



Molecular characterization of Algerian date palm cultivars using circular plasmid-like DNAs

A. Guettouchi*, N. Haider¹, I. Nabulsi¹ and N. Ykhlef²

Department of sciences of nature and life, Faculty of the sciences, University Mohamed Boudiaf, M'sila 28000, Algeria; ¹Departement of Molecular biology and biotechnology, AECS, Damascus P.O. Box 6091, Syria; ²Laboratory of Genetics, Biochemistry and vegetal Biotechnologies, Faculty of the sciences, University Mentouri, Constantine, Algeria

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Abstract

Bayoud disease of the date palm (*Fusarium oxysporum* f. sp. *albedinis*) is a vascular disease caused by a soil fungus. Twenty one Algerian date palm cultivars (20 females and 1 male) in addition to 6 genotypes of Deglet Nour were assessed for resistance (R) and susceptibility (S) to Bayoud disease using molecular markers. Specifically, the use of two circular plasmid-like DNAs (R and S plasmids) as molecular markers allowed us to detect the R cultivars. Among the 21 cultivars, we found that Baâret Ijmal is resistant.

Key words: Date palm; Bayoud, *Fusarium oxysporum* f. sp. *Albedinis*, Deglet Nour, Baâret Ijmal.

The most lethal disease affecting the North African date palm is Bayoud (*Fusarium oxysporum* f. sp. *Albedinis*), a vascular disease caused by the soil fungus *Fusarium oxysporum*. This disease attacks both mature and young palms in addition to the offshoots at their base (Saaidi 1979). According to Bensliman et al. (1994), Trifi et al. (1997) and Haider and Nabulsi (2012), the mitochondria of the date palm shelter circular plasmid-like DNAs that potentially constitute markers associated with Bayoud resistance or susceptibility. These plasmids are called R (resistant to fusariose) and S (susceptible to fusariose). Notably, it is possible to generate amplicons corresponding to every type of plasmid that is present from a sample of the total cellular DNA from the date

palm (Trifi et al. 1997). Thus, the objectives of this work were to identify resistant varieties of date palm using the molecular markers associated with Bayoud resistance.

A set of 21 beyond susceptible Algerian date palm cultivars namely, Bousaadiya (C), Deglet Nour 1 (E)^(1,2), Horra (G)², Halwa (E)² (G)¹, Rotbaya (G), Nakhlet ziane (E)², Ghars (E)¹, (G)², (A)³, Mech degla (E)^(1,2), Sennin Meftah (C), Mekarkcha(C), Kahlaya(C), Kawkawa (C), Nebgaya (C), Dfar Igat (C)¹, (G)², Helib lbel (C), Baârit djemal (C), Makkiya (A)³, Dokare (Male), Khadraya (G)², (A)³, Elmalha (C) and Hamraya (E to G)², (Table 1) and six genotypes of Deglet Nour collected from different stations viz., (Deglet Nour 1, Deglet Nour 2, Deglet Nour 3, Deglet Nour 4, Deglet Nour 5 and Deglet-Nour 6) were analyzed. (A=Aromatic, C=Common, E=Excellent, G=Good). The quality of the genotypes was considered as per the criteria given by Belguedj (2002); Hanachi et al. (1988); Belguedj and Tirichine (2011) whereas, the susceptibility of the varieties was determined by Brac de la Perrière et Benkhalifa (1991).

Total cellular DNA was extracted from frozen young leaves of adult trees according to Dorokhov and Klocke (1997) with minor modifications.

PCR was performed using two primers designed by Quenzar et al. (2001). Each reaction contained 10x

*Corresponding author's e-mail: guettouchi-ah@live.fr

PCR buffer (Eurobio), 10x MgCl₂ (50 mM) (Eurobio) and the forward primer (5' CCTTATACCAGTCGTGCTT 3', 50 μM) (Invitrogen), the reverse primer (5' AAGGCAGATATAATCGGA 3', 50 μM) (Invitrogen).

This study is based on the work of Benslimane *et al.* 1994 and 1996, in which date palm mitochondrial DNA was revealed to possess two plasmid-like DNAs called the S and R plasmids. The aim of our work was to screen 21 Algerian date palm cultivars for the presence or absence of either of the two circular plasmid-like S and R DNAs in the mitochondria of these cultivars as potential molecular markers of Bayoud disease resistance. The study was also repeated with 6 genotypes of Deglet Nour. A 373 bp fragment was generated from the S plasmid, while a 265 bp fragment was amplified from the R plasmid.

The analysis of the PCR plasmid-banding profile (Fig. 1) revealed effective discrimination of all of the

which a 373 bp fragment was generated. The second was characterized by mitochondria that possessed the R plasmid (resistant) and included the cultivars Deglet Nour 2, Deglet Nour 3, Deglet Nour 4, and Deglet Nour 5. A 265 bp fragment was amplified when the R plasmid was present.

In our study, we found that of the 21 varieties, 20 were susceptible, and only Baàrit djemal possessed mitochondria containing the R plasmid. However, the Deglet Nour variety possessed two S plasmids (373 bp fragment) in addition to an R (resistant) (265 bp fragment) plasmid and thus has the potential to be either susceptible or resistant.

There are two different genetic mechanisms of disease resistance. Monogenic resistance is based upon single genes. Flor (1942) formulated the gene-for-gene hypothesis, which stated that for each resistance gene in the host, there is a corresponding gene for avirulence in the pathogen that confers

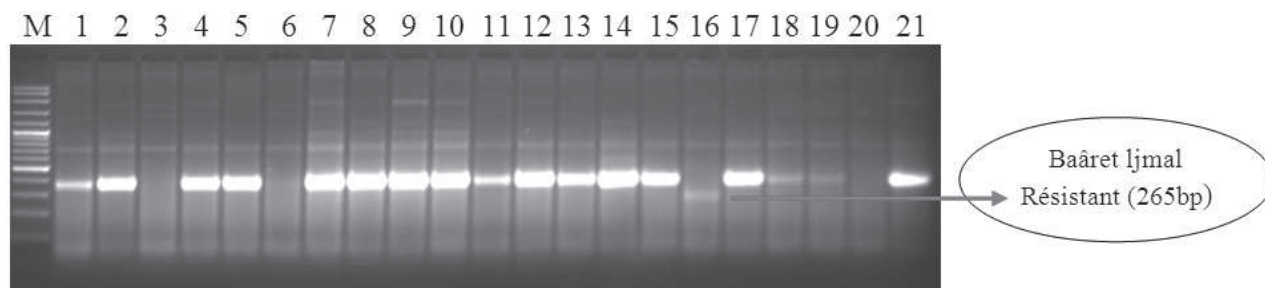


Fig. 1. PCR plasmid-banding profile distinguishing the resistant and susceptible cultivars among 21 Algerian date palm cultivars. M=Standard molecular weight size (1 kb ladder)

cultivars. The 21 varieties presented several different plasmid profiles, which were grouped into two classes as follows: The first class, which possessed the S plasmid (susceptible), from which a 373 bp fragment was generated, included Bou-Saâdiya, Deglet Nour 1, Horra, Halwa, Rotbaya, Nakhlet ziane, Ghars, Mech degla, Sennin Meftah, Mekarkcha, Kahlaya, Kawkawiya, Nebgaya, Dfar Igat, Helib Ibel, Makkiya, Dokare, Khadraya, Elmalha, and Hamraya (1-21).

The second, which included the Baàrit djemal variety, was characterized by the presence of the R plasmid, from which a 265 bp fragment was amplified.

The analysis of Figure 02 indicates that the 6 Deglet Nour genotypes displayed two different plasmid profiles, which were grouped into 2 classes: The first included the genotypes Deglet Nour 1 and Deglet Nour 6, whose mitochondria contained the S plasmid, from

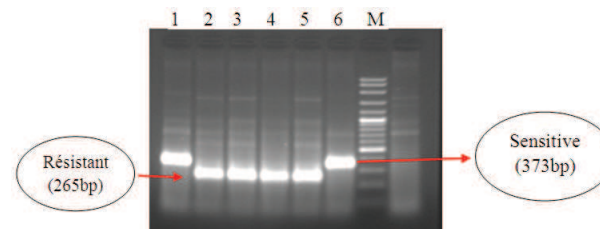


Fig. 2. PCR plasmid-banding Profile distinguishing the resistant and susceptible genotypes among 06 Algerian genotypes of Deglet-Nour to the Bayoud. M=Standard molecular weight size (1 kb ladder)

resistance and viceversa (Slusarenko *et al.* 2000). The second mechanism of disease resistance is dependent on two or more genes.

The resistance of the date palm to Bayoud does not appear to occur by a simple mechanism but is likely to be under the control of multiple genes, many of which are activated during pathogenesis. Several genes are strongly associated with cultivar resistance, including a number of nuclear genes. Additionally, the composition of the cytoplasmic environment plays a role in resistance.

Thus, this technique enables the establishment of a preliminary list of Algerian date palm cultivars that are resistant or susceptible to Bayoud disease. More studies are necessary to fully understand all of the underlying defense mechanisms and all of the genes that contribute to resistance.

Authors' contribution

Conceptualization of research (NH); Designing of the experiments (AG, IN); Contribution of experimental materials (AG, IN); Execution of field/lab experiments and data collection (AG, IN); Analysis of data and interpretation (AG, IN); Preparation of manuscript (AG).

Declaration

The authors declare no conflict of interest.

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