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Diverse phytoplasmas associated with maize bushy stunt disease in Peru

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Abstract Corn plants showing symptoms of midribs chlorosis, and leaf reddening, short internodes, ear proliferation, and plant growth reduction were collected in Peru from fields in nine localities in the provinces of Huancayo, Chupaca, and Jauja in the Junín region, and tested to verify phytoplasma presence and identity. Primers amplifying the phytoplasma ribosomal 16S and ribosomal protein genes were used. The phytoplasma presence was detected in symptomatic samples from all the surveyed areas. The sequencing of the obtained amplicons indicated the presence of '*Candidatus* Phytoplasma asteris' and '*Ca. P. pruni*'-related strains. A BLASTn search of sequenced genes showed that the two '*Candidatus* Phytoplasma' strains identified in corn shares 100% and 99.82% identity with the '*Ca. P. asteris*' strains from maize and 99.92% and 99.55% with 'Ca. *P. pruni*'-related strains, respectively. The RFLP analyses allowed to enclose these phytoplasma strains in the 16SrI-B and 16SrIII-J subgroups; however, the two phytoplasmas were, in some cases, present in mixed infection. The 16SrIII-J phytoplasma is for the first time reported associated with the maize bushy stunt disease and this represent a relevant information for the disease epidemiology towards its appropriate management in the affected area.

Keywords *Zea mays* · Phytoplasma · Shortening of internodes · Disease · Molecular detection

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Introduction

Corn is one of the main species cultivated worldwide (FAO, 2020). In Peru, both hard yellow and starchy corns are grown for human consumption as dry or wet grain (Huamanchumo de la Cuba, 2013). According to the Peruvian Ministry of Agrarian Development and Irrigation, the national production of starchy corn for 2019 was 305,198 tons, (MIDAGRI, 2021). Maize bushy stunt (MBS) is the most important disease that decreases maize production especially in Latin American cultivations, where it can decrease the production and yield of the crop up to 100% (Jones & Medina, 2020). The disease has been reported in the United States, Costa Rica (Harrison et al., 1996), Mexico (Pérez-López et al., 2016), Nicaragua (Hruska et al., 1996), Belize (Henríquez et al., 1999), Colombia (Duduk et al., 2008), and Brazil (Bedendo et al., 1997). In Peru, MBS has been reported in the departments of Ancash and Ayacucho (Nault et al., 1979, 1981) and in the Junin region (Hodgetts et al., 2009; Nipah et al., 2007). The MBS infected corn plants shows symptoms of midribs chlorosis, and leaf

reddening, short internodes, ear proliferation, and plant growth reduction (Bedendo et al., 1997). This disease was reported associated with the presence of '*Candidatus* Phytoplasma asteris' (16SrI-B subgroup) (Lee et al., 2004) and known to be transmitted by *Dalbulus maidis* (Hemiptera: Cicadellidae) (Nault & Delong, 1980). The complete genome of a Brazilian strain of this phytoplasma from corn was recently sequenced (Orlovskis et al., 2017). Phytoplasmas are pleomorphic bacteria with size ranging from 200 to 800 nm. They multiply and survive in isotonic environments including plant phloem and hemolymph of insect vectors (Bertaccini et al., 2014) and are classified using molecular tools such as nested polymerase chain reaction (PCR) on the 16S ribosomal gene followed by sequencing or restriction fragment length polymorphism (RFLP) analyses. The two methods are defining '*Candidatus* Phytoplasma' species and 16S ribosomal groups, respectively (Bertaccini & Lee, 2018; IRPCM, 2004).

Symptoms associated with MBS were observed more than ten years ago in fields located in the Mantaro Valley (Junín region) (Hodgetts et al., 2009). In the last production campaigns these symptoms were again observed in several fields located in the same area. Considering the increasing yield losses, a survey was carried out to verify the symptom incidence and the identity of the associated phytoplasmas to devise the most appropriate management measures to be adopted to reduce the MBS impact in these corn cultivations.

Materials and methods

Sample collection and disease incidence estimation Whole plants with symptoms of chlorotic streaking, shoot proliferation, anthocyanescence, dwarfism, generalized chlorosis, bud atrophy, and internode shortening (Fig. 1) were collected in the department of Junin-Peru from production fields in the localities of Sicaya, San Agustin de Cajas, Hualhuas, and San Jeronimo located in Huancayo province, Tres de Diciembre, San Juan de Iscos, Ahuac, Chupaca in Chupaca province and El Mantaro in Jauja province (Table 1; Fig. 2), between November 2018 and April 2019. A total of 114 samples from nine different areas were sampled and, after washing under tape water, 2.5 g of leaf vein tissues were stored at −20 °C for nucleic acid extraction. Disease incidence estimation was calculated in each area as the proportion of fields with the number of plants showing MBS symptoms to the total number of fields assessed multiplied by 100.

Nucleic acid extraction The DNA extraction from the plant samples was done using a CTAB method (Doyle & Doyle, 1990). For negative control, the DNAs of two symptomless plants collected in asymptomatic fields were extracted. The quality and purity of the extracted DNA were verified in a 1% agarose gel in 1X TAE buffer (40 mM Tris-Acetate, and 1 mM EDTA at pH 8.3), stained with ethidium bromide (10 ng/μl) and visualized with a Gel Doc XR+ Gel Documentation System (Bio-Rad Laboratories, Inc.). Quantification was obtained by NanoDrop One (Thermo Scientific™).

Detection of disease-associated pathogens To amplify the phytoplasma 16S rRNA gene, the universal primers P1/P7 (Deng & Hiruki, 1991; Schneider et al., 1995) were used in PCR followed by nested PCR with P1A/16Sr-SR (Lee et al., 2003, 2006), 3fwd/3rev (Manimekalai et al., 2010), R16F2n/R2 (Gundersen & Lee, 1996) and fU5/rU3 (Lorenz et al., 1995) primers. PCR with primers MBSF1/MBSR1, amplifying an ATP-dependent Zn protease gene of phytoplasmas from corn (Harrison et al., 1996) was also performed. Additionally ribosomal protein (rp) gene primers rpL2F3/rp(I)R1A specific for phytoplasmas in group 16SrI (amplicon about 1200 bp), and rp(III)F1/rp(III)R1, specifically amplifying about 800 bp of the phytoplasmas enclosed in ribosomal group 16SrIII (Martini et al., 2007) were used for phytoplasma identity confirmation. To verify the possible presence of *Spiroplasma kunkelii* the primers CSSF1/R1 (Barros et al., 2001) were used.

All PCR reactions were carried out in a 25 μL mix consisting of 2 μL of each primer (10 μM), 1 μL dNTP mix (100 μM), 2.5 μL buffer (10x), 0.2 μL Taq DNA polymerase (5 U/μL) (Invitrogen, USA) and 2 μL of diluted DNA (approx. 50 ng) with the cycling reported in the corresponding literatures. For the nested PCR amplification, the amplicons were diluted 1: 30 with sterile distilled water and 1 μL was used as a template. The presence of amplified products was verified in 1% agarose gel as described above.

Fig. 1 *Zea mays* plants with phytoplasma symptoms in production fields in Chupaca: **a** chlorotic streaking, ear proliferation; **b** anthocyanescence; **c** dwarfism, generalized chlorosis; **d** sprout atrophy and internode shortening

Identification of disease-associated pathogens The 3fwd/3rev, R16F2n/R2, and fU5/rU3 amplicons were subjected to RFLP analyses using *Tru1*I and/or*Hha*I (Fermentas, Vilnius, Lithuania) restriction enzymes under the conditions reported by the manufacturer. The obtained restriction profiles were compared with those of 19 phytoplasma strains (Table 2). The reactions were visualized in 6.7% polyacrylamide gels in 1X TAE buffer. Virtual restriction profiles were produced for 16S rRNA gene sequences of 1290 and 1248 nucleotides in agarose 4% gels using pDRAW32 program and in the *i*PhyClassifier (Zhao et al., 2009) respectively, in both cases the most informative restriction enzymes (Lee et al., 1998) were used for digestion.

Fragments of the phytoplasma 16S ribosomal and *rp* genes amplified by MBSF1/MBSR1, fU5/rU3, P1A/16SrSR, 3fwd/3rev, rpL2F3/rp(I)R1A, rp(III)F1/rp(III)R1 and CSSF1/R1 primers were purified from agarose gel using the Wizard® SV Gel and PCR Cleaning System Kit (Promega, USA) following the manufacturer's recommendations. The purified fragments from the positive samples for the 16S ribosomal gene and *rp* gene were sequenced in both directions with the same primers used for PCR. The sequences obtained were edited using BioEdit (Hall, 1999), assembled and the sequences analyzed using the Basic Local Alignment Search Tool (BLASTn) (Altschul et al., 1990) by comparing them with those deposited in the National Centre of Biotechnology Information (NCBI) GenBank database (https://www.ncbi.nlm.nih.gov/; Benson et al., 2013). The phytoplasma sequences obtained were deposited at the NCBI GenBank database under the accession numbers MW559787, MW578284, MW578287, MW578288, MW578363, and MW578290.

Multiple alignments of the consensus sequences were performed using the Cluster W program (Thompson et al., 1994). Phylogenetic analyses were carried out with MEGA v6.0 (Tamura et al., 2013) by the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980) on the 16S rRNA and *rp* gene sequences. *Acholeplasma laidlawii* and a '*Ca. P. trifolii*' strain were used as outgroups for the two genes, respectively.

Table 1 Place of collection of corn samples, symptoms, and results obtained by nested PCR for phytoplasma detection (in bold the samples sequenced and deposited in the GenBank database)

Sample code	Collection site	Symptoms*	Detection by PCR	
			MBS F1/ R1	Nested fU5/ rU3**
MSIII2, MSIV1	Sicaya - Huancayo	Chl. strip., anth.	2/2	1/2
MSI1		Chl. strip., anth., poor grains	0/1	1/1
MSI3, MSI4, MSI6, MSII2, MSII3, MSIII1, MSIII3, MSI5		Chl. strip., anth, sh. int., dwarf.	5/8	6/8
MSI2		Chl. strip., sh. int., dwarf.	0/1	1/1
MSII1		Chl. strip., anth., cob prol.	0/1	1/1
MCI1***, MCI2, MCI3	San Agustín de Cajas - Huancayo	Anth.	2/3	2/3
MHuIII1, MHuIII2, MHuIII3	Hualhuas - Huancayo	Anth.	0/3	1/3
MHuII3, MHuII4, MHuII5		Anth., cob prol.	3/3	2/3
MHuIII4, MSJIV7		Anth., sh. int., dwarf.	0/2	2/2
MHuII1		Leaf yellow, anth., sh. int., dwarf.	1/1	1/1
MSJIV2, SJIV3, MSJIV5, MSJIV6, MSJIV8	San Jerónimo - Huancayo	Anth.	2/5	4/5
MSJIV4, MSJIV11, MSJIV12***, MSJIV1, MHuII2, MSJIV9, MSJIV13		Chl. strip., anth.	2/7	4/7
MSJIV10		Chl. strip., anth., sh. int., dwarf.	0/1	1/1
M3DI1, M3DI2, M3DI3, M3DI4	3 de Diciembre - Chupaca	Chl. strip., anth., sh. int, dwarf.	0/4	1/4
MIsI2, MIsI3, MIsI4, MIsI5, MIsI6, MIsI7, MIsI8, MIsI9, MIsI10, MIsI1	San Juan de Iscos - Chupaca	Chl. strip., anth., sh. int., dwarf.	1/10	5/10
MAII2	Ahuac - Chupaca	Anth., sh. int., dwarf.	1/1	1/1
MAII1, MAII3, MAII4		Chl. strip., anth., sh. int., dwarf.	3/3	3/3
MAII5		Chl. strip., sh. int., dwarf.	1/1	1/1
MChII15, MCCIV3	Chupaca - Chupaca	Chlorotic stretch, sh. int., dwarf.	2/2	2/2
MCCIV9, MCCIV10, MCCIV11, MCCIV13 MCCIV14		Chlorotic striations, sh. int., dwarf., cob prol.	5/5	5/5
MChI1, MChI2, MChI3, MChI4***		Chl. strip., anth., sh. int., dwarf.	1/4	3/4
MChII14, MCCIII7, MCCIII7.1*** and MCCIII7.1i***, MCCIII7.2, MCCIV2***, MCCIV4, MCCIV8, MCCV2, MCCV5, MCCIII4, MCCIII8, MCCV3		Chl. strip, proliferation of buds, sh. int., dwarf., cob prol.	12/12	12/12
MChII1, MChII2, MChII4, MChII5, MChII7, MChII8, MChII9, MChII10, MChII11, MChII12, MCCIII1, MCCIII2, MCCIII3, MCCIII5, MCCIV7, MCCIV15, MCCV6		Chl. strip, sh. int., dwarf.	16/17	17/17
MCCV1, MCCV4		Chl. strip, sh. int., dwarf., cob prol.	2/2	2/2
MEMI1, MEMI2, MEMI4, MEMI5, MEMI7, MEMI9, MEMI10, MEMI12, MEMI13, MEMI14, MEMI15	El Mantaro - Jauja	Anth.	2/11	11/11
MEMI11		Anth., sh. int., dwarf.	0/1	1/1
MEMI6		Chl. strip., anth.	0/1	1/1
MEMI8		Chl. strip., anth., sh. int., dwarf.	0/1	1/1
MEMI3		Chl. strip, sh. int., dwarf.	0/1	1/1

*, Chl. strip., chlorotic stripping; anth., anthocianescence; sh. int., short internodes; dwarf., dwarfism; cob prol., corn cob proliferation

**, nested PCR was carried out on P1/P7, R16F2n/R2 and fU5/rU3 products

***, nested PCR was carried out on P1/P7 and P1A/16Sr-SR products

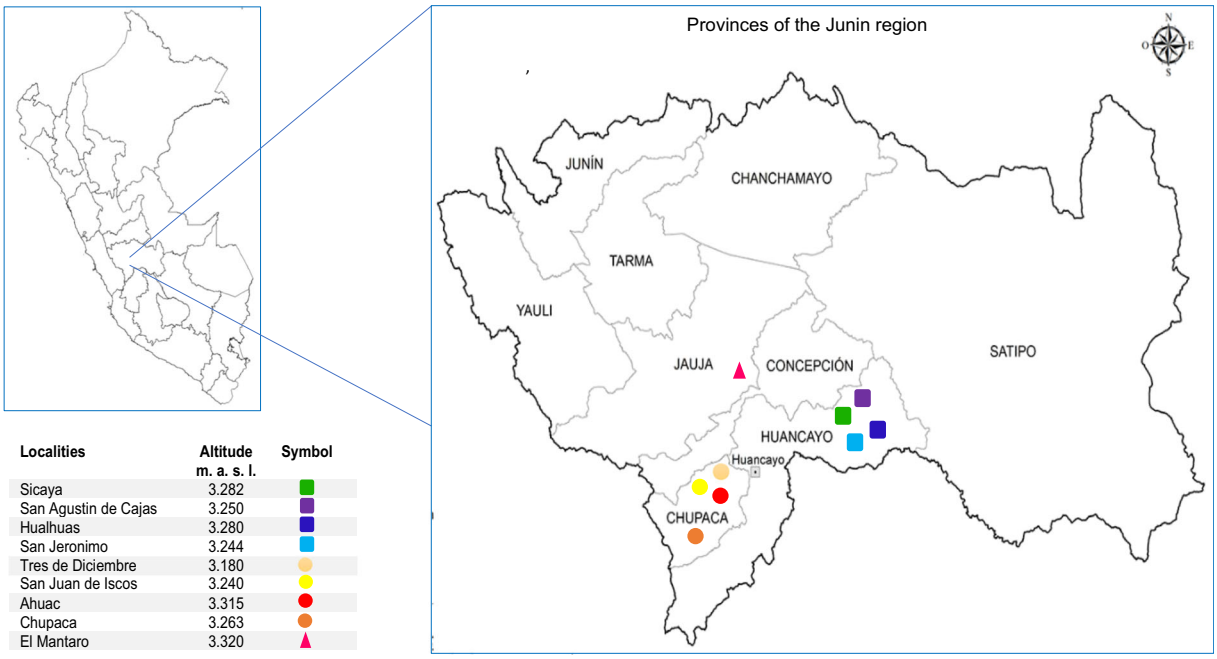


Fig. 2 Sample collection sites in the Junin region, Peru (image modified from https://imagenestotales.com/mapa-del-peru/)

Table 2 Phytoplasma strains used as reference in PCR/RFLP analyses

Phytoplasma strain (acronym)	Ribosomal subgroup	Host plant	Literature
Aster yellows strain 2192 (AY2192)	16SrI-B	Periwinkle	Bertaccini, 2014
Potato purple top (PPT)	16SrI-C	Periwinkle	Bertaccini, 2014
Witches' broom disease of lime (WBDL)	16SrII-B	Periwinkle	Bertaccini, 2014
Faba bean phyllody (FBC)	16SrII-C	Periwinkle	Bertaccini, 2014
Peach X disease (CX)	16SrIII-A	Periwinkle	Bertaccini, 2014
Green Valley X disease (GVX)	16SrIII-A	Periwinkle	Bertaccini, 2014
From *Euscelidius variegatus* (API)	16SrIII-B	Periwinkle	Bertaccini, 2014
Crepis biennis yellows (CR)	16SrIII-B	Periwinkle	Bertaccini, 2014
Goldenrod yellows (GR)	16SrIII-D	Periwinkle	Bertaccini, 2014
Chayote witches' broom (CB7/B7)	16SrIII-J	*Sechium edule*	Montano et al., 2000
Chayote witches' broom (MSCK10)	16SrIII-J	*Momordica charantia*	Montano et al., 2000
Pichris echoides yellows (PEY)	16SrIX-C	Periwinkle	Bertaccini, 2014
Alder yellows (ALY)	16SrV-C	Periwinkle	Bertaccini, 2014
Potato witches' broom (PWB)	16SrVI-A	Periwinkle	Bertaccini, 2014
Ash yellows (ASHY)	16SrVII-A	Periwinkle	Bertaccini, 2014
European stone fruit yellows (LNp)	16SrX-B	Periwinkle	Bertaccini, 2014
Flower stunting (BVK)	16SrXI-C	Periwinkle	Bertaccini, 2014
"stolbur" (STOL)	16SrXII-A	Periwinkle	Bertaccini, 2014
Molière disease (MOL)	16SrXII-A	Periwinkle	Bertaccini, 2014

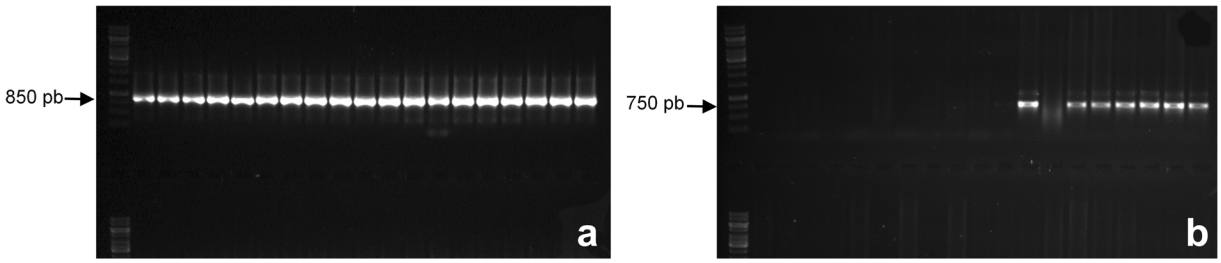


Fig. 3 PCR amplification of phytoplasmas from samples collected at the location of Sicaya: **a** the 850 bp fragments amplified with primers fU5/rU3;**b** the 750 bp fragments amplified with primers MBSF1/MBSR1. Marker on the left, GeneRuler 1 kb DNA Ladder (Thermo Scientific™)

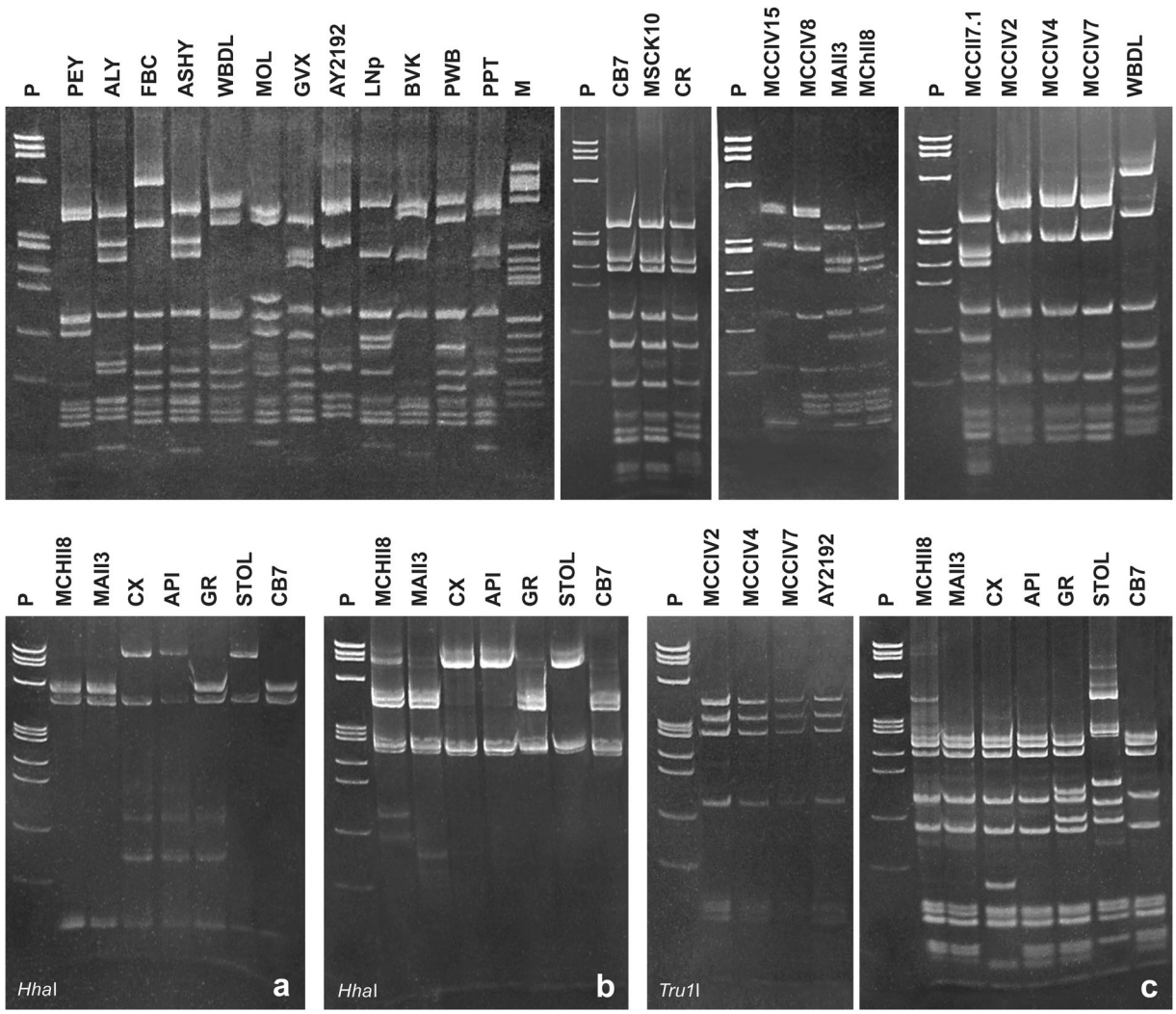


Fig. 4 Phytoplasma RFLP profiles of 16S rDNA in 6.7% polyacrylamide gels of selected amplicons from DNA samples form corn from Peru (as listed in Table 1) and phytoplasma controls (as listed in Table 2). Top lane: amplicons obtained with the primer pair 3fwr/3rev (about 1200 nt) and digested with *Tru1*I. Bottom line: amplicons obtained with primers 3fwr/3rev in (**a**) and in (**b**); **c** amplicons obtained with primers R16F2n/R2 (1248 nt). Enzymes used are listed at the bottom of each Fig. P, marker phiX174 *Hae*III digested with fragment sizes in base pairs from top to bottom of 1353; 1078; 872; 603; 310; 281; 271; 234; 194; 118 and 72

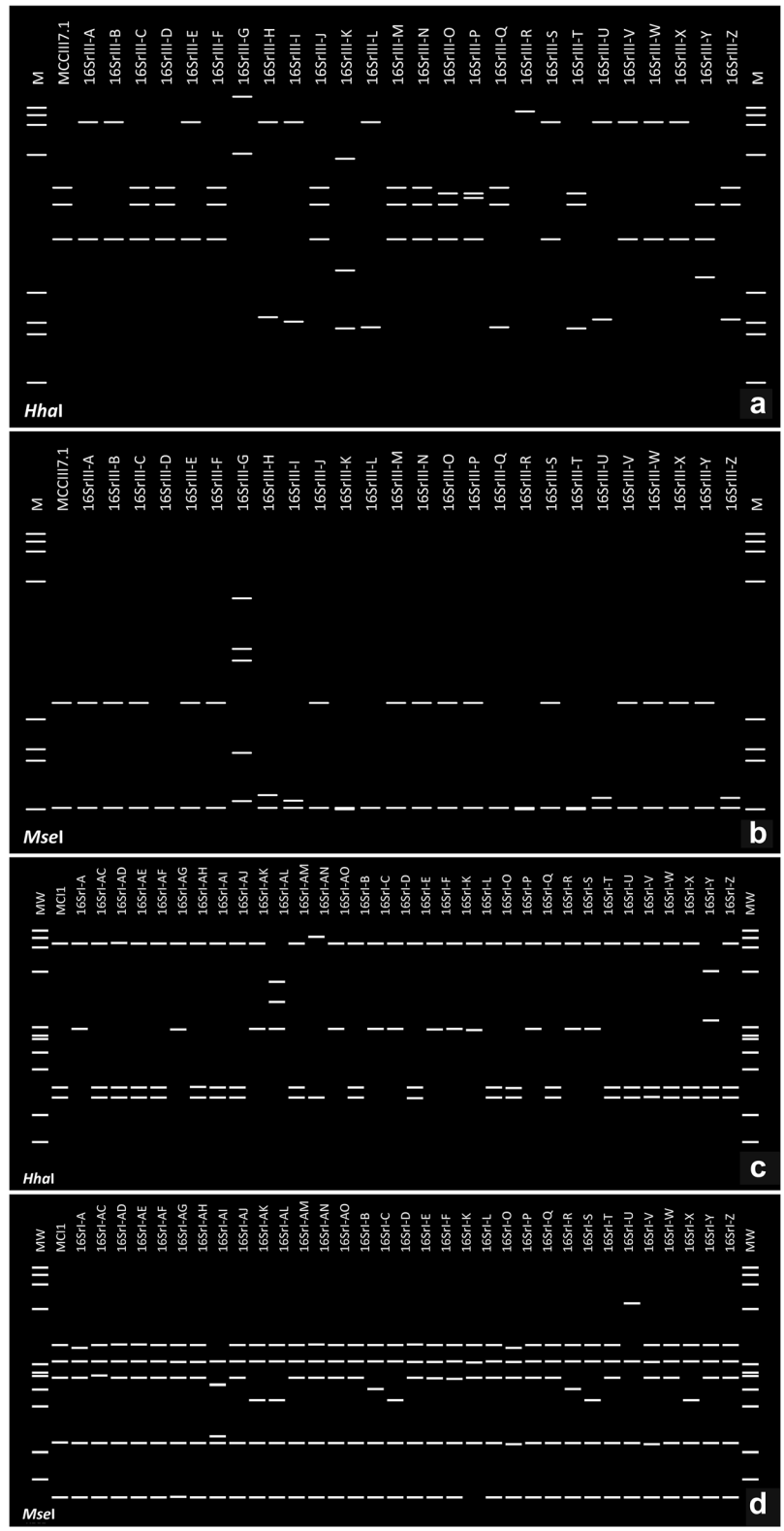
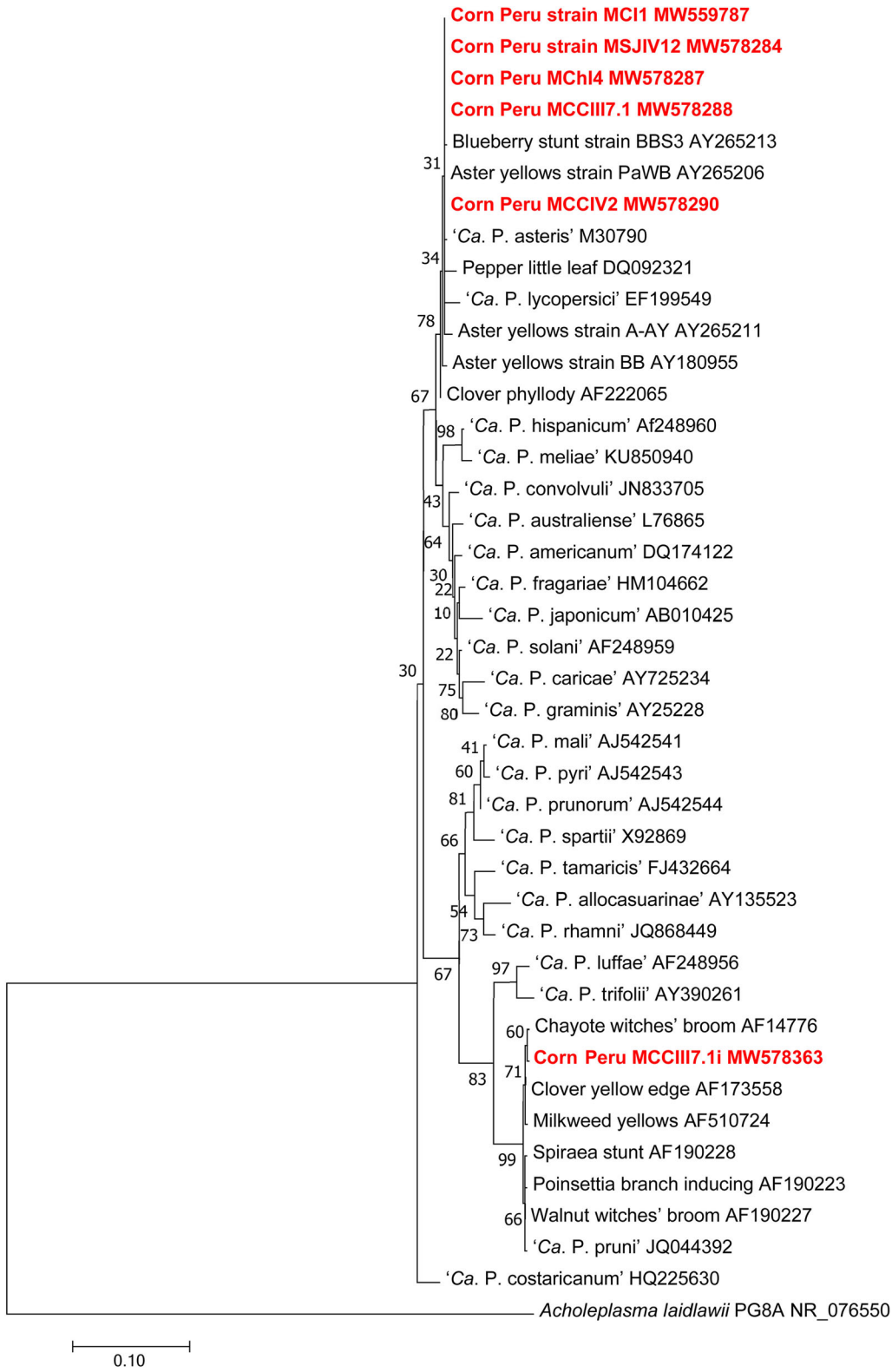


Fig. 5 Virtual RFLP analyses with *Mse*I and *Hha*I of 16Sr RNA gene sequences from corn strains compared with phytoplasmas representing ribosomal subgroups 16SrIII and 16SrI, respectively. In (**a)** and (**b**), the sequence of a 16SrIII-J strain is compared in agarose 4% gel using pDraw and in (**c**) and (**b**) a sequence of a 16SrI-B strain is compared using the *i*PhyClassifier. M and MW, PhiX174, marker *Hae*III digested


Corn Peru strain MCI1 MW559787
Corn Peru strain MSJIV12 MW578284
Corn Peru MChI4 MW578287
Corn Peru MCCIII7.1 MW578288
Blueberry stunt strain BBS3 AY265213
31
Aster yellows strain PaWB AY265206
Corn Peru MCCIV2 MW578290
'Ca. P. asteris' M30790
34
Pepper little leaf DQ092321
'Ca. P. lycopersici' EF199549
78
Aster yellows strain A-AY AY265211
Aster yellows strain BB AY180955
67
Clover phyllody AF222065
98
'Ca. P. hispanicum' Af248960
'Ca. P. meliae' KU850940
43
'Ca. P. convolvuli' JN833705
'Ca. P. australiense' L76865
64
'Ca. P. americanum' DQ174122
30
22
'Ca. P. fragariae' HM104662
10
'Ca. P. japonicum' AB010425
22
'Ca. P. solani' AF248959
30
75
'Ca. P. caricae' AY725234
80
'Ca. P. graminis' AY25228
41
'Ca. P. mali' AJ542541
60
'Ca. P. pyri' AJ542543
81
'Ca. P. prunorum' AJ542544
66
'Ca. P. spartii' X92869
'Ca. P. tamaricis' FJ432664
54
'Ca. P. allocasuarinae' AY135523
73
'Ca. P. rhamni' JQ868449
67
97
'Ca. P. luffae' AF248956
'Ca. P. trifolii' AY390261
60
Chayote witches' broom AF14776
Corn Peru MCCIII7.1i MW578363
71
83
Clover yellow edge AF173558
Milkweed yellows AF510724
99
Spiraea stunt AF190228
Poinsettia branch inducing AF190223
66
Walnut witches' broom AF190227
'Ca. P. pruni' JQ044392
'Ca. P. costaricanum' HQ225630
Acholeplasma laidlawii PG8A NR_076550
0.10

◀ **Fig. 6** Molecular analysis by maximum likelihood method of the 16S rRNA gene sequences obtained from the phytoplasmas detected in corn samples from Peru (in bold red). '*Candidatus* Phytoplasma' species officially described are used for comparison. *Acholeplasma laidlawii* is used as an outgroup. The tree with the highest log-likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Evolutionary analyses were conducted in MEGA6

Results

The MBS disease incidence was recorded to be 77% in Sicaya, 66% in San Agustín de Cajas, 56% in Hualhuas, 77% in San Jerónimo, 25% in Tres de Diciembre, 50% in San Juan de Iscos, 100% in Ahuac, 97% in Chupaca, and 100% in El Mantaro. A total of 114 plants of Cusqueado variety were collected in the different areas showing symptoms of chlorotic streaking, cob proliferation, anthocyanescence, dwarfism, generalized chlorosis, bud atrophy, and internode shortening (Fig. 1 and Table 1). The nested amplification with fU5/rU3 primers produced fragments of approximately 850 bp in 94 samples, while the MBSF1/MBSR1 primers amplified fragments of approximately 750 bp in 61 samples (Table 1 and Fig. 3). The use of CSSF1/R1 primers produced in some of the samples amplicons of approximately 700 bp, however their sequences resulted aspecific and did not allow to verify the presence of *S. kunkelii*.

Among the 114 samples 15, from different locations, were sent for sequencing using the P1A/16SrSR, R16F2n/R2 or 3fwd/3rev primers used for their amplification and subjected to RFLP analyses. The RFLP and virtual RFLP analyses of these samples with appropriate restriction enzymes (*Mse*I = *Tru1*I, and *Hha*I) on the amplicons and the sequences obtained with the phytoplasma primer pairs R16F2n/R2 and 3fwr/3rev, respectively, showed the presence of two profiles and allowed enclosing the detected phytoplasmas in subgroups 16SrI-B and 16SrIII-J (Figs. 4 and 5). A total of five fragments of approximately 1450 bp, amplified by nested PCR with primers P1A/16Sr-SR from samples collected in San Agustin de Cajas (MCl1), San Jerónimo (MSJIV12) and Chupaca (MCHI4, MCCIV2, MCCIII7.1) resulted positive to the presence of '*Ca. P. asteris*'. Further amplicon sequencing using the same primers of the sample MCCIII7.1 resulted positive to the presence of a '*Ca. P. pruni*'-related strain (Fig. 6). Further amplification and sequencing of the 15 selected samples with ribosomal protein group-specific primers confirmed the presence of the two 'Ca. Phytoplasma' species in mixed infection in two surveyed localities (Chupaca and Ahuac Chupaca, Junin). The BLASTn analysis showed that these sequences, except the one submitted to GenBank under the accession number MW578363, had a 100% identity to those of MBS phytoplasma strains from Colombia, Brazil, and Mexico (GenBank accession numbers HQ530152, CP015149, and AY265208 respectively) and to several other '*Ca. P. asteris*'-related strains. The sequence MW578363 showed 99.85% identity to *Solanum lycopersicum* phytoplasma clone TomRed (GenBank accession number KC412031) from Argentina and to other phytoplasma strains from Colombia, Bolivia, and Brazil resulting 'Ca. *P. pruni*'-related and 99.92% identity to a *Delphinium* phytoplasma strain (GenBank accession number EF514210).

The BLASTn analysis of ribosomal gene sequences showed 99.82% and 99.55% identity to the *rp* gene from '*Ca. P. asteris*' strains from maize from Brazil and oil palm from Colombia (GenBank accession numbers CP015149 and KF434318) and to '*Ca. P. pruni*'-related strains from *Cucurbita maxima* from Argentina (GenBank accession number KC412017), respectively. The dendrogram confirmed that these two '*Candidatus* Phytoplasma' strains cluster with phytoplasmas mainly identified in South American areas and belonging to the two identified 'Ca. Phytoplasma' species (Fig. 7).

Discussion

The molecular testing performed allowed the detection of phytoplasmas in the majority of the symptomatic corn samples collected in the surveyed growing areas of Central Peru (Table 1). The use of U5/U3 primers in nested PCR assays allowed verifying a widespread presence of phytoplasmas in almost all the fields indicating a very close correspondence between symptoms and phytoplasma presence. The two phytoplasmas identified were not differentiated by corn phytoplasma specific primers MBSF1/R1 (Harrison et al., 1996), however

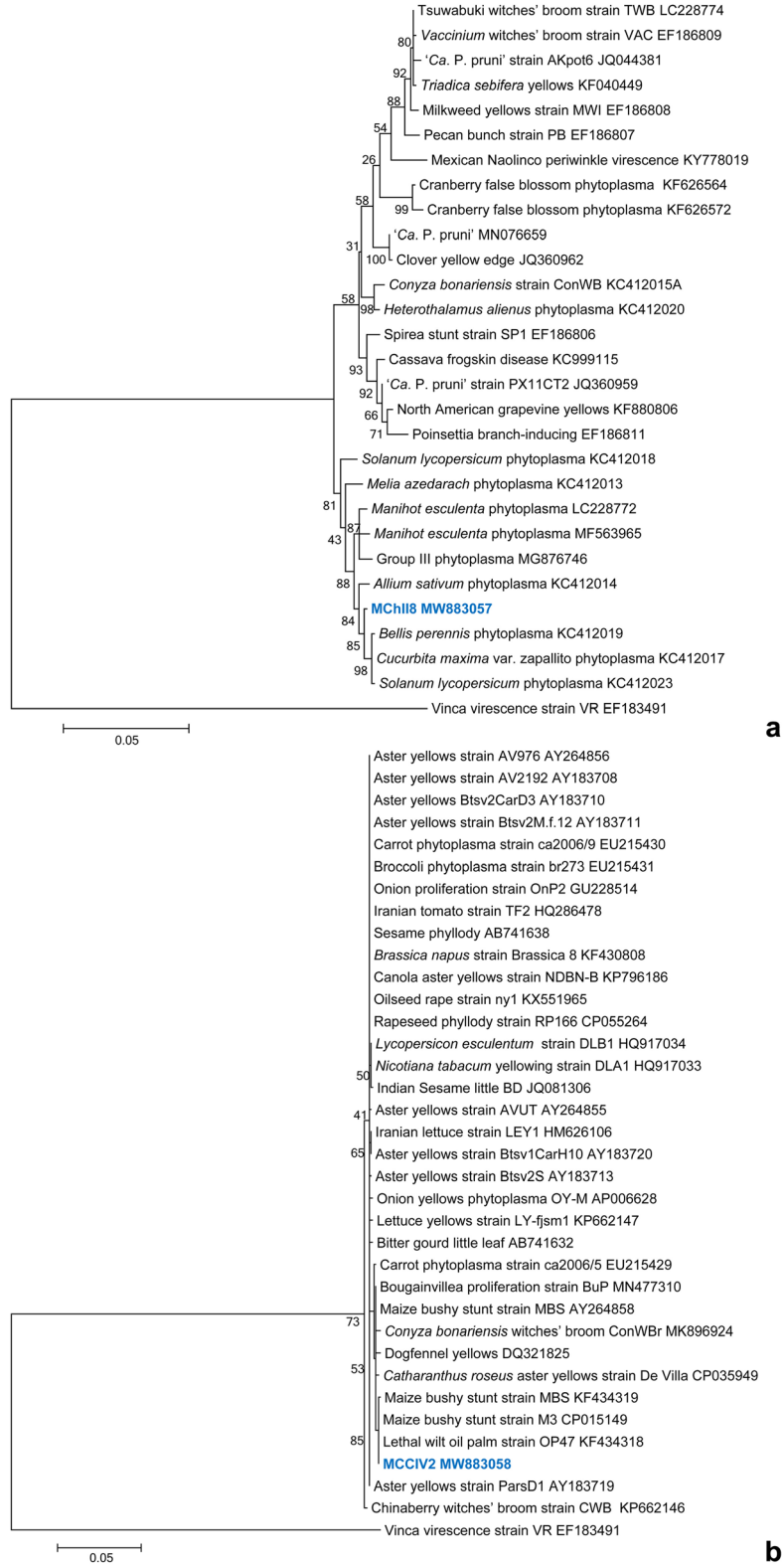


Fig. 7 Molecular phylogenetic analysis by Maximum Likelihood of the *rp* gene sequences obtained from phytoplasmas detected in corn from Peru (in bold blue). The strain from corn was compared with the *rp* gene of strains of '*Ca. P. asteris*' in a) and of '*Ca. P. pruni*' in b). '*Ca. P. trifolii*' (16SrVI-A), vinca virescence strain VR, is used as an outgroup. Analyses were conducted in MEGA6

RFLP and sequencing of the amplicons obtained with 16S rRNA gene and group specific ribosomal protein primers allowed to confirm the presence of 16SrI-B and 16SrIII-J subgroups, in some cases in mixed infection.

The presence of *S. kunkelii* was not detected in spite of the use of primers reported to be specific (Barros et al., 2001). This pathogen was identified in screening carried out in Mexico and Brazil (Davis et al., 1972; Chen & Liao, 1975; Gordon et al., 1985; Bedendo et al., 1997) in corn plants showing symptoms very similar to those found in this survey. However, these symptoms are, according to the literature, also associated with the presence of viruses and therefore are not specific and the presence of viruses was not screened since the main focus was the identification of the phytoplasmas that were already reported associated to the disease in this area (Hodgetts et al., 2009).

While the '*Ca. P. asteris*' is widely reported to infect corn on the American continent (Lee et al., 2004), this is the first detection of 16SrIII-J phytoplasmas in corn. The 16SrIII-J phytoplasma strain is widespread in other countries of South America such as Brazil (Montano et al., 2000), Argentina (Galdeano et al., 2013) and Chile (Quiroga et al., 2020) where it was detected in several herbaceous and woody horticultural species. Recently it was also identified in faba bean in Peru (Torres-Suarez et al., 2021) and insect vectors of this phytoplasma strain were identified as *Paratanus exitiosus* (Beamer) and *Bergallia valdiviana* Berg 1881 in Chile (Quiroga et al., 2019).

Both phytoplasma strains detected appear to be very closely related to strains detected mainly in Latin America, suggesting a wide distribution of similar strains in different countries and diverse crops. Considering that more than one corn cultivation cycle is performed each year in these areas, it is important to verify insect vectors presence for both phytoplasma strains. Crop rotation and a change in vector feeding preference could also play a relevant role in the infection of the 16SrIII-J phytoplasmas strain in corn. The presence of infected erratic seeds, spouting near the corn fields could maintain and increase the disease dissemination to new crops by insect vectors making very relevant the need of verification of phytoplasma seed transmission reported for corn in other areas of the world (Calari et al., 2011; Satta et al., 2019). The further verification of these epidemiologic aspects will make it possible to develop and apply well focused and environmentally friendly management tools to reduce the epidemic spread of phytoplasmas in corn also in other world cultivations recently reported also in Europe (Çağlar et al., 2019, 2021; Duduk & Bertaccini, 2006).

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Declarations

Research involving human participants and/or animals The authors certify that no special permits were required for the field-work investigations. Investigations did not involve any species endangered or protected in Peru.

Informed consent All the authors declare that the principles of ethical and professional conduct were duly followed during the execution of this research. The research was funded by Vice-Rector's Office for Research (CANON Projects, resolution N° 2771–R–2019) of the Universidad Nacional del Centro del Peru.

Conflict of interest All authors affirm that 1) there exist no actual or potential conflict of interests to disclose, 2) the manuscript is original and has not been published previously (partly or in full), and is not under review for publication elsewhere, 3) all the necessary local, national and international standards, regulations and conventions, including normal scientific ethical practices, have been duly followed and respected. Additionally, all authors have endorsed the final version of the manuscript before submission.

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