

## Pathogen profile

## ***Iris yellow spot virus (Tospovirus: Bunyaviridae): from obscurity to research priority***

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### SUMMARY

**Taxonomy:** *Iris yellow spot virus* (IYSV) is in the genus *Tospovirus*, family *Bunyaviridae*, with a single-stranded, tri-segmented RNA genome with an ambisense genome organization. Members of the other genera in the family infect predominantly vertebrates and insects.

**Geographical distribution:** IYSV is present in most *Allium*-growing regions of the world.

**Physical properties:** Virions are pleomorphic particles of 80–120 nm in size. The particle consists of RNA, protein, glycoprotein and lipids.

**Genome:** IYSV shares the genomic features of other tospoviruses: a segmented RNA genome of three RNAs, referred to as large (L), medium (M) and small (S). The L RNA codes for the RNA-dependent RNA polymerase (RdRp) in negative sense. The M RNA uses an ambisense coding strategy and codes for the precursor for the G<sub>N</sub>/G<sub>C</sub> glycoprotein in the viral complementary (vc) sense and a non-structural protein (NSm) in the viral (v) sense. The S RNA also uses an ambisense coding strategy with the coat protein (N) in vc sense and a non-structural protein (NSs) in the v sense.

**Transmission:** The virus is transmitted by *Thrips tabaci* Lindeman (Order: Thysanoptera; Family: Thripidae; onion thrips) and with less efficiency by *Frankliniella fusca* Hinds (tobacco thrips).

**Host:** IYSV has a relatively broad host range, including cultivated and wild onions, garlic, chives, leeks and several ornamentals. Some weeds are naturally infected by IYSV and may serve as alternative hosts for the virus.

**Symptoms:** IYSV symptoms in *Allium* spp. are yellow- to straw-coloured, diamond-shaped lesions on leaves and flowering scapes. Diamond-shaped lesions are particularly pronounced on scapes. As the disease progresses, the lesions coalesce, leading to lodging of the scapes. In seed crops, this could lead to a reduction in yield

and quality. Early to mid-season infection in bulb crops results in reduced vigour and bulb size.

**Control:** Resistant varieties are not available, but a limited number of accessions with field tolerance have been identified. Integrated disease management tactics, including sanitation, crop rotation, thrips management, maintenance of optimal plant vigour, soil fertility, irrigation and physical separation of bulb and seed crops, can mitigate the effect of the disease.

Virus code: 00.011.0.85.009

Useful link: <http://www.alliumnet.com/>

**Keywords:** Bunyaviridae, thrips, tospoviruses, viruses.

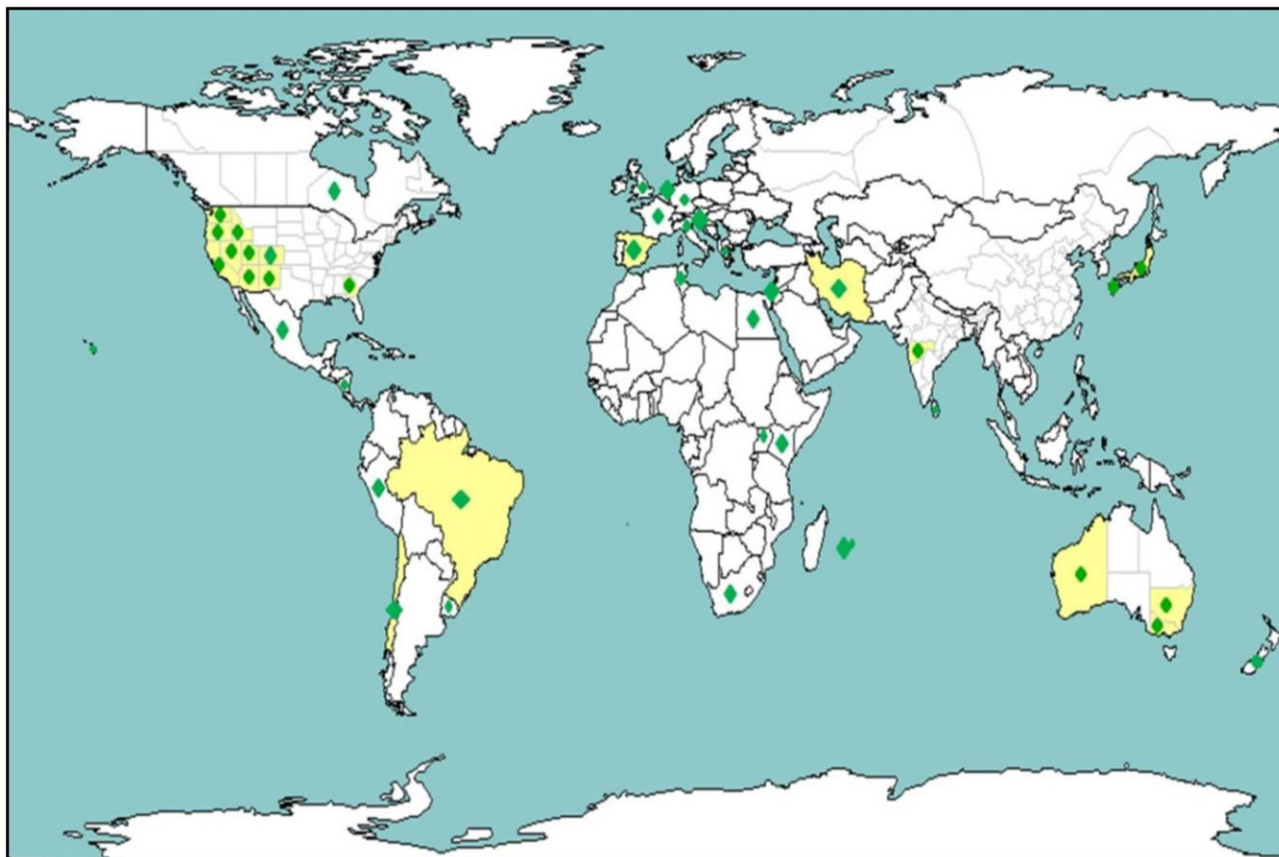
### INTRODUCTION

*Iris yellow spot virus* (IYSV) is an economically important virus and a threat to onion seed and bulb crops in many parts of the world (Gent *et al.*, 2006; Mandal *et al.*, 2012; Pappu *et al.*, 2009). Iris yellow spot (IYS) disease is most damaging to high-value onion crops, where it can reduce bulb size and seed yields (Gent *et al.*, 2004) and may cause crop losses of up to 100% (Poizzer *et al.*, 1999). IYSV is a member of the genus *Tospovirus*, family *Bunyaviridae*. Members of the genus *Tospovirus* are the only plant-infecting viruses in a family of viruses that infect vertebrates or insects. IYSV is transmitted by onion thrips (*Thrips tabaci* L.) in a persistent and propagative manner (Kritzman *et al.*, 2001; Nagata *et al.*, 1999). Recently, tobacco thrips (*Frankliniella fusca* Hind) have been shown to transmit IYSV, albeit at a lower efficiency relative to *T. tabaci* (Srinivasan *et al.*, 2012).

The disease was first reported on onion from Brazil in 1981 (de Avila *et al.*, 1981), and was characterized by chlorotic and necrotic eye spots with a green island at the centre, known as diamond eyes. In the late 1980s, similar symptoms were observed in the onion-growing regions of the Treasure Valley (southwestern Idaho and southeastern Oregon) in the USA (Hall *et al.*, 1993; Israel (Gera *et al.*, 1998), and on *Iris hollandica* in the Netherlands

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**Fig. 1** World map showing the countries in which *Iris yellow spot virus* (IYSV) has been reported. Yellow and green regions denote the presence of IYSV within a country.

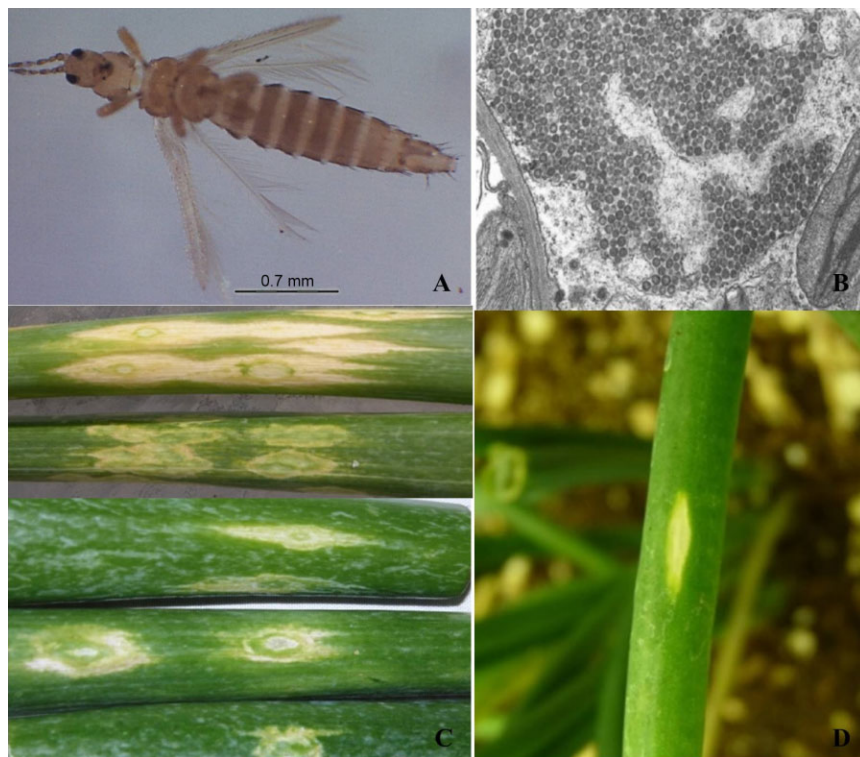
(Cortês *et al.*, 1998). In the USA, although IYSV was found in the late 1980s, it initially caused concern as a potential threat to onion production in the northwestern USA; however, it remained 'dormant' without causing much economic damage for a decade. Interestingly, since 2000, several states in the USA, beginning with Arizona, California and Utah, have reported its occurrence (Abad *et al.*, 2003; Bag *et al.*, 2009a; Evans *et al.*, 2009a, b; Moyer *et al.*, 2003), and IYSV began to re-emerge as the cause of disease epidemics in the Pacific Northwestern states of Idaho, Oregon and Washington State beginning in 2003. Since then, the virus has been reported from several more states with reports of economic losses in both onion seed and bulb crops (Gent *et al.*, 2006; Pappu *et al.*, 2009). IYSV has now been reported from other major onion-growing countries (Pappu *et al.*, 2009; Mandal *et al.*, 2012; Turina *et al.*, 2012) (Fig. 1).

The ever-increasing globalization and trade of plants, sets and bulbs, and the small size of its primary vector, *T. tabaci*, (Fig. 2A), have probably contributed to the spread of IYSV to other onion-growing regions. With improvement in diagnostics, the virus has been reported from an increasing number of countries in the last decade. The continued spread and increasing economic impact of

the virus on an economically important crop, such as onion, and the threat to other *Allium* crops, have provided impetus to gain a better understanding of its biology, epidemiology and molecular biology, and to develop management options. For several onion growers in the USA and abroad, thrips and IYSV have become major concerns and are considered important research priorities (<http://www.alliumnet.com/>), resulting in several multi-state, multi-institutional and multi-agency collaborations funded by onion grower associations, and regional and federal funding agencies.

## PHYSICAL PROPERTIES

Hall *et al.* (1993), using transmission electron microscopy, showed the presence of virions that shared similar morphological features to known tospoviruses. Virions consist of 80–120-nm pleomorphic particles (Fig. 2B) (Kritzman *et al.*, 2001). Cortês *et al.* (1998) purified the nucleocapsid protein from infected *Nicotiana benthamiana* Domin, and subsequently purified the viral RNA and showed RNA segments corresponding to three sizes: small (2.9 kb), medium (4.8 kb) and large (8.9 kb).



**Fig. 2** (A) Adult *Thrips tabaci* Lindeman (onion thrips). (B) Electron micrograph of *Iris yellow spot virus* (IYSV) from a thin section of infected *Nicotiana benthamiana* (photograph credit: Dr Abed Gera, Volcani Center, Bet Dagan, Israel). (C) Symptoms of IYSV in onion. Early symptoms display characteristically yellow- to straw-coloured chlorotic or necrotic lesions, elongated, lenticular or spindle shaped, from the mid to lower portion of the infected plant. As the disease develops and plants grow, the lesions elongate and coalesce together, completely covering the leaves and scapes. Sometimes, green tissue in the centre of the lesions is present in the shape of a diamond: when present, this is considered as a specific diagnostic symptom for IYSV. (D) Reproduction of symptoms characteristic of IYSV infection in mechanically inoculated onion plants under controlled conditions.

## SYMPTOMATOLOGY AND HOST RANGE

IYSV infection on onion plants produces a wide range of disease symptoms. Early symptoms display yellow- to straw-coloured chlorotic or necrotic lesions, elongated, lenticular or spindle shaped, from the mid to lower portion of the infected plant. As the disease develops and plants grow, the lesions elongate and coalesce, covering the leaves and scapes. Sometimes, green tissue in the centre of the lesions is present in the shape of a diamond which could be used as a diagnostic feature (Fig. 2C). Symptoms observed in the field have been reproduced in healthy onions by mechanical inoculation under controlled glasshouse conditions (Fig. 2D; S. Bag and H. R. Pappu, unpublished data). However, the rate of transmission remains relatively low (30%). Hence, the virus is maintained on experimental hosts, such as *Nicotiana benthamiana* or *Datura stramonium*. Infection and subsequent development of foliar symptoms result in a decline in photosynthetic activity and, as a result, reduced bulb size (Gent *et al.*, 2006). Later in the season, the coalesced lesions cause the scape to lodge. In other *Allium* hosts, similar symptoms were observed, whereas, in weeds, mild chlorosis, necrosis, streaking or purpling was also observed (Evans *et al.*, 2009a, b). The virus tends to be localized in and within close proximity to the lesions on leaves and scapes, and the highest virus titre is usually found in the inner leaves at the centre where thrips reside and feed (Kritzman *et al.*, 2001). In a recent study, Boateng and Schwartz (2013) determined

the virus distribution in various parts of the infected onion plants obtained from the field. IYSV could not be detected in roots, basal plates, bulb scales or dead leaves, and virus accumulation, based on the optical density, was significantly higher in younger leaves than in intermediate and older leaves under field conditions (Fig. S1, see Supporting Information). Within an individual symptomatic leaf, virus accumulation was greater in the basal section relative to the middle or top sections (Boateng and Schwartz, 2013).

In various experimental hosts (Bag and Pappu, 2009; Bulajic *et al.*, 2009; Gent *et al.*, 2006; Srinivasan *et al.*, 2011), the virus causes chlorotic ring spots, chlorotic local lesions and necrotic spots, which coalesce and lead to the early senescence of infected leaves. The symptomatology depends on the virus isolate (Bulajic *et al.*, 2009) and environmental conditions. The virus causes local infections in most experimental hosts, with only a few exceptions, such as *N. benthamiana*, where it produces necrosis in veins, stem and buds, leading to lodging of infected plants and early senescence (Bag and Pappu, 2009) (Fig. S2, see Supporting Information); in another study, it remained symptomless, but could be detected serologically (Bulajic *et al.*, 2009). In *Eustoma russellianum* Salisb., the virus causes necrosis in systemic leaf tissues (Srinivasan *et al.*, 2011). No local lesions or other symptoms developed in a number of other plant species tested (Bulajic *et al.*, 2009), including *D. stramonium* (Bulajic *et al.*, 2009). However, IYSV was shown to produce local lesions (Bag *et al.*,

2012a; Bag and Pappu, 2009), further suggesting that symptom development is dependent on isolates and environmental conditions. Isolates were characterized that differ in symptom development and disease severity in experimental hosts, suggesting the existence of biologically distinct strains (Bag *et al.*, 2012b).

Apart from the cultivated onion, IYSV has also been reported from other *Allium* spp., such as *A. galanthum* (Cramer *et al.*, 2011), *A. porrum* (Gent *et al.*, 2007; Schwartz *et al.*, 2007), *A. pskemense* (Pappu *et al.*, 2006b), *A. roylei* (Cramer *et al.*, 2011), *A. sativum* (Bag *et al.*, 2009b), *A. schoenoprasum* and *A. tuberosum* (Cramer *et al.*, 2011) and *A. vavilovii* (Cramer *et al.*, 2011; Pappu *et al.*, 2006b). In addition to *Allium* spp. and some ornamentals, several weeds have been identified that are naturally infected with IYSV. Recent reports of natural infections of weeds include *Ameranthus retroflexus*, *Chenopodium album*, *Kochia scoparia*, *Lactuca serriola* and *Tribulus terrestris* (Sampangi *et al.*, 2007), *Atriplex micrantha* and *Setaria viridis* (Evans *et al.*, 2009a, b), and *Cichorium intybus*, *Arctium minus*, *Rumex crispus* and *Taraxacum officinale* (Hsu *et al.*, 2011; Smith *et al.*, 2011). A recent search of IYSV in weeds within and next to onion fields with a high IYSV disease incidence in northern Italy failed to reveal any possible reservoir hosts (M. Turina, unpublished data). A current listing of the host range of IYSV is available at <http://www.alliumnet.org>.

## GENOME ORGANIZATION

As a typical tospovirus, the IYSV genome consists of three single-stranded RNAs—large (L), medium (M) and small (S) (Fig. 3A). The L RNA is 8880 nucleotides long and contains a single open reading frame (ORF) of 8621 nucleotides in the viral complementary (vc) strand (Bag *et al.*, 2010). The ORF potentially codes for a protein of 2873 amino acids with a predicted molecular weight of 331.17 kDa, sharing many of the features of the viral RNA-dependent RNA polymerase (RdRp) coded by L RNAs of known tospoviruses. The 5' and 3' termini of IYSV L RNA (vc) contain two untranslated regions of 33 and 226 nucleotides, respectively, and both termini have eight nucleotides conserved at the 5' terminal end (5'AGAGCAAT3') and reverse complementary to the 3' end (5'TCTCGTTA3'), another common feature of tospovirus genomic RNAs (Bag *et al.*, 2010; Nichol *et al.*, 2005).

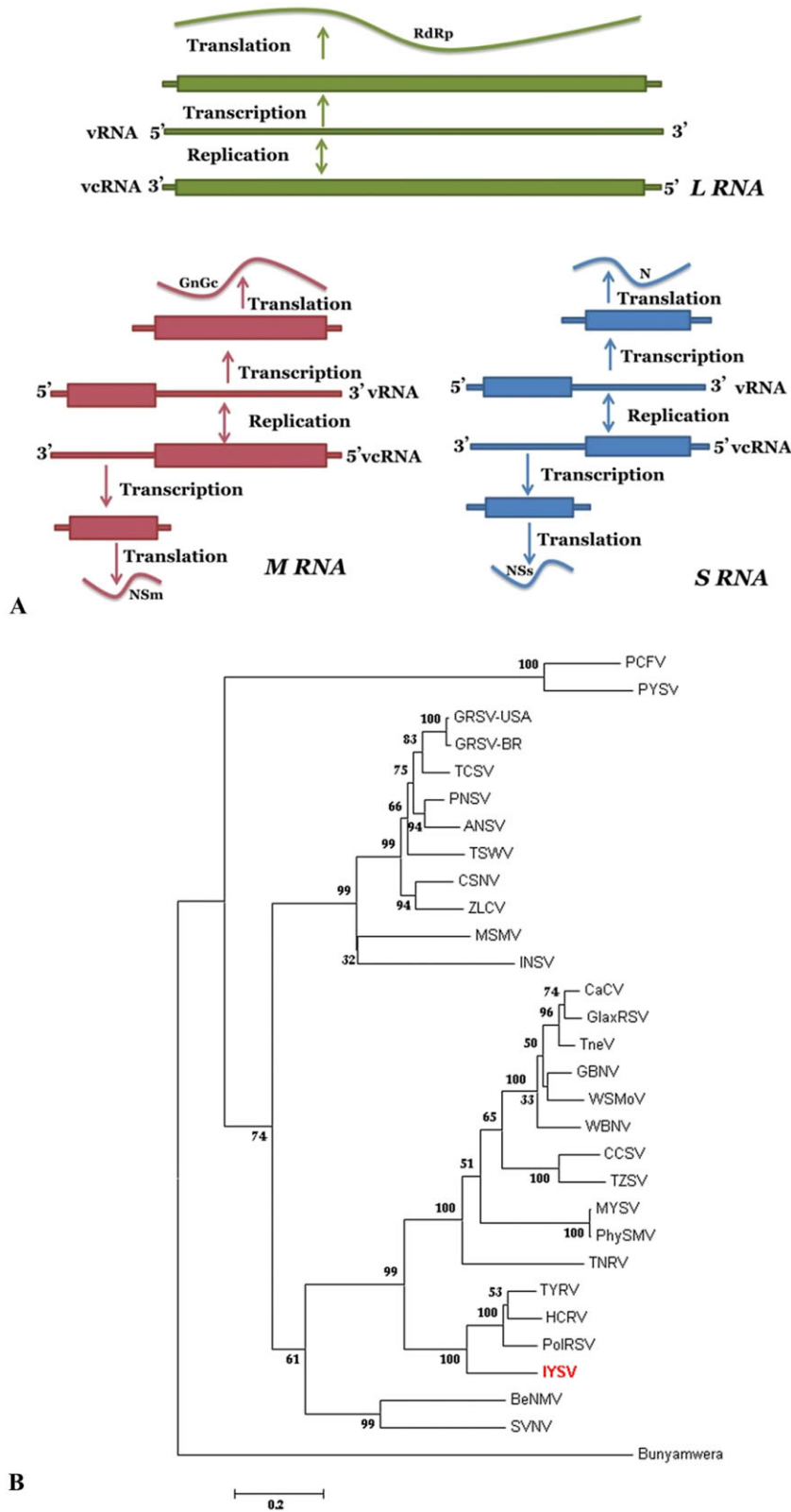
The M RNA is 4821 nucleotides long with two ORFs in ambisense arrangement expressed through two subgenomic RNAs, possibly capped by a 'cap snatch' mechanism (Duijsings *et al.*, 2001; van Knippenberg *et al.*, 2005). The smaller ORF of 935 nucleotides is located at the 5' end of the viral (v) sense strand, potentially encoding a 311-amino-acid protein with a predicted molecular mass of 34.7 kDa, coding for a non-structural protein (NSm) homologue to the NSm of *Tomato spotted wilt virus* (TSWV), which has been shown to be a movement protein.

The second ORF, the homologue to TSWV glycoprotein precursor (G<sub>M</sub>/G<sub>C</sub>), is in the vc sense, and is 3410 nucleotides in length, potentially coding for a 1136-amino-acid protein of 128.8 kDa. The two ORFs are separated by a 380-nucleotide intergenic region (Bag *et al.*, 2009c; Cortês *et al.*, 2002). The G<sub>N</sub> protein in TSWV has been shown to serve as a ligand for the virus that mediates the uptake by thrips receptors (Sin *et al.*, 2005; Whitfield *et al.*, 2005).

The S RNA segment of IYSV is 3105 nucleotides long (Cortês *et al.*, 1998) and codes for two non-overlapping ORFs in an ambisense arrangement. The ORF in v sense is 1329 nucleotides long, coding for a 50.1-kDa non-structural protein (NSs) shown to be a silencing suppressor for TSWV (Takeda *et al.*, 2002), and potentially plays a similar role in other tospoviruses. The second ORF in vc sense is 819 nucleotides long, and potentially codes for the nucleocapsid protein of 30.5 kDa (Cortês *et al.*, 1998) encapsulating each tospovirus genomic RNA.

The three IYSV genomic RNAs share several genome-wide characteristics with those of other tospoviruses. The first eight nucleotides of the 5' and 3' terminal sequences are identical for all three RNA segments. Furthermore, the three RNA segments have complementary ends forming stable panhandle termini (Fig. S3A, see Supporting Information). Both coding ORFs of S and M RNA are separated by intergenic regions rich in A and U residues with potential for internal pairing, and are probably involved in transcription termination of the subgenomic RNAs (Fig. S3B) (Adkins, 2000; Nichol *et al.*, 2005). The tetraloop motif is speculated to determine the binding of translation factors or other proteins (Clabbers *et al.*, 2014).

Based on the N gene amino acid sequence of known tospovirus species, IYSV forms a clade with recently described tospoviruses from Eurasia, such as *Tomato yellow ring virus* (TYRV), *Hippeastrum chlorotic ringspot virus* (HCRV) and *Polygonum ringspot virus* (PoIRSV), with 69.7%–72.9% and 51%–71.3% identity at amino acid and nucleotide levels, respectively (Fig. 3B). Similarly, based on various genes coded by M and L RNAs, IYSV forms a clade within the Eurasian group, distinct from the American clade (Bag *et al.*, 2009c, 2010), suggesting its European origin. Among the known IYSV isolates, the N protein amino acid sequence identity ranges between 89% and 90%. Phylogenetic analysis of the complete N protein sequences of 99 IYSV isolates available in GenBank showed that IYSV isolates formed two distinct clades (Fig. 4A). Clade I mainly consists of the Asian isolates (India, Japan, Sri Lanka), three isolates from the Oceania (Australia and New Zealand) and three isolates from the USA. Another clade consisted of the rest of the isolates from the Americas and Eurasia. Most of the isolates formed close clusters based on the collection sites, suggesting limited evolution within a given geographical region. Based on this study and an earlier study by Turina *et al.* (2012), the little variability observed in some clades and the higher variability seen in isolates from the Mediterranean area support



**Fig. 3** (A) Schematic representation of the genome organization and replication strategy of tospoviruses, showing the three RNAs: large (L), medium (M) and small (S). The rectangular boxes indicate the proteins coded. (B) Phylogeny based on amino acid sequences of nucleocapsid protein of known tospoviruses. The tospoviruses fall into two major groups based on their prevalence and distribution: Eurasia consisting of Europe, Asia and Oceania; the Americas consisting of North and South America. The representative gene sequence was obtained from the National Center for Biotechnology Information (NCBI) GenBank. Virus acronyms used in TREE are: *Groundnut ringspot virus* (GRSV); *Groundnut bud necrosis virus* (GBNV); *Impatiens necrotic spot virus* (INSV); *Iris yellow spot virus* (IYSV); *Peanut yellow spot virus* (PYSV); *Polygonum ringspot virus* (PolRSV); *Tomato chlorotic spot virus* (TCSV); *Tomato spotted wilt virus* (TSWV); *Watermelon bud necrosis virus* (WBNV); *Zucchini lethal chlorosis virus* (ZLCV); *Alstroemeria necrotic streak virus* (ANSV); *Bean necrotic mosaic virus* (BeNMV); *Calla lily chlorotic spot virus* (CCSV); *Capsicum chlorosis virus* (CaCV); *Chrysanthemum stem necrosis virus* (CSNV); *Groundnut ringspot virus-USA* (GRSV-USA); *Gloxinia tospovirus* (GlaxRSV); *Hippeastrum chlorotic ringspot virus* (HCRV); *Melon severe mosaic virus* (MSMV); *Melon yellowspot virus* (MYSV); *Pepper necrotic spot virus* (PNSV); *Peanut chlorotic fanspot virus* (PCFV); *Physalis silver mottle virus* (PhySMV); *Soybean vein necrosis virus* (SVNV); *Tomato necrosis virus* (TNeV); *Tomato necrotic ringspot virus* (TNRV); *Tomato yellow fruit ring virus* (TYRV); *Tomato zonate spot virus* (TZSV); *Watermelon silver mottle virus* (WSMoV).

the hypothesis that the origin of IYSV could be from this region and, to some extent, the clustering of isolates could be based on geographical origin.

IYSV isolates could be grouped into Netherlands (NL) and Brazil (BR) genotypes based on restriction fragment length polymorphism (RFLP; Zen *et al.*, 2005). A global analysis of 99 complete N gene sequences available in GenBank showed an almost even distribution of known IYSV isolates into NL and BR genotypes (Fig. 4B). Temporal and spatial analysis of the N protein sequences showed that the observed genetic diversity among IYSV populations could be attributed to the combined action of genetic recombination, purifying selection and genetic drift (Iftikhar *et al.*, 2014).

Localization and interaction studies of viral proteins provide important information about virus replication in their host plants. Tripathi *et al.* (2013) studied the *in vivo* localization and interaction patterns of IYSV proteins in infected *N. benthamiana*, and identified the interacting partners. IYSV genes were cloned into binary pSITE-BiFC vectors and agroinfiltrated into marker *N. benthamiana* plants expressing cyan or red fluorescent protein (CFP/RFP) in their nuclei. Bimolecular fluorescence complementation (BiFC) analysis was used to test the interactions between IYSV proteins (Fig. 5A). IYSV proteins localized in the cell periphery of cells, and homotypic and heterotypic interactions between IYSV nucleocapsid (N) and movement (NSm) proteins were observed. These interactions were further confirmed by pull-down assays. Interacting regions of IYSV N and NSm were identified by the yeast two-hybrid assay and  $\beta$ -galactosidase assay. The N protein self-association was found to be mediated through the N- and C-terminal regions making head to tail combinations. Self-interaction of IYSV NSm was shown to occur through multiple interacting regions. The N- and C-terminal regions of IYSV N interacted with an N-terminal region of IYSV NSm in a yeast two-hybrid assay (Tripathi, 2014). These studies provided new insights into the localization and interactions of IYSV proteins.

Mixed infections of commercial crops with two or more distinct tospoviruses have been reported from different cucurbits and *Allium* crops (Kunkalikar *et al.*, 2011; Mullis *et al.*, 2004). These mixed infections could be the contributing factor to the observed genetic diversity of segmented viruses, such as tospoviruses. Using *Datura*, Bag *et al.* (2012a) found potential genetic complementation between IYSV and another distinct tospovirus, TSWV, in plants that were mechanically inoculated with both viruses. In *Datura*, TSWV causes systemic infection (Fig. 5B), whereas IYSV is restricted to inoculated leaves (Fig. 5B). In contrast, when *Datura* plants were mechanically inoculated with a mixture of TSWV and IYSV, symptoms characteristic for each of the viruses (concentric ring spots for TSWV and necrotic spots for IYSV) were produced in inoculated leaves (Fig. 5B). Interestingly, more severe symptoms were observed in younger, uninoculated leaves relative to those produced by TSWV infection alone (Fig. 5B). On further molecular

analysis of different symptomatic leaves, it was found that TSWV facilitates the selective movement of the IYSV NSs gene to younger, uninoculated leaves (Fig. 5B), causing more severe systemic infection by overcoming plant defence more effectively. Further investigation revealed that, in systemically infected leaves of dually infected plants, there is a reduced level of TSWV N gene-specific small interfering RNAs (siRNAs). There was no TSWV NSs-specific siRNAs in inoculated and systemically infected leaves of dually infected plants, suggesting effective and efficient overcoming of the plant silencing mechanism under the influence of both NSs genes relative to the presence of individual NSs genes (Bag *et al.*, 2012a).

## DIAGNOSIS

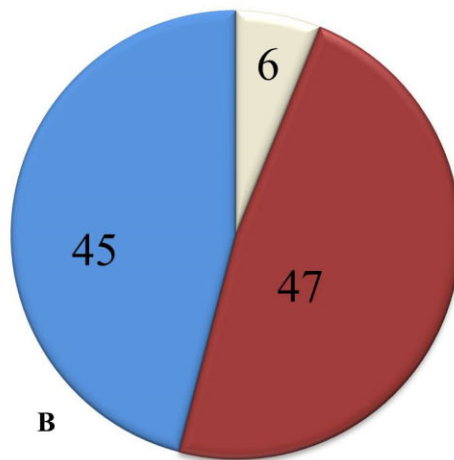
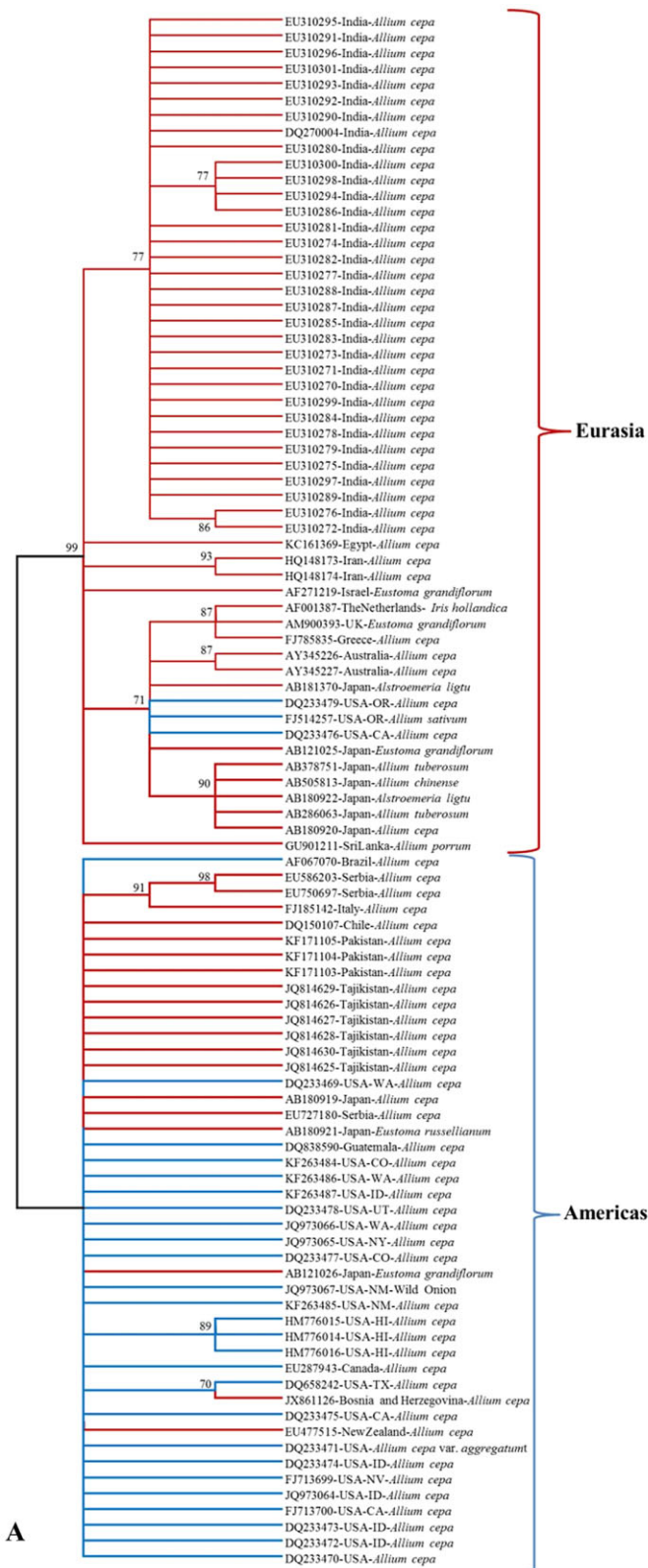
Tospoviruses generally produce necrotic and chlorotic symptoms in their host plants in both localized and systemic infections. In the case of IYSV, the virus tends to remain localized and produces characteristic symptoms as discussed above. The diamond-shaped lesions are more obvious on scapes than on leaves, and are unique to IYSV infection in onion and could be used as a diagnostic aid.

For immunodiagnosis, IYSV-specific polyclonal antibodies have been developed against the N protein and enzyme-linked immunosorbent assay (ELISA) reagents and immunostrips are commercially available for rapid and accurate diagnosis (Pappu *et al.*, 2006a). Although the N gene (and the protein) is conserved among known IYSV isolates, some differences in the efficiency among the various commercial kits that utilize different polyclonal antisera have been reported (Tomassoli *et al.*, 2009). For the detection of IYSV in individual thrips, a polyclonal antiserum was produced against the recombinant NSs protein that was expressed in *Escherichia coli*. The antiserum has been used to determine the seasonal dynamics of thrips transmitters in field-collected thrips populations (Bag *et al.*, 2014).

Molecular diagnostics based on 'traditional' PCR and real-time PCR for IYSV detection and diagnosis have been developed and the N gene was used as a target for amplification for this purpose (Chingandu *et al.*, 2012; Pappu *et al.*, 2006a). In most cases, the resulting amplicons have been sequenced, and more than 100 complete N gene sequences are now available in GenBank. This dataset on IYSV N gene sequences has facilitated some detailed genetic diversity studies on a global scale (Iftikhar *et al.*, 2014).

## TRANSMISSION AND EPIDEMIOLOGY

Of the 14 species of thrips reported to transmit tospoviruses (reviewed by Riley *et al.*, 2011; Turina *et al.*, 2012), onion thrips are the confirmed vector for IYSV (Cortês *et al.*, 1998; Hsu *et al.*, 2010; Kritzman *et al.*, 2001; Nagata *et al.*, 1999) (Fig. 2A). Recently, Srinivasan *et al.* (2012) have reported that *Frankliniella fusca* (Hinds) (tobacco thrips) can also transmit IYSV, albeit with a



**Fig. 4** (A) Phylogeny based on amino acid sequences of the nucleocapsid protein of *Iris yellow spot virus* (IYSV) available in GenBank. The evolutionary history was inferred using the unweighted pair group method with arithmetic average (UPGMA). The optimal tree with the sum of branch length = 0.76736606 is shown. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 99 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 270 positions in the final dataset. Evolutionary analyses were conducted in MEGA6. (B) Genotyping of IYSV accessions based on *in silico* restriction fragment length polymorphism (RFLP) simulation of nucleocapsid (N) gene (percentage of accessions under various genotypes).

lower efficiency, under experimental conditions. Infestation by *T. tabaci* was correlated with a higher incidence of IYSV in onion (Kritzman *et al.*, 2001). Similar to TSWV, IYSV is considered to be acquired at the larval stage and transmission occurs by second-instar larvae and adult thrips in a propagative and persistent manner (Inoue *et al.*, 2010; Ullman *et al.*, 1992a, b, c, 1993; Wetering *et al.*, 1996; Wijkamp *et al.*, 1993). In addition to being a primary vector for IYSV, onion thrips are a serious pest of onion and their leaf-feeding damage can cause up to 60% yield loss (Waiganjo *et al.*, 2008). There is no evidence that IYSV is transmitted through either onion bulbs or seeds (Boateng and Schwartz, 2013; Bulajic *et al.*, 2009; Kritzman *et al.*, 2001).

Originating in the Mediterranean region, *T. tabaci* is now a cosmopolitan pest and is present in almost every *Allium*-growing region in the world (Jones, 2005). The insect reproduces by thelytokous (Kendall and Capinera, 1990; Lewis, 1973), parthenogenesis (Moritz, 1997) and arrhenotoky (Diaz-Montano *et al.*, 2011; Kendall and Capinera, 1990). Onion thrips are polyphagous, infest plants belonging to ~25 species, but show preference for Alliaceae over other plant species. In *Allium* spp., they have been shown to have a mixed population of both sexes, in contrast with only females in other crops (Bosco and Tavella, 2010). This preferential habitat towards *Allium* spp. could be correlated with the increased number of *Allium* spp. known to be susceptible to IYSV infection. Recent reports have highlighted the genetic basis of the differential ability of specific *T. tabaci* populations to transmit TSWV (Inoue *et al.*, 2010; Jacobson and Kennedy, 2013; Jacobson *et al.*, 2013; Westmore *et al.*, 2013; Wijkamp *et al.*, 1995), and TYRV (Golnaraghi *et al.*, 2007), which might influence *T. tabaci*-IYSV transmission and virus-host specificity. Adaptation to specific *T. tabaci* populations through co-evolution could explain the recent outbreaks of the disease in various onion-growing areas in the USA and elsewhere. Because of its rapid growth cycle and abundance in both open field and glasshouse environments, *T. tabaci* usually overwinters in weeds and other vegetable and fruit crops under field conditions.

Several studies have investigated the role of transplants, crop volunteers and weeds present in and around onion fields. IYSV infection has been detected in different weeds collected from onion fields from different onion-growing regions (Evans *et al.*, 2009a, b; Hsu *et al.*, 2011; Nischwitz *et al.*, 2007; Sampangi *et al.*, 2007; Smith *et al.*, 2011), suggesting that weeds could serve as alternative hosts and potential sources of inoculum for virus transmission and infection of new *Allium* plantings. Infected trans-

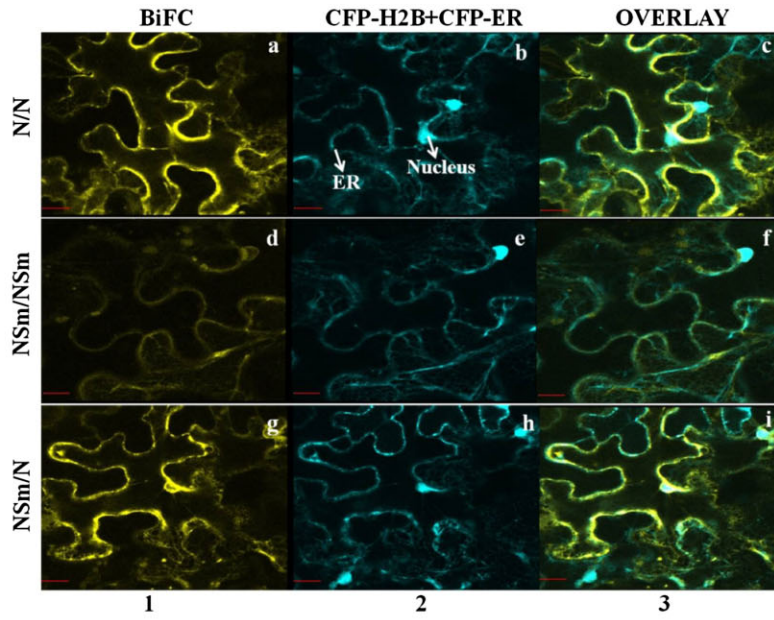
plants carrying viruliferous thrips may also be a probable cause of long-distance disease spread, especially in island states and nations, such as Hawaii (Sether *et al.*, 2010) and Mauritius (Lobin *et al.*, 2010). Sequence comparisons of the N gene suggested that IYSV in Georgia, USA might have originated in Peru from either infected onions or onions harbouring viruliferous thrips (Nischwitz *et al.*, 2006). Similarly, it has also been shown that IYSV could have been introduced into Colorado (Mahaffey and Cranshaw, 2010) and New York (Hsu *et al.*, 2011) by either infected transplants or plants carrying viruliferous thrips imported from states from which IYSV had already been reported.

Hsu *et al.* (2011) and Smith *et al.* (2011) have suggested that volunteer onions, bulbs, imported bare root transplants and weeds could be major sources of IYSV introduction into commercial onion fields of New York. The seasonal dynamics of *T. tabaci* in onion fields in Oregon suggested that the largest number of viruliferous thrips were present in the middle of July near overwintering onion fields, compared with fewer viruliferous thrips in onion fields that were located further from overwintering onion fields, suggesting that overwintering onion fields are probable sources of primary infection in this study (Bag *et al.*, 2014). As suggested by Mo *et al.* (2009), thrips typically invade onion fields early in the season, with subsequent buildup of the population during the season. A different pest and disease dynamic was described by Hsu *et al.* (2010), who found higher thrips numbers later in the season, migrating from adjacent fields that had been harvested.

## DISEASE MANAGEMENT

Although the virus has been known to infect *Allium* spp., especially onion, for over 25 years, an organized effort to develop management tactics has started only recently after the virus-induced disease outbreaks have become increasingly damaging and widespread in the western USA. Scientifically validated management tactics have just begun to emerge for IYSV. Research on the development of integrated disease management has been considered a priority area by several state-based onion grower associations, state and federal funding agencies in the USA, and support from these various sources has resulted in a nationwide organized effort of an interdisciplinary team of scientists, including virologists, plant pathologists, horticulturalists, entomologists, geneticists and breeders. As a result, various production practices that could influence disease outbreaks have been identified. Based on these observations, a risk index to estimate and predict

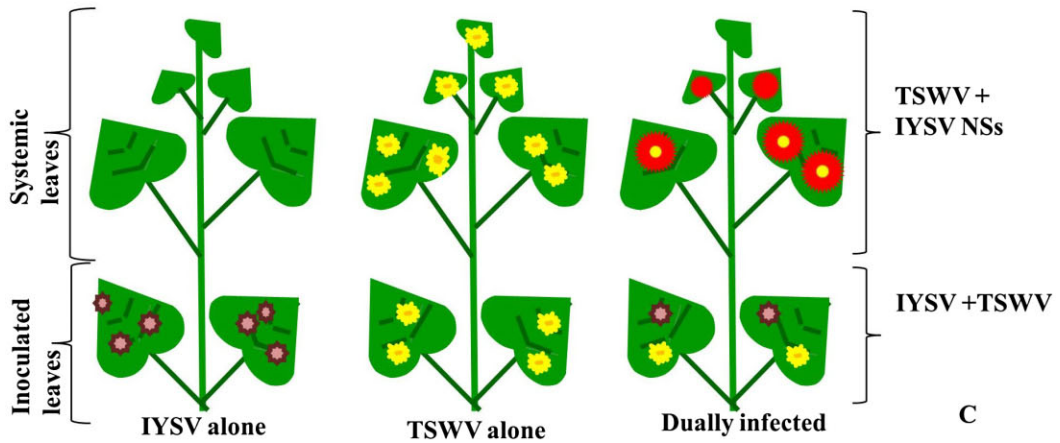




A



B



**Fig. 5** (A) Confocal micrographs showing the *Iris yellow spot virus* (IYSV) protein interactions examined. Interaction assays were performed in leaf epidermal cells of transgenic *Nicotiana benthamiana* plants expressing cyan fluorescent protein fused to the nuclear marker histone 2B (CFP-H2B) and the endoplasmic reticulum marker (CFP-ER). Shown are the interaction assay [bimolecular fluorescence complementation (BiFC), column 1], localization of CFP-H2B and CFP-ER (nucleus and ER, column 2) and a merge of all panels (overlay, column 3). Proteins listed first in the pair of interactors were expressed as C-terminal fusions to the amino-terminal half of yellow fluorescent protein (YFP). Those listed second were expressed as C-terminal fusions to the carboxy-terminal half of YFP. All protein fusions to each half of YFP were tested in all pairwise combinations, a subset of detectable interactions of which is shown here: (a–c) N/N; (d–f) NSm/NSm; (g–i) NSm/N. Micrographs shown are representative of at least 50 cells examined. Scale bar, 20  $\mu$ m. (B) Differential response of *Datura stramonium* to mechanical inoculation with IYSV and *Tomato spotted wilt virus* (TSWV). Top left: *D. stramonium* is a permissive host for TSWV, wherein infection results in concentric ring spots in inoculated leaves, and leaf curling, mottling and necrosis in systemic leaves, shown by white arrows. Top right: *D. stramonium* is a restrictive host for IYSV. IYSV infection produces small necrotic spots. Bottom left: distinctive concentric rings and necrotic spots develop in inoculated leaves of *Datura* infected with both viruses. Bottom right: more severe symptoms develop in inoculated and systemic leaves of dually infected *Datura*. Red arrows show symptoms in inoculated leaves and white arrows show symptoms in systemic infection. Insets show close-up of TSWV and IYSV symptoms in inoculated leaves. (C) Schematic representation of differential response of *D. stramonium* to IYSV and TSWV.

the likelihood of disease outbreaks has been developed and is being validated (Schwartz *et al.*, 2010).

Similar to any other insect-transmitted virus, an integrated disease management approach needs to be developed and adapted to reduce the impact of the disease. The central part of such an integrated strategy is to grow virus-resistant or virus-tolerant cultivars and to develop sound thrips management practices. For disease resistance, possible approaches include the development of resistant cultivars to either the virus or its thrips vector, or both. The evaluation of cultivars and breeding material for virus resistance must be carried out under field conditions relying on natural disease pressure, as there is no reliable and efficient method for the inoculation of onion by mechanical inoculation. Attempts to develop a screening method based on mechanical inoculation have so far yielded limited results—the disease could be reproduced under glasshouse conditions using manual inoculation (Fig. 2D), but the inoculation efficiency was no more than 30%, which is not acceptable for screening for virus resistance (S. Bag and H. R. Pappu, unpublished data); another study reported a transmission rate of 60% by mechanical inoculation (Kumar and Dhawan, 2013).

Field resistance could be manifested as reduced disease severity and/or delayed symptom development with the goal that bulb yield and grade are not negatively affected. The evaluation of onion germplasm and existing cultivars has been ongoing in several states in the USA. No resistant variety has been identified to date, but germplasm and cultivars with tolerance to the virus and to thrips feeding have been identified. Shock *et al.* (2008) and Diaz-Montano *et al.* (2010) evaluated a number of onion cultivars for resistance to *T. tabaci*, and found that two cultivars, Colorado 6 and NMSU 03-52-1, had the smallest numbers of thrips per plant, but neither cultivar was resistant to IYSV. During a further evaluation of 17 additional cultivars, none was found to be resistant to thrips and/or IYSV (Diaz-Montano *et al.*, 2010). In a study conducted by Multani *et al.* (2009), 18 winter-sown onion entries were screened for reduced IYSV symptom expression, with NMSU 03-52-1, NMSU 04-41, NMSU 04-44-1 and 'NuMex Jose Fernandez' exhibiting the fewest IYSV symptoms. Moghadam

*et al.* (2011) evaluated 13 winter-sown onion entries for IYSV symptom expression, and NMSU 05-33-1 showed a delayed symptom expression, suggesting that differences exist among entries for IYSV symptoms.

Singh (2013) evaluated the breeding materials for reduced IYSV-induced symptom expression after one cycle of selection. He found a limited number of instances in which selection was able to reduce virus damage. The effect of onion foliage colour on thrips feeding behaviour has been well documented: yellow–green-coloured foliage is less attractive than blue–green-coloured foliage to thrips (Diaz-Montano *et al.*, 2012; Jones *et al.*, 1934; Molenaar, 1984). Although foliage colour depends on nutritional level, these foliage types can be distinguished by different amounts of epicuticular waxes on leaf surfaces. Blue–green foliage carries large amounts of epicuticular waxes relative to lighter green foliage types, such as glossy (essentially no waxes) and semi-glossy (intermediate to waxy and glossy) foliage types (Damon and Havey, 2013; Molenaar, 1984). This trait is being exploited to develop onion cultivars showing less damage by thrips and IYSV. However, early research suggests that this trait may not be the sole determinant for thrips feeding, as plants with blue–green foliage have been observed with a reduced number of thrips per plant, and plants with light green foliage have been observed with a high number of thrips per plant (Singh, 2013).

Results from numerous groups have indicated that thrips populations are often lower on onions with less epicuticular wax when compared with waxier types (Diaz-Montano *et al.*, 2012; Jones *et al.*, 1934; Molenaar, 1984). This may be advantageous to growers by reducing the frequencies of insecticide sprays over the growing season. However, there is no evidence that reduced waxiness of foliage conditions resistance to thrips or IYSV. Smaller amounts of epicuticular waxes are recessive to waxier types (Molenaar, 1984). Two chromosome regions have been identified controlling the amounts of epicuticular waxes on onion foliage (Damon and Havey, 2013). Molecular markers are being identified to facilitate the backcrossing of less waxy phenotypes into diverse onion populations.

Inducers of systemic acquired resistance (SAR), such as Actigard (1,2,3-benzothiadiazole-7-thiocarboxylic acid-5-methyl-ester; Syngenta, Wilmington, DE, USA), have been used on onions under field conditions and the final disease incidence was determined. Although Actigard reduced the final disease incidence in some years, the effect was not consistent from year to year, indicating that other factors, such as environment, could influence the resistance response (Gent *et al.*, 2006). Efforts to understand the mechanism behind the SAR induction against IYSV are being carried out under controlled conditions and by using experimental hosts, *Datura* and *N. benthamiana*. With *Datura*, a local lesion host for IYSV, three descriptors were used—lesion number, lesion size and relative virus levels in Actigard-treated plants. As determined by ELISA and PCR, Actigard-treated plants showed a significant level of reduction in virus levels relative to buffer-inoculated control plants. In addition, Actigard also reduced the IYSV lesion number and size in treated *Datura* plants (Fig. S4, see Supporting Information) (Tripathi and Pappu, 2011).

In the absence of any significant host plant resistance to IYSV, management options are currently centred around cultural or production practices, such as sanitation (removal of crop volunteers, better weed management), crop rotation, straw mulching, irrigation scheduling, avoidance of the green bridge by physical separation of bulb and seed crops, fertilizer management, especially for nitrogen (Buckland *et al.*, 2013; Larentzaki *et al.*, 2008; Malik *et al.*, 2009), and sound thrips control practices through the judicious use of reduced-risk insecticides (Nault *et al.*, 2012). The use of straw mulch resulted in the reduction of thrips numbers by 30% (Schwartz *et al.*, 2009). In a recent study in Utah, USA, it was found that reduced soil nitrogen in the absence of biostimulants can reduce onion thrips densities and final disease incidence without a significant loss in yield. It also created a favourable environment for soil microbial activity, and reduced the risk of leaching soil nitrate (Buckland *et al.*, 2013). It was further suggested that adult onion thrips prefer onion leaf tissues with higher nitrogen contents caused by maximum use of nitrogen fertilizers (Buckland *et al.*, 2013; Malik *et al.*, 2009). It has been widely noted and accepted that plants that are under stress (such as moisture stress) show more disease relative to those that are not. The field tolerance (expressed as reduced symptoms) to IYSV observed in some cultivars and breeding lines could be caused by an increased tolerance to plant stress. This is a worthwhile topic for future research. Using multiple approaches, such as crop rotations, together with soil and fertilizer managements, to control thrips infestation during the season, especially in the early stages of the crop, could reduce the damage caused by both thrips feeding and IYSV infection.

The use of RNA interference (RNAi) by the expression of IYSV genomic sequences to generate transgenic plants with resistance to IYSV is an approach that could complement ongoing efforts to develop virus-resistant varieties using traditional plant breeding.

However, efficient transformation and regeneration of onion continue to be a challenge. Transgenic onion expressing an RNAi construct was developed to reduce lachrymatory synthase activity, significantly reducing the level of tear-inducing lachrymatory factor (Eady *et al.*, 2008), and this strategy could be further exploited to develop IYSV-resistant cultivars.

## SUMMARY

The initial emergence of IYSV and the lull, which was followed by resurgence and the subsequent rapid spread of IYSV in the USA, could be considered a textbook example of basic epidemiological principles at work: virus inoculum levels, levels and distribution of vector species, climatic effects, production practices, regional and international trade of onion sets and transplants may all have played an important role in virus spread and subsequent outbreaks. Although the virus was first reported in the 1980s, it remained of little concern because of its negligible impact in major onion-growing regions of the world until 2000. Virus epidemics and their rapid spread to new areas have been witnessed since then. However, the trigger of this re-emergence resulting in outbreaks is not known. New strains, new vector biotypes, increased trade of onion planting material, global warming and the expansion of the virus host range, including weeds, might have contributed to an increase in the levels of virus inoculum and its subsequent spread by thrips. Considering the continuing new reports of IYSV from many parts of the onion-growing areas of the world, and the importance of *Allium* spp. for many regional and national economies, research should continue in an attempt to understand the epidemiology and to develop control options.

In a relatively short period of time of 10 years since IYSV outbreaks became economically damaging, considerable progress has been made in our understanding of the virus and the vector, and the multitude of their interactions, in diverse managed ecosystems. A package of practical disease management recommendations and a risk index have been developed that can be used as decision-making tools for growers in some parts of the USA. This risk index must be adjusted and fine-tuned to meet the different climatic conditions and production practices in different parts of the onion-growing regions. This index was made possible because of the sustained financial support of research on this disease by onion growers, industry, state and federal funding agencies in the USA and elsewhere. This is a fine example of a public–private partnership to address a potential food security issue in a coordinated, collaborative and interdisciplinary manner.

Challenges remain however. A lack of a reliable assay based on mechanical inoculation for the infection of onion is a bottleneck, and biological studies have to rely on experimental hosts, such as *N. benthamiana*. Considering the critical role of thrips in virus spread, the options for the management of vector thrips are still fairly limited, and chemicals utilizing different chemistries are few

and research should continue in this area. In addition, the use of molecular approaches to target the developmental genes of thrips through RNAi is another fertile ground for research that could yield effective ways to intervene and interrupt the IYSV–thrips association, and thus contribute to the limitation of the role of thrips in virus outbreaks.

Overall, the significant progress made on IYSV bodes well for the future of research on this important disease of onion and its potential to reduce the impact of the disease. This is contingent upon continued adherence to the proven model that made the progress possible: a strong private–public partnership, and a sustained, long-term funding for area-wide, collaborative, interdisciplinary and mission-oriented research.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1** Distribution of *Iris yellow spot virus* (IYSV) in plants. (A) Onion plant with bulb. (B) Virus accumulation was found to be significantly greater in younger leaves (8–11) than in intermediate and older leaves. (C) IYSV was not detected in roots, basal plates, bulb scales or dead leaves.

**Fig. S2** Comparison of biologically distinct isolates of *Iris yellow spot virus* (IYSV) that differed in disease development in *Nicotiana benthamiana* under controlled glasshouse conditions. Initial symptom differentiation was superimposed for each isolate. IYSV-Idaho (IYSV-ID) and IYSV-Washington (IYSV-WA) were found to be more severe than IYSV-California (IYSV-CA). IYSV-ID and IYSV-WA

isolates had a more severe phenotype at 22 days post-inoculation (dpi) compared with the mild strain, IYSV-CA, as shown in the top panel. By 50 dpi, IYSV-ID and IYSV-WA isolates were lethal to the plants, whereas the effect on the plant infected with IYSV-CA was less severe, as shown in the bottom panel.

**Fig. S3** Secondary RNA structure of *Iris yellow spot virus* (IYSV) genomic RNAs based on minimum free energy (MFE) fold algorithm prediction using the RNA Fold Web Server (<http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi>). (A) Secondary structure of small RNA. Inset shows the panhandle structural forms in all three RNAs as a result of the presence of the complementary structure. (B) Structure of the intergenic region (IGR) of IYSV M RNA forming a stem-loop structure. The apical Y-shaped loop formed ( $dG = -63.70$ ) is shown as an enlarged image.

**Fig. S4** Acibenzolar-S-methyl (ASM)-induced resistance response against *Iris yellow spot virus* (IYSV) in *Datura stramonium* and *Nicotiana benthamiana* plants. (A, B) IYSV symptoms in *D. stramonium* plants. *Datura stramonium* leaves showing lesions induced by IYSV in buffer- and ASM-treated plants at 14 days post-inoculation (dpi). Different sets of plants were treated with buffer or ASM (0.1 mM). Seven days later, treated plants were inoculated with IYSV. The diameters of IYSV lesions were measured at 14 days post-IYSV inoculations. (C, D) Reverse transcription-polymerase chain reaction (RT-PCR) of IYSV nucleocapsid (N) gene. Relative levels of expression of nucleocapsid (N) gene of IYSV in ASM- and buffer-treated *D. stramonium* (C) and *N. benthamiana* (D) plants. Different sets of plants were treated with buffer or ASM (0.1 mM). Seven days later, treated plants were inoculated with IYSV. RT-PCR was conducted on samples collected at 14 days post-IYSV inoculations. I, IYSV-inoculated plants; B + I, buffer-treated plants inoculated with IYSV; A + I, ASM-treated plants inoculated with IYSV; H, healthy plants; A, ASM-non-inoculated plants. (E) RT-PCR of pathogenesis-related (PR-1a) genes. Detection of PR-1a genes in buffer- and ASM-treated *N. benthamiana* plants. Different sets of plants were treated with buffer or ASM (0.1 mM). A set of three plants per treatment was used for analysis. Leaf samples were harvested after 2 days of treatment, and expression of the PR1a gene was detected by RT-PCR using PR-1a gene-specific primers.