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Iris yellow spot virus in Zimbabwe: Incidence, severity and characterization of *Allium*-infecting isolates

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ABSTRACT

Iris yellow spot virus (IYSV), an emerging pathogen of alliaceous crops, has been reported in many countries worldwide. The pathogen has recently been reported infecting garlic (*Allium sativum*), leek (*A. ampeloprasum*) and onion (*A. cepa*) in Zimbabwe. A study was carried to determine its incidence, severity and distribution in Zimbabwe. IYSV disease surveys were conducted in 18 districts across six provinces. Symptomatic alliaceous leaf samples were tested for IYSV by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Furthermore, the pathogen was characterized by transmission studies, transmission electron microscopy and molecular assays. The pathogen was detected in garlic, leek, shallot (*A. aggregatum*) and onion crops in all districts, with disease incidences ranging from 26.7 to 78.8% and disease severities of up to 3.7 in some districts. Up to 70% of onion seedlings that were mechanically inoculated with sap from diseased onion plants produced typical IYSV symptoms characterized by green chlorotic tissues surrounded by tan-colored necrotic tissues. Electron microscopy studies revealed spherical enveloped particles that were 80–120 nm in diameter. Analysis of the partial nucleocapsid protein (N) gene of the Zimbabwean IYSV isolates revealed that they were at least 94.89% identical and similar to each other at nucleotide and amino acid levels. These isolates were closely related to isolates from Asia, Australia, Europe, South Africa and the USA, but were distinct from isolates from Brazil, The Netherlands and The UK. Further work to ascertain the pathogen's economic impact needs to be carried out. In addition, farmers need to be educated about the disease so that they can control it by growing resistant varieties and/or controlling the vector.

1. Introduction

Iris yellow spot virus, IYSV, an emerging pathogen of alliaceous crops, has been reported in many countries worldwide (Bag et al., 2015). The pathogen is responsible for causing significant yield losses in bulb and seed onion crops (Gent et al., 2006; Mandal et al., 2012). Besides infecting cultivated alliaceous crops, IYSV also infects wild *Allium* species, ornamental plants and several weeds (Smith et al., 2011; Bag et al., 2015). Since its detection and first report in iris (*Iris holandica*) in the Netherlands in 1998 (Cortês et al., 1998), IYSV has been reported on all continents (Pappu et al., 2009), and continues to be reported in many countries. In Africa, IYSV was first reported in Reunion (Robene-Soustrade et al., 2005). It has subsequently been reported in South Africa (du Toit et al., 2007), Kenya and Uganda (Birithia et al., 2011), Egypt (Hafez et al., 2012), Mauritius (Lobin et al., 2010) and Zimbabwe (Karavina et al., 2016a).

IYSV belongs to the genus *Tospovirus* in the family *Bunyaviridae*. The IYSV virion consists of quasi-spherical enveloped particles that are 80–120 nm in diameter. The virion has a tripartite single-stranded RNA genome consisting of large (L), medium (M) and small (S) segments. The L RNA (~8.9 kb) has a single open reading frame (ORF) and encodes the RNA-dependent RNA polymerase in negative sense (Bag et al., 2010). The M and S segments are ambisense and each encodes two proteins. The M RNA (~4.8 kb) encodes the non-structural movement (NSm) protein in the viral sense and the Gn-Gc protein in the viral complementary sense, while the S RNA (~3.1 kb) encodes the nucleocapsid (N) protein in the viral complementary sense and the nonstructural protein (NSs) in the viral sense (Cortês et al., 1998). Like other tospoviruses, IYSV is transmitted exclusively by thrips (Thysanoptera: Thripidae) in a

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circulative-propagative mode (Whitfield et al., 2005). Currently, *Thrips tabaci* Lindeman and *Frankliniella fusca* (Hinds) are the known IYSV vectors (Srinivasan et al., 2012; Bag et al., 2015). In addition to transmitting IYSV, thrips damage alliaceous crops through their feeding activities.

Thrips are the primary pests of alliaceous crops in Zimbabwe, capable of causing up to 40% yield loss (Zindoga, 2015). Most of the thrips-induced yield loss is attributed to their feeding on the crop foliage. Typical thrips damage symptoms include silvery spots that turn into white blotches along the leaves, followed by the development of silvery patches and leaf curling. This reduces the plant's photosynthetic capacity, and ultimately decreases bulb size and yield (Waiganjo et al., 2008; Munoz et al., 2014). On leafy *Allium* spp like shallots (*A. aggregatum*) and leeks (*A. ampeloprasum*), the presence of leaf lesions and necrotic spots reduces crop marketability.

In recent years, alliaceous crops with necrotic, irregularly-shaped and grey-to-bleached white leaf lesions have been found in Zimbabwe. The lesions grow and coalesce, eventually girdling and killing the plant leaf. Sometimes, the necrotic lesions surround islands of green and/or chlorotic tissues. Early and severe crop infection results in premature defoliation. These symptoms are typical of IYSV infection (Krauthausen et al., 2012; Buckland et al., 2013). Recent surveys have reported up to 60% Iris yellow spot disease (IYSD) incidence in alliaceous crops in Zimbabwe (Karavina et al., 2016a, b). However, the incidence figures were based on localized disease surveys. This study sought to determine the occurrence and distribution of IYSV in Zimbabwe. In pursuance of that, nationwide disease surveys were conducted and IYSV isolates were characterized based on symptomatology, serology, microscopy, transmissibility and molecular assays. This would provide critical information needed to develop control strategies against the IYSD.

2. Materials and methods

2.1. Disease surveys

IYSD surveys were conducted from May 2014 to November 2015 in 18 districts across six Zimbabwean provinces (Fig. 1). In each season, 101 fields were surveyed and 608 *Allium* spp. samples displaying suspected IYSD symptoms were collected. In addition, a total of 93 non-alliaceous crop samples were collected from fields where *Allium* spp were intercropped with brassicas (*Brassica* spp), Irish potatoes (*Solanum tuberosum* L.) and roses (*Rosa* spp). Sampled sites were located with global positioning system (GPS) coordinates in order to facilitate return visits. A field was considered IYSV-positive if at least one collected sample tested positive by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Young leaves displaying IYSD-like symptoms were sampled. Disease incidence and severity were visually assessed and recorded. Disease severity was scored on a scale of 0–5, based on the number and size of leaf lesions using a scale developed by Shock et al. (2008) where:

1 = No symptoms.
2 = 1%–25% of foliage diseased.
3 = 26%–50% of foliage diseased.
4 = 51%–75% of foliage diseased.
5 = 76%–99% of foliage diseased.
6 = 100% of foliage diseased (leaves completely dried).

Disease incidence (DI) was calculated as below:

$$DI = \frac{\text{Number infected plants}}{\text{Total field plant population}} \times 100$$

During the surveys, a short questionnaire on farmers' knowledge of IYSV, its vectors and control was administered.

2.2. Serological diagnosis

Leaf samples were tested for IYSV in duplicate wells using a commercial kit supplied by Loewe® Biochemica GmbH (Sauerlach, Germany) following the manufacturer's instructions. Briefly, a microtiter plate was coated with IYSV-specific coating antibody (IgG). About 0.5 g of alliaceous tissue from the middle and bottom portions of youngest leaves showing disease symptoms were excised and ground in liquid nitrogen in a pestle and mortar. Macerated plant tissues were mixed with Conjugate Buffer at a 1:20 dilution, and 0.2 ml mixture added to each microtiter well and incubated overnight. After four washes, an enzyme-labelled antibody-AP-conjugate was applied to the plate wells. In the final step, 0.2 ml of the Substrate Buffer Solution containing the dissolved substrate tablet was applied to the microtiter plate. After two hours of incubation, the reaction was visually assessed for yellow color development. Samples giving weak yellow color were subjected to reverse transcription polymerase chain reaction (RT-PCR) to confirm whether they were IYSV-infected or not.

2.3. Transmission studies

Commercial *Allium cepa* cv. Texas Grano seeds were sown in a 242-cell float tray in pine bark media, pH 5.5 ($CaCl_2$ scale). Three-week-old onion seedlings were individually transplanted to 15 cm diameter plastic pots. A week later, the seedlings were mechanically inoculated with IYSV. Briefly, IYSV-infected onion leaves were homogenized in 10 mM sodium phosphate buffer (pH 7.0), containing 0.1% sodium sulfite using sterilized pestle and mortar. Leaf debris were removed by squeezing the homogenate through a non-absorbent cotton pad. The extracted sap was gently rub-inoculated onto fully expanded onion transplant leaves. Inoculated plants were kept in an insect-proof greenhouse with 25/18 °C cycle, day/night temperatures and observed for virus symptom development over a three-week period. Twenty onion seedlings were mechanically inoculated with the virus each season and the experiment was replicated three times per season.

2.4. Electron microscopy studies

These were carried out at the Microscopy and Microanalysis Unit of the University of KwaZulu-Natal, Pietermaritzburg, South Africa. Briefly, 2 mm × 2 mm onion leaf pieces were excised from young symptomatic leaves and fixed in 3% glutaraldehyde, for 12 h. Samples were twice washed in 0.05 M sodium cacodylate for 30 min each time and then fixed in a 50:50 solution of 2% osmium tetroxide and 0.05 M sodium cacodylate for 2 h. After two 30 min washes in 0.05 M sodium cacodylate, the samples were dehydrated in graded ethanol series (10%, 30%, 50% and 70% for 10 min each; 90% and 100% for 15 min each). Samples were twice washed with 100% propylene oxide for 15 min each time to remove residual ethanol, embedded in Epon-Araldite resin mixture, and polymerized. Sectioned material was stained with uracyl acetate followed by lead nitrate and examined for virus particles under the JEOL JEM-1400 Transmission Electron Microscope (TEM).