BIOLOGIA PLANTARUM 51 (1): 169-172, 2007

BRIEF COMMUNICATION

Molecular characterization of Mauritanian date palm cultivars using plasmid-like DNAs markers

A. OULD MOHAMED SALEM*[1], S. RHOUMA**, S. ZEHDI**, M. MARRAKCHI** and M. TRIFI**

*Département de Biologie, Faculté des Sciences et Techniques, Université de Nouakchott, BP 5026, Mauritania**
*Faculté des Sciences de Tunis, Campus Universitaire, 2092 El Manar Tunis, Tunisia***

Abstract

Mauritanian date palm cultivars and progenies of two controlled crosses were analyzed according to the identity of mitochondrial plasmid-like DNAs. Starting from total genomic DNA and appropriate primers, polymerase chain reaction was designed to amplify either a 373-bp or a 265-bp fragments corresponding to the S and the R-plasmid respectively. Data proved that 5 cultivars out of 10 studied have exhibited the R-plasmid suggesting their resistance to the fusariosis. The existence of intra-cultivar variability has also been revealed in the cv. Ahmar. In addition, analysis throughout progenies of two controlled crosses suggested the strict maternal transmission of the date palms' mitochondrial genome.

Additional key words: *Phoenix dactylifera* L., fusariosis, genetic inheritance, PCR.

The date palm (*Phoenix dactylifera* L.; 2n = 2x = 36), a long lived dioecious monocotyledon is cultivated in arid and semi-arid areas for food and other commercial purposes. Despite its importance, little is known about Mauritanian date palm genetic resources. There are more 300 ecotypes suggesting a highly diversified genetic patrimony (Munier 1973). However, Mauritanian date palm groves are threatened by a fusariosis caused by the *Fusarium oxysporum* f. sp. *albedinis* (FOA) (Louvet and Toutain 1981) and no cure for the disease exists (Freeman and Maymon 2000). Hence, the elaboration of research programs aiming at the date palm's genetic resources evaluation has become imperative. In this scope, many studies have recently reported and described the use of morphological (Rhouma 1994), biochemical (Baaziz and Saaidi 1988, Ould Mohamed Salem *et al.* 2001a,b) and molecular markers (Corniquel and Mercier 1994, Sedra *et al.* 1998, Ben Abdallah *et al.* 2000, Trifi *et al.* 2000, Saker *et al.* 2000, Ouenzar *et al.* 2001, Sakka *et al.* 2004, Zehdi *et al.* 2004). These are particularly useful for cultivar identifications, genetic diversity analysis and phylogenic relationships among date palm accessions. Moreover, a significant correlation between soluble peroxidases polymorphisms, cell wall phenolic compounds and mitochondrial plasmid-like DNAs and pathogen resistance/susceptibility has been reported in date palms (El Modafar and El Boustani 2000, 2001, Ouenzar *et al.* 2001, Trifi 2001).

As part of our research programs aiming at the development of resistance reliable markers in Mauritanian date palm germplasm, we have been interested in the mitochondrial genome and in particular to mini-circular plasmid-like DNAs. These have been previously described in date palms' mitochondria and considered as a reliable early diagnostic tool (Ouenzar *et al.* 2001, Trifi 2001). Indeed, it has been demonstrated that two mini-circular DNAs have been evidenced in date palms' mitochondria: the R-plasmid (1 345 bp) found in mitochondria of fusariosis-resistant cultivars and the S-plasmid (1 454 bp) revealed in those of fusariosis-sensitive cultivars. These two plasmids differ one from the other by the absence, in the R plasmid, of a 109-bp

Received 16 November 2004, *accepted* 21 February 2006.
Abbreviations: BSA - bovine serum albumin; PCR - polymerase chain reaction; PEG - polyethylene glycol.
Acknowledgements: Authors are grateful to the 'Agence Universitaire pour la Francophonie (AUF)' and the Tunisian 'Ministère de la Recherche Scientifique, de la Technologie et du Développement des Compétences (MRSTDC)' for financial support.
The two first authors have contributed equally in this work.
[1] Author for correspondence; fax: (+222) 525 39 97, e-mail: boukhary@univ-nkc.mr

DNA segment (Benslimane *et al.* 1994, 1996). Since, only one type of these two plasmids can be found in a given cultivar, this structural difference enables, using a polymerase chain reaction (PCR) approach, an easy and fast characterization of the presence of either the S or the R plasmids.

In this study, we report the search of mitochondrial plasmid DNAs investigated in local date palm ecotypes with respect to the identity of hosted plasmid-like DNA (R or S). The inheritance type of these DNAs was also reported. The possibility of developing a large scale screening procedure is discussed in order to characterize all of the Mauritanian date palm germplasm. Such investigation would be of great interest to establish improvement and breeding programs aiming at the selection of individuals displaying both good fruit quality and fusariosis resistance.

Ten samples belonging to nine local date palm cultivars distributed throughout Atar (N: 20°32' W: 13°03') and Chinguitti (N: 20°27' W: 12°22') oases were analyzed (Table 1). The dominant cultivated ecotype in Mauritania Ahmar was also sampled from the two prospected palm groves located in the Adrar region.

Table 1. Denomination, label, origin and plasmid type of the tested cultivars (cv. Ahmar was sampled from both date palm groves, letters *a* and *c* in index refer to Atar and Chinguitti oases, respectively).

Cultivar	Label	Origin	Plasmid type
Adaghd	ADG	Atar	R
$Ahmar_a$	AHM_a	Atar	R
$Ahmar_c$	AHM_c	Chinguitti	S
Bedjeire	BDR	Chinguitti	S
Enzer	ENZ	Chinguitti	S
Lemdina	LMD	Atar	S
Sel Medina	SMD	Chinguitti	S
Tamchkrert	TMC	Atar	R
Tiguidert	TGD	Atar	R
Tijeb	TJB	Atar	R

For inheritance analysis, we used the progenies of two reciprocal controlled crosses involving parents identified as female Deglet Nour × T138 pollinator and female Deglet Bey × DF1 pollinator. 20 plantlets were analyzed from each cross.

Total cellular DNA was purified according to Dellaporta *et al.* (1984) modified as followed: 1 g of young mature leaves from each adult tree was sliced into small pieces and grounded with mortar and pestle in the presence of liquid nitrogen. The obtained powder was resuspended in 7.5 cm^3 of an extraction buffer consisting of 100 mM Tris-HCl pH 8, 50 mM EDTA, 500 mM NaCl, 1 % polyethylene glycol (PEG 6000); 0.1 % bovine serum albumine (BSA). DNA content was determined using a *GeneQuant* spectrophotometer (*Pharmacia*, Saclay, France) and its integrity was electrophoretically checked by a 0.8 % agarose minigel according to Sambrook *et al.* (1989).

In order to evidence the R and S plasmids, an appropriate primers pair identified as P1 (5'-CCTTATA CCAGTCGTGCTT-3') and P2 (5'-AAGGCAGATATA ATCGGA-3') was used. These primers are complementary to a region flanking the 109-bp DNA sequence. In fact, these primers hybridize to sequences located at approximately 128 nucleotides upstream and 137 nucleotides downstream from the 109 bp sequence in the targeted DNAs and providing amplification products either of 373 bp in the case of S plasmid or 265 bp in the case of R plasmid (Fig. 1).

PCR reactions were performed under the conditions described by Trifi (2001). Total cellular DNA (20 ng) from each sample was used as template in a total volume of 0.025 cm^3 including: 0.0025 cm^3 enzyme buffer (×10), 12.5 pmol each primer, 0.1 mM each dNTPs and 0.5 unit of *Taq* DNA polymerase (*QBIOgene*, Illkirch, France). PCR reactions were carried out in a thermal cycler (*Crocodile III*, *QBIOgene*) programmed to execute the following cycling: 1 cycle of 5 min at 94 °C as preliminary denaturation step, followed by 25 cycles, each consisting of a denaturation step at 94 °C for 3 min; an annealing step at 52 °C for 1 min and an elongation step at 72 °C for 1 min. A final extension of 5 min was systematically performed at the last cycle. After amplification, the reaction mixture was loaded on a 1.5 % agarose gel, separated by electrophoresis, stained with ethidium bromide and photographed under UV light (Sambrook *et al*. 1989).

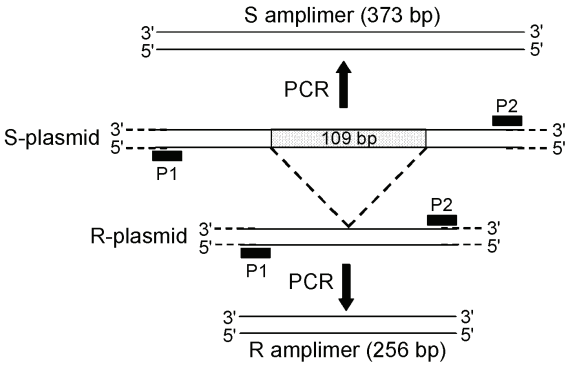

Fig. 1. Illustration of the designed strategy to amplify the region of interest in the R and S plasmid-like DNAs in mitochondria of date palm. The S plasmid gives rise to an amplimer of 373 bp while the R plasmid amplimer's corresponds to a fragment of 265 bp. The 109 bp stippled box delimits the region of the S plasmid which is not found in the R plasmid. The blackened bars refer to primers P1 and P2.

The integrity of DNAs resulting from the modified Dellaporta *et al.* (1984) protocol was shown convenient for subsequent analyses by electrophoresis (Fig. 2) since high molecular mass bands without any contamination and degradation were observed. In this case, the yields are of approximately about 60 µg per 1 g of fresh plant material. Consequently we assume that the cited protocol of Dellaporta *et al*. (1984) (the slicing of the plant

material, the addition of PEG 6000 and BSA to the extraction buffer) improved the yield and quality of DNA extracted from date palm leaves.

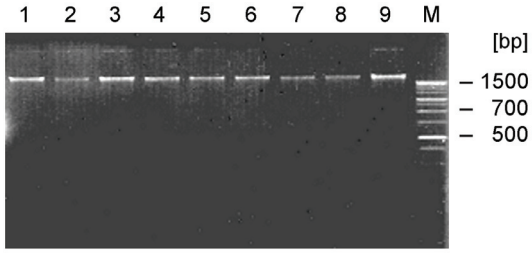

Fig. 2. Agarose gel electrophoresis of total genomic DNA from nine date palm cultivars. *1 - 9*: lanes loaded with 25 ng of DNA. *M*: 1 kb ladder size standard.

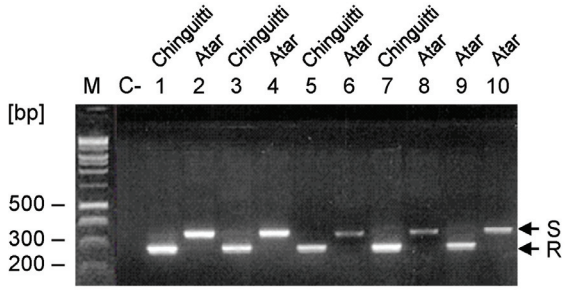

Fig. 3. Ethidium bromide 1.5 % agarose stained gel containing the 373 bp and the 265 bp fragments respectively designated S and R, amplified by PCR from total cellular DNA of the nine date palm cultivars. *Lane 1*: ENZ, *lane 2*: TGD, *lane 3*: BDR, *lane 4*: AHM*a*, *lane 5*: SMD, *lane 6*: TMC, *lane 7*: AHM*c*, *lane 8*: ADG, *lane 9*: LMD, *lane 10*: TJB (for abbreviations see Table 1); *C-*: negative control including *Taq* DNA polymerase and primers without any DNA; *M*: 1 kb ladder size standard. *Arrows* indicate the relative position of R and S plasmids.

Fig. 3 highlights the amplified products corresponding to the mini-plasmid from the date palm accessions. In fact, two banding patterns were observed since DNA bands differed in size (373 and 265 bp respectively) according to their position in agarose gels. Hence, we may assume that the mitochondrial plasmid-like DNAs constitute one of the date palms specific characteristics since they have been evidenced in Mauritanian or Moroccan and Tunisian cultivars (Ouenzar *et al.* 2001). In addition, our data proved that the Mauritanian accessions analyzed are split in two groups according to their plasmid patterns (Table 1). The first one is composed of the Chinguitti cultivars (*i.e.* Ahmar, Enzer, Bedjeire, Sel Medina) together with cv. Lemdina collected from Atar's grove. These are characterized by the S-plasmid. All the remaining cultivars exhibiting the R-plasmid are in the second group. It is worth noting that the Ahmar cultivars originated from the prospected areas have different plasmid types suggesting their discrimination despite their similar appellation. AHM_a has a product size of 373 bp (S type) and AHM_c has a product corresponding to 265 bp.

In order to determine the inheritance of the targeted DNA plasmids analysis of two controlled crosses' progenies was performed. Progenies always exhibit the maternal plasmid-like DNA. Thus, the first cross involving female of Deglet Nour cultivar with R-plasmid versus the T138 pollinator gave progenies with the R-plasmid (Fig. 4*B*), Whereas the opposite cross with Deglet Bey cultivar as female and the DF1 as the male have generated individuals with the S-plasmid (Fig. 4*A*). Consequently, we assume that a maternal transmission of the mitochondrial plasmid-like DNA is suggested in date palms.

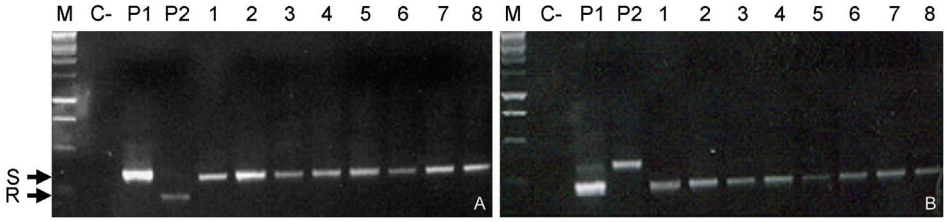

Fig. 4. Agarose gel electrophoresis of PCR products from genomic DNAs of parents (P1 and P2) and progenies (*lanes 1 - 8*) of two controlled crosses. *Panel A*. *P1*: female Deglet Bey; *P2*: male DF1. *Panel B. P1*: female Deglet Nour; *P2*: T138 pollinator. *M*: 1 kb ladder size standard ; *C-*: negative control.

The outcome of this study was to determine the reliability of date palm plasmid-like DNAs specific-PCR analyses for accurate and reliable method usable in date palm breeding programs. As a preliminary step, we have characterized a set of widely cultivated date palm cultivars collected from Mauritanian oases according to their plasmid patterns and analyzed the inheritance mode of these DNAs in date palms. Our data proved that the Mauritanian date palm cultivars studied are characterized by the exclusive presence of the S or the R-plasmid. Similar results have been reported in Moroccan and Tunisian cultivars (Ouenzar *et al.* 2001, Trifi 2001). Furthermore, these authors have demonstrated a strongly significant correlation between the hosted plasmid type and the phenotype of trees towards bayoud. Indeed, in 36 date palm cultivars analyzed by Ouenzar *et al.* (2001) and when only the S or the R plasmid was detected, the correlation was verified in 31 cases out of 35 (88 %). Comparable results were obtained by Trifi (2001) using 9 date palm cultivars. Hence, by inference to this established correlation, we may assume that Adaghd, Ahmar from Atar, Tamchkrert, Tiguidert and Tijeb cultivars exhibiting the R-plasmid are fusariosis resistant. While, Enzer, Ahmar from Chinguitti, Bedjeire, Lemdina

and Sel Medina characterized by the S-plasmid are fusariosis sensitive. In addition, since date palm is clonally propagated throughout offshoots our data proved differentiation between Ahmar cultivars sampled from Atar and from Chinguitti. In fact, according to their similar appellation, only one type of plasmid DNA would be evidenced in their mitochondria. Surprisingly, these accessions have exhibited different plasmid-like DNA patterns and therefore do not have the same material lineage. It is worth noting that only farmers' observations based on morphological and fruit parameters are taken in account in date palm cultivars' appellation. A high degree of fruit characteristics similarity is considered in the denomination of the Ahmar cultivars studied despite their differentiation according to the plasmid patterns.

On the other hand, the inheritance mode of mitochondrial plasmid-like DNA seems to be scrupulously of maternal type suggesting that these DNAs constitute an efficient marker reliable in the extrachromosomic inheritance analyses. The maternal heredity of mitochondrial genome has been reported in other species such as maize (Conde *et al.* 1979) and kiwi fruit (Chat *et al.* 1999). Moreover, results obtained with the reciprocal crosses indicate the stability of these extranuclear markers in date palm and consequently its potential use as an early and easy tool to molecularly assist the selection of clones displaying either fruit quality or fusariosis resistance.

References

Baaziz, M., Saadi, M.: Preliminary identification of date palm cultivars by esterases isoenzymes and peroxidase activities. - Can. J. Bot. **66**: 89-93, 1988.

Ben Abdallah, A., Stitti, K., Lepoivre, P., Du Jardin, P.: [Identification of date palm (*Phoenix dactylifera* L.) cultivars using randomly amplified DNA (RAPD)]. - Cahiers Agr. **9**: 103-107, 2000. [In French]

Benslimane, A.A., Hartmann, C., Ouenzar, B., Rode, A.: Intramolecular recombination of a mitochondrial minicircular plasmid-like DNA of date palm mediated by a set of short direct repeat sequences. - Curr. Genet. **29**: 591-593, 1996.

Benslimane, A.A., Rode, A., Quétier, F., Hartmann, C.: Characterization of two minicircular plasmid-like DNAs isolated from date palm mitochondria. - Curr. Genet. **26**: 535-541, 1994.

Chat, J., Chalak, L., Petit, R.J.: Strict paternal inheritance of chloroplastic DNA and maternal inheritance of mitochondrial DNA in intraspecific crosses of kiwi fruit. - Theor. appl. Genet. **99**: 314-322, 1999.

Conde, M.F., Pring, D.R., Leving, C.S.: Maternal inheritance of organelle DNAs in *Zea mays-Zea perennis* reciprocal crosses. - J. Hered. **70**: 2-4, 1979.

Corniquel, B., Mercier, L.: Date palm (*Phoenix dactylifera* L.) cultivar identification by RFLP and RAPD. - Plant Sci. **101**: 163-172, 1994

Dellaporta, S.L., Wood, J., Hicks, J.B.: A plant DNA preparation, version II. - Plant mol. Biol. Rep. **4**: 19-21, 1984.

El Modafar, C., El Boustani, E.: Relationship between cell wall susceptibility to cellulases and pectinases of *Fusarium oxysporum* and susceptibility of date palm cultivars. - Biol. Plant. **43**: 571-576, 2000.

El Modafar, C., El Boustani, E.: Cell wall-bound phenolic acid and lignin contents in date palm as related to its resistance to *Fusarium oxysporum*. - Biol. Plant. **44**: 125-130, 2001.

Freeman, S., Maymon, M.: Reliable detection of the fungal pathogen *Fusarium oxysporum* f. sp. *albedinis*, causal agent of bayoud disease of date palm, using molecular techniques. - Phytoparasitica **28**: 341-348, 2000.

Louvet, J., Toutain, G.: Bayoud. *Fusarium* wilt of date palm. - In: Nelson, P.E., Toussoun, T.A., Cook, R.J. (ed.): *Fusarium* Diseases, Biology and Taxonomy. Pp. 13-20. Pensylvania State University Press, University Park 1981.

Munier, P.: Le Palmier Dattier. [The Date Palm]. – Maisonneuve & Larose, Paris 1973. [In French.]

Ouenzar, B., Trifi, M., Bouachrine, B., Hartmann, C., Marrakchi, M., Benslimane, A.A., Rode, A.: A mitochondrial molecular marker of resistance to bayoud disease in date palm. - Theor. appl. Genet. **103**: 366-370, 2001.

Ould Mohamed Salem, A., Trifi, M., Sakka, H., Rhouma, A., Marrakchi, M.: Genetic inheritance analysis of four enzymes in date palm (*Phoenix dactylifera* L.). - Genet. Resour. Crop Evol. **48**: 361-368, 2001a.

Ould Mohamed Salem, A., Trifi, M., Salhi-Hannachi, A., Rhouma, A., Marrakchi, M.: Genetic variability analysis of Tunisian date palm (*Phoenix dactylifera* L.) cultivars. - J. Genet. Breed. **55**: 269-278, 2001b.

Rhouma, A.: Le Palmier Dattier en Tunisie: I. Le Patrimoine Génétique. [The Date Palm in Tunisia. I: Genetic Patrimony.] - INRAT, Arabesque 1994. [In French.]

Saker, M.M., Bekheet, S.A., Taha, H.S., Fahmy, A.S., Moursy, H.A.: Detection of somaclonal variations in tissue culture-derived date palm plants using isoenzyme analysis and RAPD fingerprints. - Biol. Plant. **43**: 347-351, 2000.

Sakka, H., Zehdi, S., Ould Mohamed Salem, A., Rhouma, A., Marrakchi, M., Trifi, M.: Genetic polymorphism of plastid DNA in Tunisian date palm germplasm (*Phoenix dactylifera* L.) detected with PCR-RFLP. - Genet. Res. Crop Evol. **51**: 479-487, 2004.

Sambrook, J., Fritsch, E.F., Maniatis, T.: Molecular Cloning: A Laboratory Manual. 2nd Ed. - Cold Spring Harbor Press, New York 1989.

Sedra, M.H., Lashermes, P., Trouslot, P., Combes, M.C., Hamon, S.: Identification and genetic diversity analysis of date palm (*Phoenix dactylifera* L.) varieties from Morocco using RAPD markers. - Euphytica **103**: 75-82, 1998.

Trifi, M., Rhouma, A., Marrakchi, M.: Phylogenetic relationships in Tunisian date palm (*Phoenix dactylifera* L.) germplasm collection using DNA amplification fingerprinting. - Agronomie **20**: 665-671, 2000.

Trifi, M.: [Polymorphism and molecular typing of Tunisian date palm (*Phoenix dactylifera* L.) varieties: relation with resistance towards bayoud.] - Thèse de Doctorat d'Etat ès-Sciences Naturelles, Université de Tunis El Manar, Faculté des Sciences, Tunis 2001. [In French.]

Zehdi, S., Sakka, H., Rhouma, A., Ould Mohamed Salem, A., Marrakchi, M., Trifi, M.: Analysis of Tunisian date palm germplasm using simple sequence repeat primers. - Afr. J. Biotechnol. **3**: 215-219, 2004.