Occurrence of iris yellow spot virus from onion crops in Egypt

E. E. Hafez · A. A. El-Morsi · O. A. El-Shahaby ·
A. A. Abdelkhalek

Received: 15 July 2014 / Accepted: 8 October 2014 / Published online: 24 October 2014
© Indian Virological Society 2014

Abstract Iris yellow spot virus (IYSV) is a infects onion bulb and seed crops in many countries including Egypt. Results of the mechanical inoculation revealed that, small chlorotic lesions and systemic necrosis were observed on both *Nicotiana benthamiana* and *Datura stramonium* after 10 days, while there were no symptoms were appeared on the onion plant. The viral biological transmission with *Thrips tabaci* was highly reported to be efficiently for virus transmitted. Our results confirmed the presentence of virus-like particles of a *Tospovirus* infected onion leaf using transmission electron microscopy. Both of sequence and phylogenetic analysis of N gene revealed that our viral isolate is IYSV with 95 % identity with reported Israel isolate. The sequence of N gene had three motifs: casein kinase II Phosphorylation site, *N*-myristoylation site and protein kinase C phosphorylation site. These motifs are involved in regulation, activity and stability of IYSV. To our knowledge, this is the first molecular characterization of IYSV in Egypt.

Keywords IYSV · *Allium cepa* · Virus transmission · Thin sectioning · RT-PCR · Nucleocapsid gene

Introduction

Onion (*Allium cepa* L.), is one of the most economically important vegetable crops cultivated in many governorates in Egypt [1]. It is grown mainly for its bulbs, which are stored to meet the increase in demand for both local consumption and export. The annual production and storage losses in onions as a result of diseases can range from insignificant amount to more than 50 %, depending on the location, environmental conditions and the causal agent [2].

Iris yellow spot virus (IYSV), genus *Tospovirus*, family *Bunyaviridae*, is an economically important viral pathogen of onion in many onion-growing areas of the world and is transmitted by onion thrips (*Thrips tabaci*) [3, 4]. Recently, IYSV spread rapidly in several onion-growing countries all over the world. Once, IYSV established in an area, it spread rapidly in onion crops. For example, in Colorado, its incidence increased from 6 % in 2001 to 73 % 2 years later [5].

Disease symptoms of IYSV appear as chlorotic, eye-like or diamond-shaped lesions, or necrotic, straw-colored to white, dry, elongate or spindle-shaped lesions frequently more numerous in mid-to lower portions of the scapes [6, 7].

Unlike *Tomato spotted wilt virus* that has numerous indicator hosts by mechanical inoculation, IYSV has a narrow host range, and its mechanical transmission in hosts is difficult [8]. In tospoviruses, the most common gene sequenced is the nucleocapsid gene and sequence comparisons have proven useful in the identification and classification of the viruses [9]. To date, no information is available on the nucleotide sequence of the N gene of IYSV isolates from Egypt. According to the economic importance of this virus and due to its disaster impact on the onion yield in Egypt, it became necessary to characterize and identify the virus in both infected onion field and its biologically vector, as a step to control the vigorous

E. E. Hafez (✉) · A. A. Abdelkhalek
Plant Protection and Bimolecular Diagnosis Department, Arid
Lands Cultivation Research Institute, City of Scientific Research
and Technological Applications, Alexandria, Egypt
e-mail: elsayed_hafez@yahoo.com

A. A. El-Morsi · O. A. El-Shahaby
Botany Department, Faculty of Science, Mansoura University,
Mansoura, Egypt

distribution of the virus between the Egyptian governorates.

Materials and methods

Viral isolate

Egyptian onion IYSV was previously detected in Egypt [1]. The onion tissues that showed the viral symptoms were used in this study as the source of inoculums.

Transmission studies

Mechanical transmission

IYSV-Egypt strain was maintained in *Nicotiana benthamiana* and *Datura stramonium* L., by mechanical inoculation. Mechanical transmission was performed by homogenizing IYSV-Egypt infected plant tissues with symptoms in 10 mM sodium phosphate buffer (pH 7.0), containing 0.1 % sodium sulfite [10]. Inoculated plants were kept under greenhouse conditions and observed for virus symptoms development.

Thrip transmission using viruliferous insects (*Thrips tabaci* L.)

A virus free culture of the adult thrips was grown and pre-grown were observed as individual on bean pods to lay eggs. Newly hatched larvae were collected and reared on bean pods [11]. Groups of virus-free thrips, newly hatched larvae were placed on IYSV-Egypt-infected *D. stramonium* L. plants with symptoms for 2 h and then transferred to healthy onion seedlings in another cage for 24 h. Subsequently, thrips were killed by spraying with 0.01 % Malathion and plants were observed for developing virus symptoms for 4 weeks. Uninfected, healthy onion plants grown in an insect-proof greenhouse were used as negative controls.

Electron microscopy examination

Ultrathin Sections (2×1 mm) were excised from symptomatic young leaves of onion and fixed in 2.5 % glutaraldehyde buffered with 75 mM potassium phosphate, pH 7.0, for 2 h. Samples were then rinsed in the same buffer and postfixed in 1 % OsO_4 for 3 h. After a buffer rinse, the samples were dehydrated in a graded acetone series, embedded in an Epon–Araldite resin mixture, and polymerized as described by Birithia et al. [12]. Sectioned material was stained with uranyl acetate followed by lead nitrate and examined for virus by electron microscopy.

Fig. 1 Symptoms showing development of chlorotic local lesions, veinal necrosis and drying of leaves on both mechanically inoculated *Datura stramonium* (a) and *Nicotiana benthamiana* (b)

RT-PCR detection and sequence analysis

Total RNA from healthy and infected onion tissue was extracted using RNeasy Mini Kit according to manufacturer's instructions (QIAGEN, Germany). The first strand cDNA synthesis was performed in a total volume of 25 μl . The reaction mixture contained 2.5 μl of $5 \times$ buffer with MgCl_2 , 2.5 μl of 2.5 mM dNTPs, 4 μl from oligo (dT) primer (20 pmol/ μl), 2 μg RNA and 200 U reverse transcriptase enzyme (M-MLV, Fermentas, USA). RT-PCR amplification was performed in a thermal cycler (Eppendorf, Germany) programmed at 42 °C for 1 h.

The synthesized IYSV-Egypt cDNA was subjected to PCR amplification using IYSV-N gene primers; forward (5'-TAAAACAAACATTCAAACAA-3') and reverse (5'-CTCTTAAACACATTTAACAAGCAC-3') reported by [9]. The PCR reaction conditions were initial denaturation at 95 °C for 2 min, followed by 34 cycles at 95 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min. Final extension at 72 °C for 10 min. The amplification product was sequenced directly after the purification with PCR clean up column kit (QIAGEN, Germany). The nucleotide sequences of the amplification products were deposited in GenBank and compared with those of previously reported IYSV isolates.

Results

Symptomatology of IYSV-Egypt and cytopathological effects

Small chlorotic lesions and systemic necrosis were observed on leaves of IYSV-Egypt inoculated *D. stramonium*, and *N. benthamiana* plants 10–12 days post inoculation (Fig. 1).

Fig. 2 a, b Symptoms showing development of Straw-colored, chlorotic, lenticular-shaped lesions with green islands, chlorotic, diamond shaped lesions and lesions with green islands on both naturally infected onion (a) and infected onion by transmission with viruliferous thrips (b). **c** showing electron micrograph of enveloped IYSV-Egypt virions and double-membraned structures in thin sections of Virus transmitted to onion plants by viruliferous thrips (Bar 500 nm). **d** showing RT-PCR amplification of the nucleocapsid gene of IYSV-Egypt. Lane M 100 bp DNA ladder, lane C healthy onion plant (control), lanes 1–3, *D. stramonium*, *N. benthamiana* and onion infected plant samples

The lesions gradually increased both in number and size and spread throughout the inoculated leaves. Symptoms appeared on *D. stramonium* earlier than those of *N. benthamiana*. Symptoms developed on infected onions by transmission with viruliferous thrips were identical to those observed on naturally infected plants include straw-colored, chlorotic, and necrotic lesions on leaves (Fig. 2a, b). These plants were observed for symptom development for 4 weeks, and IYSV-Egypt transmission was ascertained by RT-PCR.

In ultrathin sections of onion plants that acquired the virus through transmission by viruliferous thrips, numerous spherical particles were observed (Fig. 2c).

RT-PCR for the viral N gene

When total RNA of IYSV-Egypt infected plants (*D. stramonium*, *N. benthamiana* or onion) tissues was subjected to RT-PCR amplification using the N gene tospoviruses universal primers, an amplicon with molecular size of 1,100 bp was observed only with infected tissues of each plant species (Fig. 2d).

Nucleotide sequence and sequence analysis of nucleocapsid gene

The nucleocapsid gene of IYSV-Egypt from onion evaluated in this study was successfully amplified, sequenced and submitted to GenBank with accession number

KC161369 (Fig. 3). The sequence contains a single open reading frame (ORF) of 822 nucleotides with an ATG start codon at position 1 and a TAG stop codon at position 820, encoding for a protein of 273 amino acids. The deduced N gene polypeptide contains 33 strongly basic and 41 strongly acidic amino acids. The theoretical PIs were 9.09. The calculated molecular mass of the N gene peptide is 13.67 kDa and the full length was classified as stable [Instability Index (II): 30.46]. Phylogenetic analysis based on the deduced amino acid sequences of N gene indicated that the IYSV-Egypt is closely related to that from Israel (Acc# AAF75556, with identity 95 %).

Bioinformatics tools used to analyze and identify several motifs from the whole proteins of plant viruses. Motifs are typically 6–30 amino acids and correspond to its active site, substrate or ligand binding site and structurally important segment of proteins. In the nucleocapsid protein of IYSV-Egypt three motifs were detected. These motifs are Casein kinase II Phosphorylation site (6 sites), (N-myristoylation sites (5 sites) and Protein kinase C phosphorylation site (10 sites). These motifs are involved in regulation, activity and stability of virus.

Discussion

In Egypt onion is the third vegetable crop consumed by public and is one of the main and most economically

Fig. 3 The complete nucleotide sequence of the nucleocapsid (N) gene of IYSV-Egypt from onion is presented as DNA sequence. The deduced amino acid sequence of the protein encoded by the viral sense RNA is shown below the DNA sequence. Potential

N-myristoylation sites (6 aa), Casein kinase II Phosphorylation site (4 aa) and Protein kinase C phosphorylation site (3 aa) are *underlined*. Asterisks indicate the stop codons

important crops cultivated in many governorates. According to [13] Egypt, Russian Federation, Iran, USA, India and China were considered the top six onion-producing countries in the world. IYSV is among the important viruses that cause devastating losses by reducing either the yield and/or quality of onion crop. Tospoviruses, ascribed to the family ‘*Bunyaviridae*’ [14, 15] are serious viral pathogens affecting many important agricultural crops [16]. The annual losses due to tospoviruses are estimated at over one billion

USD [17]. In Egypt tospoviruses are rapidly spreading and expected to become a major constraint on yield potential of various crops. Studies on biological characteristics of IYSV have been limited due to difficulties in obtaining consistent and reproducible mechanical transmission and lack of indicator hosts. The results from our study with IYSV-Egypt revealed that small chlorotic lesions and systemic necrosis were observed 10–12 days post mechanical inoculation of two plant hosts, *D. stramonium* and *N.*

benthamiana. Similar chlorotic and necrotic local lesions symptoms were reported in infected *Datura* and *Nicotiana* plants [18, 19]. Symptoms that developed on infected onion plants after IYSV-Egypt transmission by viruliferous thrips were identical to those observed on naturally infected plants which include straw-colored, chlorotic, necrotic lesions and irregular lesions with occasional green islands. On the other hand, after mechanical inoculation, no symptoms were observed on the inoculated onion plants. These results were similar to those obtained by Gent and Pappu [6, 7]. In ultrathin sections of IYSV-Egypt—infected onion leaves, spherical particles were observed within membrane-bound vesicles only in symptomatic tissues of infected plants. Similar results were obtained by Kritzman and Kritzman [20, 21]. Plant tissues obtained after IYSV-Egypt transmission with viruliferous thrips gave positive results with the IYSV antiserum (data not shown). In view of this, ELISA samples were tested by PCR using the N gene specific primers. Comparative sequence homology in the present study has shown that the onion IYSV-Egypt shared maximum sequence identity with other IYSV at nucleotide (95 %) as well as amino acid (95 %) levels. Phylogenetic analyses based on amino acids IYSV N gene sequences may point to introduction of the virus into a particular region or country, the continent or the occurrence of gene flow from one country to another. Sequencing and phylogenetic analyses of more isolates would provide additional information on the implications of high genetic diversity among IYSV isolates worldwide. Thus, sequences of the N gene of IYSV-Egypt showed a close relationship with the Israeli strain. The high significant homology with the Israeli 95 % in the amino acid sequence can be attributed to a recent introduction of IYSV from one country to another. Since, the virus probably was introduced into Egypt from Israel via ornamental propagation material. However, the divergence of the amino acid sequences from those of the Egyptian virus strain 5 % may reflect adaptation to different hosts and environmental conditions. In view of the wide distribution of IYSV in onion fields in Israel and the abundance of the thrip vector on onions, it is suggested that the virus was introduced from Israel to Egypt by the thrip vector.

References

1. Hafez EE, Abdelkhalek AA, El-Morsi AA, El-Sbahaby OA. First report of iris yellow spot virus infection of garlic and Egyptian leek in Egypt. *Plant Dis*. 2011;96:594.
2. Schwartz HF, Mohan SK, Havey MJ, Crowe FJ. Introduction. In: Schwartz HF, Mohan SK, editors. *Compendium of onion and garlic diseases and pests*. 2nd ed. St. Paul: American Phytopathological Society; 2006.
3. Pappu HR, Jones RA, Jain RK. Global status of tospovirus epidemics in diverse cropping systems: successes achieved and challenges ahead. *Virus Res*. 2009;141:219–36.
4. Mandal B, Jain RK, Krishnareddy M, Krishna Kumar NK, Ravi KS, Pappu HR. Emerging problems of tospoviruses (Bunyaviridae) and their management in the Indian subcontinent. *Plant Dis*. 2012;96:468–79.
5. Gent DH, Schwartz HF, Khosla R. Distribution and incidence of iris yellow spot virus in Colorado and its relation to onion plant population and yield. *Plant Dis*. 2004;88:446–52.
6. Gent DH, du Toit LJ, Fichtner SF, Mohan SK, Pappu HR, Schwartz HF. Iris yellow spot virus: an emerging threat to onion bulb and seed production. *Plant Dis*. 2006;90:1468–80.
7. Pappu HR, Rosales IM, Druffel KL. Serological and molecular assays for rapid and sensitive detection of Iris yellow spot virus infection of bulb and seed onion crops. *Plant Dis*. 2008;92:588–94.
8. Bag S, Pappu HR. Symptomatology of Iris yellow spot virus in selected indicator hosts. *Plant Health Prog*. 2009;. doi:10.1094/PHP-2009-0824-01-BR.
9. Pappu HR, duToit LJ, Schwartz HF, Mohan SK. Sequence diversity of the nucleoprotein gene of iris yellow spot virus (Genus *Tospovirus*, Family *Bunyaviridae*) isolates from the western region of the United States. *Arch Virol*. 2006;151:1015–23.
10. Mandal B, Pappu HR, Culbreath AK. Factors affecting mechanical transmission of tomato spotted wilt virus to peanut (*Arachis hypogaea*). *Plant Dis*. 2001;85:1259–63.
11. Biriithia R, Subramanian S, Pappu HR, Muthomi J, Narla RD. Analysis of iris yellow spot virus replication in vector and non-vector thrips species. *Plant Pathol*. 2013;. doi:10.1111/ppa.12057.
12. Orion D, Frank A. An electron microscopy study of cell wall lysis by *Meloidogyne javanica* gelatinous matrix. *Rev Nematol*. 1990;13:105–7.
13. FAO (Food and Agricultural Organization of the United Nations), 2012 statistics. <http://faostat.fao.org> (2012).
14. Francki RIB, Fauquet CM, Knudson DL, Brown F. Classification and nomenclature of viruses. In: *Fifth Report of the International Committee on Taxonomy of Viruses*. Springer, Wien New York. 1991. pp. 186–199.
15. Van Regenmortel, MHV, Fauquet CM, Bishop DHL, Carstens EB, Ester MK, Leman SM, Maniloff J, Mayo MA, McGeoch DJ, Pringle DJ, Wickner RB. Virus taxonomy. classification and nomenclature of viruses. In: *Seventh Report of the International Committee on Taxonomy of Viruses*. San Diego: Academic Press; 2000.
16. Moyer JW. Tospoviruses (Bunyaviridae). In: Granoff A, Webster RG, editors. *Encyclopedia of virology*. San Diego: Academic Press; 1999. p. 1803–7.
17. Prins M, Goldbach R. The emerging problem of Tospovirus infection and nonconventional methods of control. *Trends Microbiol*. 1998;6:31–5.
18. Cortês I, Livieratos J, Derks A, Peters D, Kormelink R. Molecular and serological characterization of iris yellow spot virus, a new and distinct tospovirus species. *J Phytopathol*. 1998;88:1276–82.
19. Pozzer L, Bezerra IC, Kormelink R, Prins M, Peters D, Resende RdeO, de Avilla AC. Characterization of a tospovirus isolate of iris yellow spot virus associated with a disease in onion fields in Brazil. *Plant Dis*. 1999;83:345–50.
20. Kritzman A, Beckleman H, Alexandrow S, Cohen J, Lampel M, Zeidan M, Raccach B, Gera A. Lisianthus leaf necrosis: a new disease of lisianthus caused by Iris yellow spot virus. *Plant Dis*. 2000;84(11):1185–9.
21. Kritzman A, Lampel M, Raccach B, Gera A. Distribution and transmission of iris yellow spot virus. *Plant Dis*. 2001;85(8):838–42.