Journal of Plant Protection Research ISSN 1427-4345

RAPID COMMUNICATION

Molecular characterization of the partial coat protein gene of an *Onion yellow dwarf virus* isolate detected in garlic (*Allium sativum* L.) from the West Shewa zone of Ethiopia

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Vol. 60, No. 1: 106–111, 2020

DOI: 10.24425/jppr.2020.132205

Received: May 14, 2019
Accepted: September 2, 2019

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Abstract

Onion yellow dwarf virus (OYDV), an aphid-borne potyvirus is one of the major viral pathogens of garlic causing significant yield losses worldwide. It is found almost everywhere in the world where *Allium* species is grown. The aim of this study was to test the presence of OYDV infection in garlic from Ethiopia. The presence of the virus was tested by Reverse transcription polymerase chain reaction (RT-PCR). The direct sequencing of the PCR product produced a sequence of 296 bp. Sequence analysis showed 89.27% sequence homology with an isolate from Australia (HQ258894) and 89.29% with an isolate from Spain (JX429964). A phylogenetic tree constructed with MEGA 7.0 revealed high levels of homology with various isolates of OYDV from all over the world and thus further confirmed the identity of the virus.

Keywords: garlic, *Onion yellow dwarf virus*, partial coat protein gene, reverse transcription polymerase chain reaction (RT-PCR)

Introduction

Garlic (*Allium sativum* L.) is one of the most important vegetable crops grown throughout the world under a wide range of agro-climatic conditions. Among the cultivated *Allium* species, garlic is the second most widely used vegetable crop next to onion (Usman *et al.* 2016). It is widely used as an antibiotic, anti-diabetic, anti-cancerous, anti-oxidant and lipid lowering agent (Keusgen 2002). Viral pathogens are some of the factors causing serious damage to the yield and quality of garlic crops (Takaichi *et al.* 1998). In many cases, garlic plants are affected by multiple viruses. Potyvirus, carlavirus and allexivirus are the major viruses infecting *Allium* species. It has been estimated that these viruses can reduce yield by up to 60% (Conci *et al.* 1998). In garlic, more than eight viruses have been tested that aggravate the symptoms. However, *Onion yellow dwarf virus* (OYDV) is the major component of the virus disease complex in garlic (Takaichi *et al.* 2001).

OYDV, an aphid-borne potyvirus, is found in almost all *Allium* producing areas. It is one of the major viral pathogens of garlic that causes significant yield losses worldwide (Katis *et al.* 2012). Since garlic is a vegetatively propagated plant, once it is infected by viruses it acts as a source of virus transmision from one generation to the next (Lot *et al.* 1998). The virus is transmitted in a non-persistent manner by several species of aphids (Kumar *et al.* 2011). Depending on the virus isolate and the cultivar, the virus can cause irregular yellow stripes, flattening, downward curling, crinkling, stunted growth and bulb size reduction.

During storage, deterioration and pre-mature sprouting of the bulbs might also occur.

In Ethiopia, garlic is one of the most important vegetable crops produced by small and largescale growers for both local consumption as well as for export to Europe, the Middle East and the USA. The crop is mainly cultivated at mid and high altitudes of the country such as Adet, Ambo, Debre-work, Debre Zeit, Guder, Jimma, Sinana and other areas (Metasebia and Shimelis 1998; Getachew and Asfaw 2000). However, the production of the crop is affected by a number of factors and the total production and productivity of the crop is low (Yeshiwas *et al.* 2018). Debebe (2017) reported that garlic is infected by different viruses including some potyviruses. Therefore, the target of the present study was to identify the *Potyvirus* and detect the presence of OYDV infection in garlic samples from West Shewa zone of Ethiopia.

Materials and Methods

Sample collection

Ten garlic plants showing characteristic symptoms of OYDV infection, including yellow streaks on leaves and dwarfing were collected from the West Shewa zone, Ambo, Ethiopia in January 2018 (Table 1). The cloves from these naturally infected plants were stored at 8–10°C for 6 months for further study.

RNA extraction

Total RNA was extracted from garlic cloves using the RNeasy plant Mini kit (Qiagen, Germany) according to the manufacturer's instructions. The quantity and the quality of the RNA were analyzed in a UV visible double beam Spectro-photometer (Shimadzu-1800, Japan).

RT–PCR

For detection of OYDV previously published primers were used (Majumder and Baranwal 2014). The first strand of cDNA was synthesized using 10 µl of total RNA and reverse transcription (RT) mixture containing reverse primer of OYDV 0.2 µM, 20U M-MuLV Reverse Trancriptase (Fermentas, USA), 4 µl of 5X reaction buffer and 0.3 mM dNTPs. The total reaction mixture of 20 µl was incubated at 42°C for 45 min. The enzyme was inactivated by heating at 70°C for 10 min.

Table 1. Sample collection areas and the number of samples collected

No.*	Sample collection area	Crop	Sample No.
1	Awaro	garlic	1, 2, 3
2	Gadisa kiflo	garlic	4, 5, 6, 7
3	Gatira	garlic	8, 9, 10

*seria 1

PCR

The obtained cDNA was subjected to polymerase chain reaction (PCR) amplification using both forward and reverse primers designed to amplify the partial coat protein gene region of OYDV. PCR amplification was performed in a Bio-Rad T100 thermocycler. A 50 µl reaction volume of PCR mix contained 10 µl of first strand cDNA, 2 µM of each forward and reverse primer, and 1.5 mM of $MgCl_2$, 5 µl of 10X reaction buffer, 0.2 mM dNTPs and 5U of Taq DNA polymerase (Fermentas, Lithuania). The rest of the volume was made up with nuclease free water. The temperature profile consisted of a denaturation step at 94°C for 5 min, then 30 cycles of 45 s at 94°C, 30 s at an annealing temperature of 55°C, 1 min at 72°C and one final extension step at 72°C for 10 min.

Gel electrophoresis and sequencing of PCR product

Ten microliters of the amplified product were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide and photographed under UV illumination with an imaging system (Bio-Rad XR documentation system). Finally, the amplified product from sample no. 5 was sent for sequencing with forward primer to Barcode Biosciences, Bangalore, India. The sequencing was repeated thrice with the same sample.

Insilco analysis

The Basic Local Alignment search Tool (BLAST) program available at the NCBI website online (https://www.ncbi.nlm.nih.gov/nuccore) was used to identify related sequences. The sequence was compared with various homologous sequences of OYDV previously reported from different parts of the world available in the GenBank database (Table 2). The phylogenic neighbor-joining trees and evolutionary analysis were conducted using Molecular Evolutionary Genetics Analysis tool version 7.0 (Kumar *et al.* 2016) and BioEdit software. The phylogenetic tree was generated using the neighbor joining (NJ) method.

Results and Discussion

The RT-PCR amplification of the partial coat protein gene using gene specific primer resulted in the amplicons of ~318 bp fragments from garlic cloves (Fig. 1).

Table 2. List of *Onion yellow dwarf virus* (OYDV) isolates used in this study

Accession number	Isolate	Origin of OYDV	Host
		Ethiopia	*Allium sativum*
HQ258894	MS/SW1	Australia	*Allium sativum*
JX429964	SG1	Spain	*Allium sativum*
AJ409311	sd: Jinxiang	China	*Allium sativum*
KJ451436	RR1	India	*Allium cepa*
AB000841		Japan	
AJ307033	Xixia	China	*Allium sativum*
JN127342	Bate6	Australia	*Allium sativum*
KP862052	OV-7	India	*Allium cepa*
EU045558	Karnal	India	*Allium sativum*
AB000843		Japan	shallot
KF632714	OYDV-SW9-Arg2	Argentina	*Allium sativum*
AJ510223	Yuhang	China	*Allium sativum*
DQ519034		India	*Allium sativum*
DQ925455	OYDV-VN/L5	Vietnam	*Allium porrum*
MF925709	03-Iranian	Pakistan	*Allium sativum*
DQ925454	OYDV-VN/L4	Vietnam	*Allium porrum*
KF623535	5.L	Italy	*Allium cepa*
KF623540	27.T.Se	Italy	*Allium cepa*
MF925707	06-Chinese	Pakistan	*Allium sativum*
JX433019	OYDV-Se	Argentina	*Allium cepa*
HM473189	Egyptian	Egypt	*Allium sativum*
KT225546	OYDV-Egyptian	Egypt	*Allium cepa*
FR873734		India: New Delhi	*Allium sativum*
KU204909	Bantul_13	Indonesia: Bantul	shallot leaves
KF862691	220	Poland	*Allium sativum*
NC_005029	Yuhang	China	*Allium sativum*

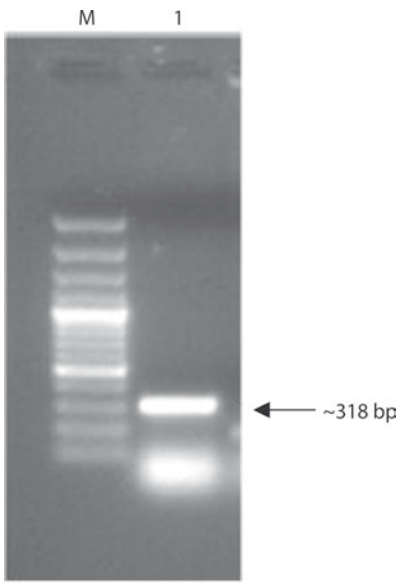

Fig. 1. Gel picture showing amplification of partial coat protein of *Onion yellow dwarf virus* (OYDV). Lane M – 100 bp DNA marker, lane 1 – OYDV

The direct sequencing of the partial coat protein gene region produced ~300 bp long nucleotide sequences. The different reactions produced read with 99–100% similarity. The sequence was analyzed by BLAST and compared with the other homologous sequences of OYDV isolates from different parts of the world. It showed 89.47% identity with a garlic isolate from Australia (GenBank accession no. HQ258894.1), 89.29% with an isolate from Spain (accession no. JX429964.1) and 89.21% with a Chinese isolate (accession no. AJ409311.1). The sequence comparison confirmed the identity of the Ethiopian isolate based on the similarity percent with homologous isolates available in the GenBank indicating the presence of OYDV in garlic from Ethiopia.

Multiple alignment of nucleotide sequences of 26 OYDV isolates available in the GenBank and the garlic isolate from Ethiopia was done using BioEdit software. The sequence identity matrix of OYDV

Table 3. Sequence identity percentage of partial coat protein gene sequence of *Onion yellow dwarf virus* (OYDV)

Seq.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1	ID																										
2	88.9	ID																									
3	88.9	97.4	ID																								
4	88.7	91.3	91.3	ID																							
5	86.4	91.3	90.6	86.0	ID																						
6	85.1	92.5	90.0	85.4	85.8	ID																					
7	85.1	90.3	89.3	85.0	85.8	92.4	ID																				
8	83.8	85.5	85.5	85.1	83.0	88.2	91.8	ID																			
9	83.2	85.0	83.6	83.1	82.2	86.3	86.0	82.5	ID																		
10	85.6	87.9	86.8	88.0	86.8	83.2	85.4	82.6	84.0	ID																	
11	84.2	86.4	85.0	83.8	84.0	87.8	87.4	85.7	88.3	85.6	ID																
12	84.6	87.5	86.1	84.5	84.0	90.6	95.6	90.7	85.9	84.2	88.4	ID															
13	84.6	87.5	86.1	84.5	84.0	90.6	95.6	90.7	85.9	84.2	88.4	100	ID														
14	85.9	86.4	85.7	86.9	92.8	81.9	81.5	79.5	83.7	91.2	83.5	82.5	82.5	ID													
15	83.9	87.2	85.8	86.0	85.1	89.6	92.8	94.6	83.8	86.0	87.8	92.8	92.8	82.9	ID												
16	85.1	87.5	87.5	89.0	88.9	83.6	84.7	83.4	83.7	90.1	84.5	85.0	85.0	93.0	86.1	ID											
17	83.5	88.2	87.5	84.5	86.1	89.6	93.5	88.9	86.6	84.2	87.0	93.5	93.5	83.9	91.3	86.0	ID										
18	83.0	82.2	81.4	81.1	81.9	84.5	83.5	82.2	81.9	82.2	84.1	84.4	84.4	81.2	84.5	82.3	83.3	ID									
19	82.7	81.8	81.1	81.5	81.5	84.2	83.1	82.5	81.5	81.8	83.7	84.1	84.1	80.8	84.1	81.9	83.0	99.6	ID								
20	84.1	86.4	85.3	87.6	86.0	82.2	83.6	80.6	83.0	90.1	83.5	82.5	82.5	91.1	83.9	93.0	83.5	81.5	81.2	ID							
21	81.2	80.4	80.4	80.0	80.1	83.1	82.0	81.4	80.8	80.7	82.6	83.0	83.0	79.7	83.0	81.5	82.6	97.0	97.4	80.1	ID						
22	81.0	82.5	81.4	80.2	81.9	83.2	83.6	81.6	81.3	83.1	85.3	83.1	83.1	81.0	84.3	82.1	84.2	79.9	79.5	81.4	78.1	ID					
23	39.1	39.2	40.0	38.2	39.2	39.3	41.8	39.3	39.3	37.6	36.9	39.7	39.7	38.9	39.3	40.0	40.0	41.1	40.7	39.2	41.4	37.6	ID				
24	37.7	38.9	39.2	37.8	38.2	37.2	40.0	37.9	37.6	36.9	36.2	38.3	38.3	37.8	37.9	38.5	38.6	39.0	38.6	37.5	39.3	36.5	92.1	ID			
25	36.7	36.4	36.1	35.4	36.8	36.2	36.5	36.5	37.2	34.4	35.5	34.8	34.8	36.4	35.5	36.8	36.2	37.9	37.6	36.1	37.9	34.4	83.1	81.0	ID		
26	39.1	38.9	38.9	38.9	38.9	38.6	40.7	38.6	39.3	36.9	37.2	39.7	39.7	38.9	39.3	40.0	39.7	40.7	40.4	38.5	41.1	36.5	91.7	92.1	81.7	ID	
27	84.6	87.5	86.1	84.5	84.0	90.6	95.6	90.7	85.9	84.2	88.4	100	100	82.5	92.8	85.0	93.5	84.4	84.1	82.5	83.0	83.1	39.7	38.3	34.8	39.7	ID

In the table, accession numbers and their corresponding countries of origin are in the following manner: 1. Ethiopia (MK812899); 2. Australia (HQ258894); 3. Spain (JX429964); 4. China (AJ409311); 5. India (KJ451436); 6. Japan (AB000841); 7. China (AJ307033); 8. Australia (JN127342); 9. India (KP862052); 10. India (EU045558); 11. Japan (AB000843); 12. Argentina (KF632714); 13. China (AJ510223); 14. India (DQ519034); 15. Vietnam (DQ925455); 16. Pakistan (MF925709); 17. Vietnam (DQ925454); 18. Italy (KF623535); 19. Italy (KF623540); 20. Pakistan (MF925707); 21. Argentina (JX433019); 22. Egypt (HM473189); 23. Egypt (KT225546); 24. India: New Delhi (FR873734); 25. Indonesia: Bantul (KU204909); 26. Poland (KF862691); 27. China (NC_005029.1)

isolates including the isolate from this study revealed that it shared 36.7% to 88.9% sequence identity with isolates from around the world (Table 3). A phylogenetic tree based on the partial coat protein gene sequence of OYDV is shown in Figure 2. Based on the analysis the new Ethiopian isolate was found to be a separate branch though clustering together with the isolates from Australia (HQ258894), Spain (JX429964), and China (AJ409311) (Fig. 2). The phylogenetic tree and BLAST analysis indicated that the Ethiopian isolate does not have much identity with the rest of the African isolates from Egypt, Nigeria or Sudan. This suggests that the Ethiopian isolate may have a different origin than the rest of the African isolates of OYDV. Germplasm exchange by international trade may be the reason for this variation (Wylie *et al.* 2014).

The partial coat protein gene sequence of the Ethiopian isolate, generated in the present study was submitted to GenBank (accession no. MK812899). To the best of our knowledge, this is the first report of OYDV in garlic from the West Shewa zone of Ethiopia. Confirmation that the local garlic is infected with OYDV will lead to the development of a management strategy. Raising virus-free plants by meristem-tip culture and

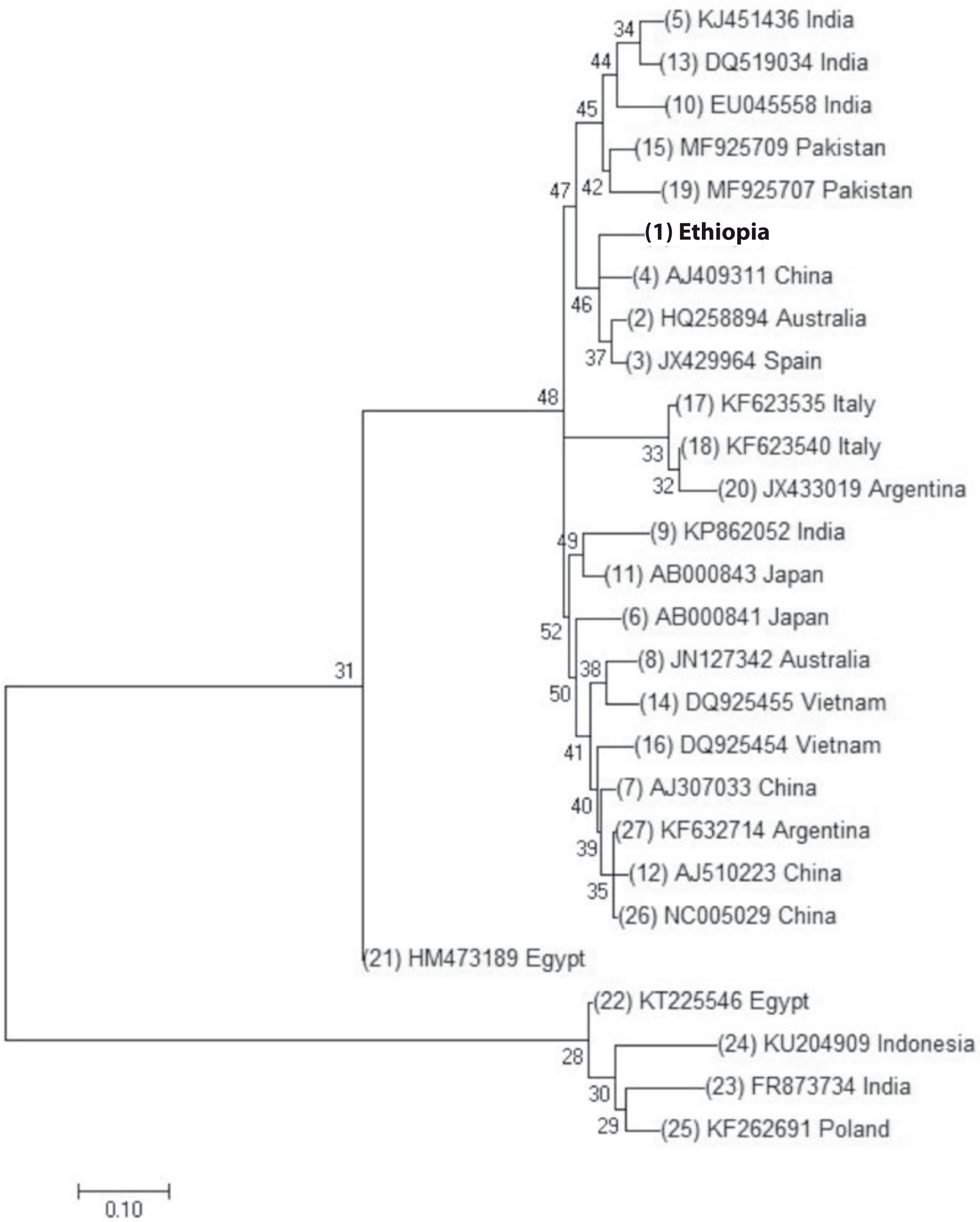

Fig. 2. Neighbor Joining tree based on the nucleotide sequences of partial coat protein gene of *Onion yellow dwarf virus* (OYDV), showing phylogenetic relationships of the Ethiopian isolate with others from different parts of the world

then multiplication of these plants under aphid-free conditions is the only method for controlling these viruses (Conci *et al.* 2010). Each step in these programs requires an assay for ensuring virus free conditions. The indexing method developed in this study will assist in these programs. This assay which has been developed will also be useful for quarantine.

Acknowledgements

Authors are thankful to the Department of Biotechnology, Sharda University, for providing the facilities.

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