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Iris yellow spot virus in onions: a new tospovirus record from India

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During 2002–03, field-infected onion (*Allium cepa*) plants, exhibiting characteristic symptoms of chlorotic spindle- or diamond-shaped lesions on the leaves and scapes with twisting or bending flower-bearing stalks, were observed in the Jalna and Nasik regions of Maharashtra, India. In the advanced stages, single spindle-shaped chlorotic lesions coalesced, leading to withering of leaves and flower-bearing stalks. The disease was transmitted to a number of virus-indicator plants by mechanical inoculation using phosphate buffer (0·01 M sodium sulfate, pH 7·0, containing 0·1% sodium sulfite). Inoculated *Nicotiana benthamiana* plants produced systemic necrotic lesions, eventually resulting in dieback and wilting of the plants, while *Nicotiana tabacum* (varieties Xanthi NC, White Burley, Samsun and GT-4) and *Nicotiana clevelandii* produced local chlorotic ring spots 3–6 days after inoculation. In *Vigna unguiculata*, necrotic local lesions developed 3–4 days postinoculation, while *Nicotiana rustica* failed to produce any symptoms and did not become infected.

Field-infected onion samples and glasshouse-inoculated plants were tested by ELISA using the following antisera: *Tobacco streak virus* (DSMZ AS-0615); *Watermelon silver mottle virus* (DSMZ AS-0118); *Iris yellow spot virus* (DSMZ AS-0528); *Tomato spotted wilt virus* (DSMZ AS-0105); *Impatiens necrotic spot virus* (DSMZ AS-0115); *Chrysanthemum stem necrosis virus* (DSMZ AS-0529); *Peanut bud necrosis virus* (ICRISAT); and *Potato virus Y* (DSMZ AS-0573), utilizing the respective positive control samples. Field-infected onion samples and the respective mechanically inoculated tobacco plants reacted strongly with *Iris yellow spot virus* (IYSV) antisera, but failed to react with any of the other antisera tested. RT–PCR using primers designed to the capsid gene and flanking sequences of IYSV (GenBank accession number AF001387) produced the expected 925-bp amplicon from infected but not healthy onions. The results of symptomatology, host range, ELISA and RT–PCR indicate that the causal agent is a strain of IYSV, which is a new report from an important onion-growing region of Maharashtra State, India. This virus has been reported as a potentially devastating pathogen of onion in Europe (Cortes *et al*., 1998), Israel (Gera *et al*., 1998) and the USA (Gent *et al*., 2004).

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First report of *Iris yellow spot virus* in onion bulb- and seed-production fields in Réunion Island

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In August 2003, leaf symptoms consisting of irregularly shaped, chlorotic or necrotic lesions were observed on onion plants (*Allium cepa*) in Réunion Island. In 2004, a total of 84 leaf samples with symptoms were collected from two fields: one used for onion seed production and one for onion bulb production. Samples were screened for the presence of three tospoviruses, *Iris yellow spot virus* (IYSV), *Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV), by DAS-ELISA using commercial antisera (PRI, Wageningen, the Netherlands; BIORAD, Marnes la Coquette, France and LCA, Blanquefort, France, respectively). Of the samples tested, 45% reacted positively (four out of six and 34 out of 78, respectively, for bulb- and seed-production fields) with the antiserum directed against IYSV; all samples tested negative for TSWV and INSV. The presence of IYSV in the serologically positive samples was verified by RT–PCR. Total nucleic acid was extracted using an RNeasy Plant Extraction kit (Qiagen). Primers based on published sequences for the nucleocapsid (CP) gene for various IYSV isolates (Cortês *et al*., 1998; Kritzman *et al*., 2000) produced negative results using RT–PCR. New primers were designed based on sequences flanking the CP gene: IYSV56U (5′-TCCTAAGTATTCACCAT-3′) and IYSV917L (5′-TAAAACTTAACTAACACAAA-3′) (sense and antisense polarity, respectively). These produced a 896-bp amplicon of the expected size, which was cloned and sequenced. The amplicon sequence was compared with other IYSV sequences using BLAST. Best matches of nucleotide identity were obtained with the CP gene of IYSV from Japan (AB121026) and Brazil (AF067070) (93 and 92% identity, respectively).

In 2004, a further survey of 10 onion bulb-production fields found that 75% of leaves with symptoms (n = 221) and 27% of bulbs (n = 64) tested positive for IYSV using ELISA. The virus was not detected in onion seed lots (n = 59; tests using crushed seeds) but was present in 15% of 45-day-old seedlings from a nursery (n = 119). Leaves showing symptoms from other *Allium* species growing in Réunion Island were screened for IYSV using ELISA. The virus was detected in leaves of leek (*Allium porrum*) (nine out of 11 samples); garlic (*Allium sativum*) (10 out of 11); and shallot (*Allium cepa* var. *ascalonicum*) (three out of three). The potential vector, *Thrips tabaci*, was widespread in all *Allium* crops surveyed, whereas *Frankliniella occidentalis* was observed only occasionally. This is the first record of IYSV in the Mascarenes Archipelago.

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