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ANATOMICAL COMPARISON OF RESPONSE TO *VERTICILLIUM DAHLIAE* INFECTION IN RESISTANT AND SUSCEPTIBLE WILD EGGPLANTS

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Abstract

To compare the anatomical response of plant stems to *Verticillium dahliae* infection, two strains of wild eggplants, Verticillium wilt-resistant *Solanum sisymbriifolium* Lam. (197) and susceptible '*daliyeqie*' (239), were used in this study. Histological observations were conducted at 0, 14, 28, 40, and 60 days post inoculation, using paraffin slices assessed via transmitted light microscopy. Quantitative and qualitative differences in stem microstructure were assessed using captured images. Transverse sections of basal stem showed reduced epidermal cell and parenchyma cell layers and xylem vessel size in stems of 197 compared with those of 239. In general, the stem microstructure of 197 was more closely arranged compared to that of 239. During pathogenic infection, similar induced resistant changes were noted in the stem microstructures of 197 and 239. However, increased lignin deposition in xylem vessels of 197 was observed after infection. In addition, stem growth in 239 was severely inhibited, and stem structure was destroyed upon pathogen infection. These inherent and induced features associated with resistance provide a more complete understanding of resistance mechanisms of wild eggplant to Verticillium wilt.

Key words: Wild eggplant, Verticillium wilt, Paraffin section, Microstructure, Basal stem.

Introduction

Eggplant (*Solunum melongena* L.) is one of the main vegetables, widely grown throughout the world. Verticillium wilt is a soil-borne and vascular disease of eggplant. This disease affects plant growth and finally reduce the production in eggplants. Thus, Verticillium wilt is one of most destructive diseases threatening eggplant production in the world (Fradin & Thomma, 2006).

Studies have reported that plant microstructure is related to resistance to Verticillium wilt, and differences in plant microstructure are associated with different disease resistance levels (Harrison & Beckman, 1982; Benhamou, 1995). On one hand, plant disease resistance is closely related to vessel type, size and thickness; the number and arrangement of fibrous cells around the vessel; and the number of crystalline cells in plants (Wang *et al*., 2002; Zhao *et al*., 2009). On the other hand, a series of changes in plant microstructure are induced after pathogen infection, such as epidermal lignification, corking of internal tissue, proliferation of vessel parenchyma cells, and the formation of gelatinous substance and tyloses to block the vessel (Smit & Dubery, 1997).

To date, reports on plant microstructure in defense responses to Verticillium wilt have mainly focused on cotton, and related research on eggplant is limited. Jiang *et al.*, (2005) reported more pith rays, xylem parenchyma cells, and vessels with thicker walls and reduced diameters in stems and taproots of resistance upland cotton cultivars compared with susceptible cultivars, which may facilitate plant resistance to *Verticillium dahliae* infection and spreading. Gu *et al*., (2011) also noted that the density of xylem parenchyma cells and vessel numbers in root and stem of resistant cotton cultivars were greater compared with those of susceptible cultivars, but the diameter of vessels exhibited the opposite trend. Some studies (Mavlanjan *et al*., 2013) indicated that root epidermal cells and vessel cells per unit area of susceptible cotton varieties were loosely arranged before *V. dahliae* inoculation, and root cortex parenchyma cell number per unit area was reduced compared with the resistant variety. After inoculation, fungal hyphae could grow rapidly in the given widened intercellular space. In eggplant, only one report (Wang *et al.*, 2002) had demonstrated that vessel type and diameter, vessel cell wall thickness and the number of crystal cells in the plants were closely related to *V. albo-atrum* resistance.

The paraffin section technique is the most widely used in conventional histological observations. It is used to observe the microstructure of normal tissues and to analyze pathologic changes in plant tissues (Li *et al.*, 2017). However, due to the specificity of plant tissue structure, different specific paraffin sectioning methods are used in different plant materials. At present, studies on paraffin sectioning technology used in eggplant, especially in wild eggplants, are limited because its sections are incomplete and difficult to observe using conventional paraffin technique.

In this study, paraffin-sectioning technologies based on normal methods were explored and optimized for eggplant basal stems. To obtain clear and complete sections, the histological changes in stems of wild eggplants with different resistance levels to Verticillium wilt were observed and analyzed after infection with *V. dahliae* to reveal anatomical features of wild eggplant and identify the main anatomical indexes associated with eggplant resistance to the pathogen. These results will provide a foundation for studies on the resistance mechanisms of eggplant to Verticillium wilt.

Materials and Methods

Wild eggplant materials: The highly resistant wild eggplant species *Solanum sisymbriifolium* Lam. (germplasm number, 197) and the highly susceptible variety '*daliyeqie*' (germplasm number, 239) were used in the present experiments. Seeds were sown in trays containing a mixture of substrates in the greenhouse under a natural photoperiod and temperature conditions. Plant seedlings were used for artificial inoculation at the four-leaf growth stage.

Artificial inoculation and sampling: A highly pathogenic isolate (QZ-S) of *V. dahliae* was screened in our previous study (Wu *et al*., 2017). For inoculum preparation, the fungus was grown in Petri dishes on potato dextrose agar (PDA) at 25°C in the dark for 3~4 d. The roots of seedlings were inoculated as described by Wu *et al.*, (2017, 2019), and water was used in a mock inoculation, which served as the control. Plants were collected at 0, 14, 28, 40 and 60 days post inoculation (dpi). Five plants of the same size were selected for each time point, and each treatment was repeated thrice. Specimens for paraffin sections were obtained from the base of the stem.

Paraffin sections: (1) *Fixation and softening*: The plant basal stem was cut into small sections (approximately 0.5~1 cm) and placed into the 95% FAA fixative (5 mL formaldehyde + 5 mL glacial acetic acid + 90 mL 70% ethanol) and 5% glycerin solution to fix at room temperature for 3~5 d. Then, the sections were placed in the mixture of glycerin and 70% ethanol (1: 1) at 35~38°C for more than 15 d. (2) *Dehydration and transparency*: Stem sections were washed with 30% ethanol 3~5 times and dehydrated with different concentrations of ethanol. First, sections were dehydrated in 50% ethanol for 30 min followed by 70%, 85% and 95% ethanol solutions for 2 h each. Finally, sections were dehydrated in 100% ethanol twice, each for 30 min. After dehydration, sections were treated with a mixture of 100% ethanol and xylene (1: 1) for 1 h followed by xylene alone (two treatments for 1.5 h each). (3) *Wax filling*: Stem sections were immersed in a proper amount of pure xylene and placed into an oven at 38°C. Then, an appropriate amount of wax was added to the xylene solution every 2 h. Next, the temperature was increased by 1~2°C every 6~12 h to 56°C in the oven. Pretreated materials were placed into another container with molten wax (56~58°C), and the wax was changed 2~3 times approximately every 12 h. (4) *Embedding*: Molten wax was poured into a carton. Then, 2~4 stem sections were immediately placed into the carton using prewarmed forceps. Then, the wax was allowed to cool at room temperature. (5) *Slicing*: Excess paraffin around the stem material was removed using a single-sided blade, and the trimmed wax block was fixed on a microtome (Germany, Leica, RM 2016). The slice thickness was adjusted to 8~10 μm. (6) *Section flattening*: Wax tape was placed smooth-side down on water (40~42°C). The wax tape was transferred to a glass slide with forceps, transferred to the oven and then stored at 40~45 °C for 3~7 d. (7) *Dewaxing*: The glass slide with wax tape was immediately immersed in xylene for 20 min then successively in a mixture of 100% ethanol and xylene (1: 1), 100% ethanol, 95% ethanol, 85% ethanol, 70% ethanol, 50% ethanol, 30% ethanol, 15% ethanol and finally distilled water, each for 2~3 min. (8) *Staining*: Sections were stained with 1% Safranine T at 30 °C for 4~6 h and then, sections were successively placed in distilled water, 15% ethanol, 30% ethanol, 50% ethanol, 70% ethanol, 85% ethanol, and 95% ethanol for 10 s each; 1% Fast green solution for 5 s; 100% ethanol twice, each for 2 s; a mixture of 100% ethanol and xylene (1: 1) for 2 min each; and xylene alone twice, each for 3~6 min. (9) *Mounting*: Neutral gum was used to mount the sections on slides. Excess gum was removed with filter paper, and then sections were put into an oven at 35~40°C to dry.

Photograph and measurement: Slices were examined and photographed using a compound optical microscope (Japan, Nikon, DM500) and camera (China, Shanghai CaiKang, CK-1000). The photos were measured using two-dimensional measurement software (DS-3000). The measurement indexes of sections included the following: xylem, vessels, pith rays, parenchyma cells, and epidermal cells (Fig. 1). For each measurement index, 3 different slices were randomly selected, and 1/12 of the area of each cross-section was used to assess the index.

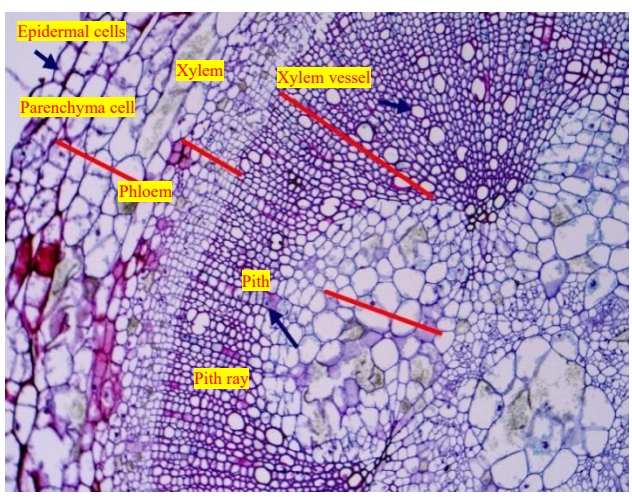

Fig. 1. Microscopic observation (10× and 40×) of paraffin-embedded stem sections of wild eggplant.

Statistical analysis: All the experiments were performed in three biological replicates for each point, including mock-inoculation (as a control) and *V. dahliae*-inoculation. Data are presented as the means ± standard deviation of 9 replicates and analyzed using SPSS 19.0.

Results

Histological observation of stem in wild eggplants: At 0 dpi, epidermis and parenchyma cells in 197 stems (Fig. 2A) are reduced compared with 239 (Fig. 2B). Larger xylem, an increased number of vessels, and longer pith rays are noted in stems of 197 compared with 239 (Fig. 2A-B). At 14 dpi, *V. dahliae* spores (the spores were stained red by Safranine T) invaded the stem phloem cells of 239 (Fig. 2D). At 60 dpi, spores invaded into the center of the medulla (Fig. 2F). Spores were not found in stems of 197 at 14 dpi (Fig. 2C), and spores invaded into the phloem in stems of 197 at 60 dpi (Fig. 2E).

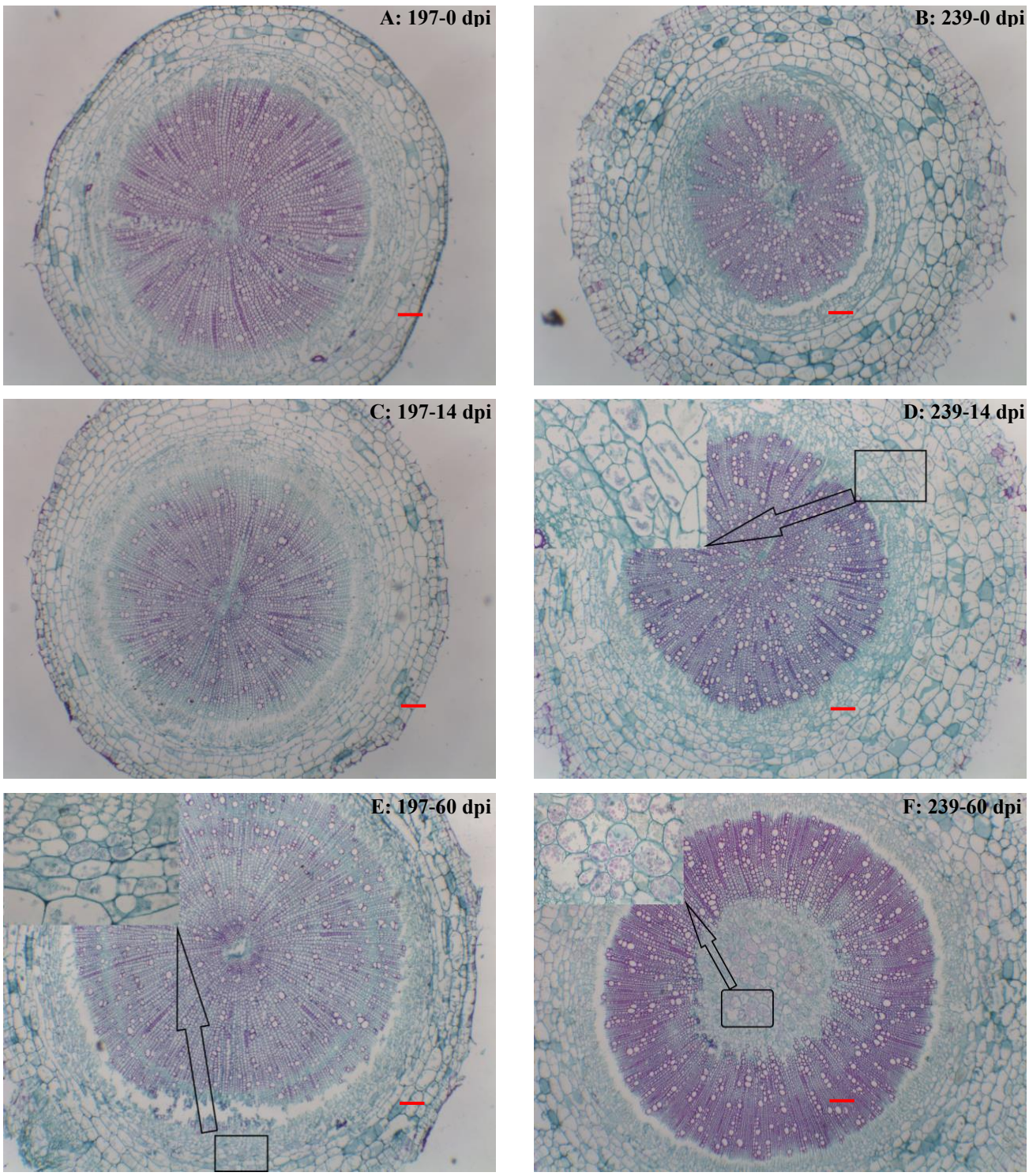

Fig. 2. Cross section of basal stem of wild eggplant 197 and 239 on different days after infection by *V. dahliae* (10× and 40×; Red scale bar is 50 μM).

Epidermal cells in stem: During the 60-day period after pathogen infection, the layers of epidermal cells in non-inoculated 197 plant stems (CK) increased slowly from 1.67 to 2.33. In contrast, the layers of epidermal cells in *V. dahliae*-inoculated 197 plant stems first decreased and then increased and the lowest value was 1.33 at 28 dpi. The number of epidermal cells per unit area exhibited the same trend as noted for epidermal cell layers. The number of epidermal cells in non-inoculated plant stems of 197 increased from 23.78 at 0 dpi to 48.44 at 60 dpi, while the number of epidermal cells of *V. dahliae*-inoculated plant stems of 197 also reached the lowest value at 28 dpi (Table 1).

In 239, the layers of epidermal cells in *V. dahliae*-inoculated plant stems increased gradually from 2.67 at 0 dpi to 6.00 at 60 dpi. However, no significant changes in the layers of epidermal cells were noted in non-inoculated plant stems within the first 14 days, but the number of layers gradually increased to 6.00 in the later period. The number of epidermal cells in non-inoculated plant stems increased from 36.56 to 95.00 within 60 days, while the number of epidermal cells in *V. dahliae*-inoculated plant stems increased from 36.56 to 56.92 within 60 days.

In general, epidermal cell numbers and layers in the stems of the resistant strain 197 were reduced compared with the susceptible strain 239, but more epidermal cells were noted in stems of 197 compared with 239, indicating that epidermal cells in stems of 197 were arranged in a more compact fashion compared with 239.

Parenchyma cells in stems: Parenchyma cell numbers and layers in *V. dahliae*-inoculated 197 plant stems were only slightly reduced compared with non-inoculated plants, but the overall trend was similar. Parenchyma cell numbers and layers in non-inoculated 239 plant stems were significantly increased compared with *V. dahliae*-inoculated plants. However, no obvious changes were observed for 60 days after pathogen infection (Table 2).

In general, the number of parenchyma cell layers in both 197 and 239 *V. dahliae*-inoculated plant stems were reduced compared with non-inoculated plants. However, parenchyma cell layers in stems of 197 were reduced compared with those of 239. However, more parenchyma cells were noted in stems of 197 which were more closely arranged compared with 239. Thus, parenchyma cell growth in stems of 197 was less affected after pathogen infection compared with 239.

Xylem in stems: A significant difference in xylem was noted between *V. dahliae*-inoculated and mock-uninoculated plant stems in both 197 and 239 plants. The xylem area in non-inoculated plant stem is increased by approximately 1.5-fold compared with *V. dahliae*-inoculated plants. Xylem area in stems of 197 is increased by greater than 2.0-fold compared with 239 after pathogen infection. During the 60-day period after pathogen infection, the xylem area in non-inoculated plant stems of 197 increased slowly, while the xylem area of *V. dahliae*-inoculated plants decreased first and then increased, reaching a minimum value at 14 dpi (1.42×10^5 μm^2). The xylem area in non-inoculated plant stems of 239 increased slowly during the early stage and increased rapidly during the later stage. However, the xylem area of *V. dahliae*-inoculated plants increased first and then decreased, and the maximum area (1.17×10^5 μm^2) was attained at 28 dpi (Table 3).

The xylem area in stems of resistant strain 197 was increased compared with the susceptible strain 239. The growth of xylem in stems of 197 and 239 was inhibited after infection with *V. dahliae*. Regarding changing trends, extent of the increase in the xylem area of resistant strain 197 was less than that noted for 239 after pathogen infection.

Xylem vessels in stems: Xylem vessel diameters in non-inoculated plant stems of 197 and 239 are slightly increased compared with *V. dahliae*-inoculated plants with the exception of the value at 40 dpi. The number of xylem vessels in non-inoculated plant stems was increased compared with *V. dahliae*-inoculated plants. At 0 dpi, the number and diameter of xylem vessels in stems of 197 were increased compared with 239. Within 60 days, xylem vessel diameter in stems of 239 was reduced compared with 197, but more xylem vessels were noted in stems of 239 compared with 197.

In general, in both the resistant and susceptible strains, no significant changes in xylem vessel diameter in stems were noted after pathogen infection, but the number of xylem vessels in plant stems increased to varying degrees. Compared with the susceptible strain 239, the number of xylem vessels in stems of the resistant strain 197 was slightly reduced, but increased diameters were observed.

Table 1. Epidermal cell measurements in stem cross-sections of wild eggplant species with different levels of resistance after infection with *V. dahliae*.

Materials	Time	Layer numbers		Cell numbers per unit area		Average cell numbers per layer	
		Non-inoculated	Inoculated	Non-inoculated	Inoculated	Non-inoculated	Inoculated
197	0dpi	1.67 ± 0.58b	1.67 ± 0.58d	23.78 ± 2.34d	23.78 ± 2.34d	15.89 ± 3.66ab	15.89 ± 3.66ab
	14dpi	2.33 ± 0.58b	1.67 ± 0.58d	29.89 ± 3.36cd	18.67 ± 1.76de	13.26 ± 3.20ab	12.17 ± 4.22abc
	28dpi	2.00 ± 0.00b	1.33± 0.58d	38.25 ± 0.35cd	15.56 ± 1.35e	19.13 ± 0.18a	12.72 ± 3.69abc
	40dpi	2.33 ± 0.58b	1.50 ± 0.50d	24.00 ± 2.00d	23.08 ± 0.37d	21.33 ± 3.71a	16.62 ± 5.60ab
	60dpi	2.33 ± 0.58b	2.67 ± 1.53cd	48.44 ± 4.07c	36.89 ± 7.07c	20.67 ± 3.61a	18.47 ± 1.50a
239	0dpi	2.67 ± 0.58b	2.67 ± 0.58cd	36.56 ± 3.53cd	36.56 ± 3.53c	14.04 ± 2.39ab	14.04 ± 2.39abc
	14dpi	2.67 ± 0.58b	2.67 ± 0.58cd	42.00 ± 2.03c	47.83 ± 1.65b	16.28 ± 3.71ab	19.83 ± 0.41a
	28dpi	2.67 ± 0.58b	4.00 ± 0.00bc	40.17 ± 0.76c	53.00 ± 2.65ab	15.33 ± 0.71ab	13.13 ± 0.88abc
	40dpi	5.50 ± 0.71a	5.00 ± 1.00ab	69.08 ± 2.24b	54.61 ± 4.30a	12.69 ± 2.04ab	11.12 ± 1.52bc
	60dpi	6.00 ± 0.82a	6.00 ± 1.00a	95.00 ± 4.24a	56.92 ± 0.82a	15.61 ± 3.81ab	10.44 ± 1.49c

Note: The different lowercase letters in the same column indicate a significant difference at the 0.05 level, the same as follows

Table 2. Parenchyma cell measurements in cross-sections of wild eggplant species with different levels of resistance after infection with *V. dahliae*.

Materials	Time	Layer numbers		Cell numbers per unit area		Average cell numbers per layer	
		Non-inoculated	Inoculated	Non-inoculated	Inoculated	Non-inoculated	Inoculated
197	0dpi	4.67 ± 0.58bc	4.67 ± 0.58ab	44.67 ± 3.21bcd	44.67 ± 3.21a	9.64 ± 0.96abc	9.64 ± 0.96ab
	14dpi	4.33 ± 0.58c	4.33 ± 0.58bc	34.67 ± 3.71e	33.00 ± 6.54d	8.14 ± 1.73bc	7.78 ± 2.23bc
	28dpi	4.50 ± 0.71c	4.00 ± 0.00bc	42.50 ± 4.24bcd	33.89 ± 1.92d	9.49 ± 0.55abc	8.47 ± 0.48bc
	40dpi	4.00 ± 0.00c	4.00 ± 0.00bc	51.61 ± 3.31ab	38.42 ± 2.73ab	12.90 ± 0.83a	9.60 ± 0.68ab
	60dpi	4.33 ± 0.58c	4.00 ± 1.00bc	38.50 ± 2.00de	41.67 ± 6.44a	8.98 ± 1.22abc	11.01 ± 4.12a
239	0dpi	5.00 ± 0.00abc	5.00 ± 0.00ab	35.56 ± 2.22de	35.56 ± 2.22cd	7.11 ± 0.44c	7.11 ± 0.44bc
	14dpi	6.00 ± 0.00a	5.67 ± 0.58a	51.56 ± 3.56ab	37.56 ± 0.77ab	8.59 ± 0.59abc	6.68 ± 0.80cd
	28dpi	6.00 ± 0.96a	5.00 ± 0.00ab	49.89 ± 1.76bc	39.33 ± 0.00ab	8.83 ± 1.15abc	7.87 ± 0.00bc
	40dpi	6.00 ± 0.00a	5.67 ± 0.58a	41.56 ± 3.37cde	37.22 ± 0.69ab	6.93 ± 0.56c	6.63 ± 0.84cd
	60dpi	5.33 ± 0.58abc	5.33 ± 0.58a	59.33 ± 3.33a	33.78 ± 3.24d	11.18 ± 0.98ab	6.41 ± 1.16cd

Table 3. Xylem measurements in stem cross-sections of wild eggplant species with different levels of resistance after infection with *V. dahlia*.

Materials	Time	Area of xylem ($\times 10^5$ s/μm^2)		Diameter of xylem vessels (μm)		Xylem vessel numbers	
		Non-inoculated	Inoculated	Non-inoculated	Inoculated	Non-inoculated	Inoculated
197	0dpi	2.01 ± 0.24cd	2.01 ± 0.24ab	39.93 ± 3.72a	39.93 ± 3.72a	35.44 ± 2.27ef	35.44 ± 2.27cd
	14dpi	2.53 ± 0.22 bcd	1.42 ± 0.08cd	34.64 ± 1.58bc	32.69 ± 2.47b	21.89 ± 1.58g	28.56 ±1.58e
	28dpi	3.34 ± 0.13ab	1.72 ± 0.65bc	37.53 ± 1.23ab	33.95 ± 1.64b	46.83 ± 2.59cde	30.11 ± 1.39de
	40dpi	3.48 ± 0.33a	2.63 ± 0.24a	33.43 ± 3.14cd	37.76 ± 0.60a	50.11 ± 3.95bc	47.17 ± 0.19b
	60dpi	3.58 ± 0.13a	2.27 ± 0.25ab	34.33 ± 2.13bc	31.77 ± 3.98b	41.44 ± 3.01def	38.67 ± 0.58c
239	0dpi	0.85 ± 0.06e	0.85 ± 0.06cd	29.05 ± 2.16d	29.05 ± 2.16bc	34.89 ± 1.58f	34.89 ± 1.58cd
	14dpi	0.61 ± 0.03e	0.94 ± 0.60d	31.83 ± 2.26cd	31.12 ± 2.38b	33.33 ± 3.61f	34.44 ± 1.84cd
	28dpi	1.62 ± 0.12de	1.17 ± 0.44cd	30.82 ± 1.30cd	31.50 ± 1.70b	56.22 ± 2.62ab	39.33 ± 0.88bc
	40dpi	1.03 ± 0.22e	1.00 ± 0.53d	25.50 ± 2.79e	31.61 ± 2.31b	52.56 ± 1.39bc	50.67 ± 1.76a
	60dpi	2.94 ± 0.19abc	0.86 ± 0.32d	31.28 ± 0.87cd	26.43 ± 0.93c	62.00 ± 3.46a	50.44 ± 3.79a

Table 4. Pith ray measurements in stem cross-sections of wild eggplant species with different levels of resistance after infection with *V. dahliae*

Materials	Time	Length(μm)		Number	
		Non-inoculated	Inoculated	Non-inoculated	Inoculated
197	0dpi	553.75 ± 17.92a	553.75 ± 17.92ab	7.22 ± 0.51cd	7.22 ± 0.51abc
	14dpi	193.31 ± 4.18e	482.98 ± 18.32b	6.78 ±0.19cd	8.22 ± 0.69abc
	28dpi	615.40 ± 29.70a	495.72 ± 14.88b	9.33 ± 0.47ab	6.78 ± 0.19abc
	40dpi	566.84 ± 31.50ab	611.55 ± 6.29a	11.78 ± 1.54a	8.83 ± 0.60a
	60dpi	512.84 ± 32.22bc	610.72 ± 59.28a	10.67 ± 1.00ab	8.67 ± 4.04ab
239	0dpi	296.29 ± 18.74de	296.29 ± 18.74cd	4.67 ±0.67d	4.67 ± 0.67bc
	14dpi	220.10 ± 14.58de	245.23 ± 35.16d	4.78 ± 0.51d	5.44 ± 0.77c
	28dpi	419.76 ± 22.53c	260.14 ± 29.14cd	7.33± 0.82cd	5.67 ± 0.67bc
	40dpi	323.76 ± 24.16d	334.76 ± 4.61cd	8.44 ± 0.19bc	5.61 ± 0.67bc
	60dpi	313.27 ± 29.32d	293.56 ± 15.97cd	11.44 ± 0.69a	6.44 ± 0.38abc

Pith rays in stems: At the same time points, pith ray length in *V. dahliae*-inoculated plant stems of 197 was increased compared with non-inoculated plants with the exception of that noted on 28 dpi. The number of pith rays in *V. dahliae*-inoculated plant stems was slightly increased compared with non-inoculated plants. In 239, pith ray length in plant stems was not significantly altered after infection, but the number of pith rays in *V. dahliae*-inoculated plant stems was reduced compared with non-inoculated plants. In addition, the length and number of pith rays in stems of resistant strain 197 were significantly increased compared with susceptible strain 239 (Table 4).

Discussion

Verticillium wilt of eggplant is ranked as one of the most destructive pathogens worldwide. In fact, most eggplant cultivars are moderately to highly susceptible to the Verticillium wilt pathogen. Wild species and relatives of eggplant are highly resistant to Verticillium wilt (Sakata *et al*., 1989), and these wild strains are abundant in Yunnan, China. This study assessed *S. sisymbriifolium* (197) and '*daliyeqie*' (239), obtained from southwest China, were highly resistant and highly susceptible to *V. dahliae*, respectively, as reported in our previous study (Wu *et al*., 2017).

Most studies focused on physiological or molecular genetic factors of pathogenesis that could lead to a better disease comprehension and eventual production of resistant plants (Song *et al*., 2020). Plants exhibit complex anatomical responses to various forms of stress induced by different biotic factors. However, the relationship between plant microstructure and resistance to Verticillium wilt is unclear, especially in wild eggplant species (or germplasms). This study is the first report on anatomical comparisons of wild eggplants in response to *V. dahliae* infection.

V. dahliae typically begins to invade the roots of the plant through the soil, and hyphae penetrate the surface of the plant roots to colonize the vascular bundles, leading to plant death (Zhao *et al*., 2014). Host plants have evolved numerous defensive mechanisms to protect themselves from invading pathogens (Jonathan *et al*., 2006), and the xylem vessels of plants are the first and important defense barriers. In this study, our findings revealed few but closely arranged epidermal cells and parenchyma cells, lignified cell walls, large xylem vessels, and numerous long medullary rays in stems of resistant wild eggplant. The above parameters might be used as inherent microstructure indexes to select plants resistant to Verticillium wilt. Importantly, some studies also demonstrated that parenchyma cell density, vessel number and vessel diameter were closely related to plant resistance to Verticillium wilt (Pennypacker, 1993; Ouellette & Chamberland, 2006).

After infection with *V. dahliae*, our studies showed that stem tissue growth was inhibited in wild eggplant. Specifically, the number of epidermal cells and parenchyma cells, xylem area and vessel diameter in *V. dahliae*-inoculated plants were reduced, and the number and length of pith rays were reduced compared with non-inoculated plants. These stem tissue alterations (especially some changes in xylem vessels) might represent an adaptive response to sustain the supply of both water and nutrients in the presence of the pathogen (Buhtz *et al*., 2017) and might coincide with a defensive response that hinders further tissue invasion by the pathogen (Eynck *et al*., 2009).

In the present study, epidermal and parenchyma cells in stems of 197 were closely arranged. In addition, a high level of cell wall lignification and long pith rays were observed, which is consistent with previous research results (Jiang *et al*., 2005). The increased cell density, the increased xylem area, and reduced cell gaps potentially reduced the invasion spread of *V. dahliae*. In addition, increased vessel diameter was noted for the resistant strain 197 compared with the susceptible strain 239. It was possible that the increased vessel diameter made it difficult to block the vessel from pathogen spread, and the normal transportation function of the plant was maintained. However, the number of vessels in stems of both wild eggplant strains increased after pathogen infection, and this finding was consistent with findings in cotton demonstrating that the increase of the number of vessels was helpful to limit the horizontal infection of hyphae (Gu *et al*., 2011; Wang *et al*., 2012).

In conclusion, the inherent resistance of tissue structure is the first natural barrier of plant resistance to Verticillium wilt and plays an important role in resistance to Verticillium wilt. In this study, the results indicated that xylem (especially the number and size of xylem vessel) is the main determinant responsible for resistance to Verticillium wilt, this is also certified by some previous experiments (Eynck *et al*., 2009; Gu *et al.*, 2011; Wang *et al.*, 2012; Buhtz *et al*., 2017). However, after pathogen infection, both resistant and susceptible plants exhibited induced tissue resistance features, such as increased lignification, vessel blockage and spore invasion, demonstrating that the plant tissue structure could only delay the speed and degree of spore invasion. The mechanism of plant resistance to Verticillium wilt is dependent on tissue structure as well as other factors, such as plant extracellular enzymes, plant secretion, cell walls and resistance genes (Song *et al*., 2020). The mechanism of eggplant resistance to Verticillium wilt is the result of complex and multiple factors and requires further research.

Conclusions

We investigated the anatomical response of basal stems to *V. dahliae* infection processes using the paraffin slice method in the Verticillium wilt resistant strain *S. sisymbriifolium* (197) and the susceptible strain '*daliyeqie*' (239). The inherent microstructure of stems of 197 was more closely arranged compared to 239. This characteristic results from the reduced number of epidermal and parenchyma cell layers and reduced xylem vessel size in stems of 197 compared with 239. However, larger xylem, more vessels, and longer pith rays were observed in stems of 197 compared with 239. During pathogenic infection, the growth of stems in 239 was severely inhibited. But increased lignin deposition was noted in xylem vessels of 197 after infection. This information will broaden our understandings of resistance mechanisms of wild eggplant to Verticillium wilt.

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