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IDENTIFICATION OF APRICOT LATENT VIRUS FROM APRICOT IN PALESTINE

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SUMMARY- A filamentous virus (Apr-47), originated from apricot cv. Mistikawi in Palestine and associated with "peach asteriod spot disease", was isolated by mechanical inoculation to *N. occidentalis*. Based on dsRNA electrophoretical pattern, coat protein size, GF 305 symptomatology, electron microscopy, RT-PCR and sequence analysis of an amplified fragment of 200 nt, the virus was identified as Apricot latent virus (ApLV). ApLV has now been recorded for the first time in Palestine..

Key words: Palestine, apricot, ApLV, RT-PCR, dsRNA

RESUME *- Un virus filamenteux (Apr-47), originaire du cultivar d'abricotier Mistikawi en Palestine et associé à la maladie "peach asteroid spot disease", a été isolé par inoculation mécanique sur N. occidentalis. Sur la base du profil électrophorétique du dsRNA, de la taille de la protéine capsidique, de la symptomatologie sur GF 305, des observations au microscope électronique, de la RT-PCR et de l'analyse des séquences d'un fragment amplifié de 200 nucléotides, il a été identifié comme un isolat de l'Apricot latent virus (ApLV). L'ApLV a donc été signalé pour la première fois en Palestine..*

Mots-clés: *Palestine, abricotier, ApLV, RT-PCR, dsRNA*

INTRODUCTION

Apricot latent virus (ApLV) was first described in Moldova from asymptomatic apricot trees cv. Silistra introduced from Bulgaria (Zemtchic and Verderevskaya, 1993; Zemtchic *et al.*, 1998). The virus was later reported from France and Italy by Gentit *et al*. (2001a). ApLV was found associated with asteroid spot disease in peach (Zemtchic *et al*., 1998). The information on the economic importance, geographic distribution and host range of ApLV is still limited.

Graft transmission test carried out with apricot cv. Mistikawi (Apr-47) to peach cv. GF305, in the course of a study for evaluating the sanitary status of stone fruits in Palestine (Jarrar *et al*., 2001), developed "*asteroid spot* disease". In that case it was decided to investigate the causal agent of the disease. Preliminary results are described below.

MATERIALS AND METHODS

To study the putative agent, purification of double stranded RNAs (dsRNAs), sap transmission to herbaceous hosts, electron microscopy, RT-PCR and coat protein analysis were performed.

dsRNAs were recovered by phenol and chloroform extraction followed by a chromatography through cellulose CF-11 column (Dodds *et al.*, 1993) from symptomatic GF305 plants.

The virus mechanically transmitted from peach cv. GF305 to herbaceous host indicators (*N. occidentalis, Cucumis sativus and Chenopodium quinoa*) using phosphate buffer pH 7.2, with the addition of 2.5 % nicotine.

Specific homologous HALV1 (5'- GGAATAGAGCCCCAAGAAG -3') and complementary CALV1 (5'- AGCAAGGTAAACGCCAAC -3') primers were used to amplify Apr-47 template. PCR was carried out

under the same conditions described by Nemchinov and Hadidi (1998). The PCR product was then run on 1.2 TBE-agarose gel at a constant voltage (100V); the amplified DNA fragment was visualized with a UV transilluminator after staining with ethidium bromide (Sambrook *et al*., 1989).

Coat protein characterization was by SDS-polyacrylamide gel electrophoresis in a discontinuous buffer solution system with stacking and separating gels (Laemmli, 1970), whereas RT-PCR was carried out as described by Nemchinov and Hadidi (1998).

RESULTS AND DISCUSSION

Polyacrylamide gel electrophoresis of Apr.47 dsRNAs extracted from GF305 plants revealed an electrophoretical pattern with a major high molecular weight dsRNA band (estimated size of about 9.5 Kbp)(data not shown), a value fitting the size range reported for members of the genus *Foveavirus* (8.7-10 Kbp) (Martelli and Jelkmann, 1998; Zhang *et al*., 1998) and ApLV (Gentit *et al*., 2001a).

The virus was transmitted from peach cv. GF305 to *N. occidentalis,* which developed symptoms 10-15 days post inoculation. Symptoms progressed from mild chlorotic veins to yellowing and finally to necrotic areas on leaf blades. Additional electron microscopy observations showed the presence of elongated particles in symptomatic plants.

RT-PCR analysis of dsRNA, extracted from Apr-47 infected GF305, resulted in the amplification of a 200 nt DNA fragment (Fig. 1). Our result was in complete agreement with previous findings indicative of the presence of ApLV (Nemchinov and Hadidi, 1998, Nemchinov *et al*., 2000).

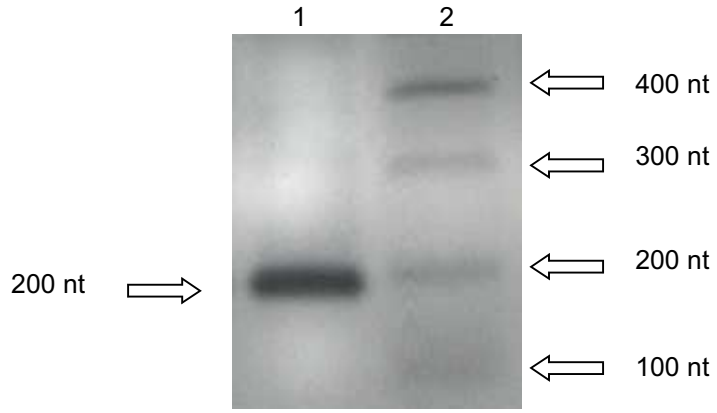

Fig. 1. Agarose gel electrophoresis of RT-PCR from dsRNA extracts from Apr47-infected GF305 (lane 1). PCR Marker is in lane 2.

Considering the dsRNA pattern, electron microscopy observations, “asteroid spot disease” on GF 305 and RT-PCR results, Apr-47 was identified as ApLV. Further results originated from the sequence analysis of an amplified fragment of 200 nt representing the partial CP ORF of the studied virus, revealed the sequence homology (data not shown) with coat protein gene of other foveaviruses. Particularly, Apr.47 was very similar to several isolates of ApLV reported previously (Nemchinov *et al.,* 2000; Gentit *et al.,* 2001b).

These preliminary results, even though they require more investigations (mainly the extension of nucleotide sequence), suggest that Apr.47 is a *Foveavirus* and that it should be considered as an isolate of ApLV.

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