Viruses Occurring in Onion Crop in Amasya Province, the Major Onion Producing Region in Turkey

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Received: 1 July 2012 / Accepted: 10 September 2012 / Published online: 16 December 2012
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Abstract Amasya is the greatest onion producing area in Turkey. Onion fields from Amasya region were surveyed for virus diseases in 2009–2011 and tested for the presence of the most important onion viruses such as *Onion yellow dwarf virus* (OYDV), *Iris yellow spot virus* (IYSV), *Leek yellow stripe virus* (LYSV), *Shallot latent virus* (SLV) and *Garlic common latent virus* (GCLV). The presence of virus diseases and their identification was ascertained through symptom observation in the fields, sap transmission to hosts, and DAS-ELISA. Based on the ELISA results, 57 out of 332 samples (17.16 %) were infected with viruses. The results showed that the highest infection was caused by OYDV (12.33 %) followed by LYSV (3.60 %). Only 1.19 % of the samples were infected with SLV, but none of the samples were found to be infected for GCLV and IYSV.

Keywords Onion · Viruses · Survey · Detection · ELISA

Onion (*Allium cepa* L.) is one of the most important field crops cultivated in Turkey, for both local consumption and export. Turkey is the third largest onion-producing country in the world after China, India and the USA, with 65,000 ha under cultivation and a total annual production almost two million tons (<http://www.fao.org/faostat-agriculture>). Amasya province, located in the middle of the Black Sea region, has the largest production of onion in Turkey.

Several factors affect onion production and ultimately the cost of onion cultivation. Onion plants are susceptible to a number of diseases caused by fungi, bacteria and viruses. The onion plants are susceptible to several viral diseases [19] and a variable number of potyviruses, carlavirus, allexiviruses and tospoviruses have been reported infecting onion plants [15].

Allium viruses cause reductions in plant vigor induce mosaics, yellow stripes, and deformations in leaves, stunting in plant, which result in drastic yield losses in infected plants [2, 4]. For instance, only *Onion yellow dwarf virus* (OYDV) can significantly reduce yields on onion and garlic, and yield losses as high as 25–54 % has been reported [5, 8, 13].

The main objectives of this study were to determine which viruses affect onion crops in the major production areas of Turkey and to determine their incidence and relative importance. The occurrence and incidence of viruses on field-grown onion in the Black Sea region of Turkey were investigated in the present study for the first time.

Field visits and collection of samples were conducted during the vegetation periods in 2010–2011. Samples were obtained from 66 fields randomly selected in each geographical area surveyed (Fig. 1). A total of 332 onion leaf samples were collected randomly from 66 fields at 25 locations, from the following four geographical districts of Amasya, Turkey; Suluova, Merzifon, Tasova and Amasya-Central. Samples were placed in labelled plastic bags and brought to the Plant Virus Research Laboratory of the Agricultural Faculty of Ondokuz Mayıs University, in Samsun, Turkey.

DAS-ELISA method was used for virus detection in onion leaf samples. The DAS-ELISA method was applied according to Clark and Adams [6] and instructions of the antisera's manufacturer (Bioreba and Agdia) for the polyclonal antisera

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Fig. 1 Map of Amasya province, a major onion producing region in Turkey, showing the main onion-growing areas surveyed for virus diseases in 2010–2011

of OYDV, *Leek yellow stripe virus* (LYSV), *Shallot latent virus* (SLV), *Garlic common latent virus* (GCLV) and *Iris yellow spot virus* (IYSV). In DAS-ELISA, leaf samples were ground (1 g leaf per 5 ml buffer) in extraction buffer (PBST: 0.13 M NaCl, 0.014 M KH_2PO_4 , 0.08 M $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$, 0.002 M KCl, pH 7.4) containing 0.05 % Tween-20, 0.1 % skimmed milk powder and 2 % polyvinyl pyrrolidone (PVP-40). Absorbance values were read at 405 nm using a microplate reader (Tecan Spectra II, Grodig/Salzburg, Austria) and also confirmed visually. All samples tested in two replicate wells and the absorbance value greater than three times that of a negative control and with a visually detectable yellow colour was rated as positive [16].

For biological isolation, saps obtained from samples by grinding leaves of infected onion plants that gave high absorbance values in ELISA tests in 0.05 M phosphate buffer (pH: 7.0) were inoculated to indicator plants; *Allium cepa*, *Nicotiana tabacum* L. “Xanthi”, *N. tabacum* L. “Samsun” and *N. tabacum* “White Burley” [7]. Five plants of each species were inoculated for each virus. After rinsing with tap water, plants were maintained in a plant growth room at 24 °C and observed for symptom development. Inoculated plants were tested for the presence of

virus by ELISA to confirm the results of previous serological testing.

In the 2010–2011 growing season, a survey was conducted in onion fields of Amasya, where virus-like symptoms were observed. The leaf samples were collected from 332 onion plants showing yellow stripes, deformation of the leaves, and stunting. Samples collected from 332 onion plants were analyzed using DAS-ELISA to determine the presence of OYDV, LYSV, SLV, GCLV and IYSV viruses in 25 areas of Amasya province of Turkey. 57 samples (17.16 %) reacted positively in DAS-ELISA with at least one of the viruses examined.

The results of serological tests showed that onion plants were infected with OYDV, LYSV and SLV in Amasya, on the other hand GCLV and IYSV were not determined in this region. Tests were conducted using several negative controls for each microplates. Therefore, the range of absorbance values of negative controls varied from 0.076 to 0.092 at 405 nm, depending on microplates and antiserum used. Positive samples gave absorbance values of 0.377–1.015 for OYDV, 0.395–0.714 for LYSV and 0.403–0.577 for SLV.

OYDV was the most prevalent virus in onion leaf samples, and 12.33 % were found to be infected with

Table 1 Detection of viruses in onion samples collected from Amasya province of Turkey during 2010–2011 by ELISA

Regions	No. of villages surveyed	No. of samples tested	Viruses ^a				
			OYDV	LYSV	SLV	GCLV	IYSV
Central	6	70	6	2	0	0	0
Suluova	9	137	24	7	4	0	0
Merzifon	6	85	5	0	0	0	0
Tasova	4	40	6	3	0	0	0
Total	25	332	41	12	4	0	0
% Infection	–	17.16	12.33	3.60	1.19	0	0

OYDV onion yellow dwarf virus, LYSV leek yellow stripe virus, SLV shallot latent virus, GCLV garlic common latent virus, IYSV iris yellow spot virus

^a Viruses were detected using enzyme-linked immunosorbent assay kits (Bioreba, Reinach, Switzerland)

OYDV, 3.60 % with LYSV and 1.19 % with SLV, but GCLV and IYSV were not detected in any sample taken from onion plants (Table 1).

The incidence of virus infections varied among regions. The highest infection rate was found in onion fields in Suluova (10.54 %), followed by fields in Tasova (2.71 %), Amasya-Central (2.40 %), and Merzifon (1.50 %).

Onion plants mechanically inoculated with extracts of ELISA-positive plants showed yellow lines on leaves three weeks after inoculation. These symptoms were similar to those that were described previously for the virus. The symptoms observed on indicator plants for OYDV corresponded to the results of DAS-ELISA. OYDV caused a yellow stripe on *A. cepa*, mosaic on *N. tabacum* L. “Xanthi” and *N. tabacum* L. “Samsun”. But the other tobacco species *N. tabacum* “White Burley” inoculated produced no symptoms. Symptoms in test plants infected with LYSV were similar to those described for OYDV. Four SLV isolates from onion, no symptoms or only slight symptoms were produced in indicator plants and could only be detected by ELISA tests.

In Turkey, onion (*A. cepa*) is largely cultivated in different geographical areas of the country. Viruses have always been a major cause of reduced quantity and quality of the worldwide *Allium* crops [17]. In the present study, the incidence and distribution of viruses in onion fields in the north regions of Turkey was investigated first time. Viruses causing mosaic, chlorosis, yellow stripe and stunting were observed in onion plants in the surveyed area in the Black Sea region in 2010 and 2011. Disease symptoms were similar to the symptoms previously reported from virus infected onion plants worldwide [1, 10, 12].

In the 332 onion leaf samples collected during surveys and tested by DAS-ELISA, three viruses (OYDV, LYSV and SLV) were found infecting onions, with OYDV being the most widespread virus. Although OYDV is one of the most important viruses infecting garlic in some areas of Turkey [9, 10] and elsewhere [11, 14, 18].

OYDV was the most widespread virus in our study. Similarly, a survey identified viruses infecting garlic, leek and onion crops in Greece. Samples of cultivated *Allium* species were collected from five districts. OYDV and LYSV were identified in 98.5 and 83.7 % of all samples, respectively, and were found in all regions [8]. A recent field survey determined, first time in Egypt, the incidence of OYDV. Field inspection, as well as ELISA, TBIA and DBIA screening tests, indicated that, the highest natural incidence (70.6 %) of the virus disease occurred in onion fields, followed by garlic (61.0 %) [1].

Our results show that onion plants in the Black Sea region of Turkey is heavily infected with OYDV whereas very low incidence levels of LYSV and SLV were found in the regions surveyed. GCLV and IYSV were not detected in any sample taken from onion. Viruses detected and the incidence of virus infections may vary among the locations [3, 20]. In this research, differences in infection rates and occurrence of viruses were also determined between viruses and locations. OYDV was found in onion plants in Suluova (17.51 %), followed by areas Tasova (15 %), Amasya-Central (8.57 %), and Merzifon (5.88 %). The incidence of LYSV in onion was 7.5 % in Tasova, 5.10 % in Suluova and 2.85 % in Amasya-Central. SLV was detected (2.91 %) only in onion leaf samples taken from Suluova region. Partially similar results were obtained by Shahraeen [17]. Based on ELISA tests, five viruses were detected in onion (OYDV, LYSV, GCLV, GarV-C and GarV-D) in Iran. OYDV was the most common with an overall average incidence of 65.5, 63.2 and 40.3 % in Hamadan, Tehran and Jiroft, respectively. LYSV (60.1 %) and GarCLV (30.7 %) were detected only in Tehran; and GarV-C (28.2 %) and GarV-D (30.1 %) were only detected in onion samples collected from the Hamadan region.

There are a few reports of virus diseases of onion in different parts of Turkey, but information on the Black Sea region is lacking. So far, only OYDV [12] and SLV [10] were determined in onion in Turkey.

The current study was the first to show the regional distribution of onion viruses in the Amasya region and to our knowledge; this is the first report of LYSV in onion in Turkey.

Acknowledgments We gratefully acknowledge the financial support of the TUBITAK (Council of Science and Technology of Turkey, Project no. 110 O 445).

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