# A MOLECULAR MARKER OF DATE-PALM (*Phoenix dactylifera* L) RESISTANCE TO BAYOUD DISEASE

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## ABSTRACT

Date-palm is one of the most important domesticated crops in the North African and the near East countries. However, date-palm plantations have been currently in danger to be completely destroyed by a vascular fusariosis (Bayoud disease) caused by the *Fusarium oxysporum* fsp *albedinis* fungus. Up today, Tunisian date-palm groves appear to be spared but for several decades, they are seriously menaced by the plague. Hence, the search of early molecular markers associated with the disease has become imperative. In this scope, two mitochondrial plasmid-like DNAs have been identified as potential markers of resistance to the bayoud disease. Here, starting from a set of Tunisian date-palm varieties, we report the availability of these molecules as reliable markers of the resistance to the plague and, how the use of PCR technology should allow a rapid and efficient diagnosis approach for identification of selected bayoud resistant individuals.

**Additional Index words**: Date-palm, Bayoud disease resistance, molecular marker

## INTRODUCTION

The date-palm is one of the most important cultivated crop in the North African marginal areas. Its utilization consists of many ecotypes clonally reproduced for their fruit quality. For instance 250 cultivars have been inspected in Tunisian oases (Rhouma, 1994) where more than 10 % of Tunisians depend on date-palm culture (Trifi, 2001). In addition, this crop is of a great socio-economic importance: first, date-palm contributes to the oases environmental stability, second is cultivated either for fruit production or many other purposes, and third it constitutes the main financial and oasiens nutritious resources. However, for several decades, this crop has been in serious danger of being completely destroyed by a vascular fusariosis locally called “Bayoud disease”. Caused by the imperfect filamentous fungus *Fusarium oxysporum* fsp *albedinis* and originated from the Moroccan plantations, bayoud disease has destroyed

several millions of Moroccan and Algerian date-palm trees (Haddouch, 1996). It is noteworthy that, up to day, Tunisian groves are speared but they are continuously threatened by this plague due to its rapid propagation into the eastward. Hence, the elaboration of a preventive strategy is imperative in order to preserve this important cultivated crop. In this scope, many markers that are correlated to the trees resistance have been reported such as isozymes (Baaziz *et al.*, 1990, Bennaceur *et al.*, 1991, Bendiab *et al.*, 1993), polyphenolics (El Hadrami *et al.*, 1996, El Idrissi-Tourane *et al.*, 1996) and mitochondrial plasmid-like DNAs (Benslimane *et al.*, 1994, Trifi *et al.*, 1997). However, the correlation between the date-palm phenotype and the described marker has not been clearly established.

As a part of our work, we became interested in the search of early molecular marker associated with the date-palm bayoud resistance. Such markers would be suitable in the rapid screening of either the field growing date-palm trees or issued from the micropropagation throughout the *in vitro* culture methods

Here, we describe how the date-palm mitochondrial plasmids constitute potential molecular markers associated with the bayoud resistance in this crop and how the polymerase chain reaction (PCR) technology allowed a powerful approach suitable in the rapid screening of selected bayoud resistant individuals.

## MATERIAL AND METHODS

### Plant material

Nine Tunisian date-palm varieties that are tested in the infested Moroccan plantation of Errachidia were used as a starting set in this study. These varieties are listed in table 1 and constitute the genotypes, mainly cultivated in Tunisian oases. The “Centre de Recherches Phoénicicoles, INRAT, Degache” kindly provided the plant material (young leaves).

### DNA purification

Total cellular DNA was extracted according to Dellaporta *et al.* (1984) method. After purification, DNA was quantified using a spectrophotometer and its integrity was determined after agarose gel electrophoresis according to Sambrook *et al* (1989).

**Primers and PCR assay**

Appropriate primers that are flanking the deleted sequence of 109 bp (Figure 1) and described by Bouachrine (1997) and Trifi (2001) were used to amplify DNA stretches. These, correspond to the two mitochondrial plasmid-like DNAs (called S and R plasmids) characterized by Benslimane (1995).

For PCR, a 25 µl reaction mixture was used containing: 0.5 µg of total cellular DNA (1 µl), 50 pM (1 µl) each primer, 2.5 µl Taq DNA polymerase standard reaction buffer, 1.5 U (0.3 µl) Taq DNA polymerase and 200 mM each dNTP (dATP, dTTP, dCTP and dGTP). The reaction mixture was overlaid with 25 µl of mineral oil to avoid evaporation during the cycling heating. PCR was then carried out in a DNA thermocycler program was as the follows a delayed step of 5 minutes denaturation at 94 °C before entering 30 cycles PCR procedure of 30 seconds at 94 °C, 1 minute at 48 °C and 1 minute at 72 °C, and final extension of 10 minutes at 72 °C. Including standard controls ensured standardization between enzyme batches and experiments. These consisted of reaction mixtures without any DNA or any enzyme and reaction mixture including the S/R recombinant DNAs characterized by Benslimane (1995).

Amplified products were electrophoresed on 1.4 % agarose gel in 0.5 TBE running buffer and detected after staining with ethidium bromide (Sambrook *et al*., 1989).

## RESULTS

Previous study of the date-palm mitochondrial DNA had evidenced two plasmid-like DNAs called the S and R plasmids that are of 1454 bp and 1345 bp respectively (Benslimane *et al*., 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 varieties: the first one is bayoud susceptible and contained the S plasmid, and the second that contains the R plasmid is bayoud resistant. This suggested that S and R DNAs could be correlated to date-palm susceptibility/resistance against the fusariosis. Our investigations were therefore developed by extending a similar study to a large number of Tunisian date-palm varieties in order to obtain a deeper insight of the relationship that exists between these plasmids and the bayoud tree's phenotype (susceptibility/resistance). As a first step, we have designed the PCR amplification process as an efficient method for rapid screening of S and R DNAs. Thus appropriate oligonucleotide

primers that are flanking the 109-bp sequence were employed to generate subfragments corresponding to each plasmid present in the tree's mitochondria (Figure 2). A 373 bp fragment is generated when the S plasmid is used as matrix, while a 265 bp fragment is amplified when the R plasmid is present in the mixture. In this case, either recombinant S and R DNA plasmids or total cellular DNA of anonymous varieties were tested as tern plats. Thus it may be assumed that the identified plasmids are present in the mitochondria of date-palm trees and constituted one of characteristics in this crop.

On the other hand, as a second research step, we have extended this technology in the evidencing of such DNAs starting from total cellular DNA purified from the Tunisian date-palm varieties. The generated plasmid amplified patterns are reported in figure 3. As expected, results exhibit that all the nine tested varieties involve mitochondrial DNA plasmids. Whereas, the banding profiles led to identify three different clusters. The first one involves varieties that have an amplified product corresponding to the S plasmid. These are the following: Boufagous, Ftimi, Khou Ftimi, Kenta, Kintichi, Goundi and Besser Hlou. The second one composed of Deglet Nour variety in which the R plasmid is detected. Surprisingly, in the third cluster including Horra variety, both of S and R plasmids have been revealed. It is noticeable that according to Saaidi (1992) and Sedra (1992), the implied varieties' response to bayoud disease was known namely "susceptible". Thus, the present analysis based on the detection of the mitochondrial plasmids agrees with the date-palm varieties' phenotype against the fusariosis for 7 out of 9 tested (77 %). In these cases only the S plasmid has been detected. However, in the remaining ones that constitute deviations the plasmid patterns consisted of only R or both of S and R molecules.

## DISCUSSION

The use of the molecular methods made possible the molecular characterization of Tunisian date-palm varieties as a short step and to the evidencing of early markers associated to bayoud disease. fortunately, our data provide evidence of date-palm mitochondrial plasmid polymorphism with respect to the bayoud-phenotype varieties. In fact, the relationship is verified in 77 % (7/9). Intriguingly, in the two remaining varieties we have detected the R plasmid or both of S and R plasmids in spite of their reported bayoud-susceptible trait. This feature could be justified by interrelations involving nuclear and mitochondrial genomes. In this case, at least two nuclear genes could be forwarded to explain the particular

phenotype of the observed deviations. These genes encode the bayoud-resistance and the plasmids-recombination, respectively. This consideration is strongly supported: first, the mitochondrial plasmids arise by recombination events are controlled by the nuclear genome (Flamand *et al*., 1993), and second a multi-gene control of date-palm bayoud-resistance/susceptibility is suggested by Sedra *et al*. (1998). Thus, our results favour the occurrence of a strong correlation between the nature of the plasmid present and the date-palms' bayoud-susceptibility/resistance. In addition, the PCR approach allow a simple, reliable and rapid method for a large scale either of naturally or *in vitro* propagated individuals screening. Since effectiveness of the availability of the presumed marker, it is obvious that our result should allow efficient selection of genotypes exhibiting both bayoud-resistance and high fruit quality.

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**Table 1.** Tunisan date-palm varieties tested in the study. ([1] : nomenclature according to Rhouma 1994; [2] : phenotype according to Saaidi 1992 and Sedra, 1992)

| Variety[1] | Label | Origin | Quality | Phenotype[2] | Plasmid |
|---|---|---|---|---|---|
| Ftimi | 1 | Djerid | Very good | Susceptible | S |
| Khou Ftimi | 2 | Djerid | Good | Susceptible | S |
| Kenta | 3 | Djerid | Very good | Susceptible | S |
| Deglet Nour | 4 | Degache | Excellent | Susceptible | R |
| Kintichi | 5 | Djerid | Appreciated | Susceptible | S |
| Boufagous | 6 | Tozeur | Very good | Susceptible | S |
| Goundi | 7 | Tozeur | Very good | Susceptible | S |
| Horra | 8 | Djerid | Aromatic | Susceptible | R/S |
| Besser Hlou | 9 | Degache | Good | Susceptible | S |

**Figure 1**: Linear alignment of S and R plasmids illustration of the designed primers used to amplify PCR products corresponding to each plasmid. Direct sequence repeats (▭); primer 1 (⇨); primer 2 (⇦)

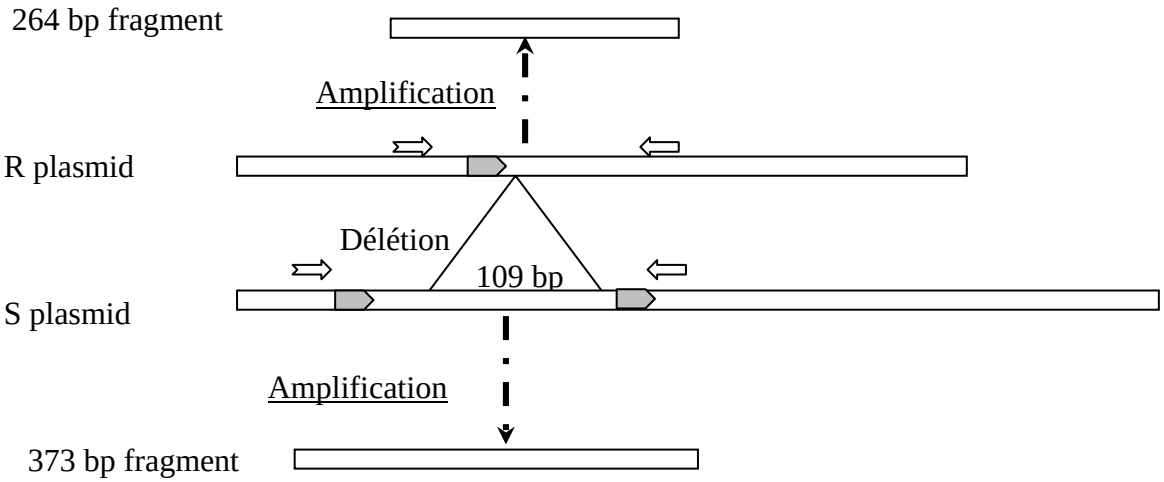

**Figure 2.** PCR process applied with the designed primers displaying the amplified products. M: Standard molecular weight size (1 kb ladder); C1 and C2: controls included, R and S: standard controls using recombinant DNAs; V1 and V2: anonymous varieties

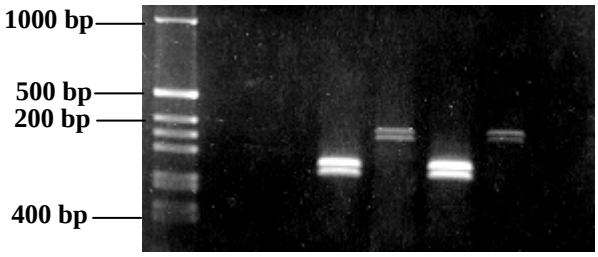


**Figure 3.** PCR plasmid-banding profiles of the implied Tunisian date-palm varieties. M: Standard molecular weight size (1 kb ladder); C: control included, R and S: standard controls using recombinant DNAs; lanes 1 to 9: varieties described in table 1.