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Short communication

# The occurrence and distribution of viruses infecting Lanzhou lily in northwest, China

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ARTICLE INFO	A B S T R A C T
<i>Keywords:</i> Lanzhou lily Lily symptomless virus Cucumber mosaic virus Lily mottle virus Mixed infections	Field surveys were conducted during 2015–16 to document the occurrence of multiple virus infections and to record predominant viruses infecting Lanzhou lily ( <i>Lilium davidii</i> var. <i>unicolor</i> ) in the major area of cultivation, northwest China. Specimens were randomly collected from crops in eight different regions and tested for <i>Lily symptomless virus</i> (LSV), <i>Cucumber mosaic virus</i> (CMV) and <i>Lily mottle virus</i> (LMoV) using a quadruplex RT-PCR assay. Results indicated a high level of LSV (98.2%), a high level of CMV (42.5%), and a low level of LMoV (1.5%). In addition, a high level of mixed infections of LSV + CMV was confirmed in Lanzhou lily; in Huangyu, north Yuzhong and Yongjing, incidence of mixed infections of LSV + CMV occurred in over 50% of plants. Interestingly, 99% of CMV-infected plants were in combination with LSV. The sequence homology of LMoV

#### 1. Introduction

Lanzhou lily (Lilium davidii var. unicolor) is a popular edible vegetable bulb, as well as a traditional medicinal plant with a 150-year cultivation history. Lanzhou lily is mostly grown in the central area of Gansu province in north-western China, and it is mainly propagated via bulbs (Fig. 1). Nearly 25% of the cultivated area at elevations of 1800-2600 m has been planted with this crop (Wang et al., 2010). In recent years it has also been successfully introduced to NingXia Hui Autonomous Region in the south, and Xinjiang Uyghur Autonomous Region in the north. Currently the area of Lanzhou lily cultivation is approximately 11,000 ha and its production is valued at RMB 2.5 billion (US\$ 375 M). The economic importance of Lanzhou lily bulb production has increased during the past decade because of a rapid increase in demand in both domestic and international markets. Currently Lanzhou lily bulbs are exported to Hong Kong, Japan, Southeast Asia, and Europe. A Lanzhou lily processing production chain has been established to the benefit of many farmers. Marketable Lanzhou lily bulbs are graded for size, weight and shape according to informal regional quality standards.

In recent years, virus infected plants exhibiting symptoms of plant stunting, have become a major problem. These infections have contributed to a deterioration in bulb quality and a reduction in yield of over 50% from 23,000 to 11,000 kg per hectare (Zhang et al., 2015a).

More than ten different viruses have been reported to infect lilies worldwide (Ryu et al., 2002). Among these lily-infecting viruses, *Lily symptomless virus* (LSV; Genus *Carlavirus*, family *Flexiviridae*), *Cucumber mosaic virus* (CMV; Genus *Potyvirus*, family *Bromoviridae*), and *Lily mottle virus* (LMoV; Genus *Potyvirus*, family *Potyviridae*), are the most commonly recorded viruses in many cultivars of lily and in particular Lanzhou lily (Asjes, 2000; Wang et al., 2010; Lim et al., 2016). All three viruses may be transmitted vegetatively and mechanically, as well as by aphids. Few studies of viral diseases in Lanzhou lily have been undertaken, in particular those induced by multiple virus infections. In view of the recently increased area of Lanzhou lily, a preliminary survey was conducted to determine the occurrence of multiple infections and to record the predominant viruses infecting Lanzhou lily in China.

#### 2. Materials and methods

#### 2.1. Survey area and specimen collection

isolate from this study (GenBank: MF781080) was similar to that previously reported in this region from Lilium

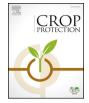
oriental. Approaches to disease control using improved virus detection methods are also discussed.

Surveys of Lanzhou lily were conducted during the growing seasons of 2015 and 2016 in northwest China over an area of approximately 2200 ha in eight major areas of cultivation in Gansu Province and NingXia Hui Autonomous Region. These included the western zone (Yongjing), the central zone (Huangyu and Xiguoyuan), the southern zone (Lintao), and the eastern zone (south Yuzhong, and north

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Fig. 1. Lanzhou lily (Lilium davidii var. unicolor): A. A crop in the field; B. Harvested bulbs.

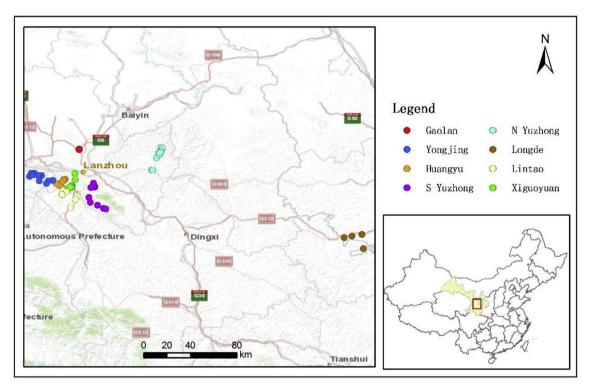


Fig. 2. Locations surveyed in 2015–16 for viral infection of Lanzhou lily (*Lilium davidii* var. *unicolor*) in eight regions in Gansu province and NingXia Hui Autonomous Region, China.

Yuzhong) of Gansu province, as well as in Longde, NingXia Hui Autonomous Region (Fig. 2). In each zone at least four specimens were randomly collected from within 4–12 lily fields located at elevations between 1737 and 2765 m. In addition, specimens were collected from fields at the Gaolan Research Station, Lanzhou, Gansu province, elevation approximately 1600 m. Three to four leaves per plant were collected, placed in separate plastic bags and labelled. Specimens were kept at 4 °C and processed within 24 h of collection.

Among the 332 specimens collected, 79% were from Gansu province and 21% from NingXia Hui Autonomous Region. In Gansu province, 12% of specimens were from the western zone, 23% from the central zone, 10% from the southern zone, 28% from the eastern zone, and 6% from the Gaolan Research Station.

Specimens of common weeds growing near Lanzhou lily crops at the Gaolan Research Station were collected to test the hypothesis that alternative plants serve as a reservoir for lily viruses. Four plants of each weed species were collected and tested for viral infection. These species included: fathen (*Chenopodium album*), shepherd's purse, (*Capsella bursa-pastoris*), Californian thistle (*Cirsium arvense syn. Cirsium setosum*), and crested wheat grass (*Agropyron cristatum*).

### 2.2. Preparation of samples for quadruplex reverse-transcription polymerase chain reaction

Samples of Lanzhou lilv and each weed species were processed and tested with the quadruplex RT-PCR assay as described previously by Zhang et al. (2015a, 2015b). Briefly, we used four pairs of primers (LSV forward, 5'- TATGGGCTTCCAATACAAC-3' and LSV reverse, 5'- TATT CGGTTTCCAGGTT-3'; CMV forward, 5'-CTTTGTAGGGAGTGAACGCT GTA-3' and CMV reverse, 5'- AGATGGCGGCAACGGATA-3'; LMoV forward, 5'-TGGCACCTCACCAAATGTA-3' and LMoV reverse, 5'-CATCAT CTGCTGTATGCCTCT-3'; and 18S rRNA forward, 5'-ATACCGTCCTAG TCTCAACC-3' and 18 S rRNA reverse, 5'-ACAAATCGCTCCACCAAC-3') to simultaneously detect the LSV, CMV, LMoV coat protein (CP) genes, and the lily 18S rRNA as a reference gene. Total RNA was extracted separately from each sample by using the RNAprep pure Plant Kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. Final elution was done with 50  $\mu$ L of RNAse free H<sub>2</sub>O. The concentration of the RNA sample was measured with a NanoDropND-1000 spectrophotometer (NanoDropTechnologies, Wilmington, DE, USA). Subsequently, the first strand of cDNA was synthesized by M-MLV reverse transcriptase with the Oligo (dT)-18 primer (TaKaRa Biotech, Dalian, China). Quadruplex PCR was carried out in  $25.0\,\mu L$ reaction volumes containing 2.0  $\mu L$  of cDNA products, 4 mM  ${\rm Mg}^{2+},$ 

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