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Occurrence of aphidborne viruses in southernmost South American populations of *Fragaria chiloensis* ssp. *chiloensis*

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Wild and cultivated *Fragaria chiloensis* ssp. *chiloensis* (Fcc) plants were collected at different locations in southern Chile in order to determine the current viral status of this native strawberry. The following aphidborne viruses (ABVs): *Strawberry mild yellow edge virus* (SMYEV), *Strawberry mottle virus* (SMoV), *Strawberry crinkle virus* (SCV) and *Strawberry vein banding virus* (SVBV), were found in wild and cultivated Fcc plants, but severe symptoms were not associated with viral infection. Furthermore, partial gene sequences of these ABV isolates were determined and displayed a high degree of conservation with virus isolates reported previously. In addition, partial gene sequences of SCV and SVBV from southernmost South American populations of Fcc are described for the first time. High-throughput parallel sequencing (Illumina) of double-stranded RNA was used to provide viral profiles of Fcc from different locations. Although strong evidence of novel viruses affecting Fcc was not found, it was confirmed that ABVs are the most frequent viruses affecting this subspecies. The information provided will help in the development of high-quality molecular tools for virus detection and control in Fcc.

Keywords: Patagonica, strawberry, *Strawberry crinkle virus*, *Strawberry mild yellow edge virus*, *Strawberry mottle virus*, *Strawberry vein banding virus*

Introduction

In southern Chile, *Fragaria chiloensis* ssp. *chiloensis* (Fcc) grows in the wild as well as in cultivation and is distinguished into two botanic forms that are recognized primarily by fruit colour. The *chiloensis* form produces a large white fruit and has been cultivated ancestrally in small fields with coastal influences at latitudes of 35–39ºS. The fields have been maintained and propagated by runners without modern horticultural practices. The *patagonica* form produces small red fruit and grows in the wild at both low (sea level) and high altitudes (up to 1850 m a.s.l.) at latitudes of 35–47ºS (Lavin *et al.*, 2000; Retamales *et al.*, 2005). Fcc is considered to be the mother of the current commercial strawberry (*Fragaria* × *ananassa*), which was produced as an interspecific cross between Fcc (from Chile) and *F. virginiana* (from North America). Because of their organoleptic qualities, great diversity of germplasm, the availability of a white-fruited form and the adaptability to different climatic conditions, Fcc has motivated researchers to collect germplasm to look for useful traits for breeding purposes (Hancock, 1990; Gambardella *et al.*, 2002).

Viruses are among the most important pathogens affecting *F.* × *ananassa*. Aphidborne viruses (ABVs) such as *Strawberry mild yellow edge virus* (SMYEV), *Strawberry mottle virus* (SMoV), *Strawberry crinkle virus* (SCV) and *Strawberry vein banding virus* (SVBV) stand out among the viruses affecting strawberry plants. Strawberry ABVs naturally infect the genus *Fragaria* and mixed infections by multiple ABVs can produce significant yield losses (Martin & Tzanetakis, 2006). Aphidborne viruses have also been found affecting Fcc in single and mixed infections (Thompson & Jelkmann, 2003; Cabrera *et al.*, 2004). These viruses could be an important constraint for the commercial development of white-fruited Fcc. Importantly, the absence of symptoms in Fcc infected with ABVs makes Fcc germplasm a potential source of viral inoculum for *Fragaria* spp. in breeding programmes (Hepp & Vera, 1990).

An understanding of the molecular variation and genetic relatedness among different isolates of a virus can provide information about the origins, dispersal patterns and geographical distribution of particular genotypes. Furthermore, sequence information from different viral isolates could also help in the development or optimization of detection methods and control strategies. Previous

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studies have demonstrated the molecular variation of ABV isolates affecting both wild and commercial strawberries (Thompson *et al.*, 2003). However, only sequences of SMYEV and SMoV (Thompson & Jelkmann, 2003, 2004) infecting the southernmost South American populations of Fcc have been reported. Therefore, a survey of viruses affecting wild and cultivated Fcc plants from Chile was carried out using ELISA, traditional and high-throughput sequencing technologies (HTS). In addition, to obtain information about genetic variation between viral isolates, partial gene sequences of ABV isolates were determined and phylogenetically analysed. As aphids are important for the spread of ABVs, the presence of the aphid *Chaetosiphon fragaefolii* in both wild and cultivated Fcc populations was also monitored.

Materials and methods

Plant material and aphid detection

Whole plants were collected from the main growing areas of wild and cultivated Fcc in Chile (Table 1). Twenty plants from each location were randomly collected during late spring and early summer. When present, 20–30 aphids were collected from different sampled plants and identified by microscopic examination. Plants were maintained in a greenhouse under controlled conditions. Insecticide (Confidor® Forte, Bayer) was applied to the plants after collection to prevent cross-viral inoculation. Young leaves were collected from each sampled plant and used for virus detection by ELISA and RT-PCR. Samples for nucleic acid extraction were frozen immediately in liquid nitrogen and stored at −80°C.

ELISA

Three completely expanded young leaves were collected from each plant. Leaf tissue (0·5 g) was homogenized in 10 volumes (w/v) of grinding buffer (20 mM Tris buffer, pH 7·4, containing 137 mM NaCl, 3 mM KCl, 2% PVP, 0·02% NaN_3 and 0·05% Tween 20) using grinding bags. Samples were tested by double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) for SMYEV, *Strawberry latent ringspot virus* (SLRSV), *Tobacco necrosis virus* (TNV), *Tomato black ring virus* (TBRV) and *Tomato ringspot virus* (ToRSV) using commercially available antibodies (BIOREBA) according to the manufacturer's recommendations. A healthy Fcc plant obtained by meristem culture was used as a negative control. The positive–negative threshold was established at an absorbance value of three times that of the negative control, which ranged from zero to an optical density of 0·10. Each sample was tested in duplicate.

Nucleic acid extraction

Total RNA was extracted using a silica-capture extraction method as described by Tzanetakis *et al.* (2007). RNA integrity was checked by electrophoresis on 1% agarose gels and by the A_{260}/A_{280} ratio using a NanoDrop spectrophotometer.

Double-stranded RNA (dsRNA) was extracted using a phenol and cellulose protocol (Tzanetakis & Martin, 2008). Essentially, fresh leaf material of all plants collected from the Cucao and Contulmo locations was pooled and frozen. Approximately 25 g of leaves from each location were ground using liquid nitrogen in a mortar and pestle and incubated at room temperature with agitation for 30 min in 45 mL 2 × STE buffer (50 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 7·5), with 10 mL 10% SDS, 30 mL water-saturated phenol, and 700 μL β-mercaptoethanol added. The supernatant was separated after 25 min of centrifugation at 10 000 ***g*** at 4°C. Twenty percent ethanol was used to bind the nucleic acids to cellulose (1 g). The dsRNA-cellulose was washed several times with STE buffer containing 18% ethanol (v/v) and the nucleic acids were eluted in 15 mL STE buffer. Nucleic acids were treated with DNAse and RNAse as described previously (Tzanetakis & Martin, 2008). Samples were then subjected to another cycle of binding to cellulose powder and washing. The dsRNA was precipitated, resuspended in 10 μL STE and separated by electrophoresis on a 1% agarose gel. Finally, dsRNA was extracted from the gel using a QuickClean DNA kit

Table 1 Virus detection in *Fragaria chiloensis* ssp. *chiloensis*

Population	Location	Botanical form	Aphid presence	ELISA SMYEV incidence (%)[a]	RT-PCR SCV[b]	RT-PCR SMYEV[b]	RT-PCR SMoV[b]	RT-PCR SVBV[b]
Vilches	35°60′00″S, 71°20′00″W	*pa*	no	7	Negative	Positive	Positive	Positive
Chillan	36°59′81″S, 72°08′19″W	*pa*	no	32	Negative	Positive	Positive	Positive
Petrohue	41°13′56″S, 72°40′06″W	*pa*	no	0	Positive	Positive	Positive	Positive
Cucao	42°59′31″S, 74°12′52″W	*pa*	yes	50	Positive	Positive	Positive	Positive
Pencahue	35°38′67″S, 71°81′01″W	*chi*	yes	30	Positive	Positive	Positive	Positive
Contulmo	38°07′95″S, 73°24′05″W	*chi*	yes	100	Positive	Positive	Positive	Positive
Puren	38°03′49″S, 73°06′60″W	*chi*	yes	100	Negative	Positive	Negative	Positive

pa: f. *patagonica*; *chi*: f. *chiloensis*; SCV: *Strawberry crinkle virus*; SMYEV: *Strawberry mild yellow edge virus;* SMoV: *Strawberry mottle virus*; SVBV: *Strawberry vein banding virus*.

[a]ELISA: incidence calculated over 20 randomly tested plants in each location using DAS-ELISA technique for SMYEV: *Strawberry mild yellow edge virus*.

[b]RT-PCR: results obtained in pooled samples for each accession.

(GenScript) and quantified with a NanoDrop spectrophotometer.

RT-PCR

Complementary DNAs (cDNAs) were synthesized using SuperScript® III reverse transcriptase (Invitrogen) as described previously by Chang *et al.* (2007) using 300 ng of RNA, 300 ng random primers, 1 × first-strand buffer, 0·5 mM dNTPs, 10 mM dithiothreitol, 16 U RNase OUT (Invitrogen) and 60 U SuperScript III in a final volume of 50 μL. The reaction was incubated for 2 h at 50°C and terminated by heating the mixture for 10 min at 70°C.

PCR amplifications for virus detection were performed using SMYEV, SCV, SVBV and AtropaNad internal control specific primers described previously by Thompson *et al.* (2003). Primer sequences for SMoV detection were D1: 5′-TAAGCGACCACGACTGTGACAAAG-3′ and D2: 5′-ATTCGGTTCACGTCCTAGTCTCAC-3′. Additionally, the primers P1: 5′-GTTTTGGTCTTAGTCGTCG-3′ and P2: 5′-TTCTACCCAGCTCCAATGAG-3′ were used for amplification of SMYEV from dsRNA (see below). Thermocycling consisted of 40 cycles of 35 s at 94°C, 40 s at either 52°C for SVBV and SMYEV, 55°C for AtropaNad or 58°C for SCV and SMoV primers, and 40 s at 72°C. PCR products were separated on a 2% agarose gel and visualized under UV light after staining with ethidium bromide. To ensure that the viral genes had been amplified, PCR amplicons were sequenced and compared with sequences in GenBank using BLASTN (Altschul *et al.*, 1997).

To verify the presence of ABVs in the dsRNA extracts, cDNA was generated from dsRNA using random primers and PCR-amplified using specific ABV primers in single PCR reactions (as described above). For identification of new viruses using dsRNA templates, double-stranded cDNA was synthesized using primers containing a random hexamer 5′-ATACATTATCATCTGAGTGA**GGTACC**NNNNNN-3′ and *Kpn*I restriction (site in bold) as previously described by Tzanetakis *et al.* (2005). Prior to cDNA synthesis, dsRNA (5 μL) was mixed with random hexamers (1 μg) and denaturated in CH_3HgOH (20 mM final concentration) for 15 min at room temperature. PCR reactions using the anchor primer 5′-ATACATTATCATCTGAGTGAGGTACC-3′ were carried out to amplify the cDNA products. For construction of cDNA libraries, PCR products were separated by gel electrophoresis and fragments larger than 500 bp were gel-extracted, purified and cloned. For Illumina sequencing, PCR products from the two Contulmo and Cucao populations were pooled, digested by *Kpn*I and prepared for HTS (Illumina) as described by the manufacturer at the Central Services Laboratory of Oregon State University.

Cloning and sequencing

All PCR products purified for sequencing were cloned in the pCR2.1-TOPO TA vector (Invitrogen). Two microlitres of the ligation solution were used to transform One Shot Mach1-T1 chemically competent cells (Invitrogen). Plasmids were isolated using QuickClean miniprep kit (GenScript) from 3-mL overnight cultures containing ampicillin. Recombinant plasmids were verified by *Eco*RI digestion. Sequencing reactions were performed at Macrogen Inc. facilities in an ABI 3730xl automatic DNA sequencer. Accession numbers of new ABV sequences submitted to GenBank are provided in Table S1.

Illumina sequencing

Cluster generation and sequencing using a Genome Analyzer System (Illumina) were performed by Oregon State University's Center for Genome Research and Biocomputing (CGRB) Core Laboratories. The 36-bp single reads were first assembled using VELVET (Zerbino & Birney, 2008), ABYSS (Simpson *et al.*, 2009), SSAKE (Warren *et al.*, 2007) and VCAKE (Jeck *et al.*, 2007) assembly software programs. CODONCODE ALIGNER v. 3.0 (CodonCode Corp.) was used to construct larger contigs. All sequences obtained were compared with database sequences using BLAST algorithms.

Phylogenetic analysis

To identify related sequences in the database, nucleotide BLAST searches were performed using SMYEV, SCV, SMoV and SVBV nucleotide sequences. CLUSTALX was used to create multiple alignments and the resulting alignments used to assemble the phylogenetic tree by the neighbour-joining algorithm (Saitou & Nei, 1987) using MEGA v. 3.1 (Kumar *et al.*, 2004). Confidence values for the groupings from the multiple alignments were derived using 1000 bootstrapped data sets (Van de Peer & De Wachter, 1993). Accession numbers of sequences used to generate the phylogenetic analysis are provided in Tables S1 & S2.

Results

ABV detection in *F. chiloensis* spp. *chiloensis*

Initial screening of the collected Fcc plants using RT-PCR and SMYEV-specific antibodies showed that this virus affects both botanical forms. However, the incidence of SMYEV was higher in the *chiloensis* form than in the *patagonica* form. In some cultivated populations 100% of tested plants were infected with SMYEV (Table 1). Samples from collected Fcc populations were also analysed by RT-PCR for the presence of other ABVs such as SCV, SMoV and SVBV (Table 1). A single band for each virus was obtained in all positive PCR reactions (data not shown). The ABVs tested were present in both Fcc forms (f. *patagonica* and f. *chiloensis*), although differentially distributed in those populations analysed (Table 1). Furthermore, all tested plants showed negative ELISA reactions for SLRSV, TNV, TBRV and ToRSV.

Independent of their origin (wild or cultivated), typical symptoms of viral infection were not evident in Fcc plants analysed from the field. However, some plants with mixed viral infection showed mild leaf chlorosis when maintained in the greenhouse under controlled conditions. Healthy and inoculated *Fragaria vesca* plants were used to compare symptom development in a sensitive plant (Fig. 1).

As strawberry ABVs are transmitted by the aphid *C. fragaefolii*, the presence/absence of this vector was recorded in the Fcc populations studied. This aphid was found in all cultivated populations but in only one of the wild ones (Table 1).

In order to search for novel viruses affecting Fcc, dsRNAs from the Contulmo and Cucao populations were extracted from pooled leaf samples. These populations were selected because they showed the highest SMYEV incidence and were also infected by SCV, SMoV and SVBV. The dsRNAs obtained showed very similar electrophoresis profiles (Fig. 2). The RT-PCR analysis of dsRNA confirmed the presence of ABVs in the two extractions (Fig. 3). Furthermore, more than 50 clones from each cDNA library were sequenced but no novel viral sequences were found. Sequence homology with SMYEV and SMoV was 84% and 8%, respectively, in the Contulmo population and 50% and 42%, respectively, in the Cucao population. Sequences from both populations showed 8% homology to plant nucleic acids.

Massively parallel sequencing technology (Illumina) was used on pooled dsRNA from the Contulmo and Cucao populations. The 4 210 264 reads generated were optimized and assembled into 498 contigs and 896 unassembled sequences. When the contigs and unassembled sequences obtained were compared to sequences in the GenBank database, 22·8% of sequences showed significant similarities with plant virus sequences, 68·9% of the sequences had no match in the databases analysed and 1·2% of sequences corresponded to environmental contamination (Fig. 4a). Analysis of contigs with homology to viral sequences identified the expected four ABVs in Fcc with 71·1% of viral sequences corresponding to SMoV, 17·6% to SMYEV, 5% to SVBV and 0·9% to SCV. Three contigs showed homology to *Aphid lethal paralysis virus* (ALPV) and 4·4% of sequences corresponded to viruses of fungi and yeasts (Fig. 4b).

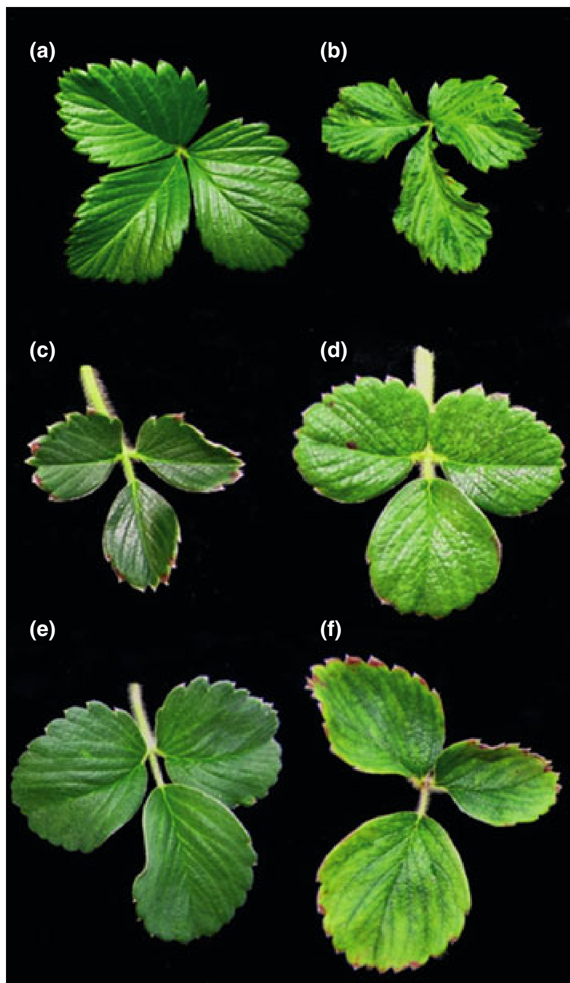

Figure 1 Symptoms of viral infection in leaves of *Fragaria vesca* and *Fragaria chiloensis* spp. *chiloensis* (Fcc). (a and b) *F. vesca.* (c and d) Fcc f. *patagonica* from Cucao population. (e and f) Fcc f. *chiloensis* from Contulmo population. (a, c and e) Leaves from healthy plants obtained by meristem culture. (b) Symptom development 3 weeks post-inoculation with *Strawberry vein banding virus*. (d and f) Leaves from plants naturally infected with *Strawberry crinkle virus*, *Strawberry mild yellow edge virus*, *Strawberry mottle virus* and *Strawberry vein banding virus*.

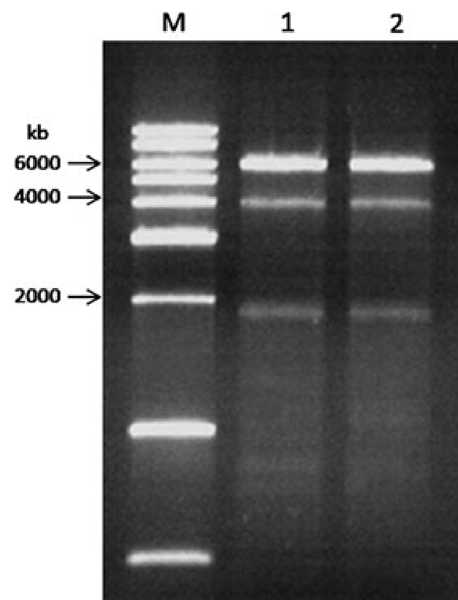

Figure 2 Double-stranded RNA (dsRNA) extracted from two *Fragaria chiloensis* spp. *chiloensis* (Fcc) populations. M: molecular weight marker (1-kb ladder). Lane 1: dsRNA extracted from Contulmo population (Fcc f. *chiloensis*). Lane 2: dsRNA extracted from Cucao population (Fcc f. *patagonica*).

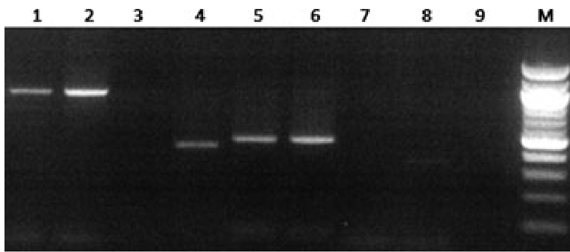

Figure 3 RT-PCR analysis of dsRNA extracted from Contulmo and Cucao *Fragaria chiloensis* spp. *chiloensis* populations. Lanes 1 and 2: amplification products using primer pair specific for *Strawberry mild yellow edge virus*. Lanes 3 and 4: amplification products using primer pair specific for *Strawberry vein banding virus*. Lanes 5 and 6: amplification products using primer pair specific for *Strawberry mottle virus*. Lanes 7 and 8: amplification products using primer pair specific for *Strawberry crinkle virus*. Lane 9: pooled PCR negative controls. Lanes 1, 3, 5 and 7: Cucao population. Lanes 2, 4, 6 and 8: Contulmo population. M: molecular weight marker (100-bp DNA ladder). Amplicons were separated on a 1·5% agarose gel.

Phylogenetic relationships between ABV isolates

Partial nucleotide sequences of Chilean ABV isolates, including 344 bp of the SCV L protein gene sequence, 231 bp of the SMYEV coat protein (CP) gene sequence, 413 bp of the SMoV polyprotein gene sequence and 381 bp of the SVBV CP gene sequence, were compared with previously reported ABV isolates from other areas of the world. Multiple alignment analysis revealed a high degree of conservation between viral isolates with few nucleotide differences observed (data not shown). To determine the evolutionary relationships between ABVs found in this work and previously reported viral isolates from strawberry species, phylogenetic trees were assembled using the corresponding nucleotide sequences. The phylogenetic analyses revealed a high level of homogeneity between the viral isolates compared. The SCV isolates were subdivided into two groups. SCV isolates Petrohue and Contulmo2 were clustered with isolates from the UK, whereas isolates Cucao, Pencahue and Contulmo1 were clustered with most of the SCV isolates from the Netherlands and Germany (Fig. 5a). Conversely, SMYEV isolates were distributed between isolates from China, making it difficult to draw any conclusions about the relationship between Chilean viral isolates (Fig. 5b). Local viral isolates of SMoV clustered separately from Chinese SMoV sequences. The isolate from the Cucao population of Fcc was different from the other Chilean isolates (Fig. 5c). The phylogeny of SVBV revealed that Chilean isolates were clustered separately from North American isolates (Fig. 5d).

Discussion

The results show that Fcc plants growing in the wild or in fields are highly infected by ABVs. Use of ELISA and RT-PCR revealed infections by SCV, SMYEV, SMoV and SVBV in the Fcc populations analysed, which is in agreement with previous reports (Herrera & Lavín, 1998; Thompson *et al.*, 2003; Cabrera *et al.*, 2004). Thompson *et al.* (2003) suggested that the high level of infection by SMoV in the Fcc population was most likely because it was uncultivated. The present results confirm this proposal but show that white-fruited cultivated Fcc plants are also highly infected by ABVs. This study presents for the first time the partial nucleotide sequences for SVBV and SCV isolates affecting both wild and cultivated Fcc populations in Chile. Previous studies reported *Nepovirus* and *Ilarvirus* infection in Fcc at low levels (Herrera & Lavín, 1998). No other economically important viruses, such as SLRSV, TNV, TBRV and ToRSV, were detected in this study.

HTS technology can be used as a diagnostic tool to identify the viral profile in infected plants and to discover possible new viruses. No novel viruses were discovered here, but the importance of ABVs in the Fcc populations

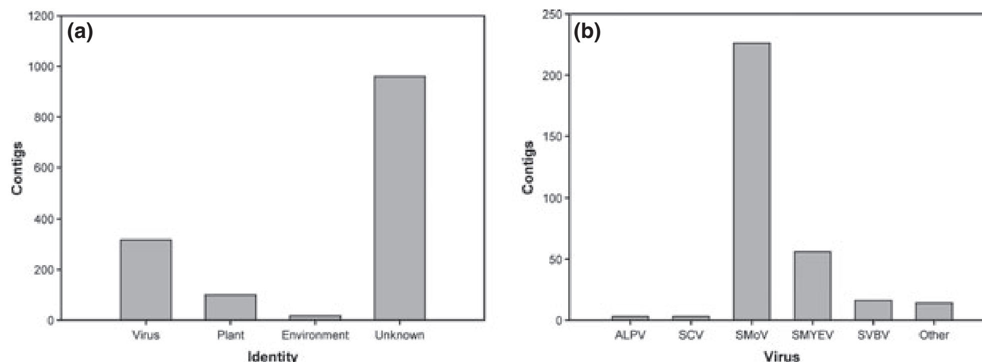

Figure 4 Identity of sequences obtained by Illumina sequencing of dsRNA from virus-infected *Fragaria chiloensis* spp. *chiloensis*. (a) Identity of all contigs obtained by Illumina after read assembly; identities of 1394 sequences were obtained by database sequence comparison using BLAST. Environment: sequences with homology to yeast, fungi and bacteria. Unknown: sequences with no match to database sequences. (b) Number of contigs with virus specific hits as identified using BLAST algorithms; 318 contigs were compared to database sequences. ALPV: *Aphid lethal paralysis virus*. SCV: *Strawberry crinkle virus*. SMoV: *Strawberry mottle virus*. SMYEV: *Strawberry mild yellow edge virus*. SVBV: *Strawberry vein banding virus*. Other: sequences with identity to yeast and fungal viruses.

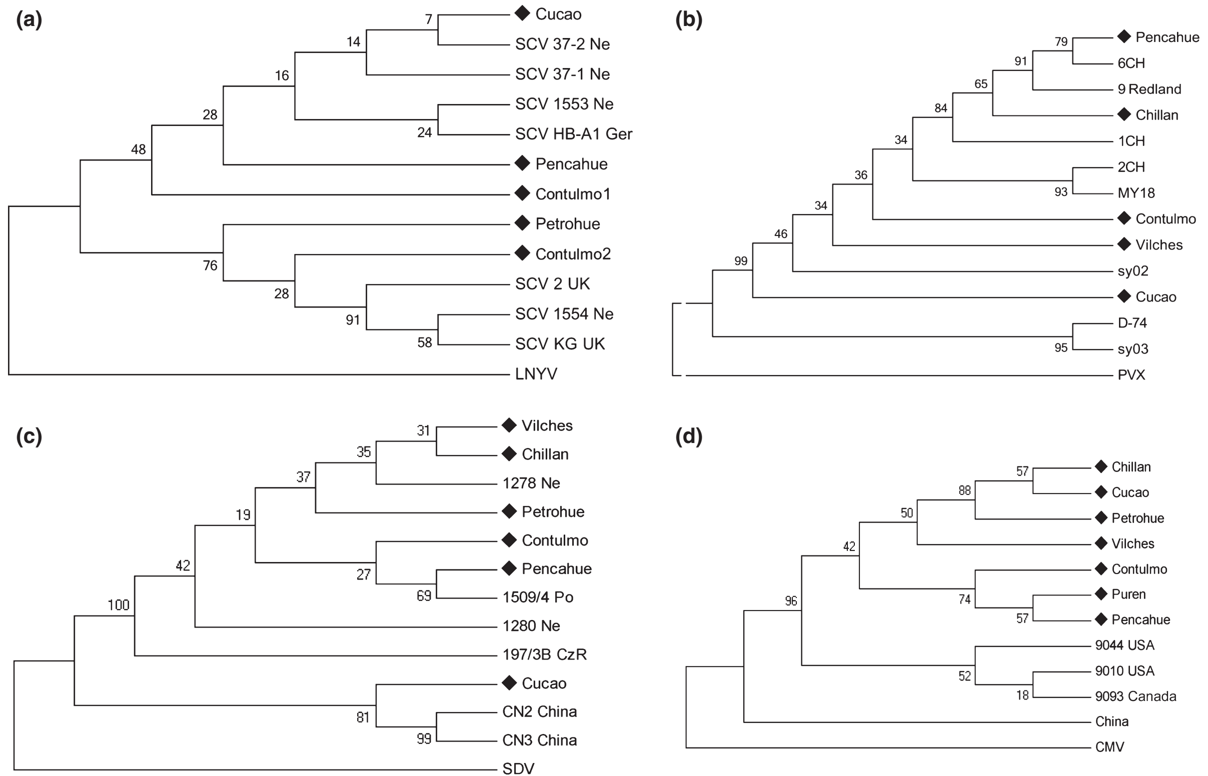

Figure 5 Phylograms of partial nucleotide sequences of aphidborne viruses affecting *Fragaria chiloensis* spp. *chiloensis* (Fcc). Consensus trees were generated using a neighbour-joining method. Nucleotide sequences from viral isolates from Chile and other areas of the world are included. Local Fcc viral isolates are named according to their provenance and are indicated by black rhombuses. The percentage reliability value given above each node indicates the frequency cluster over 1000 intermediate trees. (a) Phylogram of 344 nucleotide sequences of L protein gene of *Strawberry crinkle virus* (SCV). (b) Phylogram of 231 nucleotide sequences of CP gene of *Strawberry mild yellow edge virus* (SMYEV). (c) Phylogram of 413 nucleotide sequences of polyprotein gene of *Strawberry mottle virus* (SMoV). (d) Phylogram of 381 nucleotide sequences of CP gene of *Strawberry vein banding virus* (SVBV). *Lettuce necrosis yellow virus* (LNYV), *Potato virus X* (PVX), *Satsuma dwarf virus* (SDV) and *Cucumber mosaic virus* (CMV) were used as out-groups in (a) to (d), respectively. Previously reported sequences: Ne (Netherlands), Ger (Germany), UK (United Kingdom), Po (Poland), CzR (Czech Republic). 1CH, 2CH and 6CH (Chile; Thompson *et al.*, 2003). 9 Redland (Australia), MY18 (USA) and D-74 (Germany) (Jelkmann *et al.*, 1990; Thompson *et al.*, 2003). CN2, CN3, sy02, sy03 and China (China).

studied was confirmed. The possibility that the unknown sequences could contain sequences of viral origin cannot be excluded, but this needs further investigation. The results reported here suggest that ABVs are the most important viruses affecting Fcc and they could reduce fruit yields in white-fruited plant fields as previously observed in *F.* × *annanasa* (Martin & Tzanetakis, 2006). To determine the impact of these viruses on yield and quality characters of Fcc it will be necessary to set up field trials with known single and mixed infections of ABVs, and measure yield losses and quality.

Although the mixed ABV infections caused several symptoms in sensitive strawberry plants (*F. vesca*), infected Fcc plants generally do not show symptoms, indeed, many of the plants with mixed infections in this study grew vigorously. This can mean that farmers are not concerned about virus spread and do not use prevention strategies such as vector control or plant renewal using virus-free plants. Because the viruses found in wild Fcc plants (f. *patagonica*) were also detected in cultivated plants (f. *chiloensis*), vector movement between wild and cultivated populations could be an important factor for virus spread. The movement of the strawberry aphid (*C. fragaefolii*) between hosts can be affected by the nutritional quality of strawberry plants. Previous reports revealed that aphid migration between compatible plants was positively correlated with high nutritional quality of plants, which was important for aphid development and the reproductive fitness of the aphid colony (Underwood *et al.*, 2011). The Fcc genotype, plant–aphid compatibility and the distance between strawberry fields are all factors that need to be studied in order to obtain more information relevant to virus control strategies. Also, the high level of viral infection observed in all fields of Fcc could have resulted from horticultural practices and clonal plant production by runners.

This study reveals that the strawberry aphid *C. fragaefolii* was absent from most wild Fcc populations infected

with ABVs (Table 1). Possibly the sampling period (spring and early summer) did not coincide temporally with the occurrence of *C. fragaefolii* in wild Fcc plants, but it is also tempting to speculate that other aphid vectors could be involved in ABV spread. It has been reported that others members of the *Chaetosiphon* and *Myzus* genera can experimentally transmit SMYEV, SMoV, SCV and SVBV (Converse, 1987).

SMYEV was not detected by ELISA in any of the sampled plants from Petrohue (f. *patagonica*) but was detected by RT-PCR, suggesting that those plants had lower virus concentrations. In addition, SMYEV incidence was lower in all wild Fcc populations than in cultivated ones. This could be explained, in part, by the absence of the aphid vector or also by the defence mechanism of this botanical form.

Virus resistance genes have not been described in Fcc and the effect of virus accumulation in different populations of this taxon needs to be studied in order to identify resistant genotypes. The use of resistant cultivars is one of the most effective strategies to control viral diseases in the field (Gómez *et al.*, 2009). However, molecular mechanisms of plant–virus interaction and genetic resistance in the genus *Fragaria* remain unknown. It is possible that Fcc could be a source of tolerance to the ABVs that may be useful in breeding of *F.* × *ananassa* to reduce impact of virus infections.

The mechanism of aphid transmission of SMYEV is not completely characterized. It is the only potexvirus transmitted by aphids and the participation of a helper virus in the transmission is possible (Jelkmann *et al.*, 1990; Lamprecht & Jelkmann, 1997). Screening for viruses using HTS showed the presence of the insect virus ALPV at a low level. There is no *a priori* reason to suspect ALPV as a helper component for SMYEV. Initially, the presence of this virus needs to be confirmed by RT-PCR in a range of plants with aphid-transmissible SMYEV and then, interactions between ALPV and SMYEV could be investigated using infectious clones of SMYEV (Lamprecht & Jelkmann, 1997).

Because of the use of conserved genome sequences to construct the phylogenetic trees, low clustering significance was obtained. However, the SCV isolate from Petrohue was different from other Chilean SCV isolates (Fig. 5a). As proposed by Klerks *et al.* (2004), two phylogenetic groups were described. The results of the present study show that SCV isolates from Cucao, Pencahue and Contulmo1 were clustered in group I, while SCV isolates from Petrohue and Contulmo2 were clustered in group II.

In the phylogram of SMYEV no significant bootstrap values were obtained (Fig. 5b) and there was no relationship to geographical origin of the host plant. Three phylogenetic groups of SMYEV were described by Thompson & Jelkmann (2004) and the results of the present study showed the Chilean SMYEV isolate was related to groups II and III. The most representative isolate of group III is the MY18 strain and members of this group are non-aphid-transmissible strains such as the MY18 clone (Thompson & Jelkmann, 2004). It is possible that some of the SMYEV isolates from Chilean Fcc populations have this characteristic, but more sequence analysis and transmission studies are necessary to provide more information.

The SMoV phylogram (Fig. 5c) showed a relationship between geographically related isolates as previously described (Thompson & Jelkmann, 2003; Yang *et al.*, 2009). The isolate from Cucao was clearly different and its ancestral separation suggests that SMoV may have originated from this area. However, more evidence is necessary to confirm this.

Three phylogenic groups are proposed here for SVBV (Fig. 5d): group I, which includes viral isolates from Chile; group II, for North American isolates; and group III, for Chinese isolates. This divergence might be explained as being the result of isolation by either geographic or host factors.

This study has identified four ABVs (SMYEV, SCV, SMoV and SVBV) affecting wild and cultivated Fcc populations in Chile. These viruses are amongst the main problems affecting Fcc fields. *Fragaria chiloensis* spp. *chiloensis* is a genetic and cultural resource, but its cultivated area has been reduced. Therefore, improvement of horticultural techniques and strategies for viral disease control are essential for conservation purposes. This study provides novel ABV sequences from South American isolates and a basis for future studies to unravel the evolution of these viruses in the *Fragaria* genus.

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Supporting Information

Additional supporting Information may be found in the online version of this article:

Table S1. Accession numbers of aphidborne virus sequences incorporated to GenBank.

Table S2. Accession numbers of sequences used to generate the phylogenetic trees of aphidborne viruses.

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