Advances in Agricultural Technology & Plant Sciences
ISSN: 2640-6586

Academic Strive
Researcher's perception is our reality

Research Article *Volume 7 Issue 1*

First Report of Tomato Spotted Wilt Virus (TSWV) Infection on *Agapanthus praecox* in China

Ai XM[1,2†], Zhao LH[2†], Zhang SH[2], Zheng X[2], Zhang J[2*] and Li J[1*]

[1]Life Science College, Southwest Forestry University, China

[2]Yunnan Provincial Key Lab of Agricultural Biotechnology, Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, China

***Corresponding author:** Jie Zhang, Yunnan Provincial Key Lab of Agricultural Biotechnology, Institute of Biotechnology and Germplasm Resources, Yunnan Academy of Agricultural Sciences, Kunming, China, Email: 470917665@qq.com

[†]Equally contributed towards this article

Received Date: May 06, 2024; **Published Date:** May 15, 2024

Abstract

Tomato spotted wilt virus (TSWV), which is a member of the Orthotospovirus genus and transmitted by thrips. It has extensive host and causes significantly economic losses. In May 2023, virus-like symptoms such as ring spotted, leaf yellowing and necrosis were observed on *Agapanthus praecox* leaves in Kunming, Yunnan Province, China. Spherical viral particles with a diameter of 80-120nm were observed by TEM. The results of RT-PCR and DNA showed that the sequence of N gene for this virus shared 99.87% identity with TSWV isolate from Shandong isolate (acc. no. OQ627140.1). The obtained 777-bp consensus sequence named TSWN-N was deposited in GenBank (acc. no. OP867047.1). Phylogenetic tree analyses showed that TSWV obtained from *Agapanthus praecox* plant samples clustered together into a large clade from Guizhou. Koch's postulate and the virus was re-amplied from the inoculated plants. This is the first report of TSWV on *Agapanthus praecoxin* China and provides early warning of virus disease on medicinal materia.

Keywords: Tomato Spotted Wilt Orthotospovirus; *Agapanthus praecox*; Electron Microscopy; RT-PCR

Introduction

Agapanthus praecox subsp. Praecox Willd. is a highly valued medicinal plant of the genus Agapanthus established by L'Heritier in 1788 belongs to the family Amaryllidaceae and order Asparagales [1]. *Agapanthus praecox* is an evergreen species of genus Agapanthus, it is commonly called blue lily, agapanthus, Africa lily, or lily of the Nile [2]. It is one of the highly valued medicinal plants in South African folkloric medicine, and possesses anti-inflammatory, antioedema, antitussive, immunoregulatory, antibacterial, antifungal, andantitumor properties [3]. Diseases often occur in the planting process and cause serious economic losses. It has been reported that TSWV infecting on *Agapanthus praecox* in Australia and Africa [4,5].

TSWV is ranked second in the global top 10 plant viruses due to its importance in scientific research and economic fields [6]. It can affecting the level of plant hormones [7], and causes abnormal leaf growth, stunting and even death, significantly affecting crop yield and quality [7]. TSWV virions are typically spheroidal virions ranging from 80 to 120 nanometers in size, producing envelope structures and continuous projection layers on their outer membranes [8].

In our study, symptomless A. praecox plants leaves were received from professionally cultivated fields for regular control of virus infection in May 2023. TEM and the molecular

investigations indicated that this virus was TSWV. This work was done to fulfll Koch's postulate and verify TSWV is able to infect A. praecox plants in fields.

Materials and Methods

Samples

Samples of *Agapanthus praecox* were collected in May 2023 in Kunming, Yunnan Province, China. The five sample leaves of A. praecox were collected from diseased parts of plants and stored in a refrigerator at -80℃ on the same day for RT-PCR.

Transmission Electron Microscopy

Negative staining was performed for infected and healthy leaves and was observed under a transmission electron microscope with an accelerating voltage of 80kV [9].

RT-PCR

Total RNA was extracted from leaves using Trizol reagent (Nanjing, Norvezan Biotechnology) according to the manufacturer's protocol. The total RNA was reverse transcribed to the first-strand cDNA synthesis according to the HiScriptIII 1st Strand cDNA Synthesis Kit (Nanjing, Norvezan Biotechnology) and was amplified followed the primers in Table 1. PCR reaction system of 25 μL: 10×PCR reaction buffer (including Mg_{2+}) 2.5 μL, dNTP Mix 2 μL, 10 μmol/L upstream and downstream primers each of 0.5 μL, rTaq DNA polymerase 0.25 μL, cDNA 1.25 μL, ddH_2O 18 μL. Amplification conditions: 94℃ pre-denaturation 30s, 98℃ denaturation 10s, 58℃ annealing 30s, 72℃ extension for 45s, 35 cycles, 72℃ further extension for 10 min.

Primers	Sequence（5′-3′）
TospS-3 W	GC (a / t) GTTCCAGGGTT (a / g)
Tosp-3	AGAGCAATCGAGGCGCTA ATAA
TSWV-F	5′-ATGTCTAAGGTTAAGCTCA-3′
TSWV-R	5′-AGCAAGTTCTGCAAGTTTTG-3′

Table 1: Primers used to amplify gene sequence.

Phylogenetic Analyses

Phylogenetic analyses was conducted using MEGA version 6.0,the maximum likelihood (ML) tree was constructed with 1000 replicates as the guide value to evaluate the reliability of the resulting tree [9].

Results

TEM Detection

In May 2023, virus-like symptoms on leaves consisting of ring spotted, leaf yellowing and necrosis were observed and appeared on approximately 80% of *Agapanthus praecox* in the fieldsin Kunming, Yunnan (Figure 1A). The leaves of five samples with symptoms and symptomless were used for transmission electron microscopy (TEM) (America, FEITECNALG2Spirit) detection using negative staining [10]. The results showed that spherical particles of 80-120 nm in diameter, similar to *Orthotospovirus* (Figure 1B).

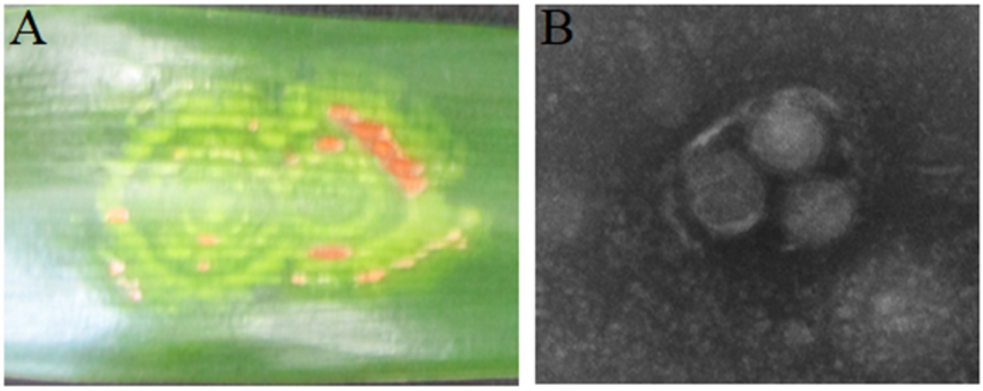
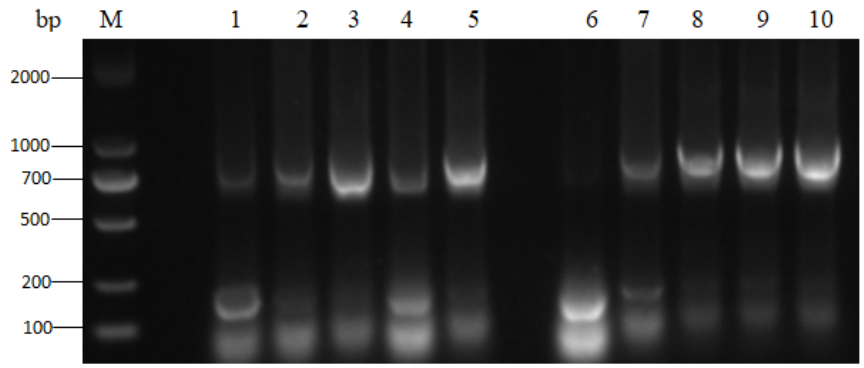


Figure 1: Electron micrograph of virus particles found in *Agapanthus praecox* leaves.

RT-PCR Detection and Sequence

In order to identify the exact virus infecting the plants, five samples of *Agapanthus praecox* with symptoms and symptomless were detected by RT-PCR (Guangdong, TCLT9620). Total RNA was extracted and reversed for RT-PCR with a pairs of universal primers for N gene of *Orthotospovirus* viruses (Table 1). A 777-bp sequence was obtained from each sample (Figure 2) and cloned into the pMD18-T vector for Sanger sequencing. BLASTn-analysis showed that the 5 amplicons were identical and shared 99.87% nucleotide sequence identity with tomato spotted wilt orthotospovirus isolate Guizhou from tobacco (acc. no. OP867047.1). Koch's

experiments also were performed and the virus from the infected plants was successfully transmitted onto healthy *A. praecoxplants* (n= 5) upon mechanical inoculation, and the plants not only developed foliar distortion symptoms but also tested positive for TSWV by RT-PCR with the N-specific primers (Table 1).



M: DL2000 DNA Maker; 1-5: the amplied fragment of TSWV using the primer Tosp-3; 6-10: the amplied fragment of TSWV using the primer TSWV-F/TSWV-R.

Figure 2: Presence of sequence in *Agapanthus praecox* plant samples by RT-PCR amplification.

Phylogenetic Analysis

Phylogenetic tree was prepared using the available amino acid sequence of N protein of *P. multiflorum* and the N gene corresponding amino acid sequence of TSWV from different regions. The results are showed that the isolate of *P. multiflorum* grouped with several TSWV isolates (e.g., LC505372.1, MK372883.1 and KP684518.1) from Korea and South China (Figure 3).

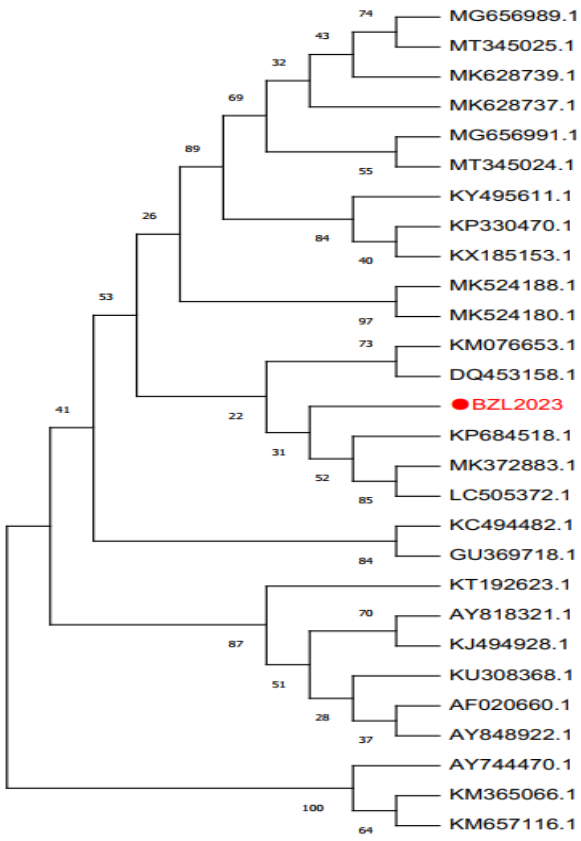


Figure 3: A phylogenetic tree constructed based on the tomato spotted wilt virus N gene sequence.

Discussion

Tomato spotted wilt orthotospovirus (TSWV), which is belonging to the genus *Orthotospovirus* of *Tospoviridae* family, and is transmitted by thrips and seed [11,12]. TSWV has widest host range, which can infect 1090 plants in 84 families [12]. It has been reported that TSWV can mainly infect vegetables: including tomato, pepper, tobacco, watermelon and lettuce [13-16]; it also infect herbage, flowers and nuts [17-19]. In this study, the results showed that A. praecox with symptoms of chlorosis, mottling, leaf yellowing and necrotic in Kunming was infected with tomato spotted wilt *orthotospovirus* based on the data of molecular identifications and electron microscopy. This is the first report of TSWV was detected on A. praecox in China.

The technology for detecting virus includes Electron microscopy (TEM), RT-PCR and ELISA. TEM is the visual way for detecting the virus. Previous studies have been reported that cucumber mosaic virus (CMV), tobacco rattle virus (TRV), ranunculus mild mosaic virus (RMMV), zucchini tigre mosaic virus (ZTMV) et al., which were observed in different host [20-22]. In this study, the virus particles were observed under TEM by negative staining for the samples, the diameter ranged from 80 to 120 nm. However, the results of TEM only identified the genus of the virus, for exact identification of the virus, RT-PCR was used for detection. Owing to the N protein is an important basis for virus classification [23]. The samples of A. praecox with have been infected by TSWV was tested using RT-PCR and NCBI sequence of N gene. A 777bp sequence was obtained from the sample, which nucleotide sequence homology was 99.87% with tomato spotted wilt *orthotospovirus* isolate Guizhou from tobaccoo. Phylogenetic tree results showed that the isolate of *P. multiflorum* grouped with several TSWV isolates (e.g., LC505372.1, MK372883.1 and KP684518.1) from Korea and South China.

This is the first report of TSWV infected A. praecox in China. Symptomatic phenotype-based field survey son some plantations in Yunnan Province indicated that the disease incidence ranged from 70% to 90%, resulting in significant loss of production of A. praecox. The result provides a new strategy for the prevention and control virus in the planting process.

Acknowledgements

This work was carried out with supporting from Yunnan Young & Elite Talents Project (YNWR-QNRC-2020 to JZ, YNWR-QNRC-2022 to LHZ), Important plan of research and development (NO. 202103AF140003), Seed industry laboratory-the project of enhancement construction in core function laboratory (NO. 202305AR340004), National Natural Science Foundation of China (No.32360639).

References

1. Duncan G (2002) Grow Agapanthus: A guide to the species, cultivationand propagationof the genusAgapanthus, National Botanical Institute, Trident Press, Cape Town, South Africa.

2. Josephine SO, Afolayan AJ (2017) Micromorphological characterization of the leaf and rhizome of *Agapanthus praecox* subsp. praecox Willd. (Amaryllidaceae). Hindawi Journal of Botany pp: 1-10.

3. Van Wyk BE (2013) MedicinalPlants of South Africa. In: 2nd (Edn.), Briza Publications, Pretoria, South Africa.

4. Bester R, Demas SU, Maree HJ (2023) First report of tomato spotted wilt orthotospovirus infecting Agapanthus (*Agapanthus praecox*) in South Africa. Plant Disease.

5. Zhang Z, Zheng K, Dong J, Fang Q, Hong J, et al. (2016) Clustering and cellular distribution characteristics of virus particles of tomato spotted wilt virus and tomato zonate spot virus in different plant hosts. Virol J 13: 11.

6. Schoelz J E, Harries PA, Nelson RS (2011) Intracellular transport of plant viruses: finding thedoor out of the cell. Molecular Plant 4(5): 813-831.

7. Liang Y, Wan J, Zhang X, Li K, Su J, et al. (2024) Comprehensive phytohormone metabolomic and transcriptomic analysis of tobacco (Nicotiana tabacum) infected by tomato spotted wilt virus (TSWV). Virus research 342: 199334.

8. Qi S, Zhang S, Islam MM, El-Sappah AH, Zhang F, et al. (2021) Natural Resources Resistance to Tomato Spotted Wilt Virus (TSWV) in Tomato (Solanum lycopersicum). Molecules 22: 10978.

9. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular biology and evolution 30(12): 2725-2729.

10. Abudurexiti A, Adkins S, Alioto D, Alkhovsky SV (2019) Taxonomy of the order Bunyavirales: update 2019. Arch Virol 164(7): 1949-1965.

11. Zhang Z, Ma X, Wu K, Zheng K, Pei W, et al. (2020) The infection cycle and epidemic characteristics of vegetable wilt in Luliang lettuce. Journal of Southwest Agriculture 33 (12): 2827-2832.

12. Li Y, Tan G, Xiao L, Zhou W, Lan P, et al. (2021) A Multiyear survey and identification of pepper- and tomato-infecting viruses in Yunnan Province, China. Frontiers in

microbiology 12: 623875.

13. Carrieri R, Sorrentino R, Lahoz E, Alioto D (2011) First report of tomato spotted wilt virus on tobacco in Campania, Italy. Plant disease 95(5): 611.

14. Pappu SS, Pappu HR, Gitaitis RD, Gay JD (1998) First report of tomato spotted wilt tospovirus infection of watermelon in Georgia. Plant disease 82(3): 351.

15. Al-Saleh MA, Al-Shahwan IM, Amer MA, Shakeel MT, Ahmad MH, et al. (2014) First report of romato spotted wilt virus in lettuce crops in Saudi Arabia. Plant disease 98(11): 1591.

16. Kwak HR, Byun HS, Choi HS, Kim HJ, Kim M (2022) First report of ranunculus mild mosaic virus in Ranunculus asiaticus in Korea. Plant disease.

17. Stanković I, Bulajić A, Vučurović A, Ristić D, Milojević K, et al. (2012) First report of tomato spotted wilt virus infecting onion and garlic in Serbia. Plant disease 96(6): 918.

18. Fang Q, Ding M, Dong J, Peng L, Zhang Z, et al. (2013) Initial report of macadamia nuts infected with tomato spot virus. Journal of Horticulture 40(2): 350-354.

19. Abirami R, Manoranjitham SK, Mohankumar S, Karthikeyan G (2022) Preponderance of mixed infection of Cucumber mosaic virus and Candidatus Phytoplasma australasia on brinjal in India. Microbial pathogenesis 169: 105596.

20. Kesharwani AK, Kulshreshtha A, Singh RP, Srivastava A, Avasthi AS, et al. (2022) First report of tobacco rattle virus infecting brassica oleracea var. botrytis (cauliflower) in India. Plant disease.

21. Chen YK, Shih PJ, Chao HY (2022) First report of mosaic disease associated with zucchini tiger mosaic virus in wax gourd (Benincasa hispida) in Taiwan. Plant disease.

22. Zeng J, Chen D, Lv C, Qin K, Zhou Q, et al. (2022) Antimicrobial and anti-biofilm activity of Polygonum chinense L.aqueous extract against Staphylococcus aureus. Scientific reports 12(1): 21988.

23. Zhu M, Van Grinsven IL, Kormelink R, Tao X (2019) Paving the Way to tospovirus infection: Multilined Interplays with plant innate immunity. Annual review of phytopathology 57: 41-62.