J. Crop Prot. 2024, 13 (2): 209-214

Short paper

Convolvulus arvensis: A new natural alternative host for '*Candidatus* Phytoplasma australasia' associated with a chickpea phyllody disease in Pakistan

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Abstract: Chickpea is an important pulse crop extensively grown in the arid and semi-arid areas of Pakistan. In April 2019, during routine data collection from chickpea fields in NIAB, Faisalabad, a few *Convolvulus arvensis* plants were observed with symptoms similar to phytoplasma infection. Four symptomatic and two asymptomatic *C. arvensis* samples were tested for phytoplasma presence using nested-PCR. BLASTn analysis of the obtained sequence and constructed phylogenetic tree confirmed that the detected phytoplasma is clustering with '*Candidatus* Phytoplasma australasia' subgroup phytoplasma, which was previously reported as associated with a phyllody disease in chickpea and many other crops in Pakistan. The present study reports that, for the first time, *C. arvensis* is a new host for this phytoplasma in Pakistan, which may act as a primary source of spreading it in important crops.

Keywords: Field bindweed, phyllody, virescence, 16SrII-D

Chickpea is an important cool-season pulse crop extensively grown in the arid and semi-arid areas of Pakistan. Regrettably, this crop is badly affected by more than 50 important and yield-limiting diseases in different parts of the world, including Pakistan (Akhtar *et al*., 2009). The situation was further aggravated in 2008 due to the prevalence of phytoplasma-associated chickpea phyllody disease in Pakistan (Akhtar *et al*., 2008). Phytoplasmas are wallless phloem-inhibiting bacteria transmitted by phloem-feeding insects and associated with severe diseases in many crops. The most characteristic symptoms associated with phytoplasmas are phyllody, virescence, stunted growth, smaller leaves, yellowing, abnormal shoots proliferation and witches' broom (Win and Jung, 2012).

In April 2019, during field surveys for screening of chickpea germplasm against phyllody disease in NIAB, Faisalabad, a few *Convolvulus arvensis* (field bindweed) plants in these fields were showing symptoms similar to phytoplasma infection (malformed, virescent and phylloid flowers). Four symptomatic and two asymptomatic *C. arvensis* samples (Fig. 1) were collected to test for phytoplasma presence. Total genomic DNA from these samples was extracted using a CTAB-based method (Doyle and Doyle,

Handling Editor: Masoud Shams-bakhsh

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Received: 19 August 2022, Accepted: 27 May 2024
Published online: 07 December 2024

1990). To amplify 16S rRNA gene from these samples, nested-PCR was carried out with universal phytoplasma primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) followed by R16F2n/R2 primers (Gundersen and Lee, 1996).

PCR products (c.1.8 and c.1.25 kbp) were obtained only from symptomatic *C. arvensis* but not from asymptomatic plants. Amplicon of R16F2n/R2 primers was purified from one sample, directly sequenced and submitted to NCBI GenBank (GenBank accession number MT119155). BLASTn analysis of the obtained consensus sequence showed > 99% identity with 16S rDNA from several phytoplasmas enclosed in the 16SrII group. A phylogenetic tree was constructed using the neighbour-joining method with MEGA7.0 (Kumar *et al*., 2016), which confirmed that the *C. arvensis* phytoplasma is clustering with '*Candidatus* Phytoplasma australasia' strains with other strains enclosed in the 16SrII-D subgroup (Fig. 2) (Table-1).

Weeds play a key role in the epidemiology of phytoplasma-associated diseases by acting as a reservoir and reproductive host of insect vectors (Akhtar *et al*., 2018; Salehi *et al*., 2018). *C. arvensis* is one of the most persistent and perennial herbaceous problematic weeds in many crops in Pakistan. Phytoplasma enclosed in several ribosomal subgroups were reported on *C. arvensis* in different countries (Salehi *et al*., 2018; Marcone *et al*., 1997). In Pakistan, phytoplasmas in subgroup 16SrII-D are the most widespread and have been associated with diseases in chickpea, tomato, flax, sesame, mungbean, tomato and Parthenium (Akhtar *et al*., 2018). The present study reports for the first time *C. arvensis* as a new host for '*Ca.* P. australasia' in Pakistan may act as source to spread this phytoplasma in important crops in Pakistan.

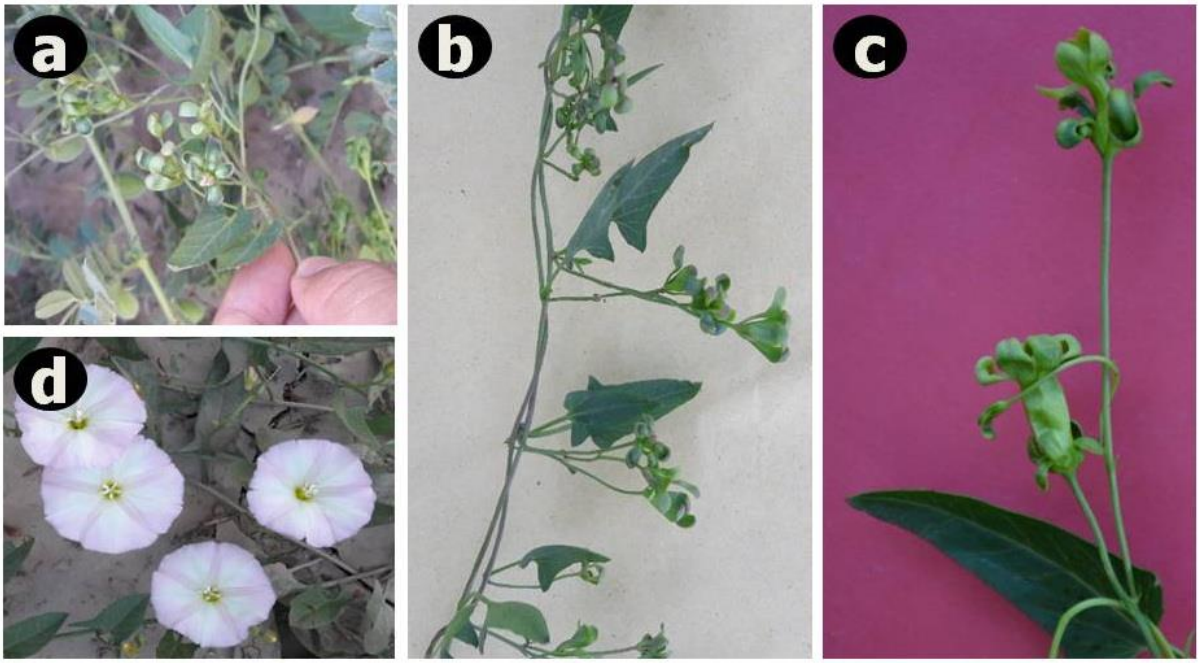

Figure 1 a-c) Malformed, virescent and phylloid flowers observed on naturally infected field bindweed plants; d) healthy flowers of *C. arvensis* plant.

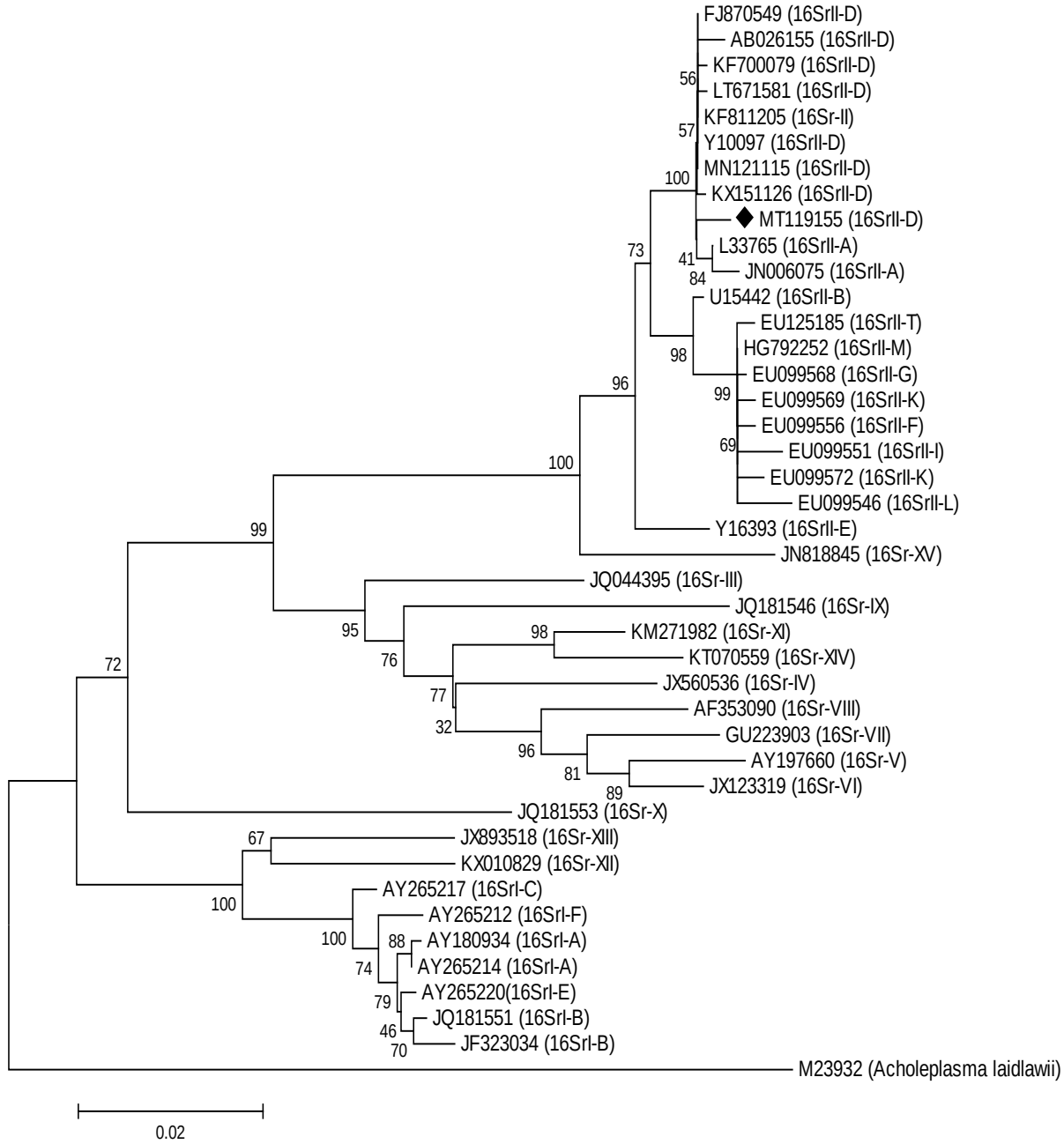

Figure 2 Phylogenetic tree based on 16S rRNA sequences showing the relationships of *C. arvensis* phytoplasma from Pakistan with reference 16Sr phytoplasma groups while *Acholeplasma laidlawii* (M23932) was used to root the tree. GenBank accession numbers are given, and phytoplasmas subgroup sequences are shown in parenthesis. Numbers on the branches are bootstrap (confidence) values of 1000 replicates. The bar indicates the number of substitutions per nucleotide position. The evolutionary distances were computed using the Kimura 2-parameter model using MEGA7.

Table 1 Phytoplasma strains used for phylogenetic analysis in this study.

Strain	Associated disease	Host	Geographical origin	16S rRNA gene classification	GenBank No.
Coconut lethal yellowing phytoplasma	Lethal yellowing	Coconut	West Indies	16Sr-IV	JX560536
Ca. Phytoplasma luffae	Witches' broom	Loofah	Taiwan	16Sr-VIII	AF353090
Peach yellows phytoplasma	Peach yellows	Peach	USA	16Sr-V	AY197660
Ca. Phytoplasma fraxini	Decline and witches' broom	Peach	Canada	16Sr-VII	GU223903
Cauliflower stunt phytoplasma	Cauliflower stunt	Cauliflower	Brazil	16Sr-XV	JN818845
Walnut witches'-broom phytoplasma	X-disease of stone fruits	Walnut	USA	16Sr-III	JQ044395
Picris echioides' yellows phytoplasma Cal	Picris echioides' yellows	Bristly Ox-Tongue	Italy	16Sr-IX	JQ181546
Spartium junceum' witches'-broom phytoplasma	Virescence and witches'-broom	*Spartium junceum*	Italy	16Sr-X	JQ181553
Willow proliferation phytoplasma	Willow proliferation-Erdos	Willow	China	16Sr-VI	JX123319
Papaya apical curl necrosis phytoplasma	Apical curl necrosis	Papaya	Brasil	16Sr-XIII	JX893518
Ca. Phytoplasma australasia	Mung Bean Phyllody	Mungbean	USA	16Sr-II	KF811205
Lychee phytoplasma	Leaf rolling and witches'-broom	Lychee	India	16Sr-XI	KM271982
Foxtail yellow decline phytoplasma	Yellow decline	Foxtail palm	Malaysia	16Sr-XIV	KT070559
Ca. Phytoplasma solani	*Bois noir*	Grapevine	Italy	16Sr-XII	KX010829
Ca. Phytoplasma aurantifolia	Tomato Big Bud	Tomato	Pakistan	16SrII-D	LT671581
Rumex bucephalophorus' dwarf phytoplasma	Fascination and dwarfing	*Rumex bucephalophorus*	Italy	16SrI-B	JQ181551
Amaranthus hypochondriacus' cladode phytoplasma	Cladodes and spica proliferation	*Amaranthus hypochondriacus*	China	16SrI-B	JF323034
Aster yellows phytoplasma strain	Aster yellows	Carrot	USA	16SrI-A	AY180934
Chrysanthemum yellows phytoplasma	Aster yellows	*Chrysanthemum frutescens*	USA	16SrI-A	AY265214
Clover phyllody phytoplasma	Aster yellows	*Trifolium repens*	USA	16SrI-C	AY265217
Blueberry stunt phytoplasma	Aster yellows	Blueberry	USA	16SrI-E)	AY265220
Aster yellows phytoplasma	Aster yellows	Leafhopper (vector)	USA	16SrI-F	AY265212
Ca. Phytoplasma aurantifolia	Phyllody	Chickpea	Pakistan	16SrII-D	FJ870549
Cactus witches'-broom phytoplasma	Witches'-Broom	Cactus	USA	16SrII-K	EU099572
Cactus witches'-broom phytoplasma	Witches'-Broom	Cactus	USA	16SrII-L	EU099546
Cactus witches'-broom phytoplasma	Witches'-Broom	Cactus	USA	16SrII-F	EU099556
Cactus witches'-broom phytoplasma	Witches'-Broom	Cactus	USA	16SrII-G	EU099568
Cactus witches'-broom phytoplasma	Witches'-Broom	Cactus	USA	16SrII-K	EU099569
Cactus witches'-broom phytoplasma	Witches'-Broom	Cactus	USA	16SrII-I	EU099551
Ca. Phytoplasma aurantifolia	Witches'-Broom	*Tephrosia purpurea*	India	16SrII-M	HG792252
Ca. Phytoplasma aurantifolia	Witches'-Broom	*Citrus aurantifolia*	France	16SrII-B	U15442
Picris echioides' phyllody phytoplasma	Phyllody	*Picris echioides*	Italy	16SrII-E	Y16393
Peanut witches'-broom	Witches'-Broom	*Catharanthus roseus*	USA	16SrII-A	L33765
Phytoplasma sp. Gph	Phyllody	*Gerbera jamesonii*	Australia	16SrII-D	AB026155
Ca. Phytoplasma australasiae	Yellow crinkle and mosaic	*Carica papaya*	Australia	16SrII-D	Y10097
Albizia lebbeck' witches'-broom phytoplasma	Witches'-Broom	*Albizia lebbeck*	Iran	16SrII-D	MN121115
Tomatillo witches'-broom phytoplasma	Witches'-Broom	Tomatillo	Mexico	16SrII-T	EU125185
Tomato big bud phytoplasma	Big bud	Tomato	India	16SrII-D	KF700079
Chickpea phyllody phytoplasma	phyllody	Chickpea	India	16SrII-D	KX151126
Ca. Phytoplasma aurantifolia	phyllody	Sesame	Thailand	16SrII-A	JN006075
Ca. Phytoplasma sp. (From this study)	Virescence and Phyllody	Field bindweed	Pakistan	16SrII-D	MT119155

Declarations

Ethics approval: This article does not contain any studies with human participants or animals performed by any authors.

Consent for publication: All authors consent to publish this research article in The Journal of Crop Protection.

Conflicts of interest: The authors declare no conflict of interest.

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پیچک صحرایی *Convolvulus arvensis*: یک میزبان طبیعی جایگزین برای "*Candidatus* Phytoplasma australasia" همراه با بیماری فیلودی نخود در پاکستان

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چکیده: نخود محصول مهمی از حبوبات است که به‌طور گسترده در مناطق خشک و نیمه‌خشک کشور پاکستان کشت می‌شود. در آوریل ۲۰۱۹ طی جمع‌آوری داده از مزارع نخود فیصل‌آباد، گیاهان پیچک صحرایی *Convolvulus arvensis* دارای علایم مشابه با آلودگی فیتوپلاسمایی مشاهده شد. چهار نمونه دارای علائم و دو نمونه فاقد علائم بیماری برای حضور فیتوپلاسما با استفاده از روش پی‌سی‌آر آشیانه‌ای بررسی شد. آنالیز بلاست ترادف‌های به‌دست آمده و بازسازی درخت فیلوژنی نشان داد که فیتوپلاسمای ردیابی شده با '*Candidatus* Phytoplasma Australasia' که قبلاً از نخود دارای علائم بیماری فیلودی و تعداد زیادی از محصولات دیگر از پاکستان گزارش شده در یک گروه قرار گرفتند. مطالعه حاضر برای اولین‌بار پیچک صحرایی را به‌عنوان میزبان جدید این فیتوپلاسما در پاکستان گزارش می‌کند و ممکن است به‌عنوان منبع اولیه آن برای انتشار در محصولات مهم باشد.

واژگان کلیدی: پیچک صحرایی، فیلودی، ویرسانس، 16SrII-D