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Susceptibility of *Arabidopsis* Ecotypes to Infection by *Iris yellow spot virus*

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Abstract

Iris yellow spot virus (IYSV; genus *Tospovirus*, family *Bunyaviridae*) is a serious constraint for onion production in the United States and several other parts of the world. A few experimental indicator hosts were reported for IYSV but there is no report of infection of *Arabidopsis* by IYSV. Establishing IYSV infection in onion by mechanical inoculation has so far proven to be inefficient resulting in numerous escapes. Using *Arabidopsis thaliana* as a host for studies on the biology and tospovirus-host interactions has an advantage of the plant's small genome size, short life cycle, and a genome that has been sequenced. Several ecotypes were evaluated for their susceptibility to IYSV and identified some where IYSV produces localized infection. Together with wild type and various mutants, the IYSV-*Arabidopsis* system could be useful for studying host-tospovirus interactions.

Introduction

Iris yellow spot virus (IYSV; genus *Tospovirus*, family *Bunyaviridae*) is a serious constraint for onion production in the United States and several other parts of the world (3,7). A few experimental indicator hosts were reported for IYSV (1) but there is no report of infection of *Arabidopsis* by IYSV. Establishing IYSV infection in onion by mechanical inoculation has so far proven to be inefficient resulting in numerous escapes. Using *Arabidopsis thaliana* as a host for studies on the biology and tospovirus-host interactions has an advantage of the plant's small genome size, short life cycle, and a genome that has been sequenced (8). *Tomato spotted wilt virus*, the type member of the genus *Tospovirus*, can infect *Arabidopsis* (2,4,5). To investigate if *Arabidopsis* could be used as a model plant for studying IYSV-host interactions, we evaluated the response of different *Arabidopsis* ecotypes to IYSV infection in a controlled environment.

Seeds of three *Arabidopsis* ecotypes, *Columbia* wild type (Col-0), *Landsberg erecta*, and *Wassilewskija*, were sown in Murashige-Skoog medium containing 0.8% agar under white light at 23°C. One-week-old seedlings were transplanted in small 28- × 55-cm plastic trays with 36 inserts having SunShine potting mixture LC1. Plants were shifted from incubator to growth chamber with a temperature of 18°C and a day-night cycle of 12 h with 160 $\mu\text{mol}/\text{cm}^2$ light intensity.

IYSV-infected datura leaves were ground in chilled 0.1 M phosphate buffer (pH 7.0) having 4% β -mercaptoethanol and the homogenate was used for inoculation of healthy *Arabidopsis* plants. Four to five fully expanded leaves of plants were dusted with carborundum powder (600 mesh) and the inoculum was applied to leaves with cotton buds. In each independent experiment, twenty plants of each ecotype were inoculated with IYSV. Mock-inoculated *Arabidopsis* plants (Fig. 1A) consisted of plants inoculated with buffer only and maintained in the same growth chamber. Mock-inoculated controls were tested before mock-inoculation and during the course of the experiment using RT-PCR for the nucleocapsid protein gene (N) of IYSV to ensure that they remained free of IYSV infection. In each independent experiment, five mock-inoculated controls were included. Each independent experiment was repeated four times.

Leaves of inoculated plants started showing wilting and purple discoloration two weeks-post inoculation. With increasing number of days-post inoculation, purple discoloration and wilting became more severe resulting in dying of leaves. Some of the plants also showed characteristic diamond-shaped lesions which is a typical symptom of IYSV infection in onion (3). Infected plants also showed chlorotic spots and yellowing of leaves (Fig. 1B).

Fig. 1. *Arabidopsis* plants showing symptoms of *Iris yellow spot virus* (IYSV) and *Tomato spotted wilt virus* (TSWV) infection: (A) healthy *Arabidopsis* plant; (B) IYSV-infected *Arabidopsis* plant showing yellowing, wilting, and purple discoloration of leaves; (C) TSWV-infected *Arabidopsis* plant showing yellowing, wilting, and purple discoloration of leaves; (D) *Arabidopsis* leaf with IYSV-induced lesion.

Leaves showing purple discoloration and wilting were collected 20 days post-inoculation and tested for IYSV infection by ELISA following kit instructions (Agdia Inc., Elkhart, IN) and RT-PCR. Using ELISA, leaves from 14 inoculated plants of each ecotype were tested in duplicate wells and absorbance was recorded at A_{405} nm. For *Columbia* ecotype, 8 out of 14 samples were positive by ELISA, and for *Wassilewskija* ecotype, 8 out of 16 samples were IYSV positive. None of the *Landsberg erecta* plants turned out positive to IYSV infection in ELISA. The average ELISA values for *Columbia* ecotype were 0.37 ± 0.13 for IYSV-inoculated plants in comparison to the healthy plants which gave an average of 0.12 ± 0.0049 absorbance value. The average ELISA value of the duplicate wells for positive IYSV-infected *Datura stramonium* control was 1.03 ± 0.108 . For *Wassilewskija* ecotype, the ELISA values for IYSV-infected plants were 1.113 ± 0.52 in comparison to the healthy controls with an average ELISA reading of 0.199 ± 0.0035 . Younger, uninoculated leaves from all ELISA-positive plants of each ecotype were tested for systemic infection with IYSV by ELISA. None of the plants showed systemic infection.

Absence of systemic infection was further verified by RT-PCR by taking one uninoculated, younger leaf from six IYSV ELISA positive plants of each ecotype. In RT-PCR, primers specific to the nucleocapsid (N) gene coded by the small RNA (S-RNA) of IYSV were used (6). The forward and reverse primers, 5' CTCTTAAACACATTTAACAAGCA 3' and 5' TAAAACAAACATTCAAACAA 3', respectively, amplified a 1100 nt fragment of the N gene (Fig. 2). PCR conditions consisted of an initial heating at 94°C for 1 min, followed by 30 cycles (denaturation at 94°C for 10 sec, annealing at 55°C for 10 sec, and initial extension at 72°C for 1 min and 10 sec), and a final extension step at 72°C for

7 min. Results of RT-PCR showed that the virus did not spread systemically to younger, uninoculated leaves. On the basis of ELISA results and RT-PCR, it was concluded that IYSV was restricted to inoculated leaves.

Fig. 2. Detection of *Iris yellow spot virus* (IYSV) in inoculated and uninoculated leaves of *Columbia* and *Wassilewskija* by reverse transcription polymerase chain reaction. Uninoculated leaves (lanes 1 to 3) and inoculated leaves from *Wassilewskija* (lanes 4 to 6); uninoculated leaves (lanes 7 to 9) and inoculated leaves from *Columbia* (lanes 10 to 12). Template-free negative control (lane 13); IYSV infected *Datura* as positive control (lane 14); O' Gene Ruler 1Kb DNA ladder (Fermentas, Glen Burnie, MD) (lane 15). Arrow indicates amplicon of expected size for IYSV N gene by RT-PCR.

These findings indicate that *Arabidopsis* ecotypes *Columbia* and *Wassilewskija* are susceptible to infection by IYSV by producing localized infection. However, the virus failed to become systemic under the conditions used. To ensure that the conditions that were used are amenable for the inoculated plants to sustain virus infection, twenty plants of *Columbia* ecotype were inoculated with *Tomato spotted wilt virus* (TSWV) as it is reported to cause systemic infection in *Arabidopsis*. As reported earlier, TSWV produced systemic infection in *Columbia* ecotype (Fig. 1C) as demonstrated by ELISA. The ELISA values for systemic infection of *Columbia* with TSWV ranged from 1.48 ± 0.85 as compared to the healthy controls which gave an average 0.125 ± 0.0056 ELISA value. This showed that the conditions we used were suitable for the systemic infection of another tospovirus, TSWV, in *Arabidopsis*.

Mechanical inoculation of onion with IYSV is inefficient and there is a need to identify other experimental hosts to use for IYSV-host interactions. Our results show that *Arabidopsis* is susceptible to infection by IYSV and could be a useful host to study the virus-host interactions. Previous reports of tospovirus infection of *Arabidopsis* include *Groundnut ring spot virus* (GRSV) and *Tomato spotted wilt virus* (TSWV), which produced systemic infection (2). Interestingly in the case of IYSV, out of the three ecotypes tested, two ecotypes of *Arabidopsis* showed localized infection by producing symptoms on inoculated leaves and infection was confined to inoculated leaves only. The differential response of *Arabidopsis* ecotypes to tospoviruses indicates that different host and/or virus factors may be involved in the outcome of interaction between *Arabidopsis* and tospovirus. It remains to be seen if other ecotypes of *Arabidopsis* produce systemic infection to the tested isolate or other isolates of IYSV. Screening of *Arabidopsis* mutants for their enhanced resistance/susceptibility to IYSV infection could provide insights into the virus infection mechanism. As IYSV produced local infection that contrasts with the systemic infection of TSWV and GRSV, future studies can be conducted to unravel the basis for this localized infection of IYSV in *Arabidopsis* in comparison to systemic infection of GRSV and TSWV.

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