STUDIES ABOUT A NEW DISEASE OF DATE PALM IN TUNISIA

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ABSTRACT

In date palms "the brittle leaf" is a new disease one, which has appeared in Tunisian date palm groves. It is spreading in the Nefta and Tozeur oases. It is also found at a low incidence in Kebili oases. Up to day, the causal agent is still unknown. In order to clarify this disease our experiments were performed starting from healthy and diseased leaf palm collected from Nefta. The sampled accessions were analysed either by R-PAGE (Return Polyacrylamide Gel Electrophoresis) or by S-PAGE (Sequential Polyacrylamide Gel Electrophoresis). The results show the presence of a small RNA band in diseased palms compared to healthy controls where no band is obtained. This RNA was only detectable at a low level in old leaves collected from symptomatic plants. Properties (Size and mobility in S-PAGE analysis) of this RNA were similar to those of viroids.

Additional Index Words: Brittle leaf disease - RNA- S-PAGE –R-PAGE - Viroids

INTRODUCTION

In Tunisia, date palms production is considerably affected by many diseases (Djerbi, 1988). The brittle leaf disease causes the death of the infected palm trees (fig.1). It was observed for the first time in 1988 by Tarkouni et al. in the oasis of Nefta. This disease infects bath adult and young trees. It induces a particular yellowing, a colour similar to that of olive oil (fig. 2). The leaflet becomes translucent and their cells loose the turgescence, this makes the leaflet easily breakable and that is why the disease was first named Broken leaf disease (Takrouni et al., 1988). Diseased trees showed reduction of the number of branches as the disease progress and never reach the maturity.

It should be noted that the decision of the authority to eradicate infected trees has an effect to slow up the propagation of the disease.

The causal agent of the disease is not yet defined. Since no viruses have been detected on diseased trees, a virus like class of pathogens is suspected.

Viroids are an independent class of plant pathogens. They are single-stranded covalently closed circular RNA molecules. Their genomes range from 246 nucleotides in *Avocado Sun Blotch Viroid* (ASBVd) (Symons, 1981) to 399 in *Chrysanthemum Chlorotic Mottle Viroid* (ChCMVd) (Navarro and Flores, 1997). They are not known to encode any proteins; hence they must rely on host enzymes for their biological function (Symons, 1997 Wan Chow Wash and Symons, 1997). Viroids cause serious diseases in economically important crops (Potato, Tomato…), fruit trees (citrus, apple, peach, grape, coconut…) and ornamental plants.

The objective of this work is to identify if the causal agent of the "Brittle leaf disease" of date palms is a viroid. Two specific techniques for viroid detection: R-PAGE (Return Polyacrylamide Gel Electrophoresis) and S-PAGE (Sequential Polyacrylamide Gel Electrophoresis) were used.

MATERIALS AND METHODS

Materials

Infected Date palms leaf samples were collected from the oasis of Nefta, Tozeur and Kebili (fig 3). The samples were collected from either young or old trees. Some samples were also collected from meristem. Healthy controls were collected from the germplasm of Degeche. Collected materials were stored at – 20°C until use.

RNA Extraction

Nucleic acids were extracted as reported by Semancik (1986). The total nucleic acids were extracted by phenol followed by an adsorption on cellulose (35 %). The adsorbed RNA were eluted by washing the cellulose and finally ethanol precipitated.

Extraction products were analysed by R-PAGE (Return Polyacrylamide Gel Electrophoresis) and S-PAGE (Sequential Polyacrylamide Gel Electrophoresis).

R-PAGE (Return Polyacrylamide Gel Electrophoresis)

Return Polyacrylamide Gel Electrophoresis was carried out as described by Schumacher et al (1986).

15 µl of each nucleic acids sample containing the dyes bromophenol, xylene cyanol and 40 % glycerol was applied on each slot of slab gel (16 x 14 x 0,15 Cm). First, electrophoresis was carried out for 2.5 hours at 46 mA, then the running buffer was replaced with a heated (70°C) solution with low salt (1:8 dilution of high salt running buffer) and electrophoresis was carried out in reverse direction for another 2.5 hours at 46 mA. Then, the gel was stained with ethidium bromide.

S-PAGE (Sequential Polyacrylamide Gel Electrophoresis)

Sequential Polyacrylamide Gel Electrophoresis was carried out as described by Flores (1986). This technique consists first, electrophoresis of extraction products on a native polyacrylamide gel (5%). When the dye bromophenol reaches the bottom of the gel, staining with ethidium bromide is performed. Second, the viroid zone delimited by two marker corresponding to the smallest known viroid and one of the greatest viroid respectively, *Avocado Sun Blotch Viroid* (ASBVd) 246 nt and *Citrus Exocortis Viroid* (CEVd) 375nt, is cut. Then placed on the top of a denaturant gel (8M Urea). After the second running, the gel was stained with silver nitrate.

RESULTS AND DISCUSSION

The survey carried on several palm date plantations around Tozeur, Nefta and Kebili for three successive years (1998 - 1999 - 2000) confirm that the disease is spreading in the Tozeur and Nefta regions where it was initially identified. The disease is also present in low incidence in the Kebili area where many new plantations have been recently established.

The results of R-PAGE and S-PAGE analysis, fig. 4 and fig. 5 respectively showed the presence of a small RNA band in diseased palms that was absent in healthy controls. This RNA was only detectable in old leaves collected from symptomatic plants (table 1). For some samples, this RNA was only detected by S-PAGE, because of its low concentration.

Table 1 : Identification of small RNA in date palm samples collected in October 1998.

Sample	Sampling description	Symptom	Circular RNA
Deglet Nour	4-5 years old.	+	+
Besser	4-5 years old.	+	+
Besser	4-5 years old.	-	-
Besser	4-5 years old. (Meristems)	+	-
Deglet Nour	4-8 years old.	+	+
Deglet Nour	2-3 years old	-	-
Kinta	6-7 years old	+	+
Tozeur	20 years old.	+	+
pollinator	20 years old (old leaves)	+	+
Pollinator	4-5 years old	-	-
Pollinator	8-9 years old	-	-
Tozeur	2-3 years old (Meristem)	-	-
Tazerzit	6-8 years old (Palms)	+	+
Tazerzit	4-5 years old (Mersitem)	-	-

The results obtained with R-PAGE and S-PAGE studies indicated that the date palm RNA band properties of either a circular single strand RNA or a double strand linear RNA of viroid RNA molecules. Viroids are potential candidate since they are small single-stranded circular RNA molecules (246-399 nt) that infect higher plants, causing diseases in crop species and resulting in important economic losses in the agricultural industry. These results are confirmed by the analysis of the samples collected in October 1999 (Table 2)

Table 2: Identification of a small RNA in date palm samples collected in October 1999.

Sample	Cultivars	Symptoms	Circular RNA
1	Khouat Alig	+	+
2	Deglet Nour	+	+
3	Besser	+	+
4	Besser	+	+
5	Alig	+	+
6	Tazerzit	+	+
7	Deglet Nour	+	+
8	pollinisator	+	+
9	pollinator	+	+
10	Khouat Alig	-	-
11	Alig	-	-
12	Tazerzit	-	-
13	Deglet Nour	-	-

A similar study based on R-PAGE and S-PAGE detection were carried on leaf samples collected in January 2000. Moreover, healthy controls from the same cultivars collected from germplasm maintained at Degache were submitted to this study, surprisingly no RNA band was identified.

It must be noted that the survey of January 2000 coincided with a cold winter in Tunisia. Since it has been well established that viroids replicate and accumulate at elevated temperatures (around and above 30°C), failure to detect the viroid – like RNA in the samples collected during the cold winter was compatible with our results.

CONCLUSION

Since date palms production records great losses as a consequence of many pathogens, we tried to identify by R-PAGE and S-PAGE techniques a potential causal agent of the brittle leaf disease.

The results available now indicate that a small RNA associated with diseased date palms are either a single strand circular or a double stranded linear RNA. Their mobility in s-PAGE and R-PAGE analysis indicates that these RNA has unusual conformation, but we can not confirm the involvement of a viroid molecule as the causal agent of disease.

The pattern of spread of the disease clearly indicated that an infectious agent was involved and therefore an eradication action should be undertaken as soon as possible.

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Fig 1 Symptoms of the brittle leaf disease on trees

Fig 2 Symptoms of the brittle leaf disease on palms

Tozeur

Nefta

kebili

Fig 4 Results of the R-PAGE showing a band in track 1 which present size and mobility similar to those of the CEVd *Citrus Exocortis Viroid* in track 2

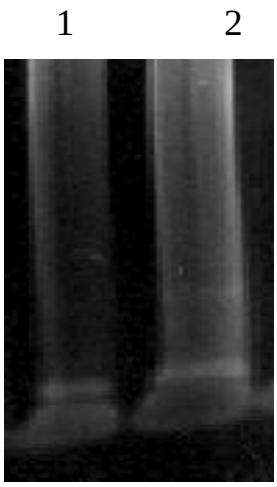

Fig 5 Results of the S-PAGE showing the presence of a band in track 2 in the zone between the smallest viroid (ASBVd) and one of the largest viroid (CEVd) in track 1