

Research Article

Occurrence and Characterization of Coat Protein Gene of *Zucchini yellow mosaic potyvirus* (ZYMV) Isolate Infecting Pumpkin (*Cucurbita pepo* L.) in Bingöl Province (Turkey)

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Abstract

Zucchini yellow mosaic potyvirus (ZYMV) belongs to family Potyviridae, which causes serious economic losses in many cucurbits from worldwide. In 2018 (September), leaf samples of pumpkin exhibiting typical viral symptoms including mosaic, blistering and wrinkling and healthy pumpkin leaf samples were collected from Bingöl and screened by Reverse- Transcription Polymerase Chain Reaction (RT-PCR) against ZYMV infection. Tested leaf samples were reacted positive resulting in an expected about 840 bp DNA fragments of partial coat protein (CP) gene of ZYMV. ZYMV-CP gene was further inserted into a pGEM-T Easy prokaryotic cloning vector and their partial nucleotide sequences and deduced amino acid were ascertained. The provided ZYMV- CP gene sequence consisted of 837 nucleotides in length coded for 279 amino acid residues of approximately 31.2 kDa. The isolate was denominated as ZYMV-Bingöl and registered with MK689858 accession number in the NCBI. The sequence of ZYMV- Bingöl CP gene were aligned with 21 isolates deposited in GenBank from different geographical location and its phylogenetic relationships were determined. Molecular analysis of the ZYMV CP gene sequence indicated the highest similarity with 100% Turkish isolate (JF317296) and the lowest with 91.64% of Korea isolate (AF062518), at nucleotide level. Moreover, phylogenetic analyses revealed that ZYMV Bingöl isolate is clustered with the Turkish- Adana isolate (JF317296) and Pakistan isolate (AB127936). ZYMV has been reported for the first time in the pumpkin plant from Bingöl province of Turkey by this study.

Key words: ZYMV (*Zucchini yellow mosaic potyvirus*), RT-PCR, cloning, characterization.

Bingöl İlinde Kabak Bitkisini (*Cucurbita pepo* L.) İnfekteleyen *Zucchini Yellow Mosaic Potyvirus* (ZYMV) Virüs İzolatının Belirlenmesi ve Moleküler Karakterizasyonu

Özet

Kabak sarı mozaik potivirüsü (ZYMV), Potyviridae ailesine ait bir virüs olup dünya çapında birçok kabakgil bitkide ciddi ekonomik kayıplara neden olmaktadır. 2018 yılı Eylül ayında, Bingöl iline bağlı kabak yetiştiriciliği yapılan alanlarda kabarıklık ve buruşukluk gibi tipik viral hastalık belirtisi gösteren bitkiler ile sağlıklı bitkilerden örnekler alınarak ZYMV enfeksiyonu bakımından Reverse Transkripsiyon Polimeraz Zincir Reaksiyonu (RT-PCR) ile testlenmiştir. RT-PCR testi pozitif reaksiyon vererek ZYMV'nin kılıf proteinine karşılık gelen yaklaşık 840 bp DNA fragmenti oluşturmuştur. Ayrıca, çoğaltılan ZYMV'nin tam kılıf protein (coat protein, CP) geni, prokaryotik klonlama vektörü olan pGEM-T Easy vektörüne klonlanmış ve genin tam nükleotid ve amino asit dizisi tespit edilmiştir. Elde edilen ZYMV-CP gen dizisinin 279 amino asiti kodladığı (yaklaşık 31.2 kDa) ve 837 nükleotidi içerdiği ortaya çıkarılmış ve bu dizi ZYMV-Bingöl izolatı olarak isimlendirilerek MK689858 erişim numarası ile gen bankasına (NCBI) kaydedilmiştir. ZYMV-Bingöl CP geninin dizisi, farklı coğrafi bölgelerden elde edilen 21 izolat ile analiz edilerek filogenetik ilişkileri belirlenmiştir. Nükleotid düzeyindeki moleküler analizlere göre ZYMV CP gen dizisi, en yüksek % 100 oranında Türkiye izolatı (JF317296) ile ve en düşük % 91.64 oranında Kore izolatı (AF062518) ile benzerlik göstermiştir. Ayrıca, filogenetik analizler, ZYMV Bingöl izolatının Türkiye-

Adana izolatu (JF317296) ve Pakistan izolatu (AB127936) ile gruplandığını belirlenmiştir. Bingöl ilinde kabak bitkisini enfekte eden ZYMV'nin bulunuşu ilk kez bu çalışmayla rapor edilmiştir.

Anahtar kelimeler: ZYMV (*Zucchini yellow mosaic potyvirus*), RT-PCR, klonlama, karakterizasyon.

Introduction

Pumpkin (*Cucurbita pepo* L.) is a cultivated plant belonging to the family Cucurbitaceae, which can grow elsewhere in different agricultural ecosystems. Thirty-two different viruses and virus-like agents have been identified, including *Zucchini yellow mosaic potyvirus* (ZYMV), which infects *Cucurbitaceae* plants (Lovisol, 1980). Since this plant pathogen was first discovered in Italy in 1973 and was provisionally called as MYSV (*Muskmelon yellow stunt virus*) (Lisa *et al.*, 1981), it has been reported in many countries in the continents of Europa, Africa, Asia, Oceania, America and Middle East (Desbiez and Lecoq, 1997).

The agent belongs to the Potyvirus which is the greatest genus among plant viral pathogen, has an infectious (+) ssRNA genome characterized by 750 nm long and 12 nm in diameter contained about 9600 nucleotides in size (Lisa *et al.*, 1981; Balint *et al.*, 1990). The viral genome also has the VPg protein (genome-linked protein) at 5' position and poly (A) tail at 3' position. ZYMV forms tubular scroll-like cytoplasmic cylindrical inclusion bodies within infectious cells (Shukla *et al.*, 1994; Adams *et al.*, 2012).

ZYMV is a worldwide plant pathogenic virus that parasitizes cucurbit plants including *Cucurbita pepo*, *C. pepo*, *C. moschata*, *C. maxima*, *Cucumis sativus*, *Cucumis melo*, *Citrullus lanatus*, and can cause substantially product losses up to 94% every year in all seasons except winter (Wang *et al.*, 1992). It has also been reported that some weeds and cultivated plants such as *Ranunculus sardous*, *Lamium amplexicaule*, *Sesamum indicum*, *Ranunculus sardous*, *Chenopodium amaranticolor*, *C. quinoa*, *Phaseolus vulgaris*, *Nicotiana benthamiana*, *Cucurbita pepo*, and *Cucumis melo* are the experimental hosts, *Melothria pendula* and *Moluccella laevis*, which are wild cucurbit species are natural hosts of ZYMV, can also serve as inoculum resources in the pathogen life table (Mahgoub *et al.*, 1997; Kheder *et al.*, 2017; Adlerz *et al.*, 1983).

Likewise other potyviruses, ZYMV is an aphid borne virus that can be horizontally transmitted in a non-persistent manner by 26 species of aphids in the *Aphididae* family. As main vectors, *Aphis gossypii* and *Myzus persicae* can be capable of carrying, spreading and transmitting this virus during feeding activity. It has also been shown that this virus can be occasionally transmitted by vertically infectious seeds (detected

serologically), mechanical inoculation (Couatts *et al.*, 2011; Tymchyshyn *et al.*, 2017; Davis and Mizuki, 1986) and can even be transmitted by leaf contact due to rubbing and crushing (Couatts *et al.*, 2013).

ZYMV-naturally infected pumpkin plants symptoms can range from mild to severe in density, which is mainly enclosed mosaic patterns from yellow to light green, yellowing, superficial foliar crinkle, filiformis in the young shoot, stunting, distorted fruits in squash, zucchini, muskmelon, cucumber, melon, and vegetable marrow. Such symptoms led to seriously product losses or render them unmarketable in Cucurbit crops (Spadotti *et al.*, 2015; Blua ve Perring, 1992).

ZYMV is determined by electron microscopy (Brandes, 1957) and symptomatological observation on indicator hosts (Lisa and Lecoq, 1984), serological methods such as ELISA (Ghanem *et al.*, 2016) and DNA-based molecular techniques such as Polymerase chain reaction (PCR) and Dot blot hybridization (Thomson *et al.*, 1995; Harper and Creamer, 1995). Nowadays, many viruses in the genus Potyvirus including ZYMV can be unequivocally detected by PCR procedure, which is broadly performed targeting the gene sequence of the viral CP protein (Simmons *et al.*, 2011; Zheng *et al.*, 2010).

In this study, the presence of ZYMV in symptomatic pumpkin plants was confirmed by the molecular techniques in Bingöl province located in the east of Turkey and the phylogenetic relationships with other isolates around the world were revealed based on the ZYMV- CP sequence.

Material and Methods

Plant material and biological cloning

Young leaves showing virus-like symptoms in pumpkin were obtained from the Beyaztoprak village of Bingöl province on September 2018. The symptoms triggered by ZYMV were also evaluated by mechanical inoculation as the conventional method in the *C. pepo*. The ZYMV suspension was empirically treated with carborundum powder and potassium phosphate buffer (PPB) (pH 7.2) on the cotyledon leaves after the seedling phase. Symptom development was observed in the climate chamber for 3 weeks. Inoculated plants were tested by RT-PCR for ZYMV infection.

Total RNA extraction, and cDNA synthesis

Because the ZYMV has an RNA genome, total RNA was extracted from nearly 0.25 g leaf

tissue according to the silica-based method described by Foissac *et al.* (2001). The asymptomatic pumpkin plant was also gathered as a negative control in the laboratory tests.

The cDNA mixture was prepared in 20 µl volume. The following components were introduced into an Eppendorf tube for the synthesis of complementary DNA (cDNA): 5 µl of purified RNA (as a template), 1 µl of 10 mM dNTP

mix, 1 µl of 20 pmol/µl the antisense primer (ZA), 5 µl of RNase free water, followed by 65 °C for 5 min and afterwards immediately immersed in ice for 5 minutes. 4 µl of 5X RT reaction buffer, 2 µl of 0.1M DTT, 1 µl of RNase inhibitor and 1 µl of RT enzyme were put onto the resulting mixture and hold at 42 °C for 50 min. Finally, the mixture was incubated at 70 °C for 15 min to discontinue the reaction.

Table 1. Accession number, location, isolate name and host of ZYMV isolates retrieved from GenBank used for phylogenetic analysis in this study

Accession no.	Location	Isolate name	Host
AJ420019	Germany	Berlin 1	<i>C. pepo</i> L.
AB458595	Syria	SYZY 1	<i>Cucurbita pepo</i> L.
FJ705272	Iran	Azr. Mak.W	<i>Citrullus vulgaris</i> L.
AB458596	Syria	SYR 3	<i>C. pepo</i> L.
FJ705263	Iran	Hor. Min. S	<i>Cucurbita maxima</i> Duch. E Lam
EF062583	Israel	AG	<i>Cucurbita pepo</i> L. 'Ma'ayan'
AB127936	Pakistan	Pak	<i>Lagenaria siceraria</i> Standl.
HM072431	Serbia	128-08	<i>C. pepo</i> L. 'Olinka'
AJ420017	Austria	Austria 12	<i>C. pepo</i> L.
AB004641	Japan	NA	<i>C. maxima</i> Duch. 'Hokoseihi'
GU586790	Brazil	ZTRICH	<i>Trichosanthes cucumerina</i>
GQ482976	India	India 1	<i>Cucumis anguria</i>
AY611021	China	CH99/116	<i>C. pepo</i> L.
AJ420020	Italy	Italy 1	?
L31350	USA	ZYMV C	<i>C. pepo</i> L.
AF062518	Korea	NA	<i>C. sativus</i> L.
AF127929	Taiwan	TW-TN3	<i>Luffa cylindrica</i> Roem.
KP872566	Turkey	C5	<i>Cucurbita moschata</i>
KP872577	Turkey	K17	<i>C. pepo</i> L.
JF317297	Turkey	ZYMV-Ah	<i>C. melo</i> L.
JF317296	Turkey	ZYMV-Ad	<i>C. pepo</i> L.
MK689858	Turkey	ZYMV-Bingol	<i>C. pepo</i> L.

Molecular detection of ZYMV- infecting pumpkin and primer pairs

The ZYMV infection was examined by RT-PCR tests in pumpkin. Primers used in the detection of ZYMV in this study were retrieved by formerly research (accession number: AB188116) and were synthesized employing the Sentegen company (Ankara/Turkey). Employed primers used were designed as sense (ZS-5'-TCAGGCACTCAGCCAACCT-3') and antisense (ZA-5'-CTGCATTGTATTACACCTAGT-3'), amplifying a CP gene region about to 837 bp.

The RT-PCR thermocycling program that amplifies the CP gene using Eppendorf Mastercycler (Hamburg, Germany) is as follows: 3 min at 94 °C (pre-denaturation), 40 cycles of 30 s at 94 °C (denaturation), 1 min at 62 °C (annealing), and 45 s at 72 °C (elongation), lastly 10 min at 72 °C for final elongation. 5 µL of cDNA were submitted to RT-PCR test in a total volume of 50 µl:

31.6 µl RNase free water, 5µl 10X PCR Buffer, 3µl 25mM MgCl₂, 1µl 10mM dNTP, 1µl 20µM of each primer pairs and using the proofreading 0.4 µl *Taq* DNA polymerase enzyme (5U/µl) (Thermo, USA).

The molecular size of PCR yield and 1 kb DNA ladder as the marker (Fermentas, Vilnius, Lithuania) was evaluated by 1.5% agarose gel electrophoresis stained with EtBr using Tris-Acetate EDTA buffer (TAE1X). The monitored DNA fragments were documented by photography with UV light (Syngene™ UV Transilluminator 2020LM).

ZYMV-Ahlat isolate (GenBank accession JF317297), from previous studies, served as a positive control, and symptomless pumpkin plant and PCR yield- free mixture were used as a negative control for diagnosis of the ZYMV.

T-A cloning and sequencing

The amplified CP gene of the expected size was recovered by using a DNA gel extraction kit

(Fermantas) according to manufacture's directions. The purified PCR products were ligated into the pGEM-T Easy plasmid vector by DNA ligase enzyme. Accordingly, a sterile PCR tube containing the following components; 2x Rapid Ligation Buffer of 5 µl, pGEM-T Easy vector of 1 µl, purified PCR product of 5 µl and T4 DNA ligase enzyme of 1 µl (Promega, USA) and was incubated at 4 °C overnight, followed by introduced into E.coli (Promega, USA) via electroschock wave (BioRad, USA). Potential recombinant plasmids were purified with the commercially supplied kit (GeneJET Plasmid Miniprep Kit, Thermo, USA).

Sequencing was carried out in both directions using the above primer sets by Iontek Company for recombinant plasmid involving the CP-DNA sequence of the ZYMV Bingöl isolate. The DNA sequence was blasted against GenBank data, was registered in NCBI (The National Center for Biotechnology Information).

Alignment and cladistic analysis

Twenty-one different ZYMV isolates were used to determine phylogenetic relationships. The sequence corresponding to 837 bp from Bingöl isolate with other isolates from the diverse host

that was online available in GenBank as illustrated in Table 1 were assembled and assessed by using the Mega 7 program. The phylogenetic dendrogram was inferred using the Neighbor-joining algorithm by 100 repetition bootstrap analysis. The evolutive interrelationships were computed with the Tamura-Nei method. The CP gene of Barley yellow dwarf virus isolate (KC900900) was designated as an outgroup for branching.

Results and Discussion

Detection of ZYMV using appropriate primer sets

A pumpkin (*Cucurbita pepo*) was collected, that characteristic viral symptom associated with mottling, mosaic and lumps, leaf distortion, down curling leaves (Fig 1), compared with healthy pumpkin which using as negative control. ZYMV infection was confirmed by RT-PCR as mentioned above. Data in Fig. 2 displayed that RT-PCR resulted in an amplification yield size of 837 bp corresponding to the ZYMV-CP gene from ZYMV-infected pumpkin. No DNA fragment healthy plant or PCR yield-free mixture using as negative control were DNA fragments displayed.



Figure 1. Symptomatic pumpkin plant identified in a garden from Bingöl province in autumn, 2018. A- General view of mosaic pattern with heavily green lumps and mottles in pumpkin plant, B- Leaves curled down from leaf edges, C- Leave with distorted and serrated edges.

Alignment and cladistic analysis

The sequence of ZYMV CP gene isolate was ascertained as 837 bp and was recorded in GenBank (with accession number MK689858), called ZYMV- Bingol. This data was used to determine the genetic diversity among Bingöl isolate and other chosen isolates in different plants. BLAST research (Basic Local Alignment Search Tool) revealed that the sequence of ZYMV-Bingol CP gene shared with 91.64-100% similarity,

the highest score with 100% of the Turkish isolate (JF317296), with the lowest score with 91.64% of Korea isolate (AF062518) and were confirmed by cladistic analyses using computer-supported program. Phylogenetic analysis of isolate presented in this study with 21 isolates from GenBank indicated that ZYMV- Bingol was same grouped with isolates identified on *Cucumis sativus* and *Lagenaria siceraria* (JF317296, AB127936) (Fig 3).

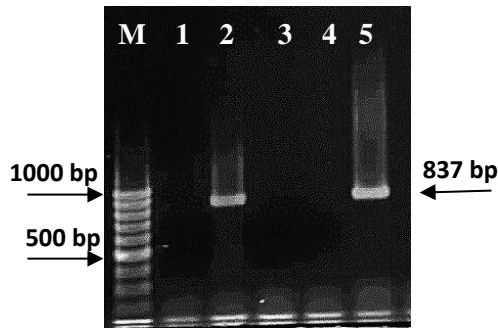


Figure 2. Agarose gel pattern displaying DNA fragment of ZYMV from individually infected pumpkin leaf tissue by RT-PCR using CP- specific primer sets. Lane M: 1 kb DNA marker Lane 1-3-4: Healthy pumpkin plants Lane 2: ZYMV isolate Lane 5: ZYMV-positive control.

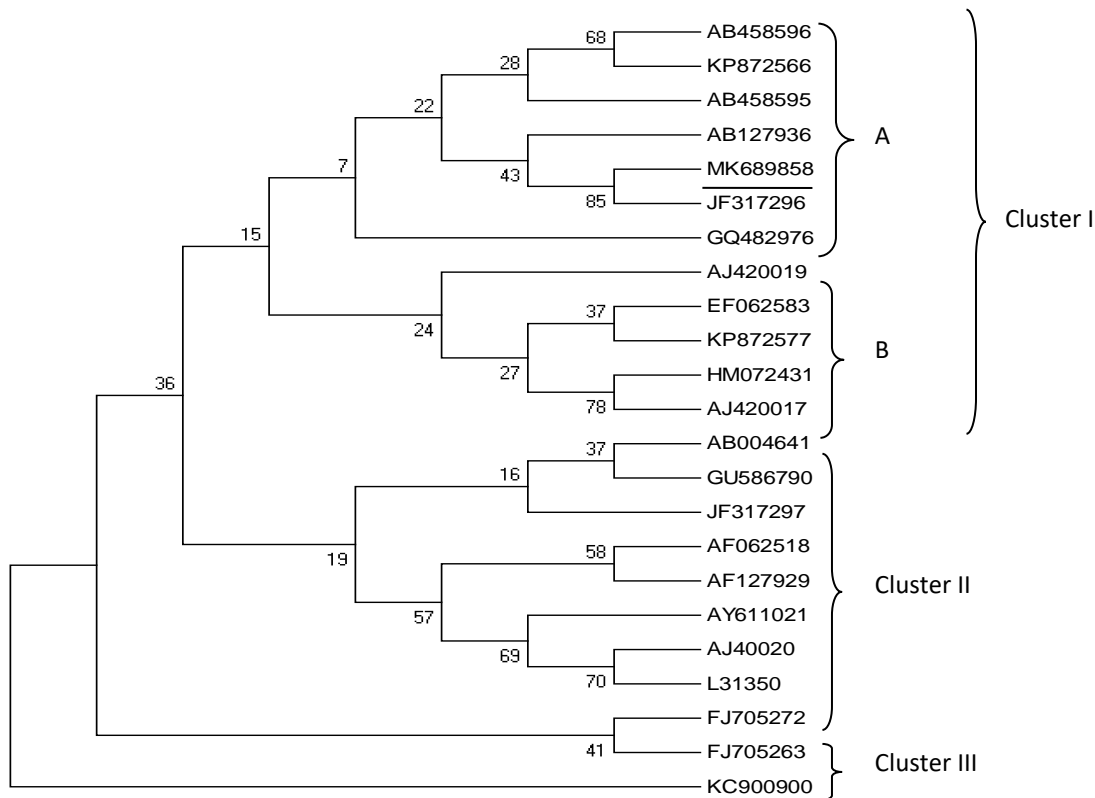


Figure 3. Phylogenetic dendrogram created by the neighbor-joining algorithm with 100 replication bootstrap value (scores at the nodes) from 21 distinct ZYMV isolates. ZYMV-Bingol isolate is underlined.

Since pumpkin (*Cucurbita pepo* L.) is consumed as fresh fruit and snacks, it is an important food for many people both in terms of nutrition and livelihood. Cucurbits are very susceptible to viruses and more than 35 viruses have been reported infecting cucurbits (Zitter *et al.*, 1996). Presently, pumpkin has substantially affected by ZYMV, followed by SqMV, CMV, WMV (Cutts *et al.*, 2011; Simmons *et al.*, 2011, 2013). ZYMV is a viral agent that is prevalent in almost all countries and leads to a severe yield loss, ruining *Cucurbitaceae* family members due to the severe epidemics.

Here, we determined the ZYMV isolate which depicted yellow-greenish severe mosaic, distortion and serrated leaves in pumpkin plants by PCR-based molecular methods from Bingöl province of Turkey, 2018. Disease symptoms observed this study were consistently similar with those reported in studies in different countries such as Czech Republic (Svoboda and Polák, 2002), Trinidad (Chinnaraja *et al.*, 2016), Sudan (Mohammed *et al.*, 2014), Egypt (Khalifa *et al.*, 2015), Saudi Arabia (Al-Saleh *et al.*, 2014), Japan (Ohtsu *et al.*, 1985), Malaysia (Fujisawa *et al.*, 1986), Western Australia (Cutts *et al.*, 2011), India

(Nagendran *et al.*, 2016), Germany, Serbia, Korea, Australia (Vucurovic *et al.*, 2012), Illinois (Jossey and Babadoost, 2008), Iran (Hosseini *et al.*, 2007), Poland (Pospieszny *et al.*, 2003) and more. About 10 years after it was first isolated, ZYMV was first recorded by Davis and Yilmaz in 1984 in Turkey and then were reported by various researchers from various geographical locations (Davis and Yilmaz, 1984; Davis, 1986; Yilmaz *et al.*, 1995).

On the other hand, ZYMV has quickly spread from east to west, from north to south in Turkey and has dominated varying proportions, unlike other Cucurbitaceae viruses in melon, cucumber, squash and watermelon from surveyed areas (Fig 4). The occurrence of ZYMV has been previously reported in numerous provinces such as Konya, Aksaray and Karaman (Yeşil, 2013, 2014; Yeşil and

Ertunç, 2012, 2013), Burdur (Çulal Kılıç *et al.*, 2016), Adana and Mersin (Kamberoğlu *et al.*, 2016; Özer *et al.*, 2012), Afyon (Yılmaz *et al.*, 1992), Hatay (Sertkaya *et al.*, 2004), Erzurum, Erzincan and Artvin (Bostan *et al.*, 2002), Gaziantep (Ozaslan *et al.*, 2006; Dağ, 2005), İzmir, Aydın, Manisa and Balıkesir (Kaya and Erkan., 2011), Ahlat (from Bitlis) (Özer *et al.*, 2012), Samsun, Sinop and Bolu (Sevik and Arli-Sokmen, 2003; Şevik and Balkaya, 2015), Amasya and Çorum (Çitir *et al.*, 1998), Tekirdağ, Edirne and Kırklareli (Köklü and Yilmaz, 2006), Tokat (Korkmaz *et al.*, 2018), Ankara (Ertunç, 1992), Antalya (KP872577, KP872571, KP872570) (Topkaya and Ertunç, 2012; 2013; 2013, respectively) (unpublished data), Turkish Republic of Northern Cyprus (Karamanlı and Kamberoğlu, 2010).



Figure 4. Map of Turkey showing the location of ZYMV fields previously detected. The ZYMV-detected province is dark blue.

In Turkey, although the virus has been identified for more than 30 years, little is known concerning its molecular feature. Most of the studies have been performed serologically, biologically and symptomatologically, but molecular characterization for its coat protein gene is very limited, besides no genomic sequence information was reported except for 2-3 studies. ZYMV can be detected with different primer sets, referred on coat protein (CP) ve Nib gene (Karamanlı and Kamberoğlu, 2010; Wang and Li, 2017; Kheder *et al.*, 2017; Nasr-Eldin *et al.*, 2016; Prieto *et al.*, 2001). We identified the ZYMV using ZS and ZA primer pairs designed specifically for the CP gene. ZYMV Bingol isolate was produced 837 bp fragment, which were equivalent to that of ZYMV-Ah (JF317297) and ZYMV-Ad (JF317297), which were harmony with that submitted Özer *et al.* (2012), Spadotti *et al.* (2015) and Topkaya and

Ertunç, (2011; 2012; 2013) (unpublished data) who successfully identified ZYMV by RT-PCR.

Cucurbita pepo with together 11 families of dicotyledons is a voluntary host for ZYMV (Romay *et al.*, 2014). The symptoms of ZYMV-Bingol isolate were characterized symptomatologically by the reaction to the pumpkin. In the surveys performed at different times, pumpkin plants were systemically infected, as expected, and exhibited foliar symptoms such as mosaic, leaf deformation and leaf blistering were exhibited, which were similar effect to naturally infected plants with ZYMV, in agreement with Wang and Le, (2017) (China) and Nasr-Eldin *et al.* (2016) (Egypt) (Fig 5). The presence of ZYMV was also confirmed in inoculated leaves by RT-PCR assay.

The origin of ZYMV, its mechanism of evolution and its geographic distribution is

important to understand the emergence of plant viruses. In the past studies, the sequence of the CP gene nucleotide was used to differentiate phylogenetic relationships, resulting in two or three groups (Zhao et al., 2003; Romay et al., 2014). ZYMV- Bingol sequence (MK689858), which has homology to previously proposed isolates, was analyzed with other representative isolates in NCBI for phylogenetic interrelationship. Phylogenetic assay of the partial sequences of the CP gene demonstrated the main three distinct clusters (named I, II and III). Cluster I are divided into two subclusters (A and B) and ZYMV-Bingöl isolate was clustered within subcluster B of cluster I as shown in Fig3. This DNA distance analysis revealed that Bingöl isolate is part of a cluster of the other Turkey (KP872566, JF317296), Syria (AB458596,

AB458595), Pakistan (AB127936) and India (GQ482976) from Middle Eastern countries. Interestingly, even though Bingöl and Bitlis (Ahlat) had a neighboring province (about 230 km between two locations) and shared an almost similar geographical region, both isolates obtained from these provinces (MK689858 and JF317297) were in the completely different cluster (Cluster I and Cluster II), respectively. In contrast, ZYMV-Ad (Adana) and ZYMV-Bingöl (Bingöl) identified in the two provinces away from each other (about 666 km between two locations) were included in the same subcluster of the same cluster (Cluster IA). This shows the variations occurring in the virus genome at the same location. This shows that rapidly ongoing variations occur in the ZYMV virus genome in the same country.

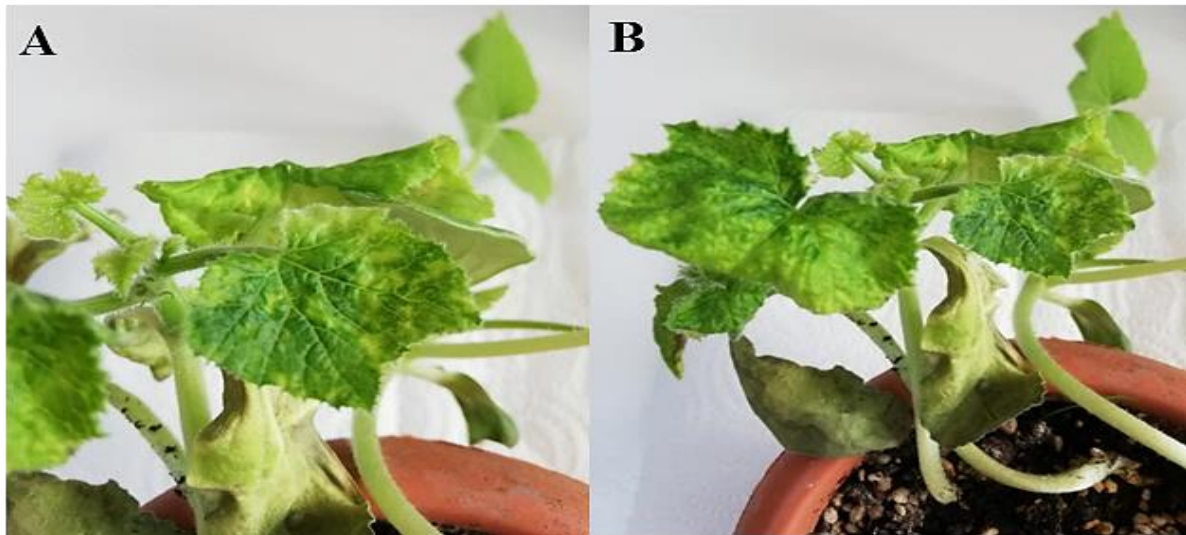


Figure 5. Symptoms induced by ZYMV in mechanical inoculated pumpkin (*Cucurbita pepo*). A- mosaic and mottle on leaves, B- severely foliar deformation.

Sequence analysis indicated that the 837 bp nucleotide sequences of ZYMV-Bingöl isolate shared with the similarity of 91.64–100% nucleotide homology with other assembled potyvirus from GenBank. These sequences had substitutions of four nucleotides at distinct points of the CP gene-specific to ZYMV-Bingöl isolate. Computational assays revealed that Timin instead of Guanine, Timin instead of Cytosine, Timin instead of Cytosine, and Adenine instead of Guanine at 22, 390, 736, and 817 positions, respectively. Also, multiple alignments showed that a conserved amino acid codon, without any change.

The cultivation of plants in the Cucurbitaceae family is not widespread and on a large scale in Bingöl province. Although there is no risk situation for ZYMV at the moment, if the cucurbit crops such as watermelon, melon,

pumpkin, cucumber are grown in large scale, necessary cultural and chemical measures should be taken to abstain ZYMV infection as the destruction of residues after harvest, prevention of planting ornamental plants as Malva and begonia served as an alternative host, planting certified tolerant or resistant varieties when available, and as well as using pesticides for vector insects (Lecoq et al., 2014).

Conclusion

According to our knowledge, this is the first record on the occurrence of ZYMV disease on *Cucurbita pepo* L. in Bingöl province of Turkey. The outcome of this study will help to better understand further research on the complexity, epidemiology and hence variety of ZYMV in Turkey.

References

- Adams, M.J., Zerbini, F.M., French, R., Rabenstein, F., Stenger, D.C., Valkonen, J.P.T. 2012. Family Potyviridae. p. 1069-1089. In: King, A.M.Q.; Adams, M.J.; Carstens, E.B.; Lefkowitz, E.J., eds. *Virus Taxonomy: Classification and Nomenclature of Viruses; Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier Academic Press, San Diego, CA, USA.*
- Adlerz, W.C., Purcifull, D.E., Simone, G.W., Hiebert, E. 1983. *Zucchini yellow mosaic virus: a pathogen of squash and other cucurbits in Florida. Proceedings of the Florida State Horticultural Society*, 96: 72-44.
- Al-Saleh, M.A., Amer, M.A., AL-Shahwan, I.M., Abdalla, O.A., Zakri, M.A. 2014. Characterization of different isolates of *Zucchini yellow mosaic virus* from cucurbits in Saudi Arabia. *African journal of microbiology*, 8(19): 1987-1994.
- Balint, R., Plooy, I., Steele, C. 1990. The nucleotide sequence of *zucchini yellow mosaic potyvirus*. Abstract of the VIIIth International Congress of Virology, 8: 84-107.
- Blua, M.J., Perring, T.M. 1992. Alatae Production and population increase of aphid vectors on virus-infected host plants. *Oecologia*, 92: 65-70.
- Bostan, H., Kaymak, H.Ç., Haliloğlu, K. 2002. Detection of *Cucumber mosaic virus* (CMV) and *Zucchini yellow mosaic virus* (ZYMV) in squash in Erzurum, Erzincan and Artvin provinces by serological and biological methods. *Journal of Turkish Phytopathology*, 31(1): 9-14.
- Brandes, J. 1957. Eine elektronmikroskopische Schnell-methode zum Nachweis faden- und stabförmiger Viren, insbesondere in Kartoffeldunkelkeimen. *Nachrichtenblatt des Deutschen Pflanzenschutz-Dienstes*, 9: 151-152.
- Chinnaraja, C., Ramkissoon, A., Rajendran, R., Tony, S.T., Ramsuhag, A., Jayaraj, J. 2016. First report of *Zucchini yellow mosaic virus* and *Squash mosaic virus* infecting cucurbits in Trinidad. *Plant Disease*, 100(4): 866.
- Coutts B.A., Kehoe, M.A., Webster, C.G., Wylie, S.J., Jones, R.A.C. 2011. *Zucchini yellow mosaic virus*: biological properties, detection procedures and comparison of coat protein gene sequences. *Archives of Virology*, 156: 2119-2131.
- Coutts, B.A., Kehoe, M.A., Jones, R.A.C. 2013. *Zucchini yellow mosaic virus*: Contact transmission, stability on surfaces, and inactivation with disinfectants. *Plant Disease*, 97: 765-771.
- Çıtır, A., Kutluk, N.D., Sağlam, N., İlbağı, H. 1998. Amasya, Çorum, Samsun ve Tokat illerinde hıyar ve kabak kültürlerinde görülen virüs hastalıklarının simptomolojik ve biyolojik yöntemlerle tanıları. Türkiye VIII. Fitopatoloji Kongresi, Ankara, s. 331-335.
- Çulal Kılıç, H., Doğan, K., Yardımcı, N., Isparta, L. 2016. Detection of *Zucchini Yellow mosaic virus* from cucurbits in Burdur Province, Turkey. *Journal of Multidisciplinary Engineering Science and Technology (JMEST)*, 3: 4.
- Dağ, D.S. 2005. Gaziantep il ve ilçelerinde Yetiştirilen Kabakgillere (Cucurbitaceae) Zarar Veren Virüslerin DAS-ELISA Yöntemiyle Saptanması. Yüksek Lisans Tezi. Gaziantep Üniversitesi.
- Davis, R.F., Yılmaz, M.A. 1984. First report of *Zucchini yellow mosaic virus* on watermelon and squash in Turkey. *Plant Disease*, 68: 537.
- Davis, R.F., Mizuki, M., K. 1986. Seed transmission of *Zucchini yellow mosaic virus* in squash. *Phytopathology*, 76: 1073.
- Davis, R.F. 1986. Partial characterization of *Zucchini yellow mosaic virus* isolated from squash in Turkey. *Plant Disease*, 70: 735-738.
- Desbiez, C., Lecoq, H. 1997. *Zucchini yellow mosaic virus*. *Plant Pathol.*, 46: 809-829.
- Ertunc, F. 1992. Ankara İlinde Kabaklarda Enfeksiyon Olusturan Viral Etmenin Teşhisi Üzerinde Araştırmalar. *Ankara Üniversitesi Ziraat Fakültesi Yayınları*, No: 1252, 13s.
- Foissac, L., Gentit, P., Svanetia-Dumas, L., Dulucq, M. J., Candresse, T. 2001. Polyvalent detection of fruit tree tricho, capillo, and foveaviruses by nested RT-PCR using degenerated and inosine-containing primers (PDO RT-PCR). *Acta Horticulturae*, 550: 37-43.
- Fujisawa, I., Hanada, T., Anang, S.B. 1986. Virus diseases occurring on some vegetable crops in West Malaysia. *Journal of Agricultural Research*, 20: 78-84.
- Ghanem, G.A.M., Noura-Hassan, M., Kheder, A.A., Mazyad, H.M., Abdel-Alim, A.I. 2016. Antiserum production, biological and serological detection of *Cucurbit yellow stunting disorder crinivirus* (CYSDV) in Egypt. *International Journal of Advanced Research*, 4(4): 1116-1128.
- Harper, K., Creamer, R. 1995. Hybridization detection of insect-transmitted plant

- viruses with digoxigenin- labeled probes. *Plant Disease*, 79: 563-567.
- Hosseini, S., Mosahebi, G.H., Koochi Habibi, M., Okhovvat, S.M. 2007. Characterization of the *Zucchini yellow mosaic virus* from squash in Tehran province. *Journal of Agricultural Science and Technology*, 9: 137-143.
- Jossey, S., Babadoost, M. 2008. Occurrence and distribution of pumpkin and squash viruses in Illinois. *Plant Diseases*, 92: 61-68.
- Kamberoğlu, M.A., Çalışkan, A.F., Desbiez, C. 2016. Current status of some cucurbit viruses in Cukurova Region (Adana and Mersin Provinces) of Turkey and molecular characterization of *Zucchini Yellow mosaic virus* isolates. *Romanian Biotechnological Letters*, 21(4): 11709-11719.
- Karamanlı, A., Kamberoğlu, MA. 2010. Survey of *Cucumber Mosaic Virus* (CMV) and *Zucchini Yellow Mosaic Virus* (ZYMV) in Turkish Republic of Northern Cyprus in Cucurbits Growth Fields. *Ç.Ü. Fen Bilimleri Enstitüsü*, Cilt: 22-23.
- Kaya, A., Erkan, S. 2011. Detection and incidence of viruses in cucurbits grown in Izmir, Aydın, Manisa and Balıkesir provinces. *Bitki Koruma Bülteni*, 51(4):387-405.
- Khalifa, M.E., El-Fallal, A.A., El-Sayed, A.K., Diab, A., Sadik, A.S. 2015. Identification of an isolate of zucchini yellow mosaic potyvirus infecting squash in Egypt. *Pakistan Journal of Biotechnology*, 12(2): 173-180.
- Kheder, A.A., Sulaiman, T.M., Ghanem, G.A.M., Tohamy, M.R. 2017. Biological, serological and molecular characterization of Egyptian *Zucchini yellow mosaic virus* isolate infecting squash plants in Fayoum Governorate. *Egyptian Journal of Phytopathology*, 45(2): 1-14.
- Korkmaz, F., Topkaya, Ş., Yanar, Y. 2018. Tokat ili kabakgil üretim alanlarında enfeksiyon oluşturan virüslerin belirlenmesi. *Gaziosmanpaşa Journal of Scientific Research*, 7(2): 46-56.
- Köklü, G., Yılmaz, Ö. 2006. Occurrence of cucurbit viruses on field-grown melon and watermelon in the Thrace region of Turkey. *Phytoprotection*, 87(3), 123-130.
- Lecoq, H., Wipf-Scheibel, C., Nozeran, K., Millot, P., Desbiez, C. 2014. Comparative molecular epidemiology provides new insights into *Zucchini yellow mosaic virus* occurrence in France. *Virus Research*, 186: 135-143.
- Lisa, V., Boccardo, G., D'agostino, G., Delavalle, G., D'aquilio, M. 1981. Characterization of a Potyvirus that causes *Zucchini yellow mosaic virus*. *Phytopathology*, 71: 667-672.
- Lisa, V., Lecoq, H. 1984. *Zucchini Yellow Mosaic Virus*. No: 282 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England, 4 p.
- Lovisolo, O. 1980. Virus and viroid diseases of cucurbits. *Acta Horticulturae*, 88: 33-82.
- Mahgoub, H.A., Desbiez, C., Wipf-Scheibel, C., Dafalla, G., Lecoq, H. 1997. Characterization and occurrence of *zucchini yellow mosaic virus* in Sudan. *Plant Pathology*, in press. *Plant Pathology*, 46(5): 800-805.
- Mohammed, H.S., Zicca, S., Manglli, A., Mohamed, M.E., El Siddig, M.A., Tomassoli, L., El Hussein, A.A. 2014. Identification and phylogenetic analysis of common pumpkin viruses in Sudan. *Journal of Plant Pathology*, 96(1): 77-84.
- Nagendran, K., Priyanka, R., Keerthana, U., Mohankumar, S., Karthikeyan, G. 2016. First report of *Zucchini yellow mosaic virus* on *Cucurbita moschata* in India. *Journal of Plant Pathology*, 98(1): 171-185.
- Nasr-Eldin, M.A., Abdelkader, H.S., Abo-Senna, A.S., Othman, B.A. 2016. Characterization and phylogenetic analysis of *Zucchini yellow mosaic virus* infecting *Cucurbita pepo* in Egypt. *Journal of American Science*, 12(3): 93-104.
- Ohtsu, Y., Sako, N. 1985. *Zucchini yellow mosaic virus* isolated from pumpkin in Milyako and Yaeyama Islands, Okinawa, Japan. *Annals of the Phytopathological Society of Japan*, 51: 234-237.
- Ozaslan, M., Aytakin, T., Bas, B., Halil Kilic, I., Didem Afacan, I., Dag, D.S. 2006. Virus diseases of cucurbits in Gaziantep- Turkey. *Plant Pathology Journal*, 5(1): 24-27.
- Özer, M., Sipahioğlu, H.M., Usta, M., Fidan, H. 2012. Cloning and sequencing of coat protein gene of *Zucchini yellow mosaic virus* isolated from squash and muskmelon in Turkey. *Turkish Journal of Biology*, 36: 423-429.
- Pospieszny, H., Cajza, M., Plewa, R. 2003. First report of *Zucchini yellow mosaic virus* in cucumber in Poland. *Plant Disease*, 87(11): 1399-1399.
- Prieto, H., Bruna, A., Hinrichsen, P., Muñoz, C. 2001. Isolation and molecular characterization of a Chilean isolate of *Zucchini yellow mosaic virus*. *Plant Disease*, 85: 644-648.
- Romay, G., Lecoq, H., Geraud-Pouey, F., Chirinos, D.T., Desbiez, C. 2014. Current status of cucurbit viruses in Venezuela and

- characterization of Venezuelan isolates of *Zucchini yellow mosaic virus*. *Plant Pathology*, 63: 78-87.
- Sertkaya, G., Sertkaya, E., Yetişir, H., Kaya, K. 2004. Investigations on incidence and transmission of *Zucchini yellow mosaic virus* (ZYMV) in cucurbits Hatay province. Proceedings of the First Plant Protection Congress of Turkey, 8-10 September 2004, Samsun, Turkey, p. 217.
- Sevik, M.A., Arli-Sokmen, M. 2003. Viruses infecting cucurbits in Samsun, Turkey. *Plant Disease*. 87: 341-344.
- Shukla DD., Ward CW., Brunt AA. 1994. *The Potyviridae*. Wallingford, UK: CAB International. 516.
- Simmons, H.E., Holmes E.C., Gildow F.E., Bothe-Goralczyk M. A., Stephenson A.G. 2011. Experimental verification of seed transmission of *Zucchini yellow mosaic virus*. *Plant Disease*, 95: 751-754.
- Simmons, H.E., Dunham J.P., Zinn K.E., Munkvold G.P., Holmes E.C., Stephenson A.G. 2013. *Zucchini yellow mosaic virus* (ZYMV, Potyvirus): vertical transmission, seed infection and cryptic infections. *Virus Research*, 176(1-2): 259-64.
- Spadotti, D.M.A., Wassano, D.T., Rezende, J.A.M., Camargo, L.E.A., Inoue-Nagata, A.K. 2015. Biological and molecular characterization of Brazilian isolates of *Zucchini yellow mosaic virus*. *Scientia Agricola*, 72: 187-191.
- Svoboda, J., Polák, J. 2002. Distribution, variability and overwintering of *Zucchini yellow mosaic virus* in the Czech Republic. *Plant Protection Science*, 38: 125-130.
- Şevik, M.A., Balkaya, A. 2015. Samsun, Sinop ve Bolu illerindeki bal kabağı (*Cucurbita moschata* Duch) popülasyonlarına ait tohum örneklerinde virüslerin tanınması ve bulunma durumlarının belirlenmesi. *Journal of Agricultural Faculty of Gaziosmanpaşa University*, 32(3): 70-77.
- Thomson, K.G., Dietzgen, R.G., Gibbs, A.J., Tang, Y.C., Liesack, W., Teakle, D.S., Stackebrandt, E. 1995. Identification of *Zucchini yellow mosaic potyvirus* by RT-PCR and analysis of sequence variability. *Journal of Virological Methods*, 55: 83-96.
- Tymchyshyn, O., Shevchenko, T., Shevchenko, O., Budzanivska I. 2017. Phylogenetic analysis of seed-transmitted isolate of *Zucchini yellow mosaic virus*. *БІОЛОГІЯ*, 2(74): 47-50.
- Vučurović, A., Bulajić, A., Stanković, I., Ristić, D., Nikolić, D., Berenji, J., Krstić, B. 2012. First report of *Zucchini yellow mosaic virus* in watermelon in Serbia. *Plant Disease*, 96: 149.
- Wang, H.L., Gonsalves, D., Provvidenti, R., Zitter, T.A. 1992. Comparative biological and serological properties of four strains of *zucchini yellow mosaic virus*. *Plant Disease*, 76: 530-535
- Wang, D., Li, G. 2017. Biological and molecular characterization of *Zucchini yellow mosaic virus* isolates infecting melon in Xinjiang, China. *Journal of Plant Pathology*, 39(1): 48-59.
- Yeşil, S. 2013. Konya, Karaman ve Aksaray illeri Kabakgil Ekim Alanlarında Görülen Virüs Hastalıklarının Serolojik ve Moleküler Yöntemlerle Tespiti ve Enfeksiyon Kaynaklarının Belirlenmesi. Doktora Tezi. Selçuk Üniversitesi.
- Yeşil, S. 2014. Virus Diseases of Edible Seed Squash (*Cucurbita pepo* L.) in Konya Province. In: Book of Proceedings Fifth International Scientific Agricultural Symposium (AGROSYM-2014), October, 23-26th, 2014, Jahorina, Sarajevo, Bosnia-Herzegovina, 226 p. ISBN: 978-99955-751-9-9.
- Yeşil, S., Ertunç, F. 2012. Virus diseases of cucurbits in Konya province. Proceedings of the Xth EUCARPIA Meeting on Genetics and Breeding of Cucurbitaceae, pp. 791-796.
- Yeşil, S., Ertunç, F. 2013. Virus diseases of Cucurbits in Karaman province. *International Journal of Ecosystems and Ecology Sciences (IJEES)*, 3(2): 235-240.
- Yılmaz, M.A., Lecoq, H., Abak, K., Baloglu, S., Sarı, N. 1992. Türkiye’de kabakgil sebze türlerinde zarar yapan virüsler. Türkiye I. Ulusal Bahçe Bitkileri Kongresi, İzmir, Bildiriler Kitabı, s. 439-442.
- Yılmaz, M.A., Baloğlu S., Özasan M., Güldür M.E. 1995. GAP Bölgesi kültür bitkilerinde belirlenen virüsler. GAP Bölgesi Bitki Koruma Sorunları ve Çözüm Önerileri Sempozyumu, Şanlıurfa, Turkey, s. 241-250.
- Zhao, M.F., Chen, J., Zheng, H.Y., Adams, M.J., Chen, J.P. 2003. Molecular analysis of *Zucchini yellow mosaic virus* isolates from Hangzhou, China. *Journal of Phytopathology*, 151: 307-311.
- Zheng, L., Rodoni, B.C., Gibbs, M.J., Gibbs, A.J. 2010. A novel pair of universal primers for the detection of *Potyviruses*. *Plant Pathology*, 59: 211-220.
- Zitter, T.A., Hopkins, D.A., Thomas, C.F. 1996. Compendium of Cucurbit Diseases. St-Paul. MN: APS Press, 87 pp.