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Virus diseases of lettuce in ankara province

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Abstract

Lettuce is one of the widely grown vegetables in Ankara province of Turkey and virus diseases cause great yield loss. Surveys were conducted to five different districts of Ankara during two years, 2014-2015 and a total of 544 samples were collected, and all were tested with the DAS-ELISA against Mirafiori lettuce big vein virus (MiLBVV), Lettuce mosaic virus (LMV), Cucumber mosaic virus (CMV), Tomato spotted wilt virus (TSVW) antisera. LMV was the predominant and unique infection detected in 2014 and was present in the 65 of the specimen but in 2015, 39 MiLBVV, 25 LMV infected isolates were detected. TSWV and CMV were not present in the research area. RT-PCR tests were performed to determine the coat protein gene regions of those viruses. Amplicons were obtained at 800 bp, 469 bp and 296 bp of LMV, MiLBVV, and LBVaV, respectively. Lettuce big vein-associated virus (LBVaV) were present in the 5 out of a total of 6 samples, as mixed infected with MiLBVV. Microscopic observations were performed to determine the presence of Olpidium spp. in the roots which is the vector of the viruses belong to Lettuce Big Vein diseases (LBV), resting spores were observed in 146 of the investigated root samples. These results indicate that LMV and LBV diseases are the most common endemic viral pathogens in Ankara's lettuce cultivation areas and the vector Olpidium spp. was also present in the research area.

Key words: Ankara, lettuce viruses, DAS-ELISA, *Olpidium* spp.

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INTRODUCTION

Lettuce (*Lactuca sativa L.*, Family: *Asteraceae*) is cultivated intensively in all parts of the world with mild climate. (Anonymous, 2019). Although Ankara is located in arid climate region, many types of vegetables are produced, among them iceberg-type lettuce cultivation stays at top (Yanmaz et al. 2015; Balkaya et al. 2016). Average lettuce production of Ankara is 75.105 tons and cultivation area is 2,65 hectares (TUIK, 2018) and all types of lettuce, including green or red leaf lettuce (*Lactuca sativa* var. *crispa*), romaine (*Lactuca sativa* var. *longifolia*), and iceberg (*Lactuca sativa* var. *capitata*), types are produced in the research area (Yanmaz et al.2015).

Plant virus infections are economiccally important and cause great yield reduction in the lettuce cultivation. Major plant virus infections are *Lettuce mosaic virus* (LMV, *Potyvirus*) which is one of the most important virus infection and cause great economic loss, *Tomato spotted wilt virus* (TSWV, *Tospovirus*), *Cucumber mosaic virus* (CMV, *Cucumovirus*), *Mirafiori lettuce big vein virus* (MiLBVV, *Ophiovirus*) and *Lettuce big vein associated virus* (LBVaV, *Varicosavirus*) viruses have been previously reported in the world (Verbeek et al. 2014). Widespread virus infection of lettuce is LMV, it is also transmitted by seeds and aphid vectors (Tomlinson, 1970). It causes severe systemic mosaic infection on lettuce plants. CMV is another infection causes the similar symptoms on lettuce leaves. It is also seed and aphid transmitted virus infection and infects very wide spectrum of plants (Palukaitis, et al. 1992).

Another group of virus diseases of lettuce is “Lettuce big-vein diseases, and are composed of two distinc virus infection, *Mirafiori lettuce big vein virus* (MiLBVV, *Ophiovirus*) and *Lettuce big vein associated virus* (LBVaV, *Varicosavirus*). The viruses cause enlargement of veins of the foliage which is defined as “big vein” symptom of lettuce. MiLBVV and LBVaV viruses, generally present in moist and heavy soils and transmitted by *Olpidium brassicae*, *O. bornovanus* or *O.virulentis* fungi (Herrera-Vasquez et al. 2009; Moreno and Fereres, 2012; Maccarone, 2013).

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TSWVis another virus infection and causes necrotic lesions on leaves, latter enlarges and causes the death of the entire plant (Pavan et al. 2008). It belongs to *Tospovirus* genus and transmitted by different *Thysanoptera* species in persistent-propagative pathway (Whitfield et al. 2005).

Lettuce viruses are detected in different vegetable production areas in Turkey, among the LMV is widespread virus infection (Ozalp, 1964; Tekinel et al., 1969; Erkan and Schlosser, 1985; Fidan and Turkoğlu, 1988; Doken et al.,1994, Kamberoglu and Alan, 2011; Karanfil et al., 2018). TSWV is only detected in southern parts of Anatolian peninsula.

Since the Ankara is the leader for lettuce production in Turkey, this research was conducted in order to determine the viruses of lettuce which cause great yield reduction.

MATERIALS AND METHODS

Virus sources: Surveys were conducted in summer in 2014 and 2015 to Ayaş, Beypazarı, Nallıhan and Cubuk districts and symptomatic and non-symptomatic plant samples were collected.

Detection of viruses by DAS-ELISA test:

All plant samples were tested for the presence of LMV, TSWV, CMV and MiLBVV by DAS-ELISA method (Clark and Adams, 1977). The tests were performed with commercial ELISA kits (Bioreba, Switzerland and Loewe, Germany) and tests were performed to according to the manufacturer's recommendations.

Nucleic acid isolations and Reverse Transcriptase-PCR amplification:

Total RNAs were extracted by using GeneJET Plant RNA Purification Kit (Thermo Sci., Lithuania) and the quantity of RNAs were estimated by Nano-Drop 2000 (Thermo Sci., Waltham, MA, USA), then stored at -80 °C. RT-PCR assays were conducted in two steps which encompassing cDNAs synthesis from total RNAs and amplifications of the CP gene regions of MiLBVV, LBVaV and LMV. cDNAs were synthesized in a 25 µl reaction mixtures containing 40 units of Reverse Transkriptase enyzme (Thermo Sci., Lithuania), 16 units of RNase inhibitor and 1.2 mM specific reverse primers of the viruses. The PCR assays were performed in 25 µl reaction volume containing 2 µl of cDNA, 1.5 units of Taq DNA polymerase (Thermo Sci., Lithuania), and, 2 mM $MgCl_2$, 2 µM each of the spesific primers.Primers used and RT-PCR conditions are shown in Table1. PCR amplification specifications consisted of 3 min denaturation at 94 °C, followed by 35 cycles of 95 °C for 1 min, primer annealing at 42 °C for 1 min and primer extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. The PCR products were electrophoresed on 1,2 % agarose gel at 100 volt for 1 h, stained with ethidium bromide and visualized under UV light by Gene Genius imaging system (UK).

Table 1. The primer sequences used in RT-PCR tests for molecular detection of LMV, MLBVV and LBVaV.

Virus name	Primer sequence 5'-3'	Fragment size (bp) and region	Denaturation temperature and time	Annealing temperature and time	Extension temperature and time	Reference
LBVaV	CGCCAGGATCTTTGATCCATCTG	296-CP	95 ºC	66 ºC	72 ºC	Whitfield et
	TTGCGACATGTTCCTCCTCATCG		30 s	30 s	45 s	al. 2005
MiLBVV	TATCAGCTCACATACTCCCTATCG	469-CP	95 ºC	61 ºC	72 ºC	Whitfield et
	CAACTAGCTCAGAATACATGCAG		30 s	30 s	120 s	al. 2005
LMV	AAGGCAGTAAAACTGATG	800-CP	95 ºC	42 ºC	72 ºC	Zerbini et
	TTTATACTACAGTCTTTA		60 s	60 s	120 s	al. 1995

Morphological observation of *Olpidium* spp:

Plant roots were examined under a light microscope to reveal the absence or presence of resting spores of *Olpidium* spp. These roots were washed in tap water and then kept in 70% ethyl alcohol for 10 seconds, and then washed again with distilled water.The cleaned root samples were stained with acid fuchsin-lactophenol solution and investigated under light microscope.

RESULTS AND DISCUSSION

Identification of virus infections in lettuce, and their prevalence and distribution in Ankara region, which is the most economically important lettuce producing area in Turkey, were carried out for the first time by this present research.

The symptoms of viruses were generally observed in the lettuce fields were unbinding of heads (Romaine type-Yedikule), excessive growth of leaf veins, small heads (icebergs) (Fig. 1 and Fig. 2), growth retardation, chlorosis and severe mosaic on leaves (green leaf lettuces). No symptoms were detected on red leaf lettuces. In this present research, LMV was the only virus detected on samples showing different severities of mosaic and necrosis, while CMV and TSWV was not present in the research area.

Figure 1. Lettuce big-vein symptom on iceberg lettuce.

Figure 2. Little head formation on iceberg lettuces.

LMV, which is widespread virus infection in the world (Zerbini et al. 1995), infects the lettuce and has some important isolates which formed three main groups: LMV-Yar (Yemen), LMV-Greek (Greece), and LMV-RoW as result of a phylogenetic analysis of isolates belonging to different geographies in the world (German-Retana et al. 2008). LMV isolates collected in the Southern Marmara Region of Turkey were shown to be seed transmitted, and were also determined as LMV-RoW strain (Karanfil and Celik, 2018). In another phylogenetic study of CP gene region of LMV isolates it was also determined as LMV-RoW group (Randa Zelyut and Ertunc, 2018). TSWV was only detected at low rates in the Eastern Mediterranean region of Turkey (Kamberoglu and Alan, 2011).

LBVaV and MiLBVV usually co-infection in field conditions and cause different necrotic symptoms in BV disease according to climatic conditions (Verbeek et al. 2013). When air temperatures are in the range of 18-22 ^{o}C, BV disease symptoms are developing, while the replication of LBVaV in above this ranges, is slowing down (Westerlund et al. 1978). MiLBVV shows typical BV disease symptoms either in single infection or mixed infection with LBVaV, but LBVaV does not show those typical symptoms in single infection (Sasaya et al. 2008). All of the collected lettuce samples were tested by ELISA with antiserum kits (Loewe, Germany) of CMV, TSWV, LMV, MiLBVV and only LMV was detected in 65 out of 220 lettuce samples collected in 2014. However, in 2015, a total of 324 samples were tested by ELISA using specific antiserums kits to CMV, TSWV, LMV, MiLBVV and 39 MiLBVV and 25 LMV infections were detected, but no reaction was obtained by TSWV and CMV antisera again (Table. 2).

Table 2. Prevalence of viruses of lettuce samples collected in 2015.

The region	Field surveyed	Samples collected	MiLBVV	LMV	CMV	TSWV	LBVaV	Infection rates
Beypazarı	39	289	29	22	-	-	6	2.71
Nallıhan	3	20	9	-	-	-	-	10.5
Ayaş	1	5	-	1	-	-	-	2.5
Çubuk	1	5	1	2	-	-	-	3
Centrum	1	5	-	-	-	-	-	0
Total	45	324	39	25	-	-	6	9.76

LBVaV is very weak in its antigenic properties, in other side, although MiLBVV virion is not stabil, its CP region is highly antigenic (Navarro et al. 2004; King et al. 2012). As result, high absorbance values in ELISA were observed in the samples collected during the cool and abundant rainy spring against MiLBVV antiserum. This suggests that the virus replicates itself more in favorable climatic conditions and in sensitive hosts such as *L. sativa* var. *capitata* (iceberg) (Sasaya et al. 2008) .

Total RNAs were tested for LMV, MiLBVV, LBVaV presence by RT-PCR with specific primers designed for coat protein gene regions. A 800 bp, 469 bp and 296 bp amplification fragments (Fig. 4,5 and 6) were obtained with 25 LMV, 39 MiLBVV and 6 LBVaV isolates respectively, and co-infection was detected in 5 samples infected by MiLBVV and LBVaV.

In the microscopic observations for determination of presence or absence of *Olpidium* spp. in the lettuce roots, their resting spores were observed in 146 samples, but no resting spore was observed in the remaining samples. They were in circular shape and generally present in the BV infected samples (Figure 3). They were determined from the samples collected from the Beypazarı region in which its groundwater level is quite high.

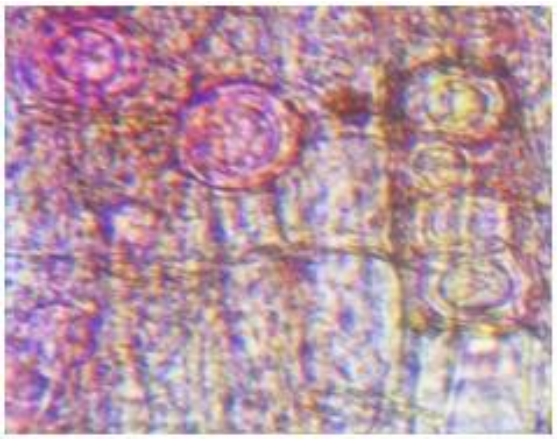

Figure 3. Resting spores of *Olpidium* spp. in the lettuce root

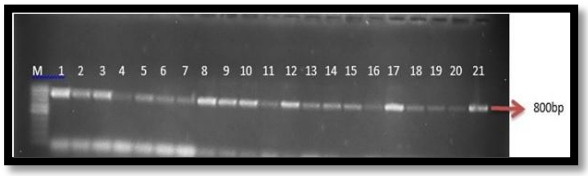

Figure 4. RT-PCR results of Lettuce mosaic virus coat protein gen sequences (800 bp) (M: Marker, 100bp DNA ladder, Thermo Scientific, 1-12: Samples collected from Ankara-Beypazarı, 13-14: Samples collected from Ankara-Cubuk, 15-21: Samples collected from Ankara-Beypazarı plato.

Figure 5. RT-PCR results of Lettuce mosaic virus coat protein gen sequences (800 bp) (M: Marker, 100bp DNA ladder, Thermo Scientific, 1-4: Samples collected from Ankara-Beypazarı, 5: Healthy lettuce 6: Water coıntrol 7-8: Samples collected fron Ankara-Nallıhan.

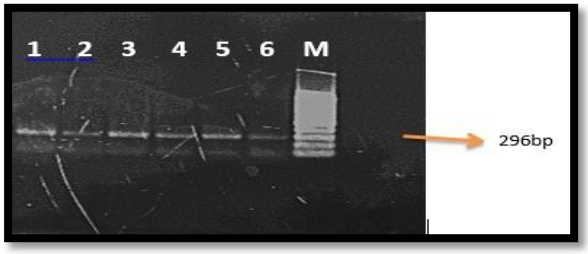

Figure 6. RT-PCR results of Lettuce big-vein virus coat protein gen sequences (296 bp) (M: Marker, 100bp DNA ladder, Thermo Scientific, 1- 6: Samples collected from Ankara-Beypazarı

CONCLUSIONS

Plant viruses causing high economic losses in different lettuce varieties cultured in Ankara were identified in this study. The virus vector, and the pathogens were identified by using serological and molecular methods. In regions where the winter is hard, the vector *Olpidium spp.* was found still present and their viruliferous zoospores infect icerbergs which highly sensitive to BV infections and curly type lettuce which sensitivity to BV is moderate. In regions where temperature rise above 27^{o} C, LMV severely infected ‘’Romaine’’ variety but only very few curly type lettuce. CMV and TSWV, which were transmitted by aphids and thrips vectors, were not detected and no vector populations were encountered. It is recommended that the species belonging to different families should be planted as rotation plants for the continuity of lettuce production in the region. Furthermore, in order to prevent the spreading of viruliferous zoospores in fields and in seedlings by irrigation, it is absolutely necessary to refrain the drainage of unsuitable field soils and avoid the cultivation of sensitive varieties in cool seasons. The use of certified virus free lettuce varieties can also be recommended to reduce the prevalence of the seed-transmitted virus diseases.

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