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SHORT COMMUNICATION

Localization of southern tomato virus (STV) in tomato tissues

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Abstract
Southern tomato virus (STV) is a dsRNA virus, which belongs to the newly formed *Amalgavirus* genus of the *Amalgaviridae* family. Currently there is no report regarding the presence of STV in tomato tissues. In this study, we performed in situ hybridization to examine the distribution of STV in host tissues. STV was found in the leaves, stems, seeds, shoot apexes and root tips of tomato and localized in the cortex tissue, vascular tissues, pith, seed coat, endosperm, cotyledon (including inner cotyledons and outer cotyledons), hypocotyls and radicles of infected tomato tissues. In addition, STV was detected in the apical part of the stems and roots for the first time. This indicates that STV is a systemic infectious virus.

Keywords Ds-RNA virus · *Amalgaviridae* · *Amalgavirus* · Virus host tissue detection · In situ hybridization

Introduction

Southern tomato virus (STV) is a member of the *Amalgavirus* (family *Amalgaviridae*), the *Amalgavirus* genus also includes *Blueberry latent virus* (BBLV), *Vicia cryptic virus* M (VCV-M), *Rhododendron virus* A (RhV), *Allium cepa amalgavirus* (AcAV), *Spinach amalgavirus* (SpAV), and *Zoostera marina amalgavirus*1 and 2 (ZmAV1 and ZmAV2) (Liu and Chen 2009; Sabanadzovic et al. 2009, 2010; Martin et al. 2011; Nibert et al. 2016; Park and Hahn 2017; Park et al. 2018). The STV genome is double-stranded RNA (dsRNA), monopartite, and about 3.5 kb in length, showing the structural features of *Totiviridae*, but close to *Partitiviridae* family in genetics (Sabanadzovic et al. 2009). It has been approved as belonging to the genus *Amalgavirus*, family *Amalgaviridae* by the International Committee on Taxonomy of Viruses (ICTV), as a representative species (Adams et al. 2014).

Since it was first characterized in 2009 (Sabanadzovic et al. 2009), STV has been reported by researchers from more than ten countries (Candresse et al. 2013; Su et al.2013; Iacono et al. 2015; Padmanabhan et al. 2015; Verbeek et al. 2015; Pecman et al. 2017; Oh et al. 2018; Turco et al. 2018). Puchades et al. (2017) have performed northern blot to detect STV in susceptible tomato plants var. Mariana. Elvira-González et al. (2017) developed a one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) for the rapid detection of STV. Puchades et al. (2017) used a digoxin-labeled RNA probe to detect nucleic acid extracts from plant leaves, fruits, roots and seeds. In 2018, a highly sensitive, STV, real-time, quantitative, PCR detection system, was described (Elvira-Gonzalez et al. 2018). As described above STV has been quantified in tomato organs, but the virus had not been visualized by RT-PCR and the dsRNA isolated.

In situ hybridization is a powerful technique for localizing a virus in a portion or section of tissue. As this technique has not been applied yet on the detection of STV, we carried out experiments to evaluate designed STV-specific RNA probes and application of the in situ hybridization technique aiming to localize STV in various tomato tissues. In this paper we present the experimental results indicating the potential diagnostic value of the techniques for localizing STV in tomato tissues.

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Material and methods

Plant materials and sample collection

A processing tomato cultivar Lige 87–5 was used as the study material. Seeds were planted in a growth chamber in conditions of 16 h light, 28 °C/8 h dark, 23 °C.

STV-negative and -positive plants were identified by RT-PCR using forward primer p42F: 5′-ATGGCTTACAATCCG ATCAC-3′ and reverse primer p42R: 5′-CTACCACCTCGA AAGCTTAG-3′ designed from the STV isolate XJ-P (Gen-Bank ID: KY228384). Tomato leaves, stems, seeds, and the apical meristems of roots and shoots were all sampled for in vitro hybridization. Leaf samples were taken from the first or second young leaves from the top of the plants. Apical meristem root and shoot tips were about 5 mm in length. Stems were also cut into 5 mm sections.

In situ hybridization

According to the sequence of STV isolate XJ-P in the cv. Lige87-5 host, a specific primer pair was designed using Vector NTI (Thermo Fischer, USA): STVprobe-F, (5′-GTG GCGCTGCTGCATTGCTT-3′) and STVprobe-R (5′-CGA AGGCCTCCTTGACTTGC-3′). Using the primers, a 300 bp fragment was amplified from the STV-positive plants, purified using a gel recovery kit (Promega Co., Madison, WI, USA) and cloned into pGEM-T Easy vector (Promega Co., Madison, WI, USA). The recombinant plasmid pGEM-STVpro was sequenced for verification, linearized with the restriction enzymes *Sal*I and *Nco*I (Promega Co., Madison, WI, USA), gel-purified and finally quantified (Thermo nanodrop nd-2000, Thermo Scientific, Wilmington, DE, USA).

Digoxigenin-labeled RNA probes were synthesized by in vitro transcription using a DIG RNA Labeling Kit (SP6/T7) (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturers' instructions.

The relevant techniques were performed using the description of the distribution of tomato chlorotic dwarf viroid in floral organs of tomato (Matsushita et al. 2011) and In situ Hybridization according to (Tsai and Harding 2013) but with some modifications. Plant sections were pre-denatured at 90 °C for 20 min, and then incubated in humidified box at 50 °C for 16–18 h. In situ hybridization was conducted with at least three plants and repeated for at least five times.

Results

The specificity and sensitivity of digoxin-labeled sense and antisense RNA probes were examined using in situ hybridization with STV infected and healthy tomato stems. Results showed that the two digoxin-labeled RNA probes both detected clear positive signals in the stems of STV-infected plants (Fig. 1A, B B), similar signals were obtained by sense and antisense probes with pre-denaturing at high temperatures whereas no signal was observed in healthy tomato stems used antisense probe (Fig. 1C) and sense probe (data not shown). No hybridization signal was obtained from infected tomato stems without pre-denaturing at high temperatures used antisense probe (Fig. 1D) or sense probe (data not shown).

STV was detected in infected tomato leaves and young stems. The STV-positive signals presented in the epidermis, palisades and spongy mesophylls at the cross section of tomato leaf samples. The hybridization signal was stronger in palisade mesophyll cells than in other tissues, which might be due to palisade being a type of strong fence structure in leaves (Fig. 2A). STV-positive signals were also found in the stem epidermis, phloem and xylem, and in the pith at the cross section of stem samples (Fig. 2B). STV was also detected in the seeds from infected tomatoes. The results showed that STV occurred in all the tissues of

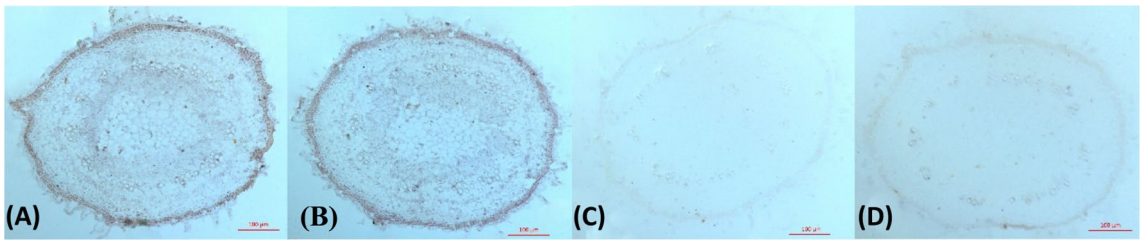

Fig. 1 Detection of *Southern tomato virus* (STV) in tomato stem: Specificity test of sense and antisense digoxin-labeled probes, with or without pre-denaturing at high temperatures. **A** Detection of STV in the infected tomato stem with the sense probe and with pre-denaturing at high temperatures; **B** detection of STV in the infected tomato stems with the antisense probe and with pre-denaturing at high temperatures; **C** detection of STV in healthy tomato stem with the antisense probe and with pre-denaturing at high temperatures; **D** detection of STV in infected tomato stem with the antisense probe and without pre-denaturing at high temperatures. Scale bar represents 100 µm in each frame

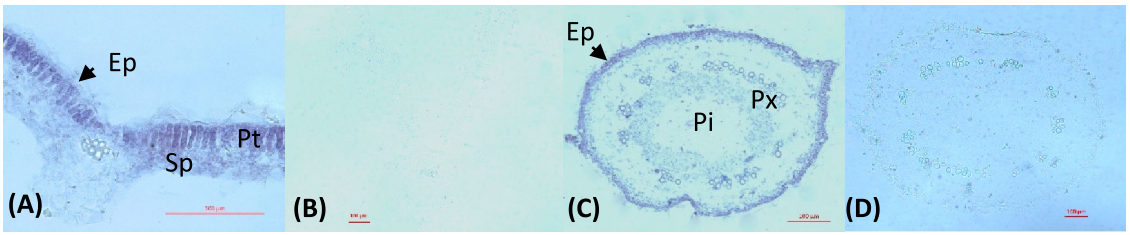

Fig. 2 Distribution of *Southern tomato virus* (STV) in tomato leaves and stems by in situ hybridization. **A** Transverse section of STV-infected tomato leaf; **B** negative control, transverse section of healthy tomato leaf; **C** transverse section of STV-infected stem; **D** negative control, transverse section of healthy stem. Ep, epidermis; Pt, palisade mesophyll; Sm, spongy mesophyll; Px, phloem and xylem; Pi, pith. Bars shows 100 µm in each frame

mature seeds, including seed coat, endosperm, and embryo (Fig.3A).

Discussion

Although, previous studies have detected STV only in seed coat and embryo by molecular hybridization and RT-qPCR (Puchades et al. 2017; Elvira-Gonzalez et al. 2018). In the present study, the technique of optical imaging analysis was used for in situ hybridization, so STV-specific signals were observed in the outer and inner cotyledons, radical, and hypocotyl of seeds from infected plants (Fig. 3A). This indicated the rapid spread rate of STV in tomato seed embryos and concurred with the vertical transmission of 70–90% as concluded by Sabanadzovic et al.(2009).

We also examined the STV in shoot and root apical meristems. The positive signals of STV were detected at the longitudinal section of the shoot apical meristem, leaf primordium, and shoot apex (Fig. 4). As shown in Fig. 4A and C, the STV-positive hybridization signal was stronger in all apical meristem cells (tunica and corpus cells) than in leaf, stem and seed tissues. Likewise, STV-positive signals were also detected in the cortex, root cap, and vascular stele of root tips (Fig. 4D and F). No distinct STV-positive signal could be detected in healthy tomato shoots and roots (Fig. 4E). These results suggest that STV can invade tomato meristematic tissues.

It had not been reported previously that the STV could be localized in the apical meristem of tomato. In our study, STV was found not only in every part of the leaves, stems and seeds, but also at the shoot and root apical meristems. This suggests that STV is a certain type of systemic infectious virus in tomato. It is well known that the long-distance transport of virus mostly occurs through the vascular system for systemic infection (Carrington et al. 1996). Because the rate of spread of virus from cell to cell lags behind the rate of meristematic cell division, shoot and root apical meristems (0.1–1 mm) are often free of virus or only infected mildly (Gosalvez-Bernal et al. 2006). The presence of STV in the shoot and root apical meristems of tomato suggests that the systemic infection of STV may be independent from the vascular bundle. STV virions have only been viewed recently (Sabanadzovic et al. 2009; Elvira-Gonzalez et al. 2018), and the ability of STV to move from cell to cell has still to be thoroughly studied.

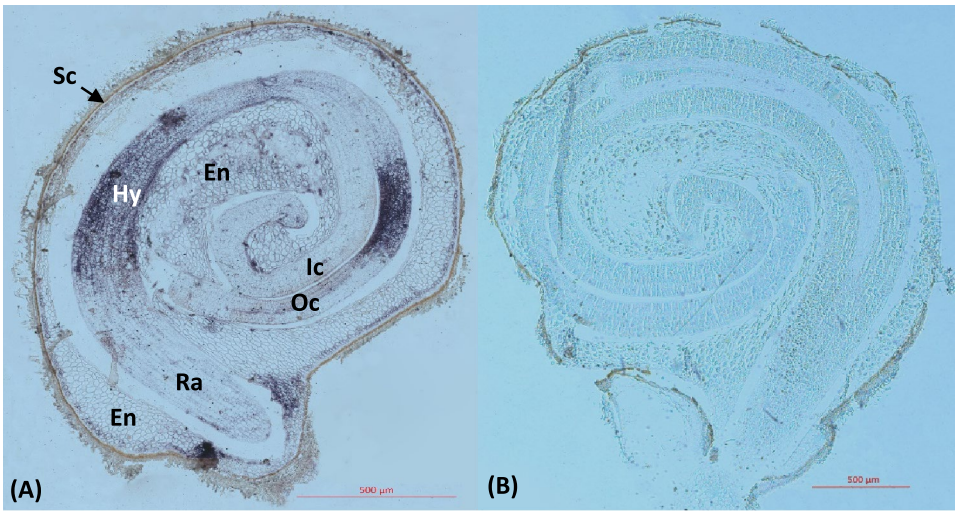

Fig. 3 Detection of *Southern tomato virus* (STV) in seeds by in situ hybridization. **A** Infected tomato seeds as a positive control; **B** healthy tomato seeds as negative control. En, endosperm; Hy, hypocotyl; Ic, inner cotyledon; Oc, outer cotyledon; Ra, radicle; Sc, seed coat. Bar represents 500 µm

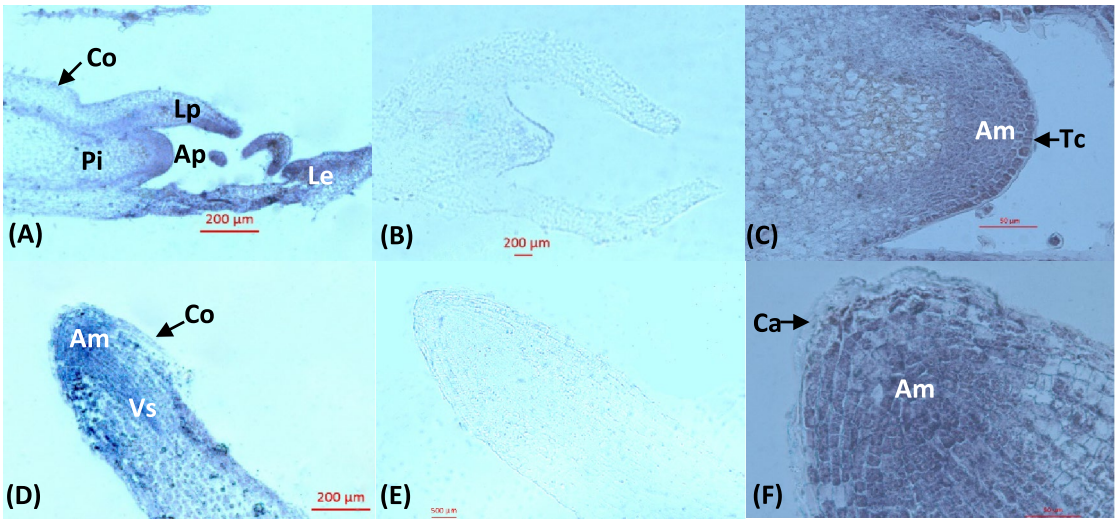

Fig. 4 In situ hybridization of *Southern tomato virus* (STV) in the shoot and root apical meristems of tomato. **A** Longitudinal section of STV-infected stem tip; **B** longitudinal section of healthy tomato stem tip, negative control; **C** magnification of the STV-infected shoot meristem; **D** longitudinal section of STV-infected root; **E** longitudinal section of healthy tomato root, negative control; **F** magnification of the STV-infected root. Ap, apex; Am, apical meristem; Ca, cap; Co, cortex; Tc, tunica cell; Le leaf; Lp, leaf primordium; Pi, pith; Vs, vascular stele. Scale bars indicate 200 µm in **A**, **B**, and **D**; indicate 50 µm in **C** and **F**; indicate 500 µm in **E**

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