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Characterization of a new nepovirus infecting apricot in Southeastern France: apricot latent ringspot virus

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Abstract

A pathogen was transmitted from apricot trees showing symptoms of viral infection to GF305 peach seedlings which reacted by stunting, shortened internodes and chlorotic mottling. The agent was transmitted to cherry, apricot, peach and plum by grafting and to several herbaceous hosts by mechanical inoculation. Isometric nepovirus-like particles of 30–31 nm diameter extracted from infected *Chenopodium quinoa* sedimented as two peaks in sucrose gradients. These particles contained two single stranded RNAs of approximately 5.9 and 7.9 kb, and a single coat protein subunit of 53.7 kDa. No cross-reactions were observed with a number of nepoviruses infecting fruit trees. Inoculation of purified particles to herbaceous or woody hosts reproduced the same symptoms caused by the original isolate. Sequencing of a 2.2 kbp cDNA clone covering the 3′ end of the small genomic RNA identified an open reading frame encoding a 317 aa N-truncated protein exhibiting significant similarities with the coat protein of nepoviruses. The 1257 nt long 3′ non-coding region showed up to about 65% homology to the equivalent region of members of the subgroup C of nepoviruses. The properties of this pathogen do not match those of any previously described nepovirus. It should therefore be considered as a new member of the subgroup C of nepoviruses, for which the name of Apricot latent ringspot virus (ALRSV) is proposed.

The nucleotide sequence reported in this work has been deposited in the EMBL databank under the accession number AJ278875.

Introduction

Apricot is one of the most important crops in south Europe and around the Mediterranean Sea. Apricot has an important economic significance in this area but is subject to various pests and diseases which can cause severe losses in production. Among these pathogens, viruses can cause serious reduction in fruit production. In France, apricot is essentially grown in the south-eastern part. Among the various fruit trees cultivated, it is becoming one of the most important and its economic impact is increasing fast. During an extensive field survey in 1994, trees of the cultivar Modesto showing dubious viral infection symptoms were observed in a commercial orchard. Trees appeared denuded, with reduced foliage and a bare and skeletal appearance. Fruits were rare but with a normal appearance. Several samples were collected from this orchard and were used for the present study, in order to identify the causal agent of these symptoms. Results of the biological, serological and molecular characterisation of a new nepovirus isolated from these plants and for which the name Apricot latent ringspot virus (ALRSV) is coined are presented here.

Materials and methods

Virus source and maintenance

The isolate obtained from the cv. Modesto was collected from a commercial orchard, graft inoculated and maintained on GF305 peach seedlings under

greenhouse conditions at the Ctifl Center of Lanxade, France. These GF305 indicator plants were tested for *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) infection by DAS-ELISA with polyclonal antibodies according to the manufacturer's instructions (Sanofi diagnostics Pasteur, Marnes-la-Coquette, France).

Bud transmission to different woody indicators in nursery

Dormant buds from various indicator plants were budded onto five homogenous 1-year clonal virus free rootstocks. At the same time, bark chips from infected symptomatic GF305 were grafted below the indicator buds. For each test, one non-inoculated plant was kept as a negative control. The following year, the plants were pruned back just above the indicator buds to force their growth. Symptoms were observed on the new flush of growth.

In 1995, the following species and cultivars were used as indicators: sour cherry (*Prunus avium*) cv. Sam and Bing grafted on Edabriz, peach (*Prunus persica*) cv. Springtime grafted on GF305, apricot (*Prunus armeniaca*) cv. Priana, Lambertin n°1 A2311 and Bergeron A115 grafted on GF305.

In 1998, a similar experiment was conducted by inoculating the same set of indicators plus plum (*Prunus domestica*) cv. Prune D'Ente P707 and Krikon grafted on *Prunus myrobalan* P1254. However, for this experiment, symptomatic GF305 peach seedlings which had been inoculated 3–4 months earlier with purified viral particles were used as inoculum.

In order to confirm virus transmission to the different indexing hosts, each of the plants of the 1995 experiment was back indexed on GF305 peach and the GF305 were tested by DAS-ELISA with the antiserum prepared against the virus (see below). The plants of the 1998 experiment were only tested by DAS-ELISA.

Mechanical transmission to herbaceous hosts

The virus was mechanically inoculated using standard techniques (Candresse et al., 1998) to several herbaceous hosts from young symptomatic leaves of GF305 plants previously inoculated with the original apricot material. All plants were grown under greenhouse conditions and observed for symptom development. Leaves from non-inoculated GF305 were used as negative control. The following species were inoculated: *Chenopodium amaranticolor*, *C. quinoa*, *C. murale*, *C. foetidum*, *Nicotiana benthamiana*, *N. tabacum*, *N. occidentalis*, *N. clevandii*, *Vigna sinensis*, *Cucumis sativus*.

In an attempt to fulfill Koch's postulates, a purified virus suspension (10 µg/ml in 50 mM phosphate buffer pH 7.2, obtained as described below) was used to inoculate the same set of herbaceous indicator plants by rubbing 10 µl of this suspension on two carborundum dusted leaves of two plants of each species. Alternatively, thirty GF305 peach seedlings were slash-inoculated with 10 µl of the same purified virus suspension. Two weeks following inoculation, GF305 plants were pruned just above the slashed area and new growth was observed for a minimum of 5 weeks. The same slash inoculated GF305 plants were used as inoculum source for further nursery inoculation studies as indicated above.

Virus purification

Chenopodium quinoa leaves showing pronounced infection symptoms, approximately one week after inoculation, were collected and stored at −20 °C until used. The purification procedure used was similar to the method described by Doz et al. (1980) for the purification of *Tomato black ring virus* (TBRV), except that the pH of the initial grinding buffer was modified to 7.8 and that two successive sucrose gradient centrifugation steps were used instead of one. The final purified virus pellet was resuspended in a minimum volume of 50 mM phosphate buffer pH 7.2 and was used for further studies. The purification yield was determined spectrophotometrically, assuming an extinction coefficient of 10 ODs at 260 nm for a 1 mg/ml solution and a light path of 1 cm, this value being compatible with the RNA content of the viral preparation as evaluated from the A_{260}/A_{280} ratio.

Electron microscopy

Purified viral preparations were placed on Formvar-coated carbon grids, stained with 1% ammonium molybdate pH 7.0 and examined with a Philips LM10 transmission electron microscope. The average particle diameter was calculated from the measurement of 30 particles.

Analysis of the virus coat protein and nucleic acid

The size of the viral coat protein was determined by electrophoresis in denaturing polyacrylamide gels as described by Laemmli (1970). The samples were prepared by boiling purified virus particles in gel loading buffer (150 mM Tris–HCl pH 7.5; 10% SDS (w/v); 25% β-mercaptoethanol (v/v)) for 5 min. Following electrophoresis, proteins were stained with Coomassie Blue.

Nucleic acids were isolated from purified virus particles by treatment with 0.5% SDS (w/v) and 5 µg proteinase K for 20 min at 50 °C followed by phenol–chloroform and chloroform extractions and ethanol precipitation. The extracted nucleic acids were recovered by centrifugation (15 min, 13,000 rpm), washed with 70% ethanol, dried and finally resuspended in denaturing loading buffer (50% formamide, 6% formaldehyde, 1X MOPS-EDTA (Sambrook et al., 1989), 3.7 ng/µl ethidium bromide), incubated 15 min at 65 °C and analyzed by electrophoresis in a 1% denaturing agarose gel containing 6.7% formaldehyde and 1X MOPS-EDTA, using 1X MOPS-EDTA as the electrophoresis buffer. The nucleic acid sizes were estimated by comparison with a set of commercial RNA markers (RNA ladder, Gibco, BRL) and with purified RNAs from *Grapevine chrome mosaic virus* (GCMV), *Lettuce mosaic virus* (LMV) and *Brome mosaic virus* (BMV).

Influence of the genome linked protein on viral nucleic acid infectivity

Nucleic acids were extracted from approximately 120 µg of purified virus particles as described by Mayo et al. (1982). The viral nucleic acids were dried and resuspended in TE buffer (10 mM Tris–HCl, pH 8, 1 mM EDTA) and adjusted to a 1 µg/µl final concentration. This viral RNA suspension was divided into two aliquots. One aliquot (20 µl) was treated with 0.5% SDS (w/v) and 0.2 µg/µl proteinase K for 10 min at 50 °C, followed by phenol–chloroform and chloroform extractions and ethanol precipitation. The resulting nucleic acid preparation was dried and finally resuspended in TE buffer at a final concentration of 1 µg/µl. The other aliquot was treated similarly except that proteinase K was omitted. The integrity of the two viral RNAs in both nucleic acid preparations were verified by 1% denaturing agarose gel electrophoresis. The two preparations were used to rub inoculate five *C. quinoa* and five *N. occidentalis* plants by rubbing approximately 1 µg of RNA on two leaves of each plant. As a negative control, two plants of each host were inoculated with TE buffer alone. Plants were observed for symptom development over the following 3 weeks.

Serological tests

Serological relationships of ALRSV with some members of the *Nepovirus* genus commonly associated with *Prunus* were determined by double antibody sandwich ELISA (DAS-ELISA). Symptomatic leaves used for these tests were collected from *C. quinoa* plants. Leaves from uninfected plants were used as a negative control. The following commercial polyclonal antibodies were used according to the manufacturer's instructions (Sanofi diagnostics Pasteur, Marnes-la-Coquette, France): *Arabis mosaic virus* (ArMV), *Cherry leaf roll virus* (CLRV), *Raspberry ringspot virus* (RpRSV), *Strawberry latent ringspot virus* (SLRSV), *Tomato ringspot virus* (ToRSV), *Tomato black ring virus* (TBRV). Other antisera for TBRV, strains ED and S, *Grapevine chrome mosaic virus* (GCMV) and *Myrobalan latent ringspot virus* (MLRSV) used were from the collection held at INRA, Bordeaux, France.

Complementary DNA (cDNA) cloning and sequence analysis

cDNA synthesis was performed on 8 µg of purified viral RNA extracted from purified viral particles as described above. First strand cDNA was synthesized the reverse transcriptase of avian myeloblastosis virus (AMV RTase). The reaction mix primed with the oligodT primers (pdT_{12-18}, Pharmacia) was used directly for second strand synthesis according to Gubler and Hoffman (1983). The double-stranded cDNAs obtained were size-selected by addition of 13.3% (w/v) PEG 8000 and 1.25 M NaCl. After stirring, for 1 h at 4 °C and centrifugation at 20,000 *g* for 10 min at 4 °C the pelleted cDNAs were washed and resuspended in sterile water. Size selected cDNAs were ligated in *Eco*RV-cut pZErO™-2 vector (Invitrogen) which was then transformed by electroporation into XL1 *E. coli* cells (Stratagene).

Recombinant plasmids were isolated by alkaline lysis (Sambrook et al., 1989) either in minipreparation scale for preliminary restriction analysis or in large scale for sequence analysis. Automated sequencing

(MWG Biotech, Germany) was used. The sequence data obtained was compiled and compared with nucleic acid sequence databases using the BLAST e-mail facility on the NCBI server (http://www.ncbi.nlm.nih..gov/BLAST). Multiple sequence alignments were performed using the ClustalW program (Thompson et al., 1994) and nepoviral sequences retrieved from the databanks. Phylogenetic analyses and phylogenetic tree reconstruction were performed with the Phylip package (Felsenstein, 1993).

Results

Transmission studies of the original viral isolate to woody indicators

Budwoods harvested from apricot trees (cv. Modesto) presenting a denuded aspect with a bare and skeletal appearance and normal but rare fruits were chip budded on GF305 indicator peach seedlings under greenhouse conditions. Two months after inoculation, the indicator plants displayed reduced vigor, short internodes leading to a severely stunted aspect, and some of their apical leaves displayed chlorotic mottling (Figure 1A). Although similar symptoms can be observed in GF305 peach seedlings induced by a number of common fruit tree infecting pathogens such as *Apple chlorotic leaf spot virus* (ACLSV), *Apple mosaic virus* (ApMV), Apricot chlorotic leaf roll phytoplasma (ACLR) and *Plum pox virus* (PPV) (Desvignes et al., 1999; Gentit et al., 1998; Nemeth, 1986), the symptoms observed on the inoculated plants were not typical of any of the above-mentioned diseases. In addition, the inoculated plants tested negative by DAS-ELISA for PNRSV and PDV.

The inoculated GF305 plants were used as inoculum source for a series of indicator plants in an orchard indexing experiment. During the spring period following their inoculation, sour cherry cv. Sam and cv. Bing reacted by displaying specific symptoms. On Sam grafted onto Edabriz, the first apical leaves, often truncated, made a rosette with some deforming chlorotic mottle. These symptoms were also associated with necrosis and twisting of the stems. Young trees had a chlorotic aspect with a weak growth (Figure 1B). On Bing grafted onto Edabriz rootstock symptoms were similar but weaker. The first leaves displayed chlorotic patterns on a part of their lamina with some deformations. These deformations corresponded mostly to enations growing on the underside. Stem tips of some plants had short internodes with a stunted aspect. New shoots produced later in the growing season usually had either attenuated or no symptoms (Figure 1C).

On inoculated apricot plants, symptoms observed were essentially a chlorotic rolling of the foliage at the end of the growth season. This symptom was associated with wilting (cv. A2311 Lambertin n°1) or a weakening of trees (cv. A115 Bergeron). These symptoms varied with rootstock, being stronger on Fereley P3297, or associated with short internodes, as with cv. Lambertin n°1 grafted on Fercino P1090. On the other hand, no remarkable symptoms were observed the year following inoculation on cv. Priana grafted on GF305. During the second year, however, these plants displayed a chlorotic rolling foliage associated with a union necrosis and a slight stem pitting of the variety (Figure 1D,E)

Studies of transmission to herbaceous hosts

Within 7–10 days following inoculation of extracts prepared from symptomatic GF305, several herbaceous indicators displayed symptoms. *Chenopodium murale*, *C. quinoa* and *C. foetidum* reacted with chlorotic and necrotic local lesions, associated with a systemic mottling and severe tip necrosis. *Nicotiana clevelandii* developed necrotic ringspots on inoculated leaves and later a systemic necrotic mosaic on un-inoculated leaves. *N. tabacum* displayed systemic chlorotic ring patterns. *N. occidentalis* developed necrotic ringspots on inoculated leaves and later a systemic embossed mosaic. *N. benthamiana* reacted with a systemic necrotic mosaic associated with an apical necrosis.

No symptoms were observed on inoculated *Vigna sinensis* and *Cucumis sativus* plants. However, the possibility of asymptomatic infection cannot be discounted for these plants at this time.

Virus purification and electron microscopy

Since the symptoms observed on both the GF305 woody indicator and the herbaceous *Chenopodium* and *Nicotiana* hosts were reminiscent of infection by a nepovirus, a protocol adapted from one previously described by Doz et al. (1980) for the purification of TBRV was used to purify the virus from symptomatic *C. quinoa* plants. Following initial positive results, the protocol was modified to include a second sucrose gradient centrifugation step in order to further improve the

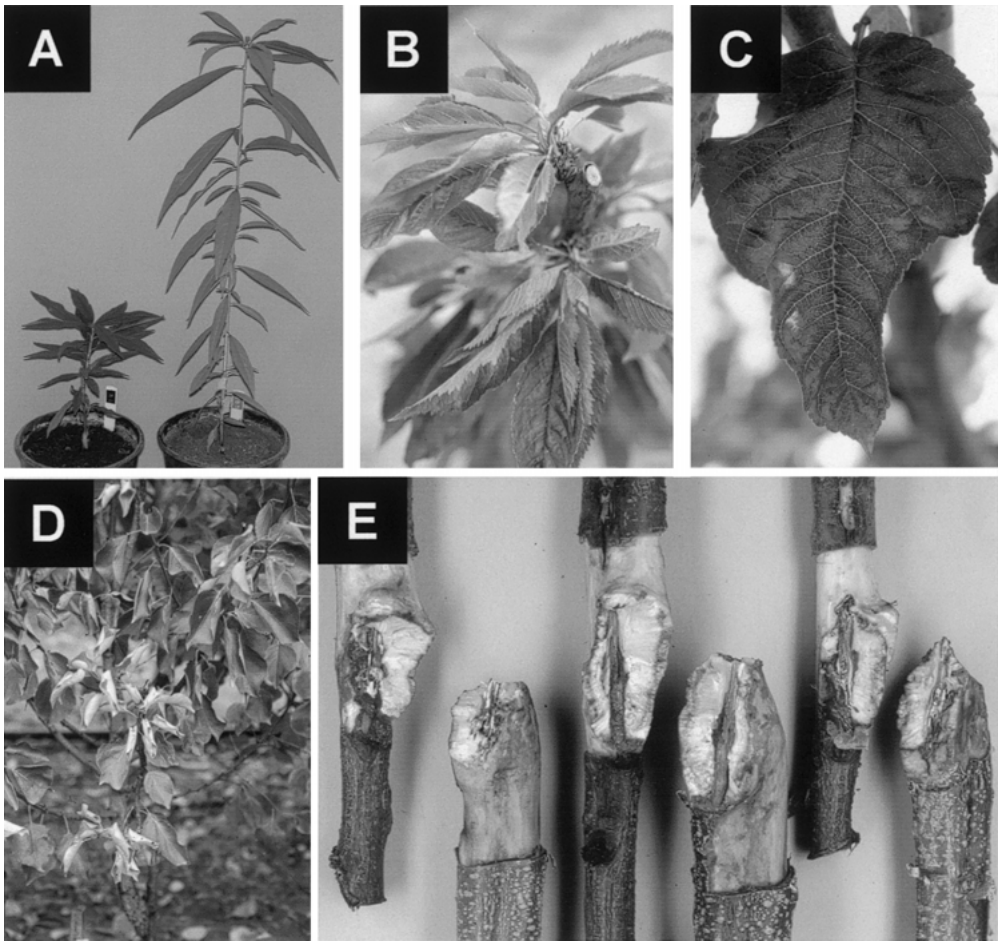

Figure 1. Symptoms induced on woody hosts by the original isolate of ALRSV. (A) symptoms on GF305 peach seedling: short internodes and chlorotic mottling are displayed 1–2 months after inoculation. (B) symptoms on sweet cherry cultivar Sam grafted on Edabriz rootstock the year following inoculation. Chlorotic mottling and rosetting sometimes associated with leaf necrosis and stem twisting. (C) symptoms on sweet cherry cultivar Bing grafted on Edabriz rootstock the year following inoculation. Chlorotic patterns on leaves associated with leaf deformations related to enations. (D,E) symptoms on apricot cultivar Priana grafted on GF305. Chlorotic and rolling foliage (D) associated with stem pitting and union necrosis (E).

purity of the viral particles recovered. Viral particles subjected to this second sedimentation step clearly separated into two clearly resolved UV-absorbing peaks. Using an absorbance value of 10 ODs for a 1 mg/ml virus suspension, yield was estimated to be around 0.8 mg virus per 100 g of fresh *C. quinoa* leaves. The purified viral preparations were used for further studies and for the production of a specific antiserum.

Following 1% ammonium molybdate staining, the viral particles observed in these preparations appeared to have a polyhedral shape and an average size of 30–31 nm in diameter, again consistent with a possible nepovirus. Two types of particles were observed, some apparently full and not penetrated by the stain and some apparently empty, penetrated by the stain (Figure 2).

Infectivity of the purified viral preparations and of purified viral RNAs

Infectivity of the purified viral preparations were first verified by inoculation of herbaceous hosts. The three *Chenopodium* and four *Nicotiana* species inoculated displayed symptoms similar to those previously observed for the original isolate from GF305 (not shown). As previously described, no symptoms were

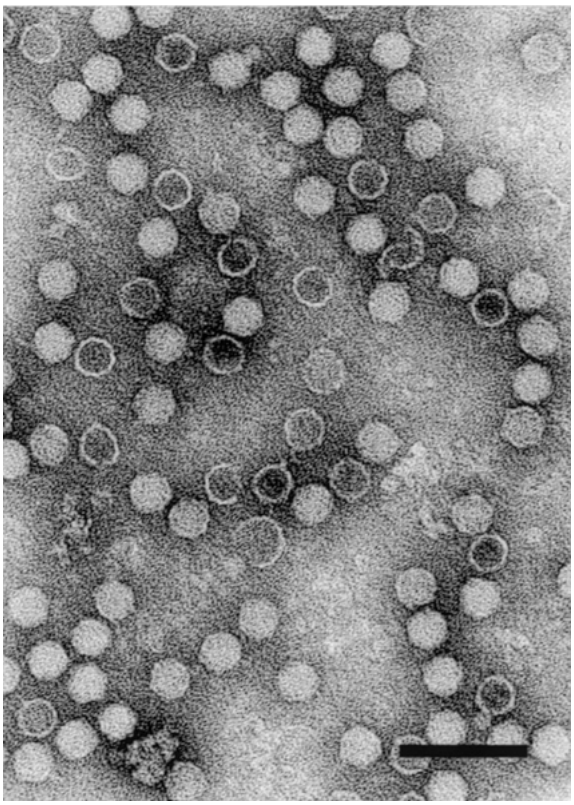

Figure 2. Electron micrograph of a purified Apricot latent ringspot virus particles preparation stained with 1% ammonium molybdate. The bar represents 100 nm.

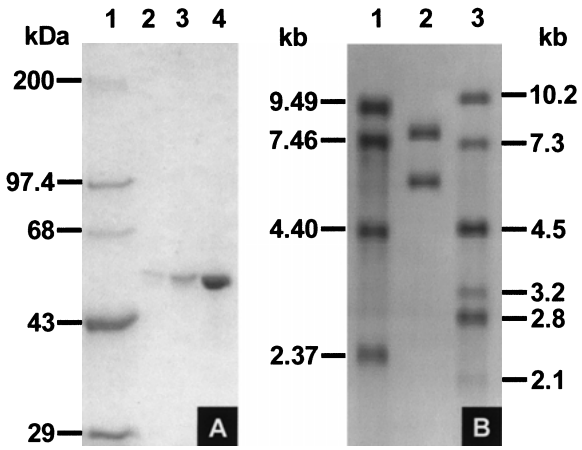

Figure 3. Analysis of the protein and nucleic acids components of ALRSV particles. (A) Analysis of the ALRSV coat protein (CP) by denaturing polyacrylamide gel electrophoresis. Lane 1, molecular weight markers. Lanes 2, 3 and 4, respectively 0.25, 1 and 4 μg of CP obtained from the purified viral preparation. (B) Analysis of the ALRSV genomic RNAs by 1% denaturing agarose gel electrophoresis. Lane 1, RNA size markers (size indicated in kilobases). Lane 2, ALRSV purified genomic RNAs. Lane 3, mixture of various viral genomic RNAs used as size markers (*Brome mosaic virus* (3.2, 2.8 and 2.1 kb), *Lettuce mosaic virus* (10.2 kb) and *Grapevine chrome mosaic virus* (7.3 and 4.5 kb)).

observed on *Vigna sinensis* or *Cucumis sativus*. The next step was to verify infectivity of the purified virus on woody hosts. Four weeks after slash inoculation with the purified viral suspension, symptoms previously described on GF305 with the original isolate were observed on 21 of the 30 inoculated peach plants. On one or two plants, young leaves had a frizzy aspect and displayed arabesque patterns along veins. Two months after inoculation, the general appearance of the GF305 plants was a stunting similar to that observed following the initial bud inoculations of GF305 (not shown).

Tests performed in the nursery with budwood collected from slash inoculated GF305 and grafted onto various indicators save similar results to those observed following inoculations performed with the original isolate. The only variety used in these assays and not previously tested with the original isolate, Prune d'Ente P707 grafted on Myrobalan, showed a weakening, with chlorotic and rolling foliage associated with a union necrosis (not shown).

Analysis of the viral coat protein and nucleic acids

Analysis of purified viral particles by denaturing polyacrylamide gel electrophoresis revealed the presence of a single coat protein with a molecular mass estimated at around 53.7 kDa (Figure 3A). Nucleic acids extracted from the purified particles were susceptible to RNase A but unaffected by DNase (data not shown), demonstrating their single-stranded RNA nature. Denaturing gel electrophoresis revealed the presence of two RNA molecules with sizes estimated at 7.9 and 5.9 kb (Figure 3B).

Since the properties of the purified viral particles were indicative of a possible member of the *Nepovirus* genus, the possibility of the presence and requirement for infectivity of a polypeptide linked to the viral RNAs (Mayo et al., 1982) was investigated. While plants inoculated with SDS-only treated RNAs displayed symptoms (3/5 *N. occidentalis* and 1/5 *C. quinoa)*, no symptoms were observed on plants inoculated with the TE buffer alone or with the nucleic acid preparation treated simultaneously with 0.5% SDS and 0.2 μg/μl proteinase K, demonstrating the requirement

for infectivity of a protease-sensitive component in the purified viral RNA preparation.

Serological analysis

In order to evaluate possible serological cross-reactions with previously known members of the *Nepovirus* genus, a fresh crude extract of infected *C. quinoa* plants was tested in DAS-ELISA with polyclonal antisera specific for nepoviruses known or suspected to infect *Prunus* species. As described in the materials and methods section, the antisera tested were against ArMV, CLRV, RpRSV, SLRSV, ToRSV, TBRV strains ED and S, GCMV, MLRSV. While all positive controls gave positive reactions, no signal was detected with the new virus.

cDNA cloning and sequence analysis

Using purified viral RNAs as template, a cDNA bank was constructed in pZErO^{TM}-2 and several clones were partially sequenced, confirming the presence of a polyA tail at the 3′ end of the genomic RNAs already inferred from the fact that cDNA synthesis was efficiently primed by an oligo-dT primer. The largest cDNA clone obtained was fully sequenced, yielding a 2208 bp virus specific sequence, excluding the 3′ terminal polyA tail. Analysis of this sequence, deposited in the EMBL databank under the accession number AJ278875, revealed the presence, in the 5′ region, of an open reading frame (ORF), the initiation codon of which was not present on the cloned fragment. Translation of this ORF yielded a N-truncated protein of 317 amino acids showing low but significant homology with the coat proteins of other members of the *Nepovirus* genus. The highest levels of similarity (28% with *Blackcurrant reversion-associated virus* (BRAV), and 26% with *Blueberry leaf mottle virus* (BLMoV)) were observed with subgroup C nepoviruses (Wellink et al., 2000; Martelli, 1975), characterized by very long 3′ non-coding sequences of approximately 1.4–1.5 kb highly conserved between the two genomic RNAs (Rott et al., 1991; Sanfacon, 1995). Significant but slightly lower levels of similarities (24–22%) were observed with other nepoviruses. In addition, the amino acids known to be conserved in the CPs of nepoviruses (Le Gall et al., 1995) were observed in this sequence, including the conserved FYGR sequence near the C-terminus of the CP. A phylogenetic tree constructed from this partial coat protein sequence is shown in Figure 4A. Very few of the branches are statistically significant as indicated by generally low bootstrap values but the new sequence clearly belong in the *Nepovirus* genus cluster.

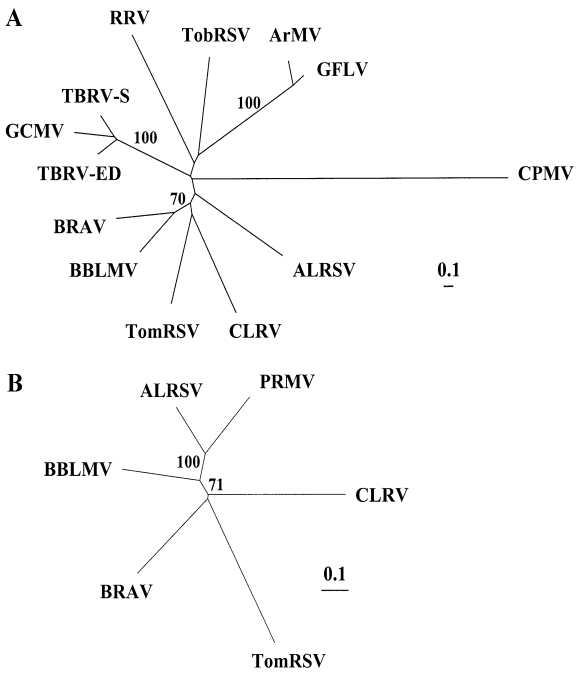

Figure 4. Phylogenetic trees describing phylogenetic affinities of ALRSV with other nepoviruses. The trees shown were constructed using distance programs (DNADIST or PROTDIST) and the FITCH program of the PHYLIP package (Felsenstein, 1993). Programs BOOTSTRAP and CONSENSE (Felsenstein, 1993) were used to evaluate the validity of the branches. TREEVIEW (Page, 1996) was used to display the trees. Bootstrap values above 50% are indicated adjacent to nodes (100 replicates). The scale bar represent the relative genetic distance. (A) Phylogenetic tree constructed using the partial coat protein sequence of ALRSV and equivalent regions from the following viruses: *Blueberry leaf mottle virus* (BLMoV, U20621); *Cherry leaf roll virus* (CLRV, S63537); *Blackcurrant reversion associated virus* (BRAV, AF020051); *Tomato ringspot virus* (ToRSV, D12477); *Tomato black ring virus* strain ED (TBRV-ED, X80831); *Tomato black ring virus* strain S (TBRV-S, CAA27694); *Arabis mosaic virus* (ArMV, X81814); *Grapevine fanleaf virus* (GFLV, X16907); *Tobacco ringspot virus* (TRSV, L09205); *Raspberry ringspot virus* (RpRSV, JQ1658); *Grapevine chrome mosaic virus* (GCMV, X15163); *Peach rosette mosaic virus* (PRMV, AF016626); *Cowpea mosaic virus* (CPMV, P03599). CPMV was used as an outgroup to generate a rooted tree. (B) Phylogenetic tree constructed from the 3′ non-coding regions of ALRSV and of other members of the subgroup C of nepoviruses. Abbreviations are as for Figure 4A. For PRMV the 3′ NCR of RNA1 (Lammers et al., 1999) was used. For CLRV the sequence with accession number S84125 was used.

In addition to the ORF, the sequenced cDNA contained a large 1257 nt long 3′ non coding region (NCR). Comparison with databanks showed this sequence to have extensive homology with the 3′ NCR of the RNA1 of *Peach rosette mosaic virus* (PRMV) (Lammers et al., 1999) (64.5% overall homology) and with the corresponding region of BLMoV (Bacher et al., 1994) (61.5%). A phylogenetic tree showing the relationships between the 3′ NCR of the subgroup C nepoviruses is shown on Figure 4B. Again, low differences in the homology levels observed resulted in low-bootstrap values for several of the branches. The branch linking the isolate studied and PRMV is, however very solid (100% bootstrap value), indicating that PRMV is currently the closest known relative to this isolate.

Discussion

The *Nepovirus* genus has a worldwide distribution (Brunt et al., 1996; Mayo and Robinson, 1996) and the principal crops affected are small fruits, fruits trees and grapevine (Mayo and Robinson, 1996; Sanfacon, 1995). In France, some of these viruses have previously been described on fruit trees (Candresse et al., 1998; Dunez et al., 1971). In the French certification scheme, the first level of control is biological indexing on GF305 peach seedlings. This indicator has been noted for its ability to reliably show symptoms when infected by a number of fruit tree-infecting viruses (Boye and Desvignes, 1986; Damsteegt, 1997; Desvignes et al., 1999). Consequently, this indicator has also been used for extended surveys of French commercial orchards. During one of these surveys several trees with dubious symptoms reminiscent of viral infection were found and indexed on GF305 which then displayed viral infection symptoms in the form of mottling and stunting due to short internodes.

Following this initial observation, a set of woody indicator hosts was artificially inoculated. The symptoms initially observed on apricot were reproduced, demonstrating the graft-transmissible nature of the disease. These indexing experiments also demonstrated that in addition to apricot and peach, cherry and plum are susceptible hosts. Purified viral preparation were obtained, which sedimented in two peaks on sucrose gradients and showed nepoviral-like particles upon electron microscope examination (Wellink et al., 2000; Mayo and Robinson, 1996).

Inoculation of purified particles to herbaceous and woody hosts reproduced all the symptoms observed on plants inoculated with the original field isolate, thus fulfilling Koch's postulates and demonstrating that the purified agent was indeed responsible for the symptoms initially observed in apricot.

Further characterization of the purified particles provided a set of results which are consistent with the agent being a new member of subgroup C of the *Nepovirus* genus. The size of the single coat protein subunit and of the two genomic RNAs (Mayo and Robinson, 1996), the presence of a protease-sensitive component in the purified viral RNAs required for infectivity (Mayo et al., 1982) as well as homologies observed between the partial coat protein sequence and the coat proteins of other members of the *Nepovirus* genus (see Figure 4A), clearly position the agent in this genus. Members of subgroup C of the *Nepovirus* genus are characterized by very large (ca. 1.4 kb) 3′ NCRs highly conserved between the genomic RNAs (Martelli, 1975; Bacher et al., 1994; Rott et al., 1991; Sanfacon, 1995; Wellink et al., 2000). Although no information is currently available on RNA1 of the new agent, the large size (1257 nt) of the 3′ NCR found in the RNA2 cDNA clone and the significant homologies observed with the 3′ NCRs of other members of the subgroup C (see Figure 4B) are very strongly indicative of a positioning of the agent in this group.

Lastly, the host range and symptomatology of the agent and the lack of significant serological cross-reactions with other nepoviruses known to infect *Prunus*, indicate that this agent should be considered a new virus. *Peach rosette mosaic virus* (PRMV) (Lammers et al., 1999) was not among the viruses evaluated for serological cross-reactions and there is currently no sequence information available for the RNA2 of PRMV, which makes comparison with the new agent somewhat difficult. However, comparison of the 3′ NCR of PRMV RNA1 with the 3′ NCR of the RNA2 of the agent showed only about 65% homology between the two sequences. Since the 3′ NCRs are invariably very highly conserved between the two genomic RNAs of subgroup C nepoviruses (Bacher et al., 1994; Rott et al., 1991; Sanfacon, 1995), it is unlikely that PRMV would show highly diverging 3′ NCRs and that the agent would in fact be PRMV. Indeed, the homology observed between the agent and PRMV is only slightly above (64.5% versus 61.6%) that observed with BLMoV, and is thus in the range observed between members of the subgroup C rather than between isolates of a virus.

All of the above results therefore lead to the conclusion that the agent should be regarded as a new member

of the subgroup C of nepoviruses for which we propose the name Apricot latent ringspot virus (ALRSV). Due to the lack of a proper detailed epidemiological study, the distribution and impact of this pathogen in its original location as well as in other fruit tree growing areas cannot be evaluated. Despite its large potential host range, ALRSV has probably a rather limited distribution: given the severe symptoms induced in several of the 'non-apricot' *Prunus* hosts, ALRSV would have been observed before if it was widely distributed. However, symptoms observed on some apricot varieties are similar to those observed with another well distributed pathogen in this area, namely the ACLR phytoplasma (Desvignes et al., 1999; Gentit et al., 1998), so that the prevalence of ALRSV in this particular crop may have been overlooked. In addition, further studies of the distribution of ALRSV may be complicated by the recent decision to try to eradicate ACLR by an extended survey of apricot orchards in the southeastern of France.

No information can be provided at this time on potential vector(s) of ALRSV. Since members of subgroup C have been reported to be either nematode (ToRSV), mite (BRAV) or pollen (CLRV) borne (Wellink et al., 2000; Martelli, 1975; Mayo and Robinson, 1996; Sanfacon, 1995), further work will be needed to determine the mode(s) of dispersion of ALRSV. Nevertheless, since PRMV appears to be the closest known relative of ALRSV and since PRMV is known to be nematode-transmitted (Eveleigh and Allen, 1982), this could also be the case of ALRSV. Similarly, the possibility that the natural host range of ALRSV could include other commercially important crops should be considered since the host range of PRMV also includes highbush blueberry (*Vaccinium corymbosum* L.) and grapevine.

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