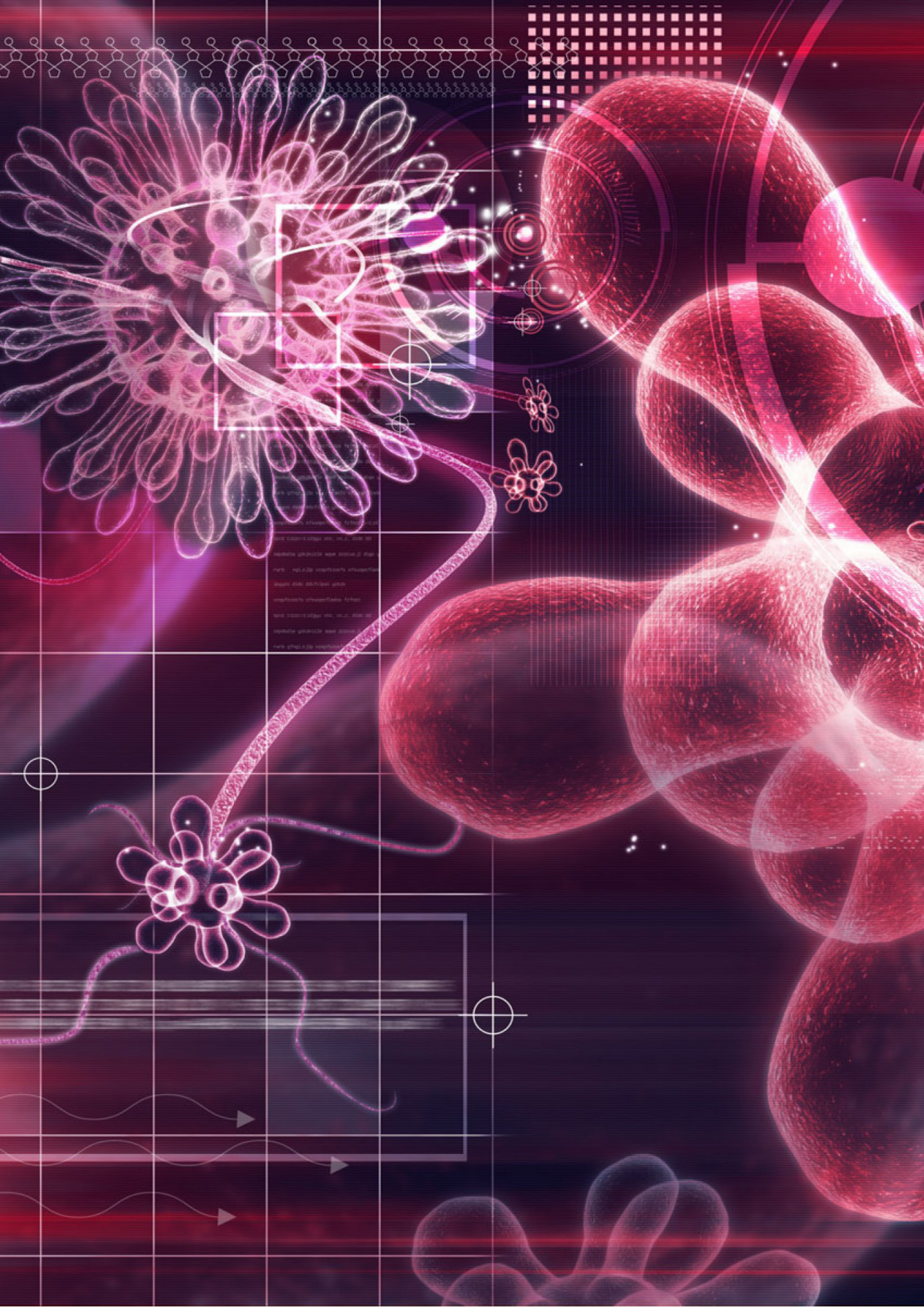

MOLECULAR IDENTIFICATION OF VIRUS CO-INFECTING WITH PHYTOPLASMA IN CARROT CROPS IN PERU

IDENTIFICACIÓN MOLECULAR DE VIRUS QUE INFECTAN CON FITOPLASMAS EN CULTIVOS DE ZANAHORÍA EN PERÚ

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ABSTRACT

Carrot is being affected by virus in co-infecting with phytoplasmas in the Mantaro Valley–Perú, producing from 20 to 98% of incidence in recent years. The aims were to identify molecularly the virus and phytoplasmas of Carrot Motley Dwarf complex, to determine the host range and mode of transmission. Carrot samples with yellowing, bruising and curling symptoms were used to extract total RNA. Molecular identifications of virus isolated from carrot were performed by the sequencing of siRNA molecules at the Genome Analyzer "Illumina's Genome Analyzer IIx", Plan-les-quater, Switzerland. These fragments of siRNAs, consisting of double-stranded RNA of 20-21 nucleotides (nt) perfectly complementary, which is generated by the presence of RNA of viruses that infect carrot, were reverse transcribed and amplified by PCR. The analysis of the readings was performed by using the "deep sequencing", the resulting readings of 20 and 24 nt were combined and each set of sequences was analyzed by BLASTn. Meanwhile, the mechanical transmission of carrot samples of similar symptoms was tested. Molecular identifications and characterizations of phytoplamas isolated from carrot were based on 16S rRNA gen using nested PCR. Methods of transmission by insect vectors were tested.The results showed that the two unrelated virus were identified: Carrot Red Leaf Virus (Polerovirus) and Carrot Mottle Virus (Umbravirus). The mechanical transmission of CMoV in four indicator plants was confirmed, too. The results showed that the phytoplasma that infects carrot in Huancayo and Chupaca belongs to 16SrIII X-disease group *(Candidatus phytoplasma pruni)*, while the phytoplasma isolated from corn Chupaca belong to AYP 16SrI Group *(Candidatus phytoplasma asteris)*. Phylogenetic analysis of the sequences, along with those obtained through BLASTn in the GenBank, determined the formation of two major monophyletic clades. From five species of insects that were identified related to carrot, three of them had phytoplasmas: *Paratanus exitiosus, Bergallia huancayoensis and Russelliana solanicola.* Only *P. exitiosus* transmitted disease. These data represent the first molecular confirmation of co-infection of phytoplasmas and virus producing similar symptoms in carrot crops in Peru.

Key word: virus, CRLV, CMoV, *Phytoplasma*, *Candidatus phytoplasma pruni*, *Candidatus phytoplasma asteris*, co-infection virus and phytoplasma, *Parathanus exitiosus,* .

RESUMEN

En estos últimos años, la zanahoria está siendo afectado por el virus en co-infección con fitoplasmas en el Valle del Mantaro-Perú produciendo 20-98% de incidencia. Los objetivos fueron identificar molecularmente los virus y fitoplasmas de zanahoria que causan el complejo del achaparramiento, para determinar el rango de huéspedes y el modo de transmisión. Se recolectaron muestras de zanahoria con síntomas de amarillamiento, morateo y encrespamiento a fin de extraer el ARN total. La identificación molecular de los virus aislados de zanahoria se realizaron por la secuencia de las moléculas de siRNA en el Genoma Analizador "Genoma Analizador IIx de Illumina", Plan-les-quater, Suiza. Estos fragmentos de siRNAs, que consta de ARN de doble cadena de 20-21 nucleótidos (nt) perfectamente complementarias, que se genera por la presencia de ARN de los virus que infectan zanahoria, fueron transcritas y amplificados por PCR inversa. El análisis de las lecturas se realizó mediante el uso de la "secuenciación profunda", las lecturas resultantes de 20 y 24 nt se combinaron y cada conjunto de secuencias se analizaron por BLASTn. Paralelamente se determinó la transmisión mecánica de virus provenientes de muestras de zanahoria con síntomas similares. Las identificaciones moleculares y caracterizaciones de fitoplasmas aislados de zanahoria se basaron en el 16S rRNA gen utilizando PCR anidada. Se ensayaron métodos de transmisión de fitoplasmas con insectos vectores. Los resultados mostraron que son dos virus no relacionados los que infectan zanahoria: Zanahoria Red Leaf Virus (Polerovirus) y virus del moteado de la zanahoria (Umbravirus). Se confirmó, también la transmisión mecánica de CMoV en cuatro plantas indicadoras. Además, se identificaron dos fitoplasmas infectando zanahoria. El fitoplasma que infecta zanahoria en Huancayo y Chupaca pertenece al grupo X-enfermedad 16SrIII *(Candidatus fitoplasma pruni)*, mientras que el fitoplasma aislado de zanahoria y maíz provenientes de Chupaca pertenecen a AYP 16SrI Grupo (*Candidatus* fitoplasma asteris). El análisis filogenético de las secuencias, comparados con los obtenidos a través de BLASTn en el GenBank, determinó la formación de dos principales clados monofiléticos. A partir de cinco especies de insectos vectoras que fueron identificados en plantas de zanahoria infectada, tres de ellos tenían fitoplasmas: Exitiosus paratanus, Bergallia huancayoensis y Russelliana solanicola. De ellas, solamente *P. exitiosus* transmitió la enfermedad. Estos datos representan la primera confirmación molecular de la co-infección de virus y fitoplasmas que producen síntomas similares en cultivos de zanahoria en el Perú.

Palabras clave: virus, fitoplasmas, zanahoria, CRLV, CMoV, *Candidatus phytoplasmas pruni, Candidatus phytoplasma asteris*, co-infection virus y fitoplasmas, *Parathanus exitiosus.*

INTRODUCTION

In the Mantaro Valley, the highland region of Peru, carrot is one of the most important vegetable crops. In recent years, this crop is being affected by a disease called "red mantle" producing from 20 to 98% of incidence and causing significant crops losses. The symptoms observed in the affected carrot plants are redness or browsing of leaves, yellowing, low rate of foliage growth, proliferation of adventitious roots, deformation and reduce the size of the root. Similar symptoms are also observed adjacent to carrot crops, assuming that pathogens have broad host range. Carrots can be affected by various pathogens such virus complex Carrot motley dwarf (CMD) known as the result of mixed infection by two viruses, the polerovirus Carrot red leaf virus and one of the umbraviruses Carrot mottle mimic virus (CMoMV) or Carrot mottle virus (CMoV) (Menzel, 2009). Also, carrots may be affected by phytoplasmas, pathogens members of the class Mollicutes that are also known to cause disease in hundreds of plant species worldwide (Liefting *et al*., 2004). The non culturable nature of phytoplasmas, erratic or temporal distribution in plants, and the low level of inoculums in the sieve tubes, is difficult to detect. Reports about others crops potentially prone to infection by phytoplasmas in Peru have been on the observation of symptoms in maize, tomato, potato, dandelion, and others (Hodgetts *et al*., 2008). Transmission of phytoplasmas between plants is by phloem-feeding insects of the order Hemiptera, primarily leafhoppers, planthoppers and psyllids (Lee *et al*., 2000). However, in the last 15 years, thanks to the development of molecular diagnostic techniques based on the isolation of 16S rRNA gene analysis of phytoplasma or virus siRNA are allowing to determine the etiology of these diseases and in depth studies, such as co infecting between pathogens such as viruses and phytoplasmas, the range of susceptible hosts, who is the transmission vector to develop fast and efficient strategies for integrated control methods of control to increase vegetable production.

Keywords: Virus, Phytoplasma, CRLV, CMoV, *carrot, Candidatus phytoplasma pruni, Candidatus phytoplasma asteris, Parathanus exitiosus.*

OBJECTIVES

- To identify molecularly the virus and phytoplasmas of Carrot Motley Dwarf complex,
- To determine the host range and mode of transmission.

MATERIALS AND METHODS

Molecular identification of viruses that cause "Carrot Motley Dwarf" in carrot

Were collected carrot with symptoms of yellowing, bruising, curling and curling + rosette (Figure 1). The methodology for the extraction of total RNA was that of Sambrook and Russell (2001). The amount and quality of RNA were checked by run agarose gel electrophoresis and the bands was visualized after staining with ethidium bromide (0,5 mg/ml) for 30 minutes.

The sequencing of siRNA molecules was realized at the "Illumina Genome Analizer IIx" FASTERIS Company SA, Plan-les-quater, Switzerland. To this end, siRNAs fragments, consisting of double-stranded RNA of 20-21 nucleotides (nt)perfectly complementary, which is generated by the presence of RNA of exogenous origin (such as viruses that infect carrots), were reverse transcribed and amplified by PCR. The analysis of the readings was performed using the "deep sequencing" methodology used by Cuellar et al. (2008) and the resulting readings of 20 and 24 nt were combined and each set of sequences was analyzed by BLASTn.

Detection of phytoplasmas on "Carrot Motley Dwarf" and other host crops

Prospective study and sampling of the disease were realized in 92 fields of carrots and other economically important crops from different farming localities in the Mantaro Valley, during 2009. Sampling was stratified and the sample was realized in 100 m2 of cultivated area in each plot. The symptoms considered were redness and yellowing of foliage, stunting, deformation and proliferation of secondary roots (Figure 2). To extract DNA from phytoplasma was used the Axygen Biosciences © kit. The extracted DNA was stored at 4 °C. The quantification of total DNA was determined using the comparative method with the molecular size marker Lambda / Hind III.

Phytoplasma detection was realized by amplification of 16S rRNA marker using the technique of nested PCR under the conditions described by Olórtegui et al. (2008). For the first DNA amplification reaction was used couple of phytoplasma universal primer PA2F: 5'-GCC AAC TAT CCG GTG GCT C -3 '/-PA2R: 5'-TTG CTA AAT GGA GTG GGC CTC-3' (expected size 1187bp). The product of this amplification was subjected to a second internal amplification with universal primer internal couple

NPA2F: 5'-ACA GCT ATG ACC AAC TGG GTG -3 '/-NPA2R: 5'-CCT GGG AAA TGG GGT ACT CG-3 '(expected size 485 bp). After the second amplification was realized electrophoretic gel run on 2% agarose. The DNA bands was visualized using the photodocument BIORAD equipped with UV trans illuminator, after staining with ethidum bromide. PCR products were sent for sequencing to the company Macrogen USA (Maryland - USA).

The phylogenetic analysis of sequences obtained were compared with the GenBank nucleotide database and analyzed using BLASTn v.2. Phylogenetic analysis identified phytoplasma sequences were realized with MEGA v.4 program. The phylogenetic tree was constructed using the "neighbor-joining" (Saitou and Nei, 1987). The distance was corrected by the nucleotide substitution method 2p Kimura and the consistency of the tree was assessed by the bootstrap method, using 1000 replicates.

Mechanical transmission of virus.

Was realized at the Greenhouse, following the methodology proposed by Menzel et al. (2009). For this, 17 species of differential plants were inoculated (Table 1), which were between 40 and 45 days after planting. The inoculum consisted of sap from infected with symptoms of curling, yellowing, bruising and yellowing + rosette. Differentials plants were inoculated for each type of symptom. The inoculated plants were placed in a greenhouse with insect-proof mesh, temperature controlled at 26 °C and 75-85% RH.

Phytoplasma transmission by insect vectors

By nested PCR was detected Phytoplasma in three insects species potential transmitters: Parathanus exitiosus, Bergallia sp. and Russelliana solanicola. By the method of capture with network were collected species of this species from infected carrot fields, located Concepción. The insects were identified at the laboratory of Entomology INIA. Finally, the insects were placed in each entomological cage (1,0 m alt. x 0,5 m wide x 0,5 m long) lined with insect-proof mesh. They were placed in each cage trays containing 100 seedling of carrot. Phytoplasmas were inoculated by placing 50 adult insects on the seedlings, leaving food for 15 days. The experimental design used was randomized complete block with the three insect treatments, respectively.

RESULTS

Molecular identification of viruses that infect carrots

The results showed that two unrelated virus were identified: Carrot Red Leaf Virus and Carrot Mottle Virus. The analysis of "deep sequencing" of siRNAs and subsequent comparison with the GenBank database indicate that the first group of sequences of viral genomes present in carrots, corresponed to Polerovirus: Carrot red leaf virus (98% similarity), reported too in Oxford (Huang et al., 2005) and the second group of sequences of viral genomes corresponded to Umbravirus: Carrot Mottle Virus group (79% similarity) reported in Germany (Menzel et al., 2008), as seen in Tables 1 and 2, respectively confirming the previous result of mechanical transmission of CMoV.

Figure 1. Carrot plants showing symptoms caused by viruses and phytoplasmas of complex Carrot motley dwarf : a) Curling. b) Yellowing. c) Bruising. d) rosette + curling.

Figure 2. Symptoms caused by phytoplasmas in different hosts: a) Deformed of roots in carrot. b) Proliferation of shoots in corn. c) Yellowing of faba beans. d) Stunt of turnip.

Table 1. Comparison of the similarity of the first sequence of virus isolated from carrot with the deposited in GenBank obtained by BLASTn.

Descripction	Nº Accession	E value	Max ident
Carrot mottle virus isolate Weddel, complete genome	FJ188473.1	$3e^{-118}$	79%
Carrot mottle virus strain UK-RG-6 putative RNA-dependent RNA	AY325514.1	$2e^{-19}$	80%
Carrot mottle virus strain UK-RG-2 putative RNA-dependent RNA polymerase gene, partial cds	AY325510.1	$1e^{-16}$	78%
Carrot mottle virus strain UK-RG-3 putative RNA-dependent RNA	AY325511.1	$3e^{-04}$	80%
Carrot mottle mimic virus isolate California RNA complete cds	FJ188471.1	0.001	85%

Table 2. Comparison of the similarity of the second sequence of virus isolated from carrot with the deposited in GenBankobtained by BLASTn.

Description	Nº Accession	E value	Max ident
Carrot red leaf virus strain UK-1, complete genome	AY695933.1	$1e^{-31}$	98%
Cucurbit aphid-borne yellows virus isolate pM0829 -3 RdRp (RdRp) and coat protein (Cp) genes, partial cds	EF063704.1	$1e^{-05}$	78%
Cucurbit aphid-borne yellows virus clone pXhy0821-5 polymerase(RdRp) and coat protein (CP) genes, partial cds	DQ973123.1	$1e^{-05}$	78%
Melon aphid -borne yellows virus isolate MABYV -TW73 RdRp protein (RdRp), coat protein (CP), and MP protein (MP) genes, partial cds	GU324114.1	$2e^{-04}$	77%
Melon aphid-borne yellows virus isolate MABYVC-TW14 RdRp protein (RdRp), coat protein (CP), and MP protein (CP) genes, partial cds	GU324113.1	$2e^{-04}$	77%

Detection of phytoplasma in carrots and other host crops.

Of 92 samples with symptoms of "red mantle", 62% had positive reaction to the marker 16S rRNA phytoplasma and 38 % were negative (Figure 3). It can be seen that only pea and quinoa were not infected by phytoplasmas.

The analysis of Table 3 indicates that it was a higher percentage of samples that had positive reaction to the phytoplasma (52.2%), indicating that the symptom is primarily associated with the presence of phytoplasmas. Then, there are signs that despite showing obvious symptoms gave negative reaction to pathogen (35.9%), we infer that these plants may be infected with viruses that also cause similar symptoms. The bands of amplified phytoplasma 16S rRNA visualized on agarose gel (Figure 4) had a size of approximately 485 bp.

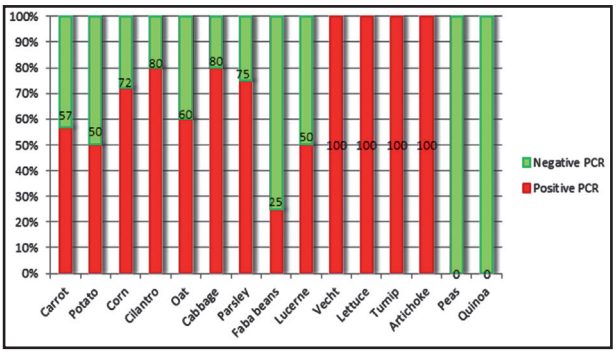

Figure 3. Percentage of positive reaction to the phytoplasma 16S rRNA marker in 92 samples of crops and bruising yellowing symptoms were collected in the Mantaro Valley during 2088 - 2009.

Table 3. Results of the PCR reaction of total samples of carrot and other species with symptoms and asymptomatic fitoplasmosis collected in the Mantaro during 2008 and 2009.

PCR Reaction	Whit *symptoms*	Asymptomatic
Positives	52,2 %	9,8 %
Negatives	35,9 %	2,2 %
Nº total samples		92

Molecular identification of phytoplasma that infect carrots

The results showed that the phytoplasma that infects carrot in the provinces of Huancayo and Chupaca belongs to 16SrIII X-disease group ***(Candidatus phytoplasma pruni)***, while the phytoplasma isolated from corn Chupaca belong to AYP 16SrI Group *(Candidatus phytoplasma asteris)*. Phylogenetic analysis of the sequences, along with those obtained through BLASTn in the GenBank, determined the formation of one monophyletic clades (Figura 5). It is observed that the two sequences from the sample *Daucus 1 phytoplasma Huancayo* (FitoJun2) and *Daucus 2 phytoplasmaChupaca* (FitoJun5) had 99% identity with eight partial sequences of phytoplasmas isolated from differents plant species from almost all of North and South America (Table 4).

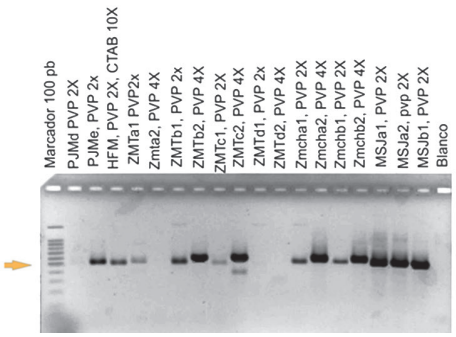

Figure 4. Agarose gel electrophoresis of amplified 16S rRNA marker of phytoplasmas obtained from samples of potato, bean, carrot and corn yellowing symptoms, from San Juan(S) and Miraflores (M) Chupaca, and Mito, Concepción (M) using nested PCR. Carriles PJMe, HFM, ZMTb1, ZMTb2, ZMTc1, ZMTc1, ZMTc2, Zmcha1, Zmcha2, Zmchb1,Zmchb2, MSJa1, MSJb1 MSJa2 results positive for the reaction. White lane: negative control.

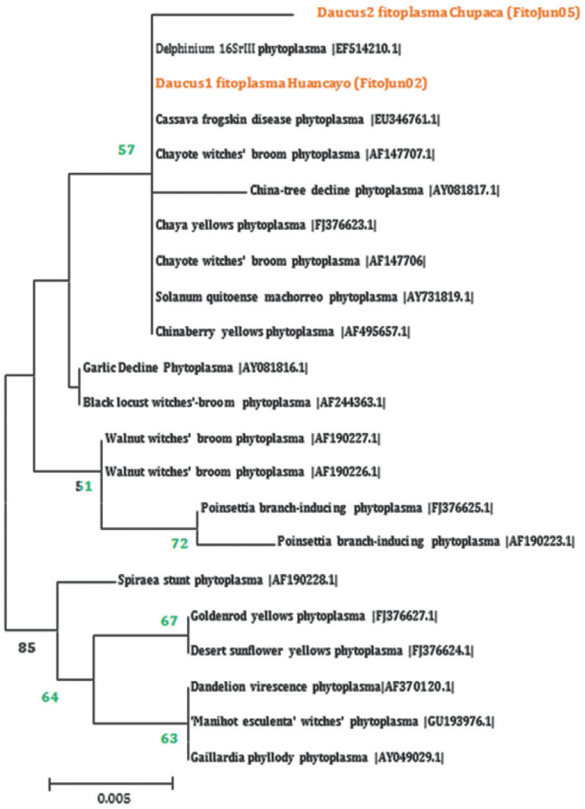

Figure 5. Phylogenetic tree constructed by the neighbor-joining method, showing the phylogenetic relationships of partial segments of the 16S rRNA gene of phytoplasmas infecting carrots in comparison with similar published (GenBank accession number). The analysis was performed with 1000 bootstrap replicates. The scale bar represents 5 substitutions per 1000 nucleotides.

Table 4. Relations of phytoplasmas obtained from GenBank by BLASTn and have 99% identity to the sequences of Daucus1 phytoplasma Huancayo (FitoJun2) and Daucus 2 Chupaca phytoplasma (FitoJun5), isolated from cultures of carrot in the Mantaro Valley.

Nombre del Fitoplasma	Nombre del huésped	Nombre científico/ familia	Especie/ grupo taxonómico	Lugar reportado	Identidad (%)	Autor, año de reporte
Chin-tree decline phytoplasmaa	Árbol chino, melia	*Melia azederach* Meliacea	***Candidatus Phytoplasma*** 16SrIII -disease group)	Argentina	99	Galdeano, 2004
Solanum quitoense machorreo phytoplasma	Naranjilla, lulo	*Solanum quitoense* solanacea	***Candidatus Phytoplasma*** 16SrIII -disease group)	Colombia	99	Alvarez *et al.*, 2007
Chaya yellows phytoplasma	Chaya	*Cnidoscolus chayamansa*/ Euphorbiacea	***Candidatus Phytoplasma*** 16SrIII -disease group)	USA	99	Lee *et al.*, 2009
phytoplasma		*Melia azederach* Meliacea	***Candidatus Phytoplasma*** 16SrIII -disease group)		99	Harrison, 2003
Chayote witches' broom phytoplasma	Chayote	*Sechium edule*) Cucurbitácea	***Candidatus Phytoplasma*** 1 SrIII -disease group)	Brasil	99	Montano *et al.*, 2000

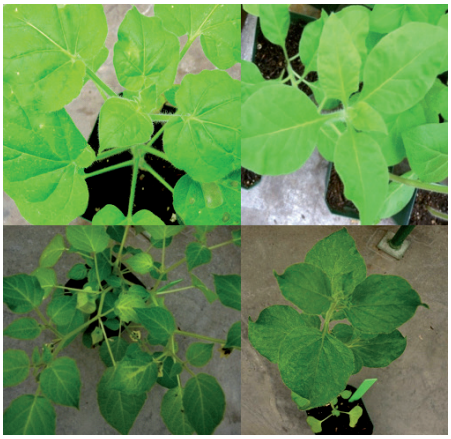

Figure 4 . Systemic symptoms caused by CMoV four indicator plants after mechanical transmission. a) Nicotiana glutinosa with soft mottled . b) N. bigelovi x N. clevelandii with soft mottled. c) Physalis floridana showing chlorotic mottle and thinning of veins. d) soft mottled Nicotiana benthamiana (ms).

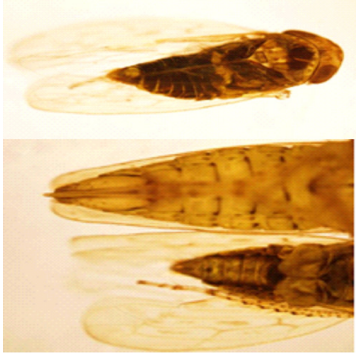

Figure 5. Dorsal view of Parathanus exitiosus transmissor of phytoplasmas in carrot collected in Concepción. a) Adult male (3.4 mm in length). b) Genital plate: female (f) and male (m).

Phytoplasma transmission by insect vectors

Five species of insects were identified in carrots and they were detected phytoplasmas cells only in three: Ruselliana solanicola, Parathanus exitiosus and Bergallia Huancayoensis.The transmission capacity of phytoplasmas infecting carrots was demonstrated only with P. exitiosus (Figure 5. It was determined by Duncan test (p = 0.05), the level of significance between treatments in the average percentage of incidence of fitoplasmosis is highly significant in Parathanus treatment (61.67%), differing significantly from the other treatments (Table 5), who did not transmit the pathogens despite containing phytoplasmas cells.

Table 5. Significance test for the average incidence percent of fitoplasmosis vector-borne of carrot in greenhouse conditions (INIA, Huancayo).

TREATMENTS (VECTORS)	AVERAGE INCIDENCE (%)	SIG *
Paratanus exitiosus	61,67	a
Russelliana solanicola	0	b
Bergallia huancayoensis	0	b
Control	0	b

DISCUSSION

The "red mantle" prospecting in carrot and determination of the hosts range

In carrot fields located in the Mantaro Valley, there has been high levels of incidence "red mantle" disease, whose symptoms caused by viruses and phytoplasma, could not initially differentiate. Also, noted that crops of economic importance surrounding the carrot fields have characteristic symptoms to those caused by phytoplasmas, making boast that they are also guests of these pathogens.

The study of molecular diagnosis of the "red mantle" in carrots and other crops of economic importance, conducted in four provinces, which includes the Valley of the Mantaro, has made it possible to confirm that the disease is widely distributed in carrot and most crops, confirming a preliminary report given by SENASA (Lenin et al., 2005), these results were contrasted with the molecular diagnostics in carrots with symptomswhere it has been determined the co - infection of the phytoplasmas and viruses CRLV and CMoV, confirming the reports given by Davis and Raid (2004).

The detection of phytoplasma in asymptomatic samples also resulted in positive reaction, allowing to infer that infected plants may have latent infection that masks the symptoms, corroborating the results obtained by Bressan et al. (2006) and Wei et al. (2004). Similarly, the molecular diagnostics using primers to amplify a segment of 16S rRNA has been widely used for the detection of various pathogenic prokaryotes, but in the case of phytoplasmas diagnosis is not easy since they may not be isolated in culture media and have under the title in the phloem. For these reasons, although the phytoplasms seriously threaten the culture of some important species, obstacles have been presented to carry out more detailed research explaining new hypotheses about this disease.

Molecular identification of virus causing "red mantle" in carrot

The analysis of the siRNAs by the 'deep sequencing' and subsequent comparison with the database of the GenBank using BLASTn, resulted in two groups of sequences, corresponding the first Carrot Red Leaf virus (98% similarity), present in Oxford (Huang et al., 2005) and the second group to the Carrot Mottle Virus (79%) reported in Germany (Menzel et al, 2008). The presence of CRLV and CMoV viruses affecting cultivated carrot in Huancayo confirm reports of Menzel et al. (2009) and Tang et al. (2009), who point out that commonly these viruses found in temperate climates caused the complex "Motley Dwarf Virus" (MDV), and partners are to produce synergy in its pathogenesis because of its form of transmission. So transmission of the CMoV or the CMoMV using the vector insect Cavariella aegopodii require the presence of the ayudador virus CRLV. This phenomenon is explained by Davis and Raid (2002), who indicate that in a doubly infected plant, the CMoV which has a single-stranded RNA, appears to be encapsidado by the protein subunits of the CRLV (a process called transcapsidacion) and therefore acquires the ability to be able to be transmitted by its vector C. aegopodii. On the other hand, the results of mechanical transmission that induced symptoms of chlorotic mottling in four differential plants Nicotiana clevelandii x N. bigelovii, N. benthamiana and Physalis floridana, have allowed to confirm the presence of CMoV, infecting carrot

plants presenting symptoms of Frizz, being one of the umbravirus which is transmitted from plant to plant by this modality, allowing a higher increase of the same owing to their dual transmission both by insects with the help of the CRLV, and mechanically by infected SAP. On the other hand, Menzel et to the. (2008) also obtained positive response when they were inoculated plants of N. benthamiana with SAP from carrot infected with CMoMV and CMoV, indicating that the two viruses may be present causing infection in complex or mixed together with the CRLV. Viruses and phytoplasmas in carrot can converge in a single plant as says it Weintraub and Phil (2010). However, the transmission of phytoplasmas with other pathogens has not been widely studied. Found that some viruses can adhere to the phytoplasma chromosome and alter its pathogenicity (Weintraub and Phil, 2010), but this does not happen in the case of Corn Stunt Spiroplasm (CSS) and the Virus from scratch fine maize (RFM) which are transmitted by the same vector Dalbulus maidis. Found that after the sequential acquisition of CSS and RFM vector first transmitted the virus and then the spiroplasma, by having this last a longer period of latency (Bosco and D'amelio, 2010). These results do not rule out the possibility of Parathanus could be transmitting the phytoplasma of carrot and CRLV virus, persistent.

Identification and molecular characterization of phytoplasmas that cause "red mantle".

The sequencing of 16S rRNA isolated from phytoplasma of carrot gene segments gave as result a lower frequency of foundations of C-G (48.8%) and greater than A-T (51.5%), confirming the innate characteristic of these pathogens have a lower percentage of C-G, such as Weintraub and Phil (2010). With respect to the relationship that have these sequences with those existing in the database of the GenBank and molecular analysis of phytoplasma sequences that affect carrot "Daucus 1 phytoplasma Huancayo FitoJun02" and "Daucus 2 phytoplasma Chupaca FitoJun05", determined that they have 99% similarity between themselves and also with eight sequences deposited in GenBank and which correspond to phytoplasmas reported in different countries of North and South America. Taxonomic location of the taxonomic group which correspond to these sequences is the 16Sr III (X-disease Group) and consequently the species Candidatus phytoplasma pruni.On the other hand, the isolated from phytoplasma in maize Zea mays phytoplasma Chupaca (MSJb2) has 99% similarity with more than 10 sequences deposited in GenBank and its taxonomic position corresponds to 16Sr I Aster Yellow Phytoplasma Group and the species Candidatus phytoplasma asteris.In other cases, the sequencing was partial with only 50 to 80 pb, and then there was a pause due to unknown factors and data which did not serve to make a bioinformatic analysis. Phylogeny. Phylogenetic analysis of these two sequences of phytoplasmas in carrot determined that they form a single clade monophyletic, together with other eight sequences of phytoplasmas reported in GenBank (99% identity), which are widely distributed in North and South America.

Transmission of phytoplasmas and viruses by insect vectors

According to Weintraub and Beanland (2006) and Weintraub and Phil (2010) as potential vectors of phytoplasma associated insects are the cicadellids and psyllids the Hemiptera order. But the fact of an insect to carry the phytoplasma as a result of his power, does not necessarily imply it as a vector, since it has been determined that there is a high specificity of transmission by vectors, defined by different factors in the vector-patogeno interaction. For example, the type of proteins found in the cell membrane of the pathogen and vector intestine microfibers that form a very specific complex, as manifest it Susuki et to the. (2009). the detection of phytoplasma in insects using PCR gave positive reaction in the P. exitiosus and B. huancayoensis as well as psillido R. solanicola, indicating that they are potential carriers of phytoplasmas.

But it has been determined as a result of several trials of transmission that only Paratanus exitiosus had capacity to transmit the Candidatus phytoplasma pruni infecting carrot. These results were evidenced by the characteristic symptoms of fitoplasmosis who presented the seedlings treated with Paratanus and contrasted with the molecular PCR test for detection of phytoplasmas. On the contrary, Bergallia and Russelliana, despite having detected phytoplasma in adults by means of nested PCR, not proved to be transmitters efficient, making presume are only carriers as a result of their diet with infected SAP.

These results allow us to infer that P. exitiosus could be transmitting phytoplasma different guests being a generalist or polyphagous pest that affects carrot, beans, potato, lettuce, corn, and at the same time

feed and move a crop to another by their easy movement between adjacent plots, would be transmitting the disease as Vargas (2001) points out.

In addition, it has been observed that carrot is grown in the Valley of the Mantaro during all seasons of the year, facilitated by the existence of irrigation water, and have noticed increased environmental temperature in recent years, these factors have influenced in the presence of a high population density of insect and host available, resulting in that the disease occurs in epidemic form.

Regarding the insect vectors of virus in carrot, it is reported that both CRLV and CMoV are transmitted by aphid Cavariella aegopodii, differentiated by the form of transmission that the first is persistently and for the second is nonpersistent in the presence of an ayudador virus, which in this case is the CRLV, with whom he meets in the same plant and has been found in the hemolymph of the vector insect. This form of transmission is explained by Davis and Raid (2002), who indicate that in a mixed infection, (ss) strand RNA of the CMoV, which does not encode the formation of protein subunits (sup) for the protein coat, is encapsidada by the sub units of the CRLV protein and therefore acquires the ability to be transmitted by its vector C. aegopodii. However, although each virus is capable of infecting plants alone, is not discarded the possibility that two or more unrelated viruses come together in a same plant carrots, including the presence of a phytoplasma and that together they are transmitted in parallel (Bosco and D'amelio, 2010). The presence of the vector C. aegopodii in the Valley of the Mantaro has been reported by Valencia et to the. (1975) as a pest of carrot and registered by SENASA in recent years, confirming the presence of these viruses and their transmission through this vector.

CONCLUSIONS

- The Carrot Motley Dwarf complex is a disease caused by viruses and phytoplasmas that is widely distributed in the provinves of Huancayo, Chupaca, Concepción and Jauja comprising the Mantaro Valley in Peru.
- Molecular identification of viruses that infect carrots in this region determined that two unrelated virus are infecting carrot: Carrot Red Leaf Virus (Polerovirus) and Carrot Mottle Virus (Umbravirus).
- The mechanical transmission confirmed the presence of CMoV who caused symptoms of mottle in four differential plants as N. bigelovii x N. clevelandii, N. glutinosa, N. benthamiana and P. floridana.
- Molecular identification based on DNA sequences of 16S rRNA gene region identified a phytoplasma infecting carrot in Huancayo and Chupaca corresponding to Candidatus phytoplasma pruni (X-disease 16SR Group III).
- Phylogenetic analysis of the sequences identified a monophyletic group formed by the two phytoplasma carrot isolates from Huancayo (Daucus 1 phytoplasma Huancayo) and Chupaca (Daucus 2 phytoplasma Chupaca) with a 0.5% genetic distance between them.
- From five species of insects that were identified related to carrot, three of them had phytoplasmas: Paratanus exitiosus, Bergallia huancayoensis and Russelliana solanicola. Only P. exitiosus transmitted disease.
- These results confirm the first molecular identification about co-infection of virus and phytoplasmas producing similar symptoms in carrot crops in Peru.

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