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Survey and molecular detection of phytoplasmas associated with potato in Romania and southern Russia

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Abstract In recent years, emerging phytoplasma diseases of potato (*Solanum tuberosum* L.) have increasingly become important in central and eastern Europe. Accurate identification of phytoplasmas and their insect vectors is essential to developing effective management strategies for diseases caused by these plant pathogens. Potato phytoplasma diseases in Europe were for a long time diagnosed only on the basis of visual symptoms. However, this approach is not very reliable and the use of modern molecular techniques such as polymerase chain reaction (PCR) is required in order to accurately determine the etiology of these phytoplasma diseases. A survey and identification of phytoplasmas associated with potato crops in Romania and southern Russia were conducted based on modern molecular techniques. Symptomatic potato plants were collected from several fields and tested for phytoplasmas by PCR. Also, selected crops and weeds in the vicinity of these potato fields were sampled and tested for phytoplasmas. Stolbur ("*Candidatus* Phytoplasma solani"; 16SrXII-A) was the only phytoplasma detected in potato and adjacent crops, including tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), and beet (*Beta vulgaris*). This phytoplasma was also detected in weeds, particularly *Convolvulus arvensis, Cuscuta* sp., and *Euphorbia falcata*. Genotyping of obtained stolbur isolates on *tuf* genes revealed that they all had the same RFLP profile corresponding to the tuf-type 'b' (VK Type II). Stolbur-affected potato plants produced a large number of spongy tubers that resulted in commercially unacceptable potato chips upon processing.

Keywords Genotyping · PCR · Potato · RFLP · Stolbur phytoplasma · *Tuf* gene

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Introduction

Phytoplasmas, previously called mycoplasma-like organisms (MLO), are unculturable, phloem-limited and insect-transmitted plant pathogens, and have been associated with diseases in hundreds of plant species, including many important food crops, ornamentals, and trees (Seemüller et al. 1998). In recent years, emerging phytoplasma diseases of potato (*Solanum tuberosum* L.) have increasingly become important in many potato producing areas around the world.

Serious epidemics of purple top disease of potato, caused by phytoplasma infections, have recently occurred in North America (Leyva-López et al. 2002; Khadhair et al. 2003; Lee et al. 2004; Munyaneza 2005; Munyaneza et al. 2006; Rubio-Covarrubias et al. 2006; Secor et al. 2006; Olivier et al. 2009; Munyaneza et al. 2008b; 2009; 2010a; b; Santos-Cervantes et al. 2010), Central and South America (Secor and Rivera-Varas 2004; Jones et al. 2004), central and eastern Europe (Linhartova et al. 2006; Paltrinieri and Bertaccini 2007; Bogoutdinov et al. 2008; Girsova et al. 2008; Fialova et al. 2009), and New Zealand (Liefting et al. 2009). Countries severely affected by phytoplasma diseases of potato include Russia, Romania, Mexico, Guatemala, and the United States. These emerging potato diseases are causing significant yield losses and a reduction in tuber and seed quality (Munyaneza 2005; Munyaneza et al. 2007a; Paltrinieri and Bertaccini 2007). Potato stolbur phytoplasma has quarantine status in the European Union (EPPO/CABI 1996).

Based on visual symptoms, the diseases caused by phytoplasmas in potatoes can be classified in two groups: aster yellows and potato witches'-broom (Salazar and Javasinghe 2001; Slack 2001). The aster yellows group has different names, including purple top wilt, haywire, apical leafroll, bunch top, purple dwarf, yellow top, potato hair sprouts, stolbur, potato phyllody, and potato marginal flavescence (Rich 1983; Banttari et al. 1993; Salazar and Javasinghe 2001; Slack 2001). Potato phytoplasmas in the aster yellows group occur worldwide. In Europe, there are numerous phytoplasmas of the aster yellows group that infect various plant species, but only stolbur phytoplasma ("*Candidatus* Phytoplasma solani") has been associated with potato (Cousin and Moreau 1977; Cousin and Smith 1988; EPPO/CABI 1996; Fialova et al. 2009). However, other phytoplasmas have recently been reported on potato in central and eastern Europe (Paltrinieri and Bertaccini 2007; Bogoutdinov et al. 2008; Girsova et al. 2008). The potato witches'-broom disease occurs in Europe, Asia, and North America, and is usually of minor economic importance (Brcak et al. 1969; Harrison and Roberts 1969; Maramorosch et al. 1970; Hodgson et al. 1974; Rich 1983; Khadhair et al. 1997; 2003; Slack 2001).

Symptoms in potato plants infected with phytoplasmas in the aster yellows group usually include upward rolling of the top leaves with reddish or purplish discoloration, proliferation of buds, shortened internodes, swollen nodes, aerial tubers, and early senescence (Fig. 1). Symptoms caused by the stolbur phytoplasma are similar to those caused by other aster yellows group phytoplasmas; however, they are often more severe and can make plants wilt and die soon after they exhibit initial infection symptoms (EPPO/CABI 1996). In addition, stolbur-infected tubers often produce chips with a discolouration defect, rendering them unmarketable (Fig. 2).

Potato phytoplasma diseases in many parts of the world, including central and eastern Europe, were for a long time diagnosed only on the basis of visual symptoms, presence of insects-vectors and/or with the help of electron microscopy of infected phloem tissues (EPPO/CABI 1996; Girsova et al. 2008; Fialova et al. 2009). However, as different micro-organisms can produce almost identical symptoms in different potato cultivars and in different plant species, visual symptomatology of phytoplasma infection is not a very reliable characteristic and the use of modern molecular techniques such as polymerase chain reaction (PCR) is essential to accurately determine the etiology of phytoplasma diseases. Based on modern classification of phytoplasmas, which uses sequence comparisons within the 16S–23S rRNA region (Lee et al. 1998; 2000; Davis and Sinclair 1998), at least eight groups of phytoplasmas have so far been identified on potato around the world: aster yellows(16SrI), peanut witches'-broom

Fig. 1 Potato plant exhibiting typical symptoms of stolbur phytoplasma infection. Symptoms include upward rolling of the top leaves with reddish or purplish discoloration, proliferation of buds, shortened internodes, and swollen nodes

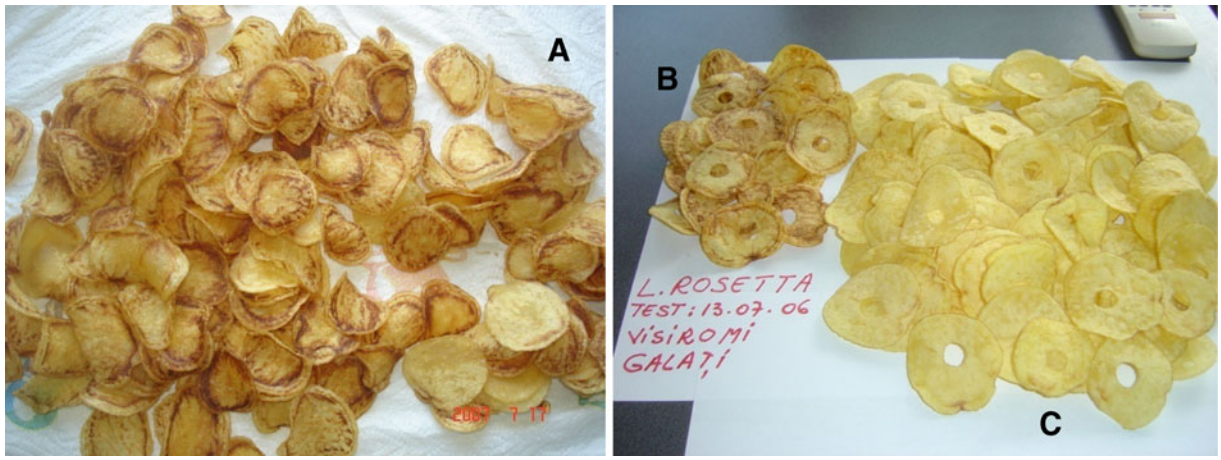

Fig. 2 Fried chips processed from tubers produced on stolbur-infected potato plants and exhibiting browning discoloration defect (**a** and **b**), and chips from tubers produced on healthy potato plants (**c**)

(16SrII), X-disease (16SrIII), clover proliferation (16srVI), apple proliferation (16SrX), stolbur (16SrXII), Mexican periwinkle virescence (16SrXIII), and American potato purple top wilt(16SrXVIII) (Lee et al. 1998; 2000; 2006; Paltrinieri and Bertaccini 2007; Santos-Cervantes et al. 2010).

Furthermore, characterization of phytoplasma strains may play an important role in elucidating etiology and epidemiology of diseases caused by these plant pathogens and developing effective management strategies for these diseases. For example, recent genotyping of stolbur isolates from grapevine using non-ribosomal *tuf*-marker allowed distinction of three genotypes of stolbur phytoplasma (Langer and Maixner 2004). These three tuf-types were found to be associated with different wild host plants of stolbur, including nettle (*Urtica dioica*) with type 'a', bindweed (*Convolvulus arvensis*) with type 'b', and hedge bindweed (*Calystegia sepium*) with type 'c'. Tuf-types have also been associated with insect vectors of stolbur (Kaul et al. 2009). Association of stolbur tuf-types with cultivated crops, wild host plants and insect vectors may lead to accurate identification of the sources of inoculum for the different stolbur genotypes infecting crops, in addition to their insect vectors (Langer and Maixner 2004; Fialova et al. 2009; Kaul et al. 2009; Radonjic et al. 2009).

Little is known on etiology and epidemiology of phytoplasma diseases of potato in central and eastern Europe. Effective management strategies for these diseases will not be realized until these plant pathogens and their insect vectors are correctly identified and their epidemiology is elucidated. The objective of the present study was to survey and identify phytoplasmas associated with potato crops in Romania and southern Russia, based on molecular techniques. In addition, impact of potato phytoplasma diseases on the tuber processing quality was investigated. Moreover, selected weeds and crops in the vicinity of the surveyed potato fields were sampled and tested for phytoplasmas to investigate whether they may constitute potential sources of inoculum for these plant pathogens. Furthermore, genotyping was performed for stolbur isolates identified in phytoplasma-infected potato plants and weed species.

Materials and methods

Survey sites

The study was conducted during the 2008 and 2009 potato growing seasons in Romania and southern Russia. In 2008, the survey was conducted in and around two potato fields in Fundulea and Csikszereda in southern and northern Romania, respectively. In 2009, the survey was conducted in two potato fields in Radovanu, southern Romania. In Russia, three potato fields were selected for the study in 2008 and were located in Gulkevichi (Krasnodar Region), as well as in Azov and Mayak (Rostov Region). In 2009, three study sites in Russia were again located in Gulkevichi, Mayak, and Azov, in addition to a potato field in Niva (Rostov Region).

Potato plants with phytoplasma infection symptoms were sampled three times during the growing season: at

vegetative and bloom stages, as well as at close to harvest. In addition, weeds and other crops exhibiting phytoplasma-like symptoms in the vicinity of the surveyed potato fields were collected. These plants included tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), eggplant (*Solanum melongena*), beet (*Beta vulgaris*), and corn (*Zea mays*), and weeds exhibiting symptoms resembling those of phytoplasma infection were collected as well. Typical symptoms in phytoplasma-infected potatoes included upward rolling of the top leaves with reddish or purplish discoloration, proliferation of buds, shortened internodes, swollen nodes, aerial tubers, witches'-broom, and early senescence (Fig. 1). Symptoms in weeds and other crops included stunting, proliferation of buds, reddish and/or yellowish discoloration of leaves, leaf rolling, witches'-broom, malformation of flowers, and wilting. Asymptomatic plants were collected as well.

The plant samples were wrapped in aluminium foil immediately after collection and caution was taken to avoid overlapping of the leaves and to press out the air inside the foil wrap to better preserve the plant material. Samples were transferred to a cooler and shipped to the laboratory of FITOLAB Plant Pest Diagnostic and Advisory Ltd. (Budapest, Hungary) for phytoplasma testing.

Phytoplasma detection, identification, and genotyping

Nucleic acid extraction DNA extraction from plants was performed using the CTAB method of Daire et al. (1997) with a slight modification. Main midribs were excised and 1 g of fresh or frozen (−20°C) tissue was ground with semi-automated, ball-bearing grinding homogenizer (Homex 6, Bioreba, Switzerland) in 7 ml of 3% CTAB extraction buffer (3% cetyltrimethylammonium bromide, 100 mM TrisHCl, 20 mM EDTA, 1.4 M NaCl pH 8, 0.2% 2-merchaptoethanol). 700 µl of homogenised sap was placed into a 1.5-ml centrifugation tube and incubated for 20 min at 60°C. An equal volume of chloroform was added to each tube and mixed gently for 1 min, then centrifuged for 10 min at 10,000 g; the aqueous phase was collected. The nucleic acid was precipitated with an equal volume of isopropanol and collected with a 10 min centrifugation at 10,000 g. The supernatant was discarded and the pellet was washed with 1 ml of 70% ethanol and dried at room temperature. The pellet was re-suspended in 500 µl TE buffer (10mMTris pH 7.6, 1 mM EDTA pH 8).

Phytoplasma detection and identification Nested PCR was performed with phytoplasma universal primer pairs P1/P7 (Deng and Hiruki 1991; Smart et al. 1996), followed by R16F2n/R16R2 (Lee et al. 1995) or fU5/rU3 (Lorenz et al. 1995) to amplify phytoplasma 16SrDNA, or 16SrDNA plus spacer region. Positive DNA controls were included in the tests. Reaction mixture without DNA templates was used as negative control. Nested PCR products obtained with R16F2n/R2 primers were digested with restriction enzymes *Tru1*I, *Rsa*I, *Alu*I, *Ssp*I, *Taq*I, *Hha*I, and *Hpa*II (Fermentas, Vilnius, Lithuania) according to the manufacturer's instructions. The restriction fragment length polymorphism (RFLP) patterns were run in 2.5% agarose gel and visualised under UV light with the application of reference controls (EAY 16Sr-B, FD-D 16SrV-C, and MOL 16SrXII-A, provided by A. Bertaccini, DiSTA, Bologna, ITALY and AY27 16SrI-A, CPh 16SrI-C, PaWB 16SrI-D, and CP & PWB 16SrVI-A, obtained from I.-M. Lee, USDA-ARS Beltsville, USA).

Genotyping of stolbur isolates on tuf genes Tuf-type characterization was performed for the 122 isolates of the plant samples that tested positive for stolbur. The stolbur isolate samples were amplified in a nested PCR with fTuf1/rTuf1 and fTufAy/rTufAy primers and then digested with *Hpa*II restriction enzyme for stolbur genotype identification, according to Langer and Maixner (2004). The RFLP profiles were compared with reference strains 1925 (tuf-type 'a') and GGY (tuf-type 'b'), obtained from M. Maixner, Julius Kühn-Institut, Bernkastel-Kues, Germany.

Sequencing of stolbur isolates Amplicons were selected among amplified products from PCR testing with R16F2n forward and R16R2 reverse primer pairs and sequenced. Selected PCR-amplified products in 25 µl final volume were run in 1.5% agarose gel. The obtained PCR fragments were excised from the agarose gel and the fragments of each sample were purified by gel extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. PCR products were then sequenced using ABI BigDye Terminator chemistry on ABI automated sequencers (Applied Biosystems, California, USA), and in both directions to minimize PCR artefacts, ambiguities and base-calling errors. After pairwise

alignment of forward and reverse sequencing outputs, the consensus sequences of R16 fragments were analyzed. The obtained sequences were compared with sequences in the NCBI Nucleotide Database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Assessing tuber processing quality (fry test)

To assess tuber processing quality, selected potato tubers (var. Lady Rosetta) from both asymptomatic and symptomatic plants were harvested from the surveyed fields and processed into fried potato chips at both Star Foods/Frito-Lay processing plant near Bucharest, Romania, and the laboratory of FITOLAB Plant Pest Diagnostic and Advisory Ltd. (Budapest, Hungary). Standard Frito-Lay procedures for chip frying and defect assessment were used (Munyaneza et al. 2007b; c).

Results

Romania survey In Romania, a total of 187 plant samples were collected and tested in 2008. The samples included 32 potato plants and 151 weeds, in addition to 4 corn plants (Table 1). In 2009, a total of 210 plant samples (including 121 potato, 3 corn, and 2 tomato plants, in addition to 84 weeds) were collected and tested (Table 1). Weeds collected and tested during both years include *Convolvulus arvensis* (bindweed), *Cirsium arvense*, *Lathyrus tuberosum*, *Cuscuta* sp. (dodder), *Euphorbia virgata*, *Bromus* sp., *Anthemisia australiaca*, *Chenopodium album*, *Sorghum halepense*, *Dactylis glomerata*, and *Xanthium strumarium*. Only stolbur phytoplasma ("*Ca*. Phytoplasma solani"; 16Sr XII-A group) was detected in potato, tomato, and weed samples. In 2008, the phytoplasma was detected in 16.7% of potato samples collected in southern Romania. Stolbur phytoplasma was detected in one of 27 *C. arvensis* samples tested. No phytoplasma was detected in samples collected in northern Romania in 2008. In 2009, 28.1 and 22.6% of potato and weed samples, respectively, tested positive for stolbur phytoplasma (Table 1). Among the sampled weeds, 13 out of 49 *C. arvensis* and 6 out of 6 *Cuscuta* sp. plants tested positive for stolbur. In addition, two tomato plant samples collected and tested for phytoplasmas were positive for stolbur. Twenty three of the 380 stolbur-positive plants (6%) were symptomless, mainly weed species.

Russia survey In Russia, a total of 77 plant samples (including 33 potato, 5 pepper, 2 tomato, 1 onion, and 7 eggplant plants and 29 weeds) were collected and tested for phytoplasmas in 2008 (Table 2). A total of 100 plant samples were collected and tested in 2009, including 54 potato plants (Table 2). In addition, 2, 4, 1, and 1 carrot (*Daucus carota*), corn, beet, and tomato plants, respectively, were included in the year 2009 samples. Weeds tested in both years include *Bilderdykia convolvulus*, *C. arvensis*, *Thlaspi arvense*, *C. arvense*, *C. album*, *Cuscuta* sp., *Lathyrus* spp., *Euphorbia falcata*, *Capsella bursa-pastoris*, and *Taraxacum officinale*. Stolbur (16Sr XII-A) was detected in potato,

Table 1 Potato, weed, and other crop samples were collected in Romania and tested for phytoplasmas by polymerase chain reaction (PCR). Detection and identification of stolbur phytoplasma were confirmed by restriction fragment length polymorphism (RFLP) and sequencing

Year	Host	Field location[a]		
		Fundulea	Radovanu	Csikszereda
2008	Potato	2/12	–	0/20
	Other crops	0/2	–	0/2
	Weeds	1/127	–	0/24
2009	Potato	–	34/121	–
	Other crops	–	2/5	–
	Weeds	–	19/84	–

[a] Number of stolbur positive samples/number of samples tested

Table 2 Potato, weed, and other crop samples were collected in Russia and tested for phytoplasmas by polymerase chain reaction (PCR). Detection and identification of stolbur phytoplasma were confirmed by restriction fragment length polymorphism (RFLP) and sequencing

Year	Host	Field location[a]			
		Azov	Gulkevichi	Mayak	Niva
2008	Potato	2/6	2/10	11/17	–
	Other crops	0/1	–	10/14	–
	Weeds	0/16	1/10	1/3	–
2009	Potato	7/8	6/19	14/19	7/9
	Other crops	–	0/4	2/2	0/2
	Weeds	2/4	0/5	3/15	0/11

[a] Number of stolbur positive samples/number of samples tested

pepper, tomato, eggplant, beet, *C. arvensis*, *Cuscuta* sp., and *E. falcata* samples. Also, aster yellows phytoplasma (16SrI-B) was detected in one sample of *Cuscuta* sp., collected in Mayak. Stolbur was detected in 22.1 and 44.2% of potato samples in 2008 and 2009, respectively (Table 2). All tomato and beet samples tested positive for stolbur, whereas 3, 5, and 1 out 5, 7, and 2 pepper and eggplant plant samples tested positive for stolbur. Among weeds, 5 out of 15, 2 out of 6, and 1 out of 4 samples of *C. arvensis*, *Cuscuta* sp., and *E. falcata*, respectively, tested positive for stolbur. Eleven of the 177 stolbur-positive plants (6.2%) were asymptomatic, mostly weed species.

Stolbur genotyping Ninety six of the 122 stolbur isolate samples were amplified in a nested PCR with fTuf1/rTuf1 and fTufAy/rTufAy primer pairs. All the *Hpa*II RFLP profiles obtained from the 96 stolbur isolate samples collected from both Romania and Russia were referable to the stolbur tuf-type 'b' described by Langer and Maixner (2004) (Table 3; Fig. 3).

Sequencing of stolbur isolates Sequence data of stolbur isolates identified in different plant species from Romania and Russia were submitted to the NCBI Genbank (GenBank Accession Nos. HM449995, HQ108386- HQ108389, HM449996-HM450002, and HQ108390-HQ108394; Table 4). Almost all the 16S rDNA gene sequences (1185 bp) of stolbur isolates, from potato, tomato, and weed samples in Romania as well as of isolates identified in potato, pepper, tomato, eggplant, beet, *C. arvensis*, *Cuscuta* sp, and *E. falcata* in South Russia shared 100% homology with the Russian potato purple top phytoplasma (GenBank Accession No. EU344886), Iranian purple top phytoplasma (GenBank Accession No. EU661607), and red clover stolbur phytoplasma (GenBank Accession No. EU814640). Only two samples isolated from potato and *C. arvense* in south Romania (GenBank Accession Nos. HQ108386 and HQ108388) showed 1 bp difference from the above 16S sequences at the same position (Table 4).

Tuber quality A large number (27%) of potato tubers collected from phytoplasma-infected plants had a spongy texture compared to those from healthy plants, but no visual symptoms were observed in the tuber flesh upon slicing. When processed into fried

Table 3 Tuf-typing of stolbur isolates from different host plants and locations in Romania and Russia. Phytoplasma detection was performed by nested PCR with universal primer pairs P1/P7-R16F2n/R2, followed by RFLP with *Tru*1. Tuf-typing was performed with primer pairs FTuf1/RTuf1-fTufAY/rTufAY, followed by RFLP with *Hpa*II

Location	Host	Number of stolbur isolates genotyped	Tuf-Typing (Tuf-type 'b' or VK-II Type)
Radovanu, Romania	*Convolvulus arvensis*	13	13
	Cuscuta sp.	6	5
	Solanum lycopersicum	2	2
	Solanum tuberosum	35	24
Mayak, Russia	*Beta vulgaris*	1	1
	Capsicum annuum	3	3
	Convolvulus arvensis	1	1
	Cuscuta sp.	2	2
	Euphorbia falcata	1	1
	Solanum lycopersicum	3	2
	Solanum melongena	5	5
	Solanum tuberosum	1	0
Azov, Russia	*Convolvulus arvensis*	2	2
	Solanum tuberosum	1	1
Gulkevichi, Russia	*Solanum tuberosum*	6	5
	Convolvulus arvensis	1	1
Niva, Russia	*Solanum tuberosum*	2	1
Total	–	122	96

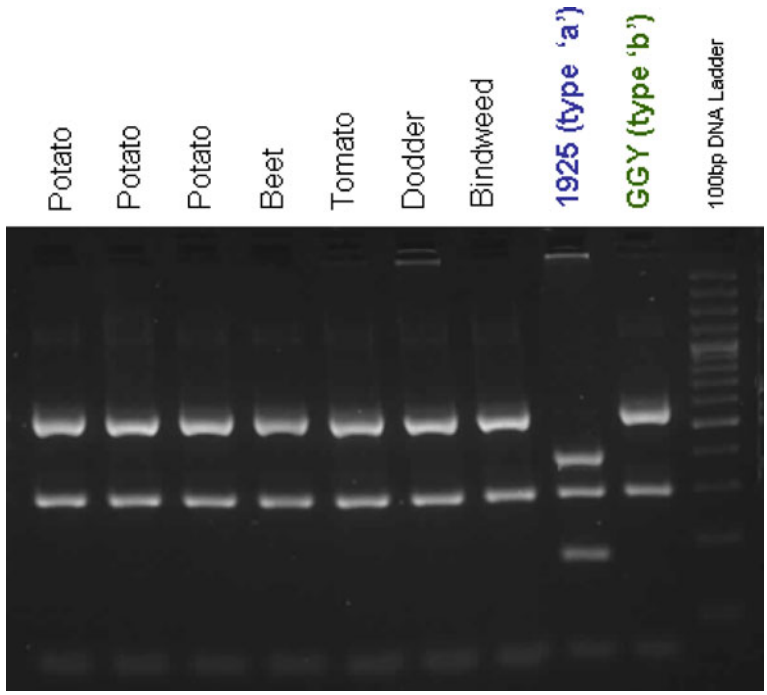

Fig. 3 RFLP patterns of DNA fragments from different plant samples amplified with fTufAy/rTufAy primers and digested with *Hpa*II, in addition to reference stolbur strains (1925 reference of tuf- type '**a**' and GGY reference of tuf-type '**b**'). Marker: 100 bp DNA Ladder and agarose gel (2.5%)

chips however, tubers from symptomatic plants produced chips with a browning discolouration defect (Fig. 2). Although, the discolouration patterns in the fried chips appeared different from those reported in zebra chip potato disease (Munyaneza et al. 2007b; c; 2008a), potato chips resulting from tubers produced by stolbur-infect potato plants were commercially unacceptable as well.

Table 4 List of GenBank accession numbers of 16S rDNA sequences obtained from stolbur-infected plant samples collected from different locations in Romania and southern Russia. The samples were amplified with R16F2n/R16R2 PCR primers (Lee et al. 1995)

Plant samples	Collection location	Phytoplasma	Accession numbers
Convolvulus arvensis	Fundulea, Romania	*Ca.* Phytoplasma solani	HM449995
Solanum tuberosum leaves	Gulkevichi, Russia	*Ca.* Phytoplasma solani	HM449996, HM449997
Convolvulus arvensis	Gulkevichi, Russia	*Ca.* Phytoplasma solani	HM449998
Solanum lycopersicum	Mayak, Russia	*Ca.* Phytoplasma solani	HM449999
Solanum melongena	Mayak, Russia	*Ca.* Phytoplasma solani	HM450000
Solanum tuberosum tubers	Mayak, Russia	*Ca.* Phytoplasma solani	HM450001, HM450002
Solanum tuberosum leaves	Fundulea, Romania	*Ca.* Phytoplasma solani	HQ108386
Solanum tuberosum leaves	Fundulea, Romania	*Ca.* Phytoplasma solani	HQ108387
Convolvulus arvensis	Fundulea, Romania	*Ca.* Phytoplasma solani	HQ108388
Cuscuta sp.	Fundulea, Romania	*Ca.* Phytoplasma solani	HQ108389
Solanum tuberosum leaves	Mayak, Russia	*Ca.* Phytoplasma solani	HQ108390
Beta vulgaris	Mayak, Russia	*Ca.* Phytoplasma solani	HQ108391
Euphorbia falcata	Mayak, Russia	*Ca.* Phytoplasma solani	HQ108392
Solanum tuberosum leaves	Niva, Russia	*Ca.* Phytoplasma solani	HQ108393
Solanum tuberosum leaves	Azov, Russia	*Ca.* Phytoplasma solani	HQ108394

Discussion

Yellows type symptoms caused by phytoplasmas, especially stolbur, have been known in Europe and in the European region of Russia and Ukraine since the early 1940s. The stolbur disease was first described by Suhov and Vovk (1949) in the former Soviet Union. Presence of stolbur was reported on different solanaceous crops, including potato, tomato, pepper and eggplant. Stolbur was also observed inducing severe symptoms on tobacco, as well as on a number of weed species in several countries in south and central Europe (Klinkovski 1957). In the last century, the host range of this phytoplasma has been thoroughly studied by several authors in Switzerland (Bovey 1956), Romania (Savulescu and Pop 1956), Hungary (Szirmai 1956; Petróczy 1962; Kuroli 1970), Czechoslovakia (Bojňanský 1959; Valenta et al. 1961), France (Marchoux et al. 1969; Marchoux and Rougier 1987) and Italy (Martelli et al. 1969). Throughout the 1950–1960s, stolbur only occasionally caused epidemics on potato, tomato and pepper. Since 2002, however, the importance of potato stolbur has been increasing in central and eastern Europe. Just in the last few years, severe phytoplasma outbreaks have been reported in potato fields in several countries, including Czech Republic, Hungary, Romania, and Russia, causing significant yield loss (30 to 80% potato crop loss) and a reduction in tuber processing and seed quality (Paltrinieri and Bertaccini 2007; Bogoutdinov et al. 2008; Girsova et al. 2008; Lindner and Haase 2008; Lindner et al. 2008; Fialova et al. 2009; JE Munyaneza, unpublished data).

Effective management of these diseases requires accurate identification of phytoplasmas affecting the potato crop and their insect vectors. Historically, phytoplasmas in eastern and central Europe were identified based on visual plant symptoms. However, the use of molecular techniques is currently the only reliable means to accurately determine the etiology of these emerging diseases. Unfortunately, only a few studies on detection and identification of potato phytoplasmas based on molecular techniques have been conducted in central and eastern Europe to confirm the identity of phytoplasmas associated with diseases in potatoes in this part of the world. It is not until very recently that Girsova et al. (2008) confirmed for first time detection of both stolbur (16SrXII-A) and aster yellows (16SrI-A and B) phytoplasmas in several potato growing regions of Russia by molecular procedures, with stolbur being more prevalent in the region. Similarly, little information is available on detection and identification of phytoplasmas associated with potato in Romania (Lindner et al. 2008).

Based on PCR testing, results of the present study indicated that stolbur phytoplasma ("*Ca.* Phytoplasma solani") was the most important phytoplasma associated with potato crops in Romania and southern Russia, supporting the recent reports by Bogoutdinov et al. (2008) and Girsova et al. (2008) in Russia, and to some extent by Lindner et al. (2008) in Romania. During this study, only stolbur phytoplasma was detected in potato, tomato, pepper, and eggplant. In addition, this phytoplasma was detected in beet plant samples. The phytoplasma was detected at a high rate in potato plants from all surveyed fields in both years of study. Among weeds, stolbur was detected in plant samples of *C. arvensis*, *Cuscuta* sp., and *E. falcata*. However, *C. arvensis* had the highest infection rate of the phytoplasma in both countries. Moreover, the analyzed *Hpa*II RFLP profiles of *tuf* gene of the 96 stolbur isolates collected from both Romania and Russia revealed the same profile corresponding to the tuf-type 'b' (VK Type II) which has been associated with *C. arvensis* (Maixner et al. 1995; Langer and Maixner 2004; Fialova et al. 2009), suggesting that this weed constitutes a major source of inoculum for the stolbur strain affecting potato and other solanaceous crops in Romania and Russia. This wild host plant of stolbur is abundant and widespread in both countries.

Sequencing results of 16S rRNA gene, as the conventional phytoplasma marker, showed very low variability among stolbur isolates of above mentioned crops and weeds collected from Romania and Russia. This finding pointed out the need to use others molecular markers to differentiate potential stolbur strains (Cimerman et al. 2009; Fialova et al. 2009). Further characterization of stolbur strains from several other plant species and identification of potential insect vectors of stolbur phytoplasma in potatoes of both Romania and Russia are in progress.

During the present study, it was documented that stolbur-infection in potatoes severely affected potato tuber processing quality. A large number of tubers from stolbur-infected potato plants produced fried

chips that were defective and unmarketable. However, these tubers did not exhibit characteristic symptoms of zebra chip disease, a newly emerging and destructive disease of potato in the Americas and New Zealand (Munyaneza et al. 2007b; c; Munyaneza et al. 2008a; Liefting et al. 2008; Secor et al. 2009; Crosslin et al. 2010). Although above-ground plant symptoms of stolbur phytoplasma infection in potato resemble those caused by zebra chip, the tuber symptoms are different. Unlike zebra chip, fresh tubers from stolbur-infected plants were spongy and did not show any symptoms upon slicing. However, these tubers produced fried potato chips that had a browning defect, especially in the vascular ring. In contrast, fresh tubers from zebra chip-affected potato plants exhibit collapsed stolons, dramatic browning of vascular tissue concomitant with necrotic flecking of internal tissues and streaking of the medullary ray tissues, all of which can affect the entire tuber. Upon frying, these symptoms become more pronounced and resulting chips show very dark blotches, stripes, or streaks, making them commercially unacceptable (Munyaneza et al. 2007b; c; Secor et al. 2009; Crosslin et al. 2010; Miles et al. 2010). Zebra chip has recently been associated with a previously undescribed species the bacterium liberibacter, designated "*Candidatus* Liberibacter solanacearum" (syn. "*Ca*. Liberibacter psyllaurous") and transmitted by the potato psyllid *Bactericera cockerelli* (Sulc) (Munyaneza et al. 2007b; c; Hansen et al. 2008; Liefting et al. 2008; Munyaneza et al. 2008a; Liefting et al. 2009; Secor et al. 2009; Crosslin et al. 2010).

In summary, results of the present study indicated that stolbur was the major pathogen associated with phytoplasma diseases of potato in Romania and southern Russia. The phytoplasma was also detected in other solanaceous crops and weeds in the vicinity of the surveyed potato fields. In addition, t*uf* genotyping revealed that all stolbur isolates collected from potato and other plants were of tuf-type 'b', a genotype known to be associated with *C. arvensis*. These results suggest that this weed more likely constitutes a major source of inoculum for the stolbur strain affecting potatoes in Romania and southern Russia. Tubers from stolbur-infected potato plants produced fried chips with a browning discoloration, rendering them unmarketable. Further studies to determine insect vectors of stolbur phytoplasma in major potato production areas in both countries are underway. Information from this study will help potato producers in this region of Europe reduce damages caused by stolbur phytoplasma by developing effective management strategies targeted at this plant pathogen and its insect vectors.

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