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# Detection of Tomato Yellow Ring Orthotospovirus in North Khorasan Province, Iran

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## Abstract

Tomato is one of the most important vegetables in the world, cultivated both in the field and in greenhouses. Orthotospoviruses, are among the most economically important pathogens of tomato and cause significant damage to this crop worldwide. Tomato spotted wilt orthotospovirus (TSWV) and Tomato yellow ring orthotospovirus (TYRV) are two species of orthotospoviruses that have been described as potential threats to tomatoes worldwide. In a study of tomato infection in greenhouses in North Khorasan province (Bojnord) with Tomato yellow ring orthotospovirus, plants with symptoms of yellowing and necrosis of leaves and necrosis of stems were observed. Infection of these samples with TYRV was confirmed using test plants, DAS-ELISA, and reverse transcription (RT)-PCR. Infection of *Petunia hybrida* and *Chenopodium quinoa* with this virus was localized, while *Nicotiana benthamiana*, *N. glutinosa*, *Datura stramonium*, and Moneymaker tomato had systemic infection. Using DAS-ELISA and a specific antibody (prepared against the nucleocapsid protein) to detect TYRV, Iris yellow spot orthotospovirus, TSWV, Impatiens necrotic spot orthotospovirus, Groundnut bud necrosis orthotospovirus, Groundnut ring spot orthotospovirus, Tomato chlorotic spot orthotospovirus, and Chrysanthemum stem necrosis orthotospovirus, *N. benthamiana* plants with systemic infection were examined and confirmed to be infected with TYRV only. In the study of samples using RT-PCR, TYRV-specific primers and total RNA extracted from infected *N. benthamiana* leaves, a fragment of approximately 825 bp in size, which included the nucleocapsid protein gene, was amplified. This fragment was sequenced (HQ154131) and a study of amino acid sequence similarity in GenBank showed that the amino acid sequence of this fragment is 99% similar to the amino acid sequence of TYRV from Fars (ABH07703), Tehran (AAV98587), and Markazi (ACT09488) provinces in Iran. This is the first report of TYRV from North Khorasan province (Bojnord) in Iran.

***Key Words:*** *TYRV, Orthotospovirus, Bojnord, Tomato*

## Introduction

Tomato is one of the most consumed vegetables in the world due to its nutrients, minerals, and antioxidants (Du et al. 2017). The area harvested of tomato in the world and Iran in 2023 is reported to be approximately 5.4 million and 74,000 hectares, respectively. In 2023, based on the latest available data, China, India, Türkiye, the United States, and Egypt are the top five tomato producing countries in the world, accounting for more than 60% of global tomato production (FAO, 2023). Tomato production is for both domestic consumption and international trade, so it is available throughout the year (Caruso et al., 2022). Monoculture conditions of tomatoes cause the emergence of many pathogens, thus threatening the quantitative and qualitative yield of production (Hanssen et al., 2010l; Caruso et al., 2022). Tomato plants are susceptible to more than 200 diseases caused by different pathogens, both in the field and after harvest (Singh et al., 2017). Fungi, oomycetes, bacteria, phytoplasmas, viruses, and viroids cause the most important tomato diseases. Plant viruses, which are one of the main causes of diseases affecting food production worldwide, are estimated to have an economic impact of more than $30 billion per annum (Sastry & Zitter, 2014; Caruso et al., 2022). The genus *Orthotospovirus*, which belong to the family *Tospoviridae* and infect more than 1000 plant species of 82 families, cause serious damage to agriculture worldwide by infecting economically important plants (Wang et al., 2022). Yellow spots, mottling, wrinkle, withering, and even death are common symptoms of plants infected by the *Tospoviridae* family (Pappu et al., 2009). Thrips transmit members of the *Tospoviridae* family in a persistent manner (Oliver and Whitfield, 2016). These viruses have a wide host range. For example, *Tomato spotted wilt orthotospovirus* (TSWV) infects over 900 species belonging to more than 90 monocotyledonous and dicotyledonous plant families (Pappu et al., 2009, Wang et al., 2022). Orthopoviruses have been known to infect various plant species in Iran (Golnaraghi et al., 2001; Ghotbi et al., 2005; Beikzadeh et al., 2012b). The host range and geographic distribution of species of the genus *Orthopovirus* are increasing (Pappu et al., 2009) and in recent years, new species belonging to this genus have been proposed (Zarzynska-Nowak). One of these species is *Tomato yellow ring orthotospovirus* (TYRV), which was first identified on tomato in Iran (Hassani-Mehraban et al., 2005), and later detected in other plants such as potato, soybean, chrysanthemum, cineraria, gazania, anemone, and alstroemeria in Iran (Hassani-Mehraban et al., 2007; Rasoulpour and Izadpanah, 2007; Golnaraghi et al., 2008; Beikzadeh et al., 2012a; Mortazavi et al., 2013). The virus has also been reported

from Poland and Kenya (Birithia et al., 2012; Zarzyńska-Nowak et al., 2016) and has a wide host range, including ornamentals, weeds, and economically important crops in the Solanaceae and Fabaceae families. Tomato yellow ring orthotospovirus infects crops grown in the field and protected environments (Karavina, 2022). In most plants, this virus causes necrotic lesions on leaves and stems, which leads to a decrease in the quality and quantity of the products. On infected tomato fruits, bright yellow ring patterns are formed (Hassani-Mehraban et al., 2005). This study was conducted to detect this virus in tomato greenhouses in North Khorasan province, Iran.

**Materials and Methods**

During this study, greenhouse-grown tomato plants with symptoms of yellowing and necrotic leaves resembling those associated with orthotopoviruses, were observed in North Khorasan province (Figure 1).

Figure 1. Symptoms of infection on tomato leaves

Fresh leaves of the infected plants were ground with cold 0.01M phosphate buffer (pH 7.0) containing 0.1% sodium sulfite. The plant sap was mechanically inoculated onto the test plants. The inoculated plants were maintained for one to two weeks under normal greenhouse conditions for symptom expression (Hassani-Mehraban et al., 2005). The symptomatic plants were tested by double antibody sandwich (DAS)-ELISA using polyclonal antisera (Wageningen university) against the eight of the most important orthotopoviruses: TYRV, TSWV, *Iris yellow spot orthotopovirus* (IYSV), *Impatiens necrotic spot orthotopovirus* (INSV), *Groundnut bud necrosis orthotopovirus* (GBNV), *Groundnut ringspot orthotopovirus* (GRSV), *Tomato chlorotic spot orthotopovirus* (TCSV), and *Chrysanthemum stem necrosis orthotopovirus* (CSNV). The absorbance of the yellow reaction mixture at 405 nm is then read using an ELISA plate reader (Clark and Adams, 1977; Williams et al., 2001; de Ávila et al., 1990). The presence of TYRV in ELISA-positive *Nicotiana benthamiana* plants with systemic symptoms was further confirmed by conventional reverse transcription (RT)-PCR using specific primer [N-start: 5'-ATGGCTACCGCACGAGTG-3'(F) and N-stop: 5'-TTAAAATGCATC-3'(R)] complementary to the N gene. The primer pair supposed to amplify about 825 bp fragment (Hassani-Mehraban et al., 2005). Total RNA was extracted from *N. benthamiana*-infected plants using Trizol reagent as recommended by the manufacturer's instructions (Invitrogen) and used as template for cDNA synthesis. PCR reactions were performed by pre-heating at 94ºC for 5 min followed by 30 cycles of 30 sec of denaturation at 92ºC, 30 sec of annealing at 55ºC and 1 min for extension at 72ºC. Finally, the amplified DNA was incubated at 72ºC for 7 min to accomplish a final extension. The PCR reactions were subjected to electrophoresis in 1% agarose gel in TAE buffer and then stained with ethidium bromide (Pozzer et al., 1999; Eiras et al., 2001). The PCR product was purified (GFX™ PCR DNA and Gel Band Purification Kit; GE Healthcare UK Limited) and sequenced (Sanger et al., 1977). Sequence data were compared with those available in GenBank, using NCBI/BLAST, to search for related sequences.

**Results**

The results obtained from experimental host range studies by mechanical inoculation are summarized in Table 1. Systemic infection of inoculated *N. benthaminana* were confirmed using DAS-ELISA. Of the eight antibodies prepared against the N protein of different orthotospoviruses used in this test, the TYRV antibody reacted positively with extracts prepared from *N. benthaminana* samples (Figure 3). No positive reactions were observed with antibodies to other orthotospoviruses.

The infection of *N. benthamiana* with TYRV was confirmed using PCR. In the PCR test using primers designed based on the N gene of this virus, this gene was amplified and after electrophoresis in agarose gel, a DNA fragment of about 825 bp was observed. No band was observed in the negative control (healthy) sample (Figure 4).

Symptoms observed on the collected samples included yellowing and necrosis of tomato leaves. The characteristic symptoms of plant infection with orthotospoviruses are usually diverse and include chlorotic and necrotic lesions, mottling, leaf deformation, systemic necrosis, vein yellowing, and ringspot (Peters and Goldbach, 1995; Kritzman et al., 2001; Ghent et al., 2004; Ghent et al., 2006). The collected tomato samples showed symptoms similar to those of orthotospoviruses infection. These samples were identified as infected with orthotospoviruses using host range, serology, and polymerase chain reaction tests.

Table 1. Reaction of several host plants to virus infecting tomato after mechanical inoculation.

| Test Plant | Symptoms | |
|---|---|---|
| | local | systemic |
| *Petunia hybrida* | NL | – |
| *Nicotiana benthamiana* | CS | Mo, LD, LN |
| *Datura stramonium* | CS | Mo, NS |
| *Chenopodium quinoa* | NL | – |
| *N. glutinosa* | NS | NS |
| Moneymaker tomato | NS | LY, LN |

CS: chlorotic spot, LD: leaf deformation, LN: leaf necrosis, LY: leaf yellowing Mo: mottling, NL: necrotic lesion, NS: necrotic spot, -: no reaction

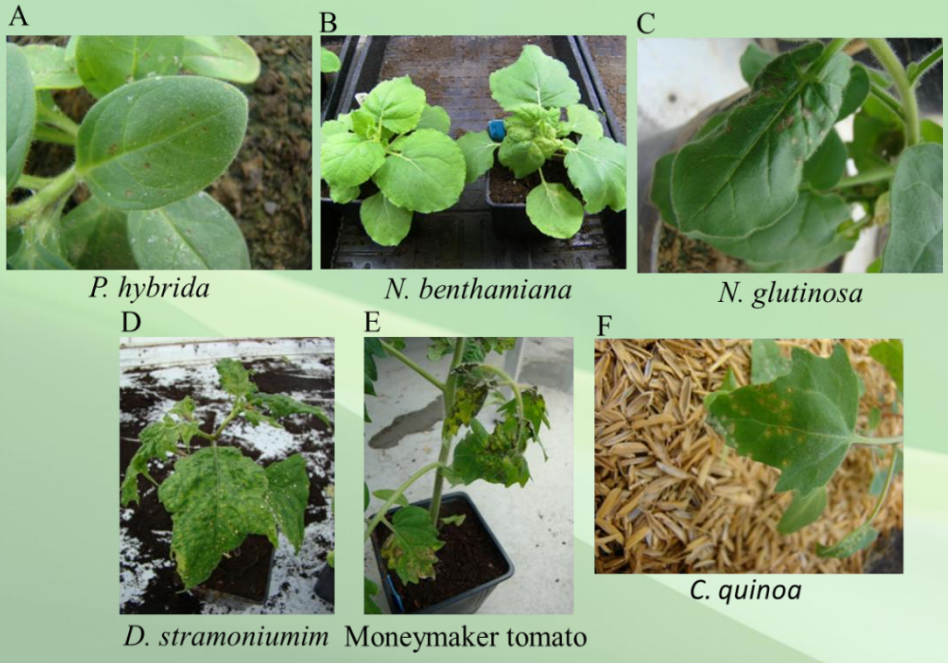


Figure 2. Symptoms of infection on test plants

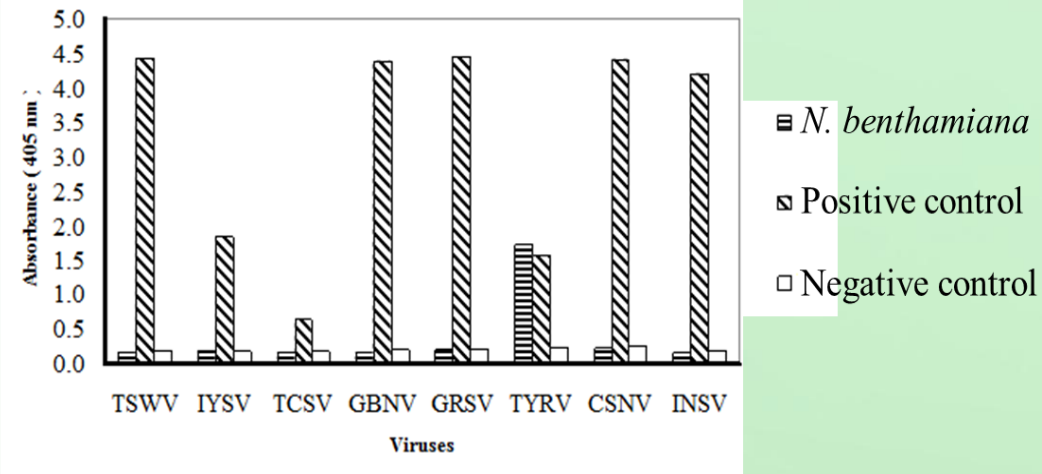


Figure 3. Mean absorbance of *N. benthaminana* sample, positive and negative controls at 405 nm

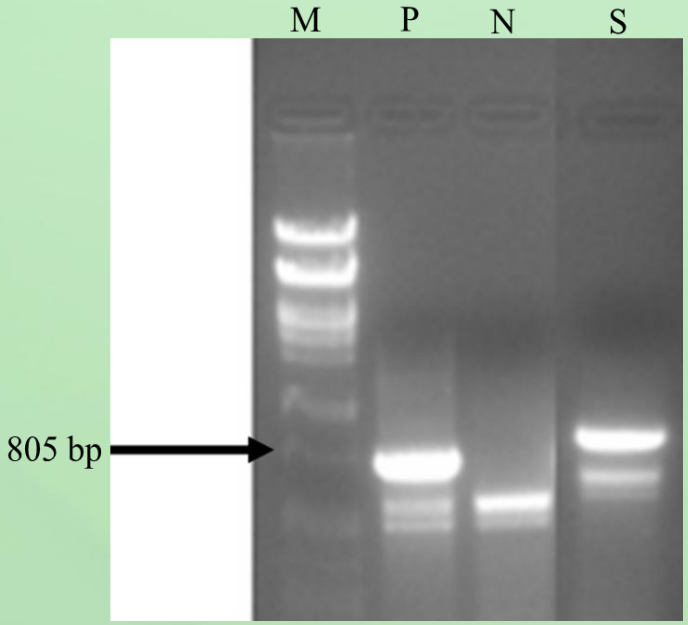


Figure 4. PCR result (M: marker, P: positive control, N: negative control, S: *N. benthamiana*)

**Discussion**

Host range studies showed that the tomato pathogen is mechanically transmitted. Symptoms of infection on *Petunia hybrida* and *C. quinoa* appeared as localized necrotic spots, while on *N. benthamiana*, *N. glutinosa*, *D. stramoniumim* and Moneymaker tomato they were systemic (Figure 2). Therefore, symptomatology and host range indicates that the samples examined were infected with an orthotospovirus and our results are consistent with several prior studies (Bezerra et al., 1999; Hassani-Mehraban et al., 2005; Rasoulpour & Izadpanah, 2007; Dong et al., 2008; Zheng et al., 2008; Ciuffo et al., 2009; Hassani-Mehraban et al., 2010).

The N-start primer is identical to the first 18 nucleotides of the N gene of TYRV from Iran with accession number AY686718, and the N-stop primer is complementary to the last 12 nucleotides of this gene in this virus. This gene has 825 nucleotides (Hassani-Mehraban et al., 2005), so it is expected that a fragment of 825 bp will be amplified in the PCR test. The amino acid sequence of the N protein reported in this study has been submitted to GenBank under Acc. No. HQ154131. A BLAST search showed that this fragment had 99% identity to TYRV from Markazi (GenBank Acc. No. ACT09488) and Fars (GenBank Acc. No. ABH07703) provinces and TYRV-t from Tehran (GenBank Acc. No. AAV98587) province in Iran. Since the amino acid sequence similarity of the N protein between a new virus and other confirmed orthotospoviruses must be less than 90% to be confirmed as a new species (de Ávila et al., 1990), the isolate examined in this study is not a new species and is confirmed as a Tomato yellow ring orthotopovirus solate based on the amino acid sequence similarity of the N protein.

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