0.10	4/7	Black Bousthammi, White Bousthammi, Boukhani, Tadmainte
RAPD-UBC-145	5	1.22	5/7	Black Bousthammi, White Bousthammi, Boufeggous ou Moussa, Boukhani, Tadmainte
RAPD-UBC-578	4	1.50	5/6	Black Bousthammi, White Bousthammi, Boufeggous ou Moussa, Tadmainte
RAPD-UBC-594	14	0.64	3/6	Iklane, Sairlayalate, Tadmainte
ISSR-Mic 19	11	1.37	6/6	Black Bousthammi, White Bousthammi, Boufeggous ou Moussa, Iklane, Sairlayalate, Tadmainte
		1.01	2/6	Black Bousthammi, Iklane

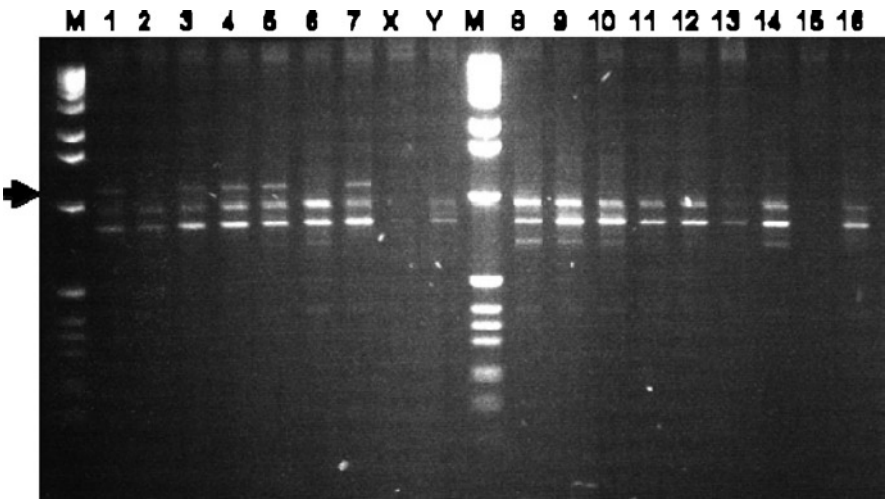

Fig. 25.3 DNA fragments amplified using RAPD-UBC-145 primer. Ethidium bromide-stained agarose gel of amplification fragments. Resistant cvs. *1* Black Bousthammi, *2* White Bousthammi, *3* Tadmainte, *4* Iklane, *5* Sairlayalate, *6* Boufegous ou Moussa, *7* Boukhani, X and Y males. Susceptible cvs. *8* Ahardane, *9* Boufegous, *10* Bourar, *11* Bouskri, *12* Deglet Noor, *13* Jihel, *14* Bouittob, *15* Mejhoul, *16* Outokdime. M: fragments of molecular weight markers (λ/EcoR1/Hind III.BAP). The *arrow* indicates the discriminative marker

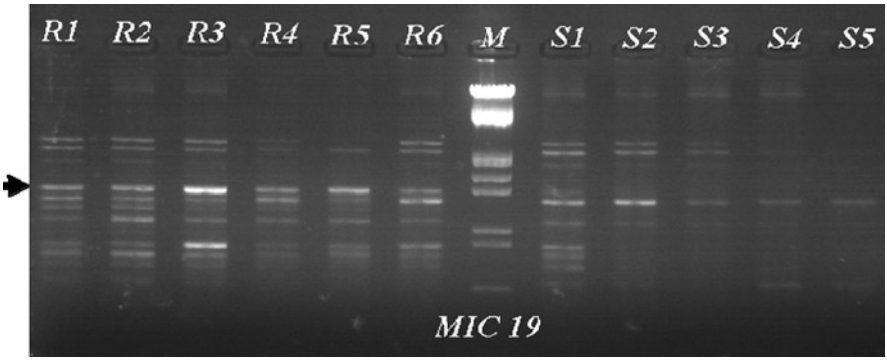

Fig. 25.4 DNA fragments amplified using ISSR Mic19. Ethidium bromide-stained agarose gel of amplification fragments. Resistant cvs.: *R1* Black Bousthammi, *R2* Iklane, *R3* Tadmainte, *R4* Sairlayalate, *R5* Boufegous ou Moussa, *R6* White Bousthammi; Susceptible cvs.: *S1* Boufegous, *S2* Oum N'hale, *S3* Jihel, *S4* Hafs, *S5* Ademou, *S6* Belhazit (non visualized). *M* fragments of molecular weight markers (λ/EcoR1/Hind III.BAP). The *arrow* indicates the discriminative marker

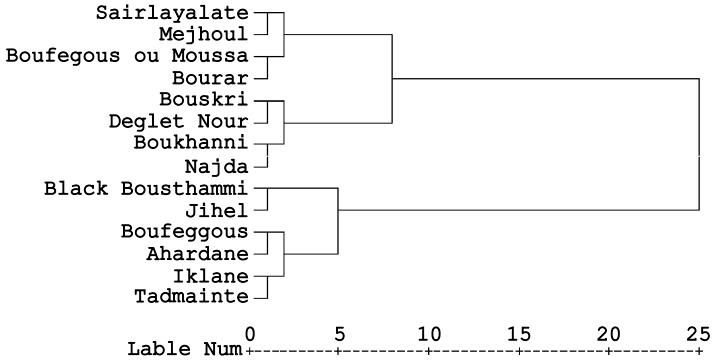

Fig. 25.5 Dendrogram of 14 Moroccan date-palm cultivars constructed by genetic distance using 79 ISSR markers. Clustering was with the UPGMA method. Resistant cvs.: Black Bousthammi, Boufegous ou Moussa, Boukhani, Iklane, Najda, Sairlayalate and Tadmainte. Susceptible cvs.: Ahardane, Boufegous, Bourar, Bouskri, Deglet Nour (Deglet Noor), Jihel and Mejhoul (Medjool)

25.4 Conclusion and Prospective

Traditional and modern genetic improvement in date palm need extended time periods and considerable funds. Therefore, they can be assisted by molecular markers that give better and more efficient research strategies. Several research results cited in this chapter show the use of molecular markers as tools to evaluate genetic diversity and genotyping of date-palm cultivars. Based on statistical analysis, Sedra (2007c) reported certain informative molecular markers which are associated with specific phonological characters in date palm. Previous study of date-palm mitochondrial DNA gave

evidence of two plasmid-like DNAs that seem to be linked to bayoud-disease resistance (Benslimane et al. 1996) but these markers cannot distinguish both cultivars studied (Trifi 2001). Each marker corresponds to one part of date palm DNA and the genome has the size estimated to 1.7 pg and it is constituted of more than 10^{12} nucleic bases. These data seem to suggest that the higher the number of markers used the greater the probability to achieve more precise results. Our research using several hundred RAPD and ISSR primers allowed identifying several markers as candidates which can distinguish partially or totally between resistant and susceptible cultivars of date palm. The difficulty and relatively weak efficiency were probably due to the nature of the genetic status of resistance. These preliminary research results open new doors to explore the use of molecular technologies in the development of a breeding program of date palm in order to rapidly select new cultivars desired by farmers, and fruit more in demand by different markets. They also may provide an area of research and a construction program of the date palm genetic map.

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