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The New Report of *Artichoke latent virus* (ArLV) From Globe Artichoke in Turkey

Türkiye'de Enginarda *Artichoke latent virüsü* (ArLV)'nün Yeni Bildirimi

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Globe Artichoke, Turkey, ArLV, DAS-ELISA, RT-PCR

ABSTRACT

In recent years, some of artichoke growers in Aegean region of Turkey stated that globe artichoke (*Cynara cardunculus L.* subsp. *scolymus* (L.) Hayek) plants in their fields have showed virus-like symptoms. So, in order to identify viruses infecting globe artichoke, a survey was conducted between April 2011 and March 2012 and totally 50 globe artichoke samples were collected from the production areas in the mentioned region from which was provided more than 30 % of total globe artichoke production in Turkey. The samples were analyzed by DAS-ELISA, RT-PCR and biological indexing methods for *Artichoke latent virus* (ArLV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV), *Broad bean wilt virus* (BBWV) and *Raspberry ringspot virus* (RpRSV). TMV, TSWV, BBWV and RpRSV were not detected in all of samples tested. In RT-PCR, amplicons of the expected size (ca. 282 bp) were obtained from 9 out of 35 symptomatic plant samples bearing virus-like symptoms. The amplified and sequenced 9 PCR products (Accession No: KC622053) showed 86 % nucleotide sequence identity with "*Artichoke latent virus* mRNA unknown function (GenBank Accession No:X87255.1)" isolate in BlastN analysis. Moreover, when mechanically inoculated with the sap from plant samples, that were positive for ArLV in RT-PCR, selected test plants produced the typical symptoms of ArLV infection. As to the result of the literature review on the topic in question, this is the latest report of natural ArLV infection from globe artichoke in Turkey.

Anahtar Sözcükler:

Enginar, Türkiye, ArLV, DAS-ELISA, RT-PCR

ÖZET

Son yıllarda Ege Bölgesi'ndeki bazı enginar üreticilerinin tarlalarındaki enginar bitkilerinde (*Cynara cardunculus L.* subsp. *scolymus* (L.) Hayek) virüs benzeri belirtiler olduğunu belirtmeleri üzerine, enginar bitkilerinde enfeksiyon oluşturan virüslerin tanılanması için Nisan 2011 ve Mart 2012 tarihleri arasında bir survey çalışması yürütülmüş ve Türkiye enginar üretiminin %30'dan fazlasının sağlandığı üretim alanlarından toplam 50 örnek alınmıştır. Alınan örnekler DAS-ELISA, RT-PCR ve biyolojik indeksleme yöntemleri kullanılarak *Artichoke latent virus* (ArLV), *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV), *Broad bean wilt virus* (BBWV) ve *Raspberry ringspot virus* (RpRSV) varlığı açısından testlenmiştir.Testlenen tüm örneklerde TMV, TSWV, BBWV ve RpRSV enfeksiyonlarının olmadığı tespit edilmiştir. RT-PCR çalışmalarında virüs benzeri belirtiler gösteren 35 örneğin 9'unda 282 bp'de ArLV amplikonları elde edilmiştir. Sekanslanan 9 PCR ürünü (Accession No: KC622053) ile yürütülen BlastN analizlerinde "*Artichoke latent virüs* mRNA unknown function (GenBank Accession No:X87255.1)" izolatı ile % 86 oranında benzerlik olduğu bulunmuştur. Ayrıca, RT-PCR çalışmalarında bu virüs ile enfekteli oldukları saptanan örnekler kullanılarak yapılan mekanik inokulasyon sonucunda, bazı test bitkilerinde ArLV enfeksiyonuna özgü tipik simptomların ortaya çıktığı görülmüştür. Mevcut bilgilerimize göre, bu çalışmadaki bulgular Türkiye'de enginar bitkilerinde ArLV'nin doğal enfeksiyonunun güncel durumunu yansıtan kayıt niteliğindedir.

INTRODUCTION

Globe artichoke [*Cynara cardunculus* L. subsp. *scolymus* (L.) Hayek], belonging to the *Asteraceae* (*Compositae*) family, is a traditionally grown vegetable in Mediterranean basin and has several uses including biomass production, paper pulp, human consumption and human health with pharmaceutical compounds (Ciancolini, 2012).

The globe artichoke is produced on about 125 351 hectares all around the world and total production is about 1 634 219 tons (FAO, 2014). Egypt, Italy, Spain, Peru and Argentina are top five countries of the world production. Turkey is ranked 11th in the world with 32 173 tons. In Turkey, artichoke production areas were located in western parts of Turkey, mainly in Aegean and Eastern Marmara regions.

Many pathogens can infect globe artichoke as in all cultivated crops and these infections can be lead to serious economic losses and reduce yield and quality. Martelli and Gallitelli (2008) mentioned that 24 viruses can cause diseases on globe artichoke. It is reported that the *Artichoke latent virus* (ArLV), *Artichoke yellow ringspot virus* (AYRSV), *Cucumber mosaic virus* (CMV), *Tobacco mosaic virus* (TMV) and *Artichoke Italian latent virus* (AILV) can be isolated singly or in mixed infections from globe artichoke production areas in many countries of Europe and in Mediterranean basin (Gallitelli *et al.*, 2004; Acquadro *et al.*, 2010). The findings from these studies revealed that the majority of these viruses were transmitted by infected propagation materials and some insect vectors.

In recent years, it has been observed certain symptoms caused by viruses and yield reduction in some of the artichoke plantations in especially İzmir and Aydın provinces of Aegean region. Due to the lack of information about viral agents affecting the globe artichoke in Turkey, the present study was carried out to determine viral agents and their incidence in the important globe artichoke production areas of Aegean region of Turkey.

MATERIAL and METHODS

To find out the viral infections in globe artichoke production areas, the surveys were conducted between April 2011 and March 2012 in Izmir and Aydın provinces which provided more than 30 % of total globe artichoke production of Turkey. Leaf tissues from 35 symptomatic and 15 symptomless plants were collected from the fields that globe artichoke plants with virus-like symptoms were present. Most common symptoms in symptomatic plants were chlorotic mottling or mosaic and distortion on leaves, the reduction in head size and the retardation in plant growth (Figure 1).

a) Mosaic on leaves, b) Reduction in head size, c) Dwarfing in plants

Figure 1. Some symptoms of ArLV on globe artichoke plants.

The samples collected were tested for the presence of *Tobacco mosaic virus* (TMV), *Tomato spotted wilt virus* (TSWV), *Broad bean wilt virus* (BBWV) and *Raspberry ringspot virus* (RpRSV) by DAS-ELISA according to Clark and Adams (1977) using diagnostic kits (Bioreba AG, Switzerland and Loewe® Biochemica GmbH, Germany) in accordance with the instructions of manufacturers.

The detection of *Artichoke latent virus* (ArLV), TMV, TSWV, BBWV and RpRSV also conducted with RT-PCR

and total RNAs were extracted according to Foissac *et al.* (2005) silica capture method from fresh leaves of plant samples. The synthesis of complementary DNA (cDNA) was performed by Fermentas First Strand cDNA Synthesis Kit according to manufacturer's protocol.

The assays were performed with highly specific primers to *Artichoke latent virus* ArLV5BL: 5'-GAT CTA GCG ATA CAC ATG CAC AAC C-3' and ArLV3BL: 5'- CGC TCA AGC TCT CGA ACT AAC TGA AC-3' (Lumia *et al.,* 2003). RT-PCR assays for TMV, TSWV, BBWV and RpRSV were performed with using highly specific primers designed by Silva *et al.* (2008), Lotos *et al.* (2009), Ferriol *et al.* (2011) and Treiber (2010), respectively.

The amplified and sequenced PCR products compared with other world isolates using BlastN. A maximum likehood dendrogram was constructed using Mega 5.10 software with ClustalW alignment method (Tamura *et al.*, 2011; Noveriza *et al.*, 2012) with the aim of determining the homology of our sequences to other world isolates and 21 well known members of *Potyvirus* genus.

Moreover, the sap from plant specimens that were positive for ArLV in RT-PCR was mechanically inoculated to certain indicator plants including *Chenopodium amaranticolor*, *C. quinoa, Nicotiana benthamiana*, *N. clevelandii* and *Petunia hybrida* (DPV, 2012; PVO, 2012). Following symptom development, symptomatic plants were tested with DAS-ELISA and RT-PCR assays to confirm the viral agent of the infections in these plants.

RESULTS and DISCUSSION

DAS-ELISA and RT-PCR assays revealed that the specimens in our study were not infected with TMV, TSWV, BBWV and RpRSV. Due to the lack of commercial ELISA kits against ArLV, RT-PCR and biological indexing were used for its identification.

The results of RT-PCR tests performed with all samples for TMV, TSWV, BBWV and RpRSV matched substantially with DAS-ELISA tests. In RT-PCR for ArLV amplicons of expected size (ca. 282 bp) were obtained from 9 symptomatic plant specimens (Figure 2) that had symptoms of chlorotic mottling or mosaic and distortion on leaves, the reduction in head size and the retardation in plant growth regarded as typical symptoms of ArLV on globe artichokes by Martelli and Gallitelli (2008). The mean incidence of diseased plants ranged from 2 % to 5 % in the artichoke fields. Sequenced PCR products were submitted to NCBI GenBank (Accession No: KC622053) and expressed 86 % nucleotide sequence identity with "*Artichoke latent virus* mRNA unknown function (GenBank Accession No:X87255.1)" isolate in BlastN analysis.

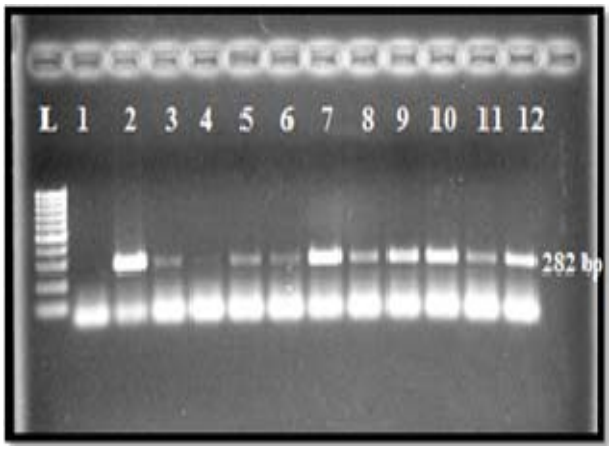

Figure 2. Detection of ArLV with RT-PCR. Lane 1 shows negative control, 2 positive control and 3, 5-12 positive samples.

According to the RT-PCR test results, a phylogenetic tree was constructed by Mega 5.10 software in order to search for homology to other isolates (Figure 3).

After mechanical inoculation of leaf sap from infected artichoke samples, necrotic local lesions occurred on the leaves of *Chenopodium amaranticolor* and *C. quinoa* while *Nicotiana benthamiana*, *N. clevelandii* and *Petunia hybrida* exhibited systemic mottling or mosaic (Martelli and Gallitelli, 2008; DPV, 2012; PVO, 2012) After the formation of symptoms, RT-PCR tests were conducted to confirm ArLV infection on indicator plants. RT-PCR and mechanical inoculation assays provided consistent results on that ArLV was the causal agent of the symptoms mentioned on globe artichoke plants in Izmir province.

ArLV is a ssRNA contained virus in 730 nm long (van Regenmortel, 2000; Gallitelli *et al.,* 2004). It can transmitted by *Myzus persicae*, *Brachycaudus cardui* and *Aphis fabae* in non-persistent manner (Rana *et al.,* 1982). ArLV has very limited host range and *C. scolymus*, *C. cardunculus*, *C. syriaca* and *Petunia hybrida* were natural hosts of this virus (Gallitelli, 1991).

First time ArLV was isolated from symptomless artichoke plants in California by Costa *et al.* (1959). The previous studies indicated that ArLV was economical important factor that can be restricted the globe artichoke production in Mediterranean basin and spread over large areas. It was indicated that due to the transmission of virus by aphid vectors and vegetation production materials, infection rate was very high (Rana and Russo, 1980; Ortega *et al.,* 2000; Pasquini and Barba, 2003).

To our knowledge, this is the first report of natural ArLV infection of globe artichoke in Turkey.

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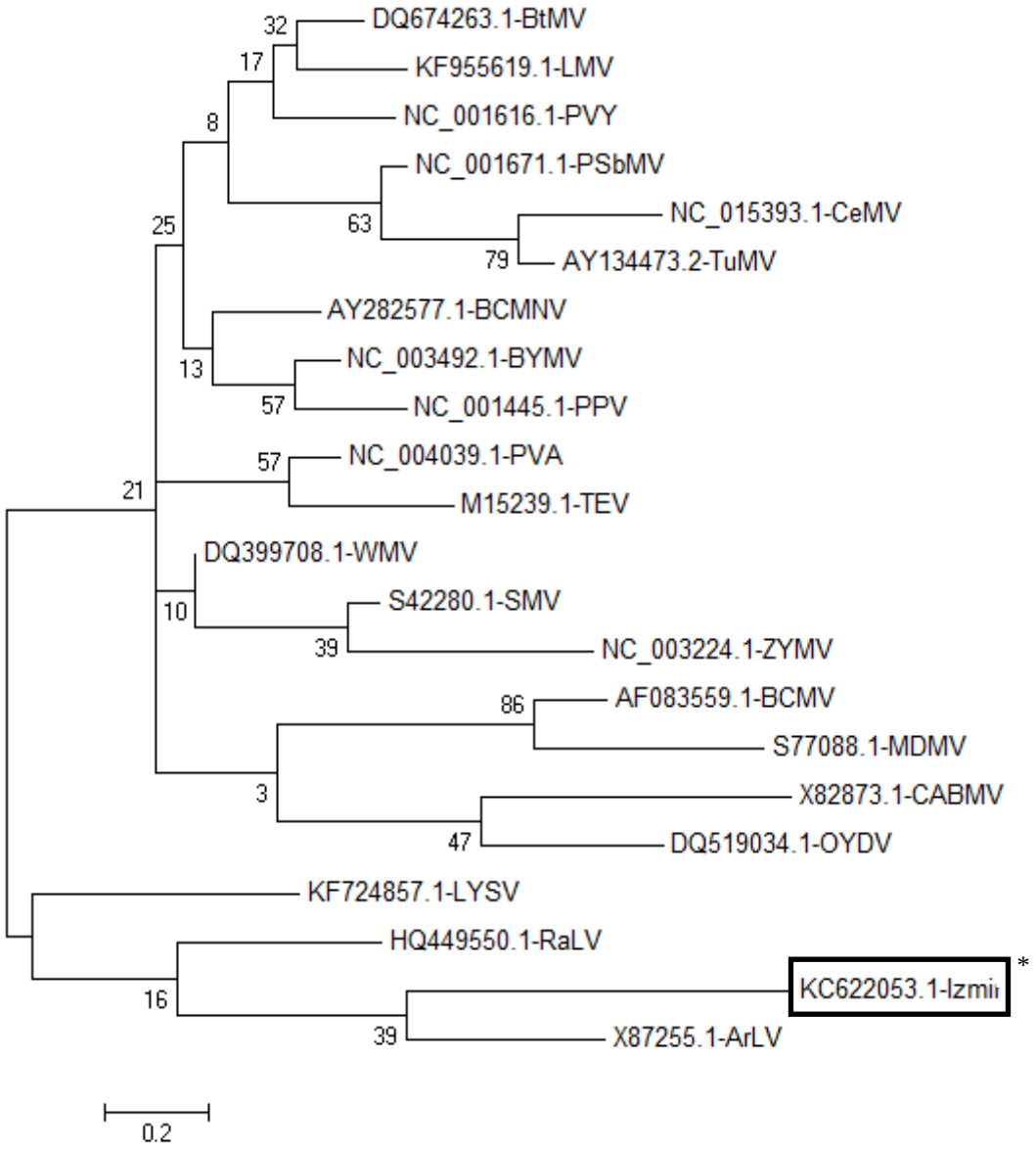

* KC622053.1 accession number indicates ArLV isolate obtained in this study.

Figure 3. Constructed Maximum Likelihood Dendogram with 22 members of Potyvirus, based on Kimura-2 parameter model with using Mega 5.10 software. The dendogram was bootstrapped 500 times.

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