Distribution of southern tomato virus in tomato tissues

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Research Article

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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. Up to now, there is no report on the presence of STV in tomato tissues. In this study, we performed in situ hybridization to examine the distribution of STV in host tissues. The 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 infection disease for tomato.

Full Text

Southern tomato virus (STV) is a member of Amalgaviridae amalgavirus. Except for STV, A. amalgavirus genus also includes the Blueberry latent virus (BBLV), Vicia cryptic virus M (VCV-M), Rhododendron virus A (RhV), Allium cepa amalgavirus (AcAV), Spinach amalgavirus (SpAV), and Zostera marina amalgavirus (ZmAV) [7, 8, 10, 13, 14, 17, 18]. STV genome is a 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 [17]. Therefore, the genus Amalgavirus of family Amalgaviridae was approved by the International Committee on Taxonomy of Viruses (ICTV), with as a representative species [1].

Since first characterized in 2009 (Sabanadzovic et al., 2009), tomato STV has been reported by researchers from more than 10 countries [2, 6, 11, 12, 15, 19, 21, 22]. Sabanadzovic et al. (2009) and Puchades et al. (2017) have performed northern blot to detect STV in susceptible tomato plants. Elvira-González et al. (2017) developed a one-step reverse transcription loop-mediated isothermal amplification (RT-LAMP) to examine STV rapidly. Puchades et al. (2017) used a digoxin-labeled RNA probe to detect nucleic acid extracts from plant leaves, fruits, roots and seeds [16]. In 2018, Elvira-gonzález et al. established a real-time quantitative PCR detection system, highly sensitive in STV detection [4]. With above technologies, STV was quantified in tomato organs of roots, stems, leaves, fruits and seeds, but the virus has not been visualized by the common technology of RT-PCR and the dsRNA is not isolated. In situ hybridization is a powerful technique in localizing a virus in a portion or section of tissue, while it is not used for STV detection till now. Here, we designed STV-specific RNA probes and conducted in situ hybridization technique to localize STV in different tomato tissues. This study would provide a valuable addition to diagnostic techniques for localizing STV in tomato tissues.

A processing tomato cultivar Lige 87−5 was used as plant materials in the study. The seeds were planted in a growth chamber under the condition of 16 h light, 28°C/8 h dark, 23°C. STV-negative and -positive plants were identified by RT-PCR. Tomato leaves, stems, seeds, and the apical meristems of roots and shoots were all sampled for in vitro hybridization. Leaf samples were the first or second top young leaves; apical meristem were the root and shoot tips of about 5-mm in length. The stems were cut into 5-mm sections.
According to the sequence of Lige 87 - 5 cv. hosts STV isolate XJ-P (GenBank accession number KY228384), a specific primer pair (STVprobe-F, (5'-GTGGCGCTGCTGCATTGCTT-3'and STVprobe-R (5'-CGAAGGCCTCCTTGACTTGC-3') was designed by Vector NTI (Thermo Fisher, USA). Using the primer, 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 SalI and NcoI (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 Roche’s DIG RNA Labeling Kit (SP6/T7) (Roche Molecular Biochemicals, Indianapolis, IN, USA) according to the manufacturers’ instructions.

According to the description in reference distribution of tomato chlorotic dwarf viroid in floral organs of tomato [9, 20] and In situ Hybridization (Tsai and Harding, 2013) but with some modifications. The sections were pre-denatured at 90°C for 20 min, and then incubated in humidified box at 50°C for 16–18 h. In vitro hybridization was conducted for at least three plants and repeated for five times or more. The specificity and sensitivity of digoxin-labeled sense and antisense RNA probes were examined by in situ hybridization with STV infected and healthy tomato stems. And results showed that the two digoxin-labeled RNA probes both detected clear positive signals in the stems of STV infected plants (Fig. 1a, 1b), but not in healthy tomato stems (Fig. 1c), and hybridization signals from infected stems were observed hardly without pre-denaturing at high temperatures (Fig. 1d). The two probes were both effective, and subsequent experiments used the antisense STV RNA probe only.

The STV was detected in infected tomato leaves and young stems by in situ hybridization. The STV positive signals presented in 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 was a type of strong fence structure of leaf (Fig. 2a). In situ hybridization STV positive signals were also found in stem epidermis, phloem and xylem, pith at the cross section of stem samples (Fig. 2b). STV was also examined in the seeds from infected tomatoes by in situ hybridization. Results showed that STV occurred in all the tissues of mature seeds, including seed coat, endosperm, embryo (Fig. 3a). However, previous study only detected STV in seed coat and embryo by molecular hybridization and RT-qPCR [4, 16]. In the present study, optical imaging technique analysis was used 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.

We also examined the STV in shoot and root apical meristems by in situ hybridization. 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 Fig. 4c, 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 showed in the cortex, root cap, and vascular stele of root tips (Fig. 4d, 4f). But 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.

Previously, STV has not been localized in the apical meristem of tomato. In our study, STV was found in not only every parts of leaves, stems and seeds, but also the shoot and root apical meristems. This suggests that STV is a type of systemic infection virus in tomato. It is well known that the long-distance transport of virus mostly occurs through the vascular system for systemic infection [3]. Because the spread of viral from cell to cell has not so high rate as meristematic cells' division, shoot and root apical meristems (0.1-1 mm) are often free of virus or infected mildly [5]. So the presence of STV in the shoot and root apical meristems of tomato suggests that the systemic infection of STV may be independent from vascular bundle. But STV virions have not be viewed until now [4, 17], and the ability of STV move cell to cell will be a long-standing puzzle.

**Declarations**

**Conflict of interest**

All authors were obtained from all individual participants included in the study and they have no conflict of interest.

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**References**


**Figures**

**Figure 1**

Specificity test of sense and antisense digoxin-labeled probes with tomato stem. a, detection of southern tomato virus (STV) in the infected tomato stem with sense probe and with pre-denaturing at high temperatures; b, detection of STV in the infected tomato stems with the sense 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.

**Figure 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.
Figure 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.

Figure 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 500μm in e.