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Short Communication

Validation of chip grafting inoculation assay to assess the resistance of *Solanum* species against phytoplasma

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Abstract

Big bud caused by several different phytoplasmas is an emerging threat to tomato production worldwide. The development of resistant varieties would be an effective approach to manage this problem, but it requires an appropriate screening technique. Recently, we have described a simple and efficient chip graft inoculation assay (CGIA) for the first time to screen tomato germplasm against Tomato leaf curl New Delhi virus. The present study was conducted to first validate the CGIA for phytoplasma transmission, then to assess the resistance of 74 genotypes belonging to different *Solanum* species against 16SrII-D phytoplasma. CGIA success rate and phytoplasma transmission was 100% since all the grafts survived and phytoplasma was detected in these plants using nested polymerase chain reaction. No genotype was found resistant as all the grafted plants showed typical disease symptoms. In addition to phytoplasma transmission, CGIA can be used for better understanding the plant–phytoplasma interactions and biology of phytoplasmas in tomato.

Keywords: chip grafting, phyllody, phytoplasma, *Solanum* spp, tomato

Introduction

Tomato is reported to be infected by many microorganisms worldwide, including phytoplasmas (Akhtar *et al.*, 2018). Phytoplasmas are a group of plant pathogenic wall-less phloem inhabiting prokaryotes (El-Sisi *et al.*, 2017) naturally transmitted by phloem-feeding insect species of the order Hemiptera (Akhtar *et al.*, 2016). Phytoplasmas can also be transmitted through grafting and by vegetative propagation (Omar and Foissac, 2012). Phytoplasma diseases of tomato have been reported worldwide (Du *et al.*, 2013). The phytoplasma infected tomato plants show different symptoms such as big bud, proliferation of lateral shoots and phyllody (Xu *et al.*, 2013).

Breeding tomato cultivars resistant to phytoplasmas or their insect vectors would be an effective tool to manage phytoplasma diseases (Kumari *et al.*, 2019). However, to date, no information is available about phytoplasma resistance in tomato. Moreover, finding resistance sources requires an appropriate, efficient and reliable screening technique. Recently, we have described a more resource-effective chip grafts inoculation assay (CGIA) for the screening of tomato germplasm against Tomato leaf curl New Delhi virus (Akhtar *et al.*, 2019). In this study, we describe the validation of CGIA and resistance potential of different *Solanum* species against 16SrII-D phytoplasma that will facilitate faster development of disease-resistant cultivars.

Experimental

The 16SrII-D phytoplasma isolate used in this study was described by Akhtar *et al.* (2018) (GenBank accession:

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Table 1. Response of different *Solanum* species against phyllody disease after chip grafting

Species/entry	Type/source	LP	DS
Solanum galapagense S.C. Darwin & Peralta			
LA0317 (TGRC 10)	D/TGRC	23	+++
LA1306 (TGRC 24)	ID/TGRC	22	+++
Solanum arcanum Peralta			
LO6122 (AVRDC 22)	ID/AVRDC	25	+++
Solanum neorickii D.M. Spooner, G.J. Anderson & R.K. Jansen			
LA2727 (TGRC 14)	ID/TGRC	22	+++
Solanum pimpinellifolium L.			
LA0722 (TGRC 8)	Wild/TGRC	23	+++
LA1261 (TGRC 9)	D/TGRC	22	+++
LA2184 (TGRC 7)	D/TGRC	25	+++
LA2340	?/TGRC	23	+++
LO2707 (AVRDC 25)	SD/AVRDC	25	+++
LO3715 (AVRDC 24)	SD/AVRDC	24	+++
LO4166 (AVRDC 26)	ID/AVRDC	22	+++
Solanum habrochaites S. Knapp & D.M. Spooner (LA1777) introgression lines in the background of cv. E-6203 (LA4024)			
LA3925	?/TGRC	23	+++
LA3930	?/TGRC	21	+++
Backcross recombinant inbred lines (*Solanum lycopersicum* E-6203 × *S. pimpinellifolium* LA1589)			
LA4157	?/TGRC	23	+++

+++ = Severe disease symptoms; LP = latent period; DS = disease severity; D = determinate; ID = indeterminate; SD = semi-determinate; TGRC = Tomato Genetic Resources Centre, USA; AVRDC = Asian Vegetable Research and Development Centre, Taiwan; ? = not known.

LT671581) maintained on tomato variety 'Nagina' in a glasshouse at Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. Plant material comprised 74 genotypes of different *Solanum* species (Tables 1 and 2). Seeds of each genotype were sown in three large earthen pots, maintained in an insect-free glasshouse at NIAB and plants were thinned to three plants per pot 4 weeks post-germination. At the age of 6 weeks, test plants were inoculated following the CGIA protocol (Akhtar *et al.*, 2019). Firstly, a slit of one inch was made one to two inches below the tip on the main stem of the seedlings using a scalpel (Fig. 1(a)). Small pieces of about 2.54–3.81 cm long tender stem/twig from a 16SrII-D phytoplasma-infected source plant were cut and dipped in sterile distilled water. A thin layer of the cell wall (bark) was removed from two opposite sides of each portion of the small piece to expose the cambium layer (Fig. 1(b)). This chip was then inserted into the slit of the test plant (Fig. 1(c) and (d)) and covered with parafilm (Fig. 1(e)). Parafilm wrapping was degraded/removed after 10 d and success or failure of the graft union was assessed visually. A graft was determined to be successful if the inserted chip retained its green colour and had fused to the rootstock at the graft union edges (Fig. 1(f)). Each grafted plant was observed to record disease severity data. Leaf samples were collected 7, 12 and 15 d after grafting to confirm the transmission/presence of the phytoplasma using nested-PCR (nested-polymerase chain reaction). Total DNA was extracted from symptomatic and healthy plant leaves of tomato test genotypes using the Cetyltrimethyl ammonium bromide (CTAB) method (Doyle and Doyle, 1990). Nested-PCR was carried out using primer pairs P1 (5′-AGAGTTT GATCCTGGCTCAGGATT-3′) and P7 (5′-CGTCCTTCAT CGGCTCTT-3′), followed by R16F2 (5′-ACGACTGCTGCT AAGACTGG-3′) and R16R2 (5′-TGACGGGCGGTGTGT ACAAACCCCG-3′) (Deng and Hiruki, 1991; Lee *et al.*, 1993).

Discussion

The CGIA successfully transmitted 16SrII-D phytoplasma to all the grafted plants of different *Solanum* species. Chip grafting and disease transmission were 100%, as all the inserted chips retained their green colour and fused to the graft union edges of rootstock as earlier reported by Akhtar *et al.* (2019). Symptoms developed from 17 to 30 d post-inoculation (DPI) depending upon the tested genotypes. Parafilm wrapped around the graft was

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Table 2. Response of different *S. lycopersicum* genotypes against phyllody disease after chip grafting

Entry	Type/source	LP	DS	Species/entry	Type/source	LP	DS
Ailsa Craig	ID/TGRC	23	+++	LA 3845B	D/TGRC	22	+++
B-L-35	D/TGRC	22	+++	LO 6203 (AVRDC10)	D/AVRDC	21	+++
Bright Valley	D/TGRC	21	+++	LO 6170 (AVRDC11)	D/AVRDC	22	+++
Canada-25	D/EFUP	20	+++	LO2527	ID/NARC	20	+++
CLN 1462A	D/AVRDC	21	+++	Lyp-1	D/AARI	21	+++
CLN1621F	D/AVRDC	21	+++	Nagina	D/AARI	18	+++
CLN1621L	D/AVRDC	20	+++	Naqeeb	D/AARI	21	+++
CLN2037E	ID/AVRDC	22	+++	NCEBR-5	D/TGRC	22	+++
CLN 2366A	D/AVRDC	23	+++	NCEBR-6	D/USA	21	+++
CLN 2413R	D/AVRDC	20	+++	NC HS-1	D/TGRC	22	+++
CLN 2418A	?/AVRDC	23	+++	PAK0010988	D/Bulgaria	22	+++
CLN 2545A	D/AVRDC	19	+++	PAK0011009	D/NARC	20	+++
CLN 2445 B	D/AVRDC	21	+++	PAK0011020	ID/NARC	21	+++
CLN2498D	D/AVRDC	20	+++	Pakit	ID/ AARI	17	+++
CLN3024A	D/AVRDC	23	+++	Picdenato	D/AARI	20	+++
CLN3078G	D/AVRDC	21	+++	PRN-28-10	ID/NIAB	22	+++
CLN3078I	D/AVRDC	20	+++	Roma	D/ AARI	21	+++
CLN3125E	D/AVRDC	17	+++	Riogrande	D/EFUP	20	+++
CLN3150A-5	D/AVRDC	22	+++	RM-350-09	ID/NIAB	22	+++
CLN3205B	D/AVRDC	23	+++	RM 400	ID/NIAB	21	+++
CLN3212A-25	D/AVRDC	20	+++	Romaking	D/NARC	24	+++
F8-145 (AVTO1323)	D/AVRDC	21	+++	Rowpac (LA 3214)	ID/TGRC	17	+++
CLN3241H-27	SD/AVRDC	20	+++	Salar	ID/AARI	21	+++
CLN3241R	SD/AVRDC	22	+++	SUNDAR	ID/AARI	19	+++
Galia	D/GWP	18	+++	T-1359	D/Syngenta	21	+++
Jaklin	D/Bulgaria	22	+++	UC-134 (LA1714)	?/TGRC	23	+++
LA1226 (TGRC1)	ID/TGRC	17	+++	V: 06232	ID/?	20	+++
LA1286 (TGRC3)	wild/TGRC	23	+++	West Virginia 63	ID/ TGRC	21	+++
LA1673 (TGRC2)	ID/TGRC	17	+++	21,354	?/TGRC	20	+++
LO 2875 (AVRDC9)	ID/AVRDC	18	+++	88,572	D/AARI	23	+++

+++ = severe disease symptoms; LP = latent period; DS = disease severity; D = determinate; ID = indeterminate; SD = semi-determinate; NIAB = Nuclear Institute for Agriculture and Biology, Pakistan; AARI = Ayub Agricultural Research Institute, Faisalabad, Pakistan; NARC = National Agricultural Research Council, Pakistan; TGRC = Tomato Genetic Resources Centre, USA; AVRDC = Asian Vegetable Research and Development Centre, Taiwan; EFUP = Establishment of facilitation unit for participatory vegetable seed and nursery production programme, Pakistan; GWP = Gujranwala Pakistan; ? = not known.

removed 10 DPI. Phytoplasma infection was confirmed in the samples collected 12 and 15 DPI and the nested-PCR product of 1.2 kbp was amplified (online Supplementary Fig. S1). However, phytoplasma was not detected in the samples collected after 7 DPI from the grafted and healthy control plants. None of the grafted genotypes were highly resistant, resistant or tolerant (Tables 1 and 2). After 25–40 DPI, severe phyllody symptoms similar to the source plant were observed in grafted plants (Fig. 1(g)).

Knowledge of the resistance level of tomato germplasm is important in the resistance breeding programmes. However, resistant germplasm identification requires an efficient and reliable screening method to clearly identify their resistance level. Grafting is considered a more standardized and reliable method to transmit viruses and phytoplasmas from infected to healthy plants (Akhtar *et al.*, 2019). The CGIA validated under the present study has several potential advantages as it is fast, easy, needs no special expertise, requires much less space, and is sensitive enough to screen tomato germplasm for phytoplasma resistance. The major advantage of the CGIA over other grafting methods is that our developed method allows one infected plant to provide a lot of chips (scions) as compared to a 1:1 ratio of the scion to rootstock in the case of side and cleft

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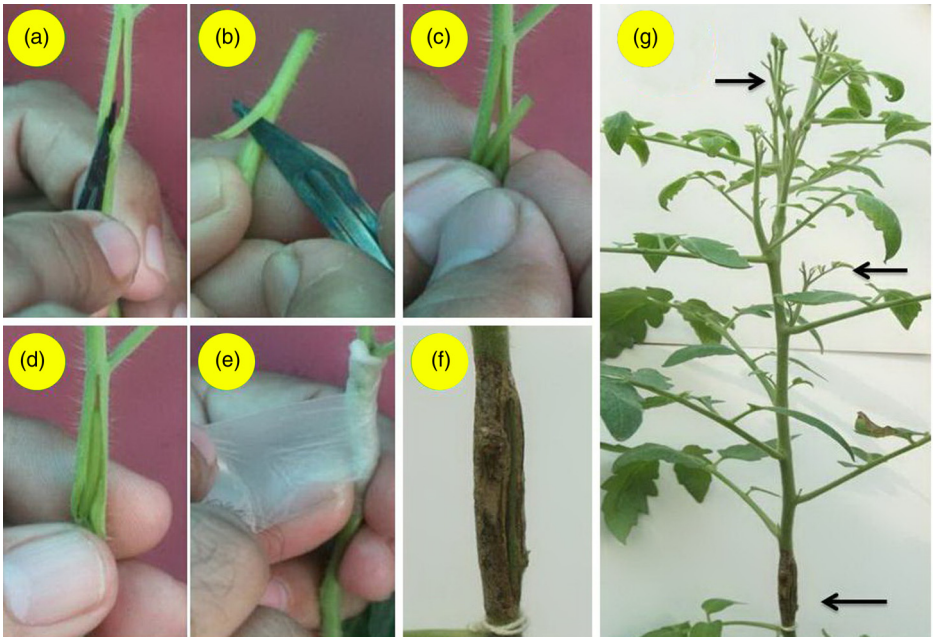

Fig. 1. Different steps involved in CGIA: (a) slit below the tip on the main stem; (b) phytoplasma infected small piece (chip) of stem cut from source plant; (c & d) insertion of chip into the slit of the test plant; (e) slit covered with parafilm; (f) inserted chip fused to the rootstock.

grafting techniques. Moreover, a chip used in this study does not require a leaf or bud which is the principle of previously used methods (Akhtar *et al.*, 2019). The CGIA, therefore, offers a tool for efficient assessment of resistance to this disease across a range of *Solanum* germplasm to develop high-yielding and tolerant genotypes/hybrids to limit phytoplasma spread in the field. Also, the CGIA can be used to graft multiple stems or branches of the rootstock plants and even they can be grafted using two different scions. Multiple grafts on single rootstock plants can increase the 'dose' of phytoplasma.

Here, for the first time, we have documented the response of a high number of genotypes of different *Solanum* species against phytoplasma. The results clearly show that no sources of resistance to 16SrII-D phytoplasma are available in the tested genotypes. In future, more genotypes of *Solanum* species will be screened using the CGIA to find resistant sources, with a view to developing resistant tomato varieties. This assay also has the potential to be extended to better understand the plant–phytoplasma interaction.

Supplementary material

The supplementary material for this article can be found at https://doi.org/10.1017/S1479262121000137.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

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