Research Article

Ekin
Journal of Crop Breeding and Genetics

4(2):8-19, 2018

www.ekinjournal.com

Ekin International biannual peer-reviewed journal

Poaceae Weed Hosts of *Yellow dwarf viruses* (YDVs) in the Trakya Region of Turkey

Havva ILBAGI[1*] Ahmet CITIR[1] Adnan KARA[1] Meryem UYSAL[2]

[1] Department of Plant Protection, Faculty of Agriculture, Namık Kemal University, Tekirdağ, 59030, Turkey
[2] Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya, 42030, Turkey

[*] Corresponding author e-mail: hilbagi@nku.edu.tr

Citation:
Ilbagi H., Citir A., Kara A., Uysal M., 2018. Poaceae Weed Hosts of *Yellow dwarf viruses* (YDVs) in the Trakya Region of Turkey. Ekin J. 4(2):8-19, 2018.

Received: 30.04.2018 ***Accepted: 06.06.2018*** ***Published Online: 30.07.2018*** ***Printed: 30.07.2018***

ABSTRACT

Trakya Region of Turkey has been one of the important cereal growing areas in Turkey. Previously sporadic and temporary infections of *Yellow dwarf viruses* (YDVs) have been reported in some parts of Turkey. YDV diseases on cereals however have been prevailing and causing yellowing, dwarfing, reddening and the reduction of grain yield on cultivated cereals since 1999 in the Trakya Region. YDV have been identified and their incidence and the rate of infections were investigated. *Barley yellow dwarf virus-PAV* (BYDV-PAV) was diagnosed as the most virulent and dominant one as *Cereal yellow dwarf virus-RPV* (CYDV-RPV) was also identified as another important virus in the area. In order to determine sources of YDVs and their over summering and overwintering hosts among the Poaceae weed species 326 symptomatic weed leaf samples and 82 intact weed plants were collected from road sides and hedge grows of cereal fields in 2010. In second year 357 weed leaf samples, 13 voluntary cereal leaves and 50 intact weed plants were also collected from same sites. Separately 7 aphid species were identified and 5 of them were used for vector transmission tests of YDVs from potted intact weeds to indicator barley (cv. Barbaros) seedlings. As a result of aphid transmissions from 15 weed species, 156 symptomatic barley leaf samples and from 6 weed species, 50 symptomatic barley samples were obtained in 2010 and 2011 respectively. So, totally 902 leaf samples were obtained from 42 weed, 3 voluntaries and 1 indicator barley species. DAS-ELISA and RT-PCR tests on 326 weed samples revealed the corresponding incidence rates were 54.60% for BYDV-PAV, 7.05% for CYDV-RPV, 5.52% for PAV+RPV, 14.41% for the other YDVs and being 81.59% total rate of virus incidence in weed samples in 2010. Test results on 370 leaf samples also revealed the incidences of BYDV-PAV as 14.86%, CYDV-RPV as 10.81%, PAV+RPV as 7.56% and the other YDVs as 48.91% totally being 82.16% rate of virus incidence from weed and voluntary cereal samples in 2011. Aphid transmitted barley samples revealed the similar incidences of viruses too. For molecular characterization the genomic region containing coat protein (CP) regions of BYDV-PAV and CYDV-RPV were amplified from selected weed species and samples by RT-PCR method. Specific DNA fragments in the sizes of 531 bp and 400 bp were amplified from 45 BYDV-PAV isolates from 24 weed species and 34 CYDV-RPV isolates from 15 weed species respectively. The selected DNA fragments of BYDV-PAV and CYDV-RPV were purified and sequenced for the determination of nucleotide sequences of CP genes of both virus isolates. Partial nucleotide sequences of 20 Turkish PAV weed isolates were determined and compared with other nine BYDV-PAV isolates in databases. Phylogenetic analysis of obtained and published nucleotide and amino acid sequences revealed the identity ranged from 86.67 - 99.80% and 70.05 - 99.40% respectively. Partial nucleotide sequences of 6 CYDV-RPV isolates were also compared with seven isolates of CYDV-RPV isolates in GenBank/EMBL. The nucleotide and amino acid sequences revealed the identity ranged from 80.44 - 95.86% and 62.50 - 93.33% identities respectively. To our knowledge, this is the first report of YDV's in Poacea weed hosts in Turkey.

Keywords**:** YDVs, BYDV-PAV, CYDV-RPV, weed host, cereal

Introduction

Trakya Region of Turkey has been one of the important cereal growing areas in Turkey. Almost one million ha of arable land covers 65% of the region has been allocated for field crops and cereal production. Annual average precipitation has been 590 mm, providing necessary moisture under dry farming for cereal production. Grain yield usually varies for the unsuitable weather conditions as well as the pest and diseases in the area. Beside important fungal diseases, sporadic and temporary infections of *Yellow dwarf viruses* (YDV) namely *Barley yellow dwarf virus-PAV* (BYDV-PAV) and *Cereal yellow dwarf virus-RPV* (CYDV-RPV) and their vectors on cereals were reported in some parts of Turkey (Bremer and Raatikainen 1975). YDV diseases on cereals however have been prevailing since 1999 in the Trakya Region (Ilbagi 2003). In addition to Trakya Region YDV diseases and *Wheat dwarf virus* (WDV) infections were also reported in 15 other cereal producing provinces of Turkey (Pocsai *et al.,* 2003; Ilbagi *et al.*, 2003). Those YDV diseases on winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), triticale (X *Triticosecale* Wittmack) and bird seed (*Phalaris canariensis* L.) caused yellowing, dwarfing, reddening and the reduction of grain yield and quality. Viruses of YD diseases have been identified and their incidence and the rate of infections were investigated (e.g., Ilbagi *et al.,* 2005; Ilbagi *et al.,* 2008). Up to now 8 YDV species were named and classified into Luteoviridae family (King *et al.,* 2011). Among them a luteovirus species *Barley yellow dwarf virus-PAV* (BYDV-PAV) was diagnosed the most virulent and dominant one as *Barley yellow dwarf virus-MAV* (BYDV-MAV) was found moderately virulent. *Cereal yellow dwarf virus-RPV* (Polerovirus, Luteoviridae) was also identified as another important virus in the area. The other YDVs of Luteoviridae family were weakly virulent and found in lower incidences. Control strategies and the prevention of YDV disease epidemics in field condition have been included in the assessments of host plants, environmental conditions, viruses and vector aphid species (D'Arcy and Burnett 1995). Beside cultivated cereal species D'Arcy (1995) complied and listed 96 annual, 2 biannual and 111 perennial Poaceae weed host in the world. Later on Poaceae weed host as a sources of YDV inoculums were reported in different countries by Garret and Dendy (2004) in the USA, Pokorny (2006) in Chech Republic, Bisnieks *et al.,* (2004) in Latvia and Sweden, Bakardjieva (2006) in Bulgaria. At the same time Ilbagi (2006) identified common reed (*Phragmites communis* Trin.) as over summering and overwintering host of BYDV-PAV, CYDV-RPV, *Maize dwarf mosaic virus* (MDMV) and *Sugarcane mosaic virus* (SCMV) in the Trakya Region of Turkey. Güncan (2010) suggested effective weed control for their being sources of YDVs, as well as competition with cultivated cereals for plant nutrients and water. YDVs are phloem-limited and obligatorily transmitted viruses in a persistent manner by a number of aphid species. Halbert and Voegtlin (1995) reported and described the biology of 25 aphid species as the vector of YDVs however 10 of them are commonly found on cereal fields. In the case of aphid vectors infestation in Turkey Kinacı and Yakar (1984) reported the presence of *Rhopalosiphum padi* L. and *Rhopalosiphum maidis* Fitch. four provinces of Central Anatolian Region 4 aphid species were identified as the vectors of BYDV's by Çalı and Yurdakul (1996) as in Trakya Region Özder and Toros (1999) identified 7 aphid species in wheat fields in Tekirdağ Province. As long as the presence of direct interactions between viruses and vectors, aphids and host plants beside weed hosts it is utmost important to investigate aphid vectors too (Power and Gray 1995). YDVs and their aphid vector specificity has been considered as a rule not the exception since the work of Rochow and Muller (1971). Merely names of some aphids and the term of BYDV-strains changed into YDV species. In order to determine sources of YDV's and their over summering and overwintering hosts among the Poaceae weed species this study was initiated in 2009. For this purpose survey trips to 12 districts of Trakya Region have been done and Poaceae weed and weed leaf samples were collected. For the identification of YDV's, DAS-ELISA and RT-PCR tests were used and nucleotide sequence and phylogenetic analysis were implemented. By employing nucleotide sequences of 531 bp fragments of code protein gene of BYDV-PAV isolates and 400 bp fragments of code protein gene of CYDV-RPV isolates obtained from Poaceae weeds phylogenetic trees were constructed and compared them with Gene Bank accessions of both viruses.

Material and Methods

Survey studies and sampling: Extensive survey studies were implemented daily by travelling at least 72 Km up to 160 Km distances from Tekirdağ in the Trakya Region where 12 counties were visited in May and June 2010 and 2011 as exhibited in Figure 1.

Totally 829 symptomatic weed plants and weed leaf samples were collected from road side verges, hedge grows, banks of creeks and fallowed cereal fields. Herbariums of intact weeds were made for

their identifications. Weed leaf samples were packed into polyethylene bags and maintained in deep freeze working at -27°C until their usage for identification of YDV's. 82 symptomatic intact weed plants however collected and transplanted into sterile mixture of soil, sand and compost (1:1:1:) filled 3 L pots maintained into insect proof cages in 2010 and similarly 50 plants were transplanted in 2011.

Aphid collections and identifications: Weeds and voluntary cereal plants were examined for aphids. Whenever possible, aphids were identified at the sampling sites. Otherwise they were collected with their colonized plants by wrapping into papers and packed in polyethylene bags, brought to laboratory. Apterous aphid colonies free from their parasites, were collected into bottles containing 70% ethanol for identification later under Olympus SZ51 Stereo microscope. Rest of the aphids were transferred and cultured on potted healthy wheat (cv. Pehivan, and Attila 12) and barley (cv. Barbaros) plants grown in sterile greenhouse conditions. So 7 aphid species were identified,5 of them were cultured for aphid transmissions of YDV's and maintained in insect proof cages as suggested by Halbert and Voegtlin (1995)

Indicator plant and aphid transmission: Barley (cv. Barbaros) was selected as indicator plants of YDV's. Seeds were sown into 500 cc pots filled with sterilized mixture of soil, sand and compost (1:1:1) having 6 seeds in each pot. So 300 pots of indicator barley seedlings were grown in 2010 and repeatedly 300 pots of barley were grown in 2011. Aphid transmissions were made as suggested by Du *et al.,* (2007) by collecting apterous individuals into petri dishes by using camel hair brush and placing them on transplanted weeds for accusation of YDV's and let them feeding for 2 days. So, 1 plant was allocated for each aphid species from which 5 aphids per plant and totally 25 aphids from 5 species were used for transmission in each pot. After accusation period, aphids were transferred to indicator plants as 5 aphids per plant as one plant saved for control. After 5 days of inoculation period aphids were killed by spraying Marshall-25 insecticide and maintained them in insect proof greenhouse conditions at 20, 25°C for the exhibition of virus symptoms.

ELISA Procedures: Totally 901 leaf samples were tested with polyclonal antibodies (manufactured by AGDIA Inc.; Elkhart IN, USA) for the presence of BYDV-PAV, BYDV-MAV and CYDV-RPV viruses by employing Double Antibody Sandwich Enzyme-Linked Immunosorbent Assays (DAS-ELISA) as described by Clark and Adams (1977) and the procedure suggested by AGDIA Inc.

Nucleic acid isolation from YDV infected samples: Obtained 901 weed and aphid transmitted cereal leaf samples were subjected to isolation of the viral nucleic acid by employing the total nucleic acid extraction method described by Falke *et al.,* (2000).

cDNA synthesis: First strands cDNA molecules were obtained from total isolated RNA's of the code protein gene fragments belong to BYDV-PAV and CYDV-RPV by using Omniscript reverse transcriptase synthesize Kit (Fermentas; Vilnius, Lithuania). For each reaction 2 µl total RNA, 1 µl primer pairs (100 pmol/µ) were used and processed according to the manufacturer's instructions.

RT-PCR amplifications: Primer pairs for BYDV-PAV (5'-CCAGTGGTTRTGGTC-3' antisense) and (5'-GTCTACCTATTTGG-3' sense) as designed by Robertson *et al.,* (1991) were used for the amplification by RT-PCR. Amplified fragments were 531 bp long and corresponded to BYDV-PAV genome nucleotides between 2938 and 3469. The PCR reaction for BYDV-PAV consisted of 3 µl 10x PCR buffer, 2 µl $MgCl_2$ (25mM), 1 µl dNTP (10mM), 2 µl primer1, 2 µl primer2, 1 µl Taq DNA polymerase enzyme, 1 µl cDNA and 13 µl RNase free water. The amplification protocol for BYDV-PAV was as fallows; initial denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 1 min, 43°C for 1 min, 72°C for 1 min. and the final extension step at 72°C for 10 min in a Techne thermal cycler. PCR products were analyzed by electrophoresis in 2% agarose gel, stained with ethidium bromide (EtBr) and viewed under UV illumination in a gel documentation system (Vilber Lourmet; Marne La Vallee Cedex 1, France).

Similarly, primer pairs for CYDV-RPV (5'-ATGTTGTACCGCTTGATCCAC-3'antisense) and (5'-GCGAACCATTGCCATTG-3'sense) as designed by Deb and Anderson (2007) were used for the amplification by RT-PCR. Amplified fragments were 400 bp long and corresponded to CYDV-RPV genome nucleotides between 3275-3675. Those primer pairs for all viruses were obtained from IDT Inc. Coralville, Iowa, USA. The PCR reaction for CYDV-RPV consisted of 3µl 10x PCR buffer, 2 µl $MgCl_2$ (25mM), 1µl dNTP (10mM), 0.5µl primer 1, 0.5µl primer 2, 0.3µl Taq DNA polymerase enzyme, 2 µl cDNA, 15.7 µl RNase free water. The amplification protocol for CYDV-RPV was as follows; Initial denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 30 sec, 60°C for 45 sec, 72°C for 1 min and the final extension step at 72°C for 10 min in thermal cycler. PCR products were analyzed by electrophoresis in 2% agarose gel, stained with EtBr and viewed under UV illumination in a gel documentation system (Vilber Lourmet; Marne La

Vallee Cedex 1, France). By employing proper primer pairs for the viruses of BYDV-MAV, BYDV-RMV and BYDV-SGV fragments consisting necessary compounds and following similar protocols PCR products were obtained and analyzed for their identifications too.

Sequencing of RT-PCR products: For sequence analysis, PCR products of BYDV-PAV and CYDV-RPV were purified from agarose gels by employing QIAquick gel extraction kits manufactured by MBI Fermentas; StLeon-Rot, Germany. Purified gels were sequenced in accordance with the manufacturer's protocol at Refgen Biotechnology Company, Ankara, Turkey. Obtained nucleotides sequences of both BYDV-PAV and CYDV-RPV isolates were aligned with Mega5 Program. The alignments were used as input data to construct phylogenetic trees with the Neighbor-Joining Distance method implemented in Mega 5.0 Program Tamura *et al.,* (2011) and compared with International Gene Bank accessions.

Results and Discussion

During the survey studies, 326 weed leaf samples from 14 annual, 3 biannual and 9 perennial totally 26 weed species were collected in 2010. Beside leaf samples 82 symptomatic intact weed plants from 15 species were also obtained and transplanted to pots for aphid transmission tests. In addition to 13 leaf samples from 3 voluntary cereal species, 357 weed leaf samples from 21 annual 1 biannual, 10 perennial species were collected. So total 370 leaf samples, 50 symptomatic intact weed plants were obtained and transplanted to pots for aphid transmission in 2011. By evaluating the distribution of weed species in 12 districts revealed that Hayrabolu was the most infested district with 21 weed species as Kırklareli Central District was found the least infested district with 7 weed species. In confirmation of our results most of those species were reported as the competitive weeds in cereal fields in Turkey (Güncan 2010). Collected and identified aphid species are listed in Table 1. *Metopolophium dirhodum* (Walker) was collected from 8 districts as infested on 8 weed species. *Rhopalosiphum padi* L. was in second place collected from 7 districts and found infested on 7 weed species. *Rhopalosiphum rufiabdominalis* (Sasaki) and *Sitobium fragariae* (Walker) were found in Hayrabolu as infested on *Bromus arvensis* and *Avena sterilis* respectively. These findings confirmed the observations of Kinaci and Yakar (1984), Cali and Yurdakul (1996) and the results of Ozder and Toros (1999) whom they identified same aphid species in cereal fields in Tekirdağ Province in the Trakya Region. As considered being predominant vectors of YDVs by Lister and Ranieri (1995) 5 aphid species; *Rapholosiphum padi* L., *Rapholosiphum maidis* Fitch., *Stobion avenae* Fabricus, *Schizaphis graminum* Rondeni and *Metopolophium dirhodum* Walker were employed for the aphid transmission tests.

Aphid transmission test results are shown in Table 2, in which 156 barley plants exhibited systemic symptoms and revealed the 79 out of 156 (50%) presence of YDV's in 2010 as 15 out of 50 (30%) of them had YDV's in 2011.

Among those weed species *Avena sterilis* was identified the best sources of YDVs as *A. barbata* and *Hordeum bulbosum* were found the least important sources of YDV's. *R. padi* was determined the most efficient vector which verified the Halbert and Voegtlin (1995)'s results. Test results of ELISA and RT-PCR implemented in 2010 are displayed in Table 3. The results revealed that; 178 of 326 weed samples (54.60%) had BYDV-PAV, 23 of 326 (7.05%) had CYDV-RPV, as 18 out of 326 (5.52%) had the mixture of BYDV-PAV+ CYDV-RPV and 45 of 326 (14.41%) of them found infected with other YDV's. So, totally 266 out of 326 (81.59%) samples from 22 poaceae weed species were identified as potential over summering and overwintering hosts of YDV's.

Four species of weeds; *Gastridium ventricosum*, *Lolium temulentum, Phleum bertolonii*, and *P. subulatum* had no virus at all. Obtained results revealed that BYDV-PAV was found as the dominant virus species on weed samples confirming the results of previous works of Ilbagi (2003), Ilbagi *et al.,* (2003), and Pocsai *et al.,* (2003) on cereal crops in 2010. The results of ELISA and RT-PCR tests implemented in 2011 were exhibited in Table 4.

It revealed the presence of BYDV-PAV at the rate of 54 of 369 (14.86%), CYDV-RPV as 40 of 369 (10.81%), mixture of BYDV-PAV+CYDV-RPV diagnosed as 28 of 369 (7.56%) and the other YDV's as 181 out of 369 (48.91%). Thus, totally 303 out of 369 (82.16%) incidence of viruses taken place on Poaceae weeds in Trakya Region of Turkey. Among the voluntary cultivated cereal only oat samples found infected with viruses. Among the weeds, *Aegilops cylindrical, A. geniculata* and *A. neclecta* were found free from viruses. All the outcomes in two years confirmed the results about the rates of YDV disease incidences on cereal crops reported by Ilbagi (2003), Ilbagi *et al.,* (2003) and Pocsai *et al.,* (2003). Two of virus free 7 weed species *Gastridium ventricosum* and *Aegilops cylindrica* however were listed as the susceptible hosts to Luteoviridae viruses by D'Arcy (1995). A perennial weed *Phalaris aquatica* was determined the most important source of YDV inoculum as *Bromus tomentellus, Avena fatua, Avena sterilis* and *Echinochloa crus-galli* followed it.

Our results in this study confirmed the findings of Garret and Dendy (2004)'s 4 wide spread grass species of being the sources of YDV's inoculum in the USA, Pokorny (2006)'s findings of *Echinochloa crus-galli, Seteria pumila* and *Phalaris canariensis* as the sources of BYDV-PAV in Chech Republic, Bakardjieva *et al.,* (2006)'s findings of *Elymus repens*, *Avena fatua* and *Sorghum halepense* as being sources of YDV diseases of cereals in Bulgaria. Obtained results in this study also confirmed findings of Bisnieks *et al.,* (2004)'s about the *Festuca elatior, Lolium perenne* and *Dactylis glomerata* as sources of BYDV-PAV and CYDV-RPV in the summer crops of cereals in Latvia and Sweden. Our findings in this study about Poaceae weed hosts and YDV infections on cereals in the Trakya Region confirmed the finding of Ilbagi (2006) about the widespread perennial weed *Phragmites communis* (*Phragmites australis*) being the over summering and overwintering host of BYDV-PAV, CYDV-RPV as well as MDMV and SCMV too.

Being the most important YDV on cereals in Turkey BYDV-PAV deserved the investigation about its molecular features. So partial CP gene sequences of 20 Turkish PAV isolates obtained from weed species were aligned and compared with the published sequences of 9 isolates of PAV available in the GenBank⁄EMBL databases. Multiple sequence alignments and pair wise sequence comparisons were performed BioEdit Software. The results of phylogenetic analysis demonstrated that the PAV isolates divided into two major groups as shown in Figure 2. In the first group, 14 weed isolates of PAV clustered with the other PAV isolates from China, Iran, Sweden, New Zealand, Pakistan and USA available in the Genbank databases by forming two subgroups. The other 6 isolates from 6 weed species of *A. stolonifera, L. rigidum, V. ciliate, A. sterilis, B. hordeaceous,* and *B. scoparius* were clustered among themselves in second group and they also had two subgroups. Sequences analysis among all the PAV isolates included the nucleotide identities was 86.67 - 99.80%. The lowest level of identity was 86.67% between Priekuli1 isolate from Sweden (Acc.No. AJ563415.1) and TR-AGR isolate of *A. stolonifera*, while the highest level identity was 99.80% between Yolo274 isolate from USA (Acc. No. DQ631850.1) with TR-VULM isolate of *V. myosurus*, TR-PHR1 isolate of *P. austrialis*, TR-CYNO isolate of *C. echinatus* by confirming Rastgou *et al.,* (2005), Pakdel *et al.,* (2010)'s results. The Cluster I included that the highest nucleotide identities were 94.63-99.80% isolates between 06KM25 isolate Chine (Acc. No. EU332333.1) and Yolo274 isolate USA (Acc. No. DQ631850.1) with TR-PHR1, TR-HMUR, TR-VULM, TR-PHR1 of weed isolates while Cluster II included that the highest nucleotide identities were 86.67 - 90.05% between Priekuli1 with TR-AGR isolate and Yolo274 with TR-VULC isolate respectively. Nevertheless, Cluster II included that the comparisons among themselves of the PAV isolates in this study revealed that the nucleotide identities were 87.67-100.00%. The lowest level of identity was 87.67% between TRAQUA1 isolate of *P. aquatica* and TR-LP-ER isolate of *L. perenne.* The highest level of identity was 100% between TR-VULM isolate of *V. myosurus*, TR-PHR1 isolate of *P. austrialis* and between TR-VULM isolate of *V. myosurus*, TR-CYNO isolate of *C. echinatus*. PAV isolates grouped according to their hosts, not grouped according to their geographical distribution or their genetic diversity as described by Bisniek *et al.,* (2004), Mastari *et al.,* (1998). Amino acid multiple sequence alignment revealed the lowest level of identity was 70.05% between Priekuli1 isolate from Sweden (Acc.No. AJ563413.1) and TR-AQUA1 isolate of *P. aquatica,* while the highest level of identity was 99.40% between Yolo274 isolate from USA and TR-VULM isolate of *V. myosurus*, and TR-PHR1 isolate of *P. austrialis*.

The identified nucleotide sequences of 6 Turkish RPV weed isolates were also aligned and compared to sequences of 7 isolates of CYDV-RPV available in GenBank⁄EMBL. Multiple sequence alignments and pair wise sequence comparisons were performed using BioEdit Software. The results of the phylogenetic analysis demonstrated that the RPV isolates were divided in two major groups as shown in Figure 3. The sequences of the RPV isolates that were analyzed uncovered that the nucleotide identities were 80.44 - 95.86%. The lowest level of identity was 80.44% between RPV-TR2 (Acc. No. KR005847) and RPV 05P4b02 isolate (Acc.No. DQ988088.1) while the highest level of identity was 95.86% between RPV-TR2 and RPV 44P4b04 isolate (Acc.No. DQ988108.1). The studied 6 weed isolates from this study were clustered in the first group with 3 RPV isolates from the USA (Acc.No. DQ988108.1, Acc.No. EF521839.1, Acc.No. DQ988105.1). The lowest level of nucleotide identity was 91.46% for RPV-TR2 and RPV 046 (Acc.No. EF521839.1). The highest level of identity was 95.86% between RPV-TR2 and RPV 44P4b04. The other 5 Turkish isolates, except RPV-TR2, formed second subgroups among themselves. The lowest nucleotide identity of the latter isolates was 80.99% between RPV-TR3 (Acc.No. KT923454), RPV 05P4b02 (Acc.No. DQ988088.1) and RPV 010 (Acc.No. EF521830.1). while the highest identity level was 95.04% between RPV-TR6 (Acc.No.

KT923457), RPV 44P4b04 and RPV 046. Bisniek *et al.,* (2004) and Mastari *et al.,* (1998) reported that host plant species play an important role in genetic diversity of BYDVs, which is in accordance with our findings. In parallel to the variations of the nucleotide sequences among the RPV isolates, the same variations are also visible in the amino acids sequences that indicates a lowest identity level of 62.50% between RPV-TR2 and RPV 44P4b04.

The molecular, serologic, as well as the transmission tests conducted in this study revealed that Poaceae weeds species might be reservoirs of *Yellow dwarf viruses* (YDVs). This investigation, because it identifies the potential sources of BYDV-PAV, CYDV-RPV, BYDV-MAV, BYDV-SGV, BYDV-RMV inoculum, provided the means for an effective control of viral infections in Trakya, for example by controlling the Poaceae weed hosts. To our knowledge, this is the first report of YDV's in Poacea weed hosts in Turkey.

Acknowledgments

We would like to thank to Turkish Scientific and Technological Research Council (TÜBİTAK) for the valuable support to Project 109O522, from part of its results this work was obtained.

Table 1. Aphid vectors of *yellow dwarf viruses* (YDVs) of cereals and their infested weed species in Trakya Region of Turkey.

Name of Aphid species	District Name	Names of weeds aphids were obtained
Rhopalosiphum padi L.	Edirne Central	*Avena sterilis* L.
	Ipsala	*Phragmites australis* (Cav) Trin. Exsteudel
	Uzunköprü	*Echinochloa crus-galli* (L.) P. Beauv.
	Lüleburgaz	*Avena fatua* L.
	Pınarhisar	*Avena sterilis* L.
	Tekirdağ Central	*Phragmites australis* (Cav) Trin. ExSteudel
	Çorlu	*Avena sterilis* L.
Rhopalosiphum maidis L.	Tekirdağ Central	*Bromus sterilis*
	Çorlu	*Phragmites australis* (Cav) Trin. ExSteudel
Rhopalosiphum rufiabdominalis (Sasaki)	Hayrabolu	*Bromus arvensis* L.
Sitobion avenae (Fab.)	Ipsala	*Hordeum murinum* L.
	Pınarhisar	*Bromus tectorum* L.
	Saray	*Avena fatua* L.
Sitobion fragariae (Walker)	Hayrabolu	*Avena sterilis* L.
Metopolophium dirhodum (Walker)	Ipsala	*Avena sterilis* L.
	Lalapaşa	*Avena sterilis* L.
	Kırklareli Central	*Hordeum bulbosum* L.
	Lüleburgaz	*Avena fatua* L.
	Pınarhisar	*Hordeum murinum* L
	Tekirdağ Central	*Phalaris aquatic* L.
	Çorlu	*Bromus hordeaceus* L.
	Malkara	*Avena sterilis* L.
Schizaphis graminum (Ron)	Tekirdağ Central	*Avena barbata* Pott ex Link

Table 2. Aphid transmission test results of YDVs to cv. Barbaros Barley by using 5 aphid species and verified by DAS-ELISA and RT-PCR tests in 2010 and 2011.

Year	Number of weed species	Number of plants as virus sources	Number of barley plants YDV's transmitted to				Total number of infected barley plants	Rate of infection
			PAV	RPV	PAV+RPV	Other		
2010	15	82	55	1	5	12	79	50%
2011	6	50	6	4	3	2	15	30%

Table 3. *Yellow dwarf virus* (YDV) disease incidences within naturally infected Poaceae weed species determined by using DAS-ELISA and RT-PCR tests in 2010 in the Trakya Region of Turkey.

Name of weed species	Number of samples	Number of weed samples *yellow dwarf virus* (YDVs) or their mixtures detected				Total number of samples YDV's detected
		BYDV-PAV	BYDV-PAV	PAV+RPV	Other YDV's (MAV-RMV+SGV)	
Aegilops triuncialis	1	1	-	-	-	1
Agrostis stolonifera	1	1	-	-	-	1
Alopecurus aequalis	3	3	-	-	-	3
Avena fatua	20	12	-	3	5	20
Avena sterilis	50	37	1	3	10	51
Bromus arvensis	42	21	4	4	2	31
Bromus hordeceaus	5	4	1	-	-	5
Bromus sterilis	39	21	2	2	7	32
Bromus tectorum	14	10	-	-	1	11
Bromus tomentellus	18	12	1	1	4	18
Cynosorus echinatus	2	2	-	-	-	2
Descampsia caespitosa	3	3	3	-	-	6
Echniochloa crus-galli	10	4	-	1	4	9
Gastridium ventricosum	1	-	-	-	-	-
Hordeum bulbosum	3	1	-	-	-	1
Hordeum murinum	2	1	-	-	-	1
Lolium perenne	11	5	2	-	-	7
Lolium rigidum	16	8	2	1	5	16
Lolium temulentum	4	-	-	-	-	-
Phalaris aquatica	10	8	-	2	-	10
Phleum bertolonii	1	-	-	-	-	-
Phleum subulatum	1	-	-	-	-	-
Phragmites australis	53	19	2	1	7	29
Poe trivialis	4	2	2	-	-	4
Sorghum halepense	9	-	3	-	2	5
Vulpia ciliata	3	3	-	-	-	3
Total 26 species	326	178	23	18	47	266
		54.60%	7.05%	5.52%	14.41%	81.59%

Table 4. *Yellow dwarf virus* (YDV) disease incidences determined by employing DAS-ELISA and RT-PCR tests within the naturally infected weed species and voluntary cereal plants in 2011 in Trakya Region of Turkey.

Names of weed species and voluntary cereals	Number of samples	Identified number of *yellow dwarf viruses* and their mixtures				Total number of viruses identified
		BYDV-PAV	CYDV-RPV	PAV+RPV	Other YDV's (MAV-RMV+SGV)	
Aegilops cylindrica	1	-	-	-	-	-
Aeligops geniculata	1	-	-	-	-	-
Aegilops neclecta	3	-	-	-	-	-
Aegilops triuncialis	2	-	1	-	1	2
Alopecurus myosuroides	7	3	1	-	3	7
Alopecurus rendlei	1	-	-	-	1	1
Apera spica venti	4	1	1	1	2	5
Arrhenatherum elatius	2	-	-	-	2	2
Avena barbata	8	2	2	1	4	9
Avena fatua	1	-	-	-	-	-
Avena sativa (voluntary)	10	2	2	-	11	15
Avena sterilis	42	9	3	3	22	37
Bromus hordeceaus	12	1	2	-	4	7
Bromus rigidus	4	1	-	1	2	4
Bromus scoparius	4	1	-	-	1	2
Bromus sterilis	31	8	6	2	11	27
Bromus tectorum	11	-	-	-	9	9
Cynodon dactylon	3	-	-	-	1	1
Dactylis glomerata	2	-	-	-	2	2
Dasyprum villosum	3	1	-	-	-	1
Echinochloa crus-galli	1	-	-	-	1	1
Elymus repens	13	-	-	-	1	1
Hordeum bulbosum	8	-	-	-	6	6
Hordeum murinum	34	3	4	5	16	28
Lolium perenne	8	1	1	-	3	5
Lolium rigidum	42	6	4	4	20	34
Phalaris aquatica	16	8	1	5	9	23
Phleum exaratum	25	3	3	3	10	19
Phragmites australis	29	1	1	1	23	26
Poa trivialis	19	2	8	1	4	15
Secale cereal (voluntary)	1	-	-	-	-	-
Sorghum halepense	18	1	-	1	12	14
Triticum aestivum (voluntary)	2	-	-	-	-	-
Vulpia ciliate	1	-	-	-	-	-
Vulpia myuros	1	1	-	-	-	1
Total 35 species	370	55	40	28	181	304
		14.86%	10.81%	7.56%	48.91%	82.16%

Figure 1. Twelve districts in the Trakya Region of Turkey where YDVs investigated Totally 829 symptomatic weed plants and weed leaf samples were collected from road.

Figure 2. Constructed Phylogenetic tree of 20 Turkish BYDV-PAV isolates with 9 PAV isolates in database.

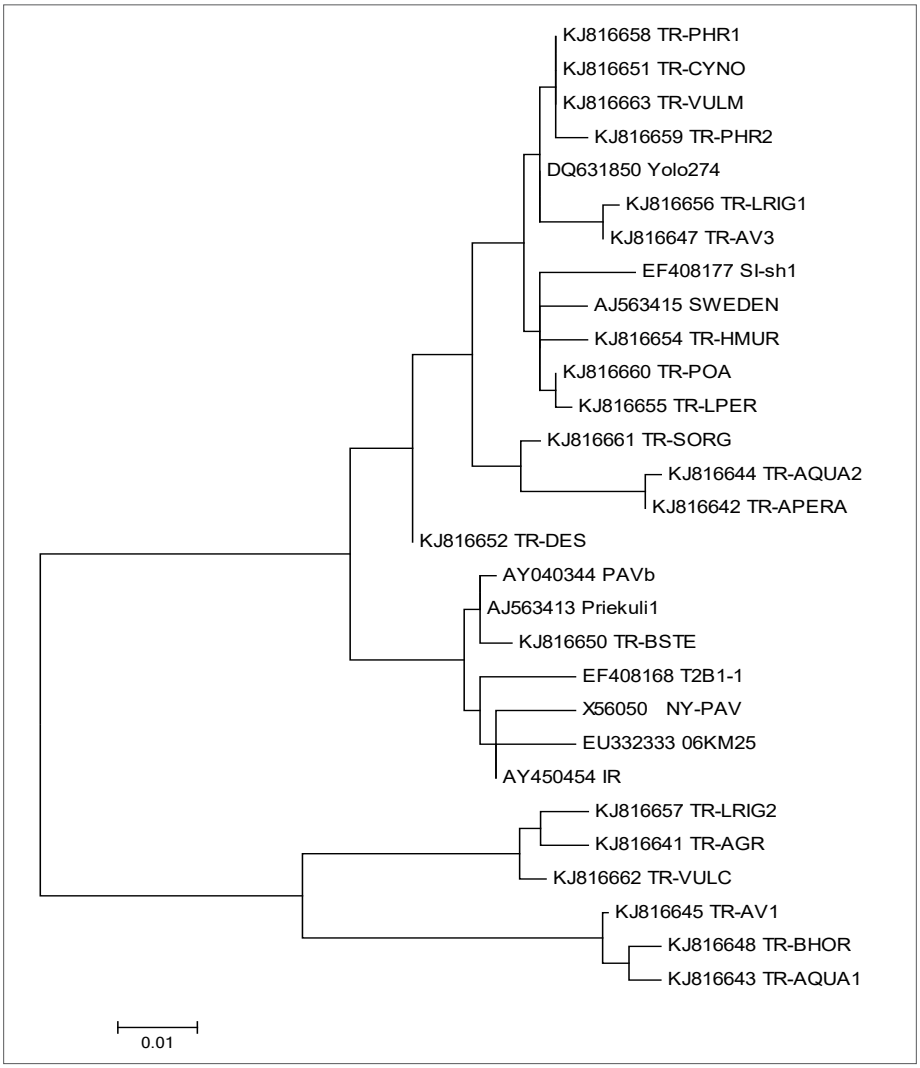
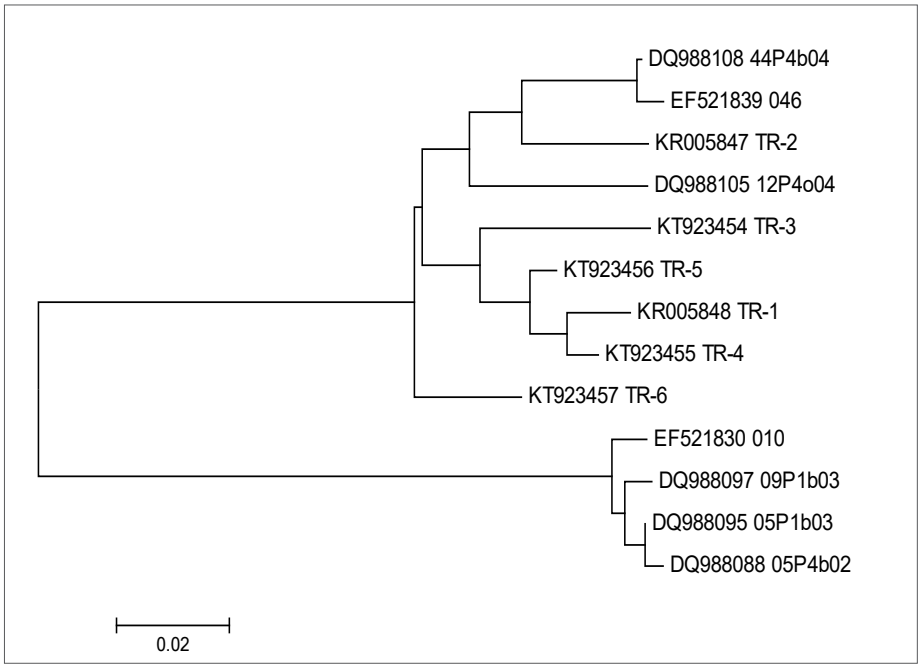


Figure 3. Constructed Phylogenetic tree of 6 Turkish CYDV-RPV isolates with 7 RPV isolates in database.



References

Bakardjieva N, Krasteva C, Habekuss A and Rabenstein F, (2006). Detection of cereal viruses and study of aphid population in Bulgaria. Institute of Plant Protection J. 43:499-501.

Bisnieks M, Kvarnheden A, Sigvald R and Valkonen JPT, (2004). Molecular diversity of the coat protein-encoding region of *Barley yellow dwarf virus*-PAV and *Barley yellow dwarf virus*-MAV from Latvia and Sweden. Arch.Virol. 149: 843-853.

Bremer K and Raatikainen M, (1975). Cereal disease transmitted or caused by aphids and leafhopper in Turkey. Ann. Acad. Sai. Fenn. A. IV. Biologica 203:1-14.

Clark MF and Adams AN, (1977). Characteristics of the Microplate Method of Enzyme-linked Immunosorbent Assay for the detection of plant viruses. J. Gen. Virol. 34: 475-483.

Çalı S and Yurdakul S, (1996). Investigation on virus diseases of wheat in Central Anatolia. Abstracts, 5th International Wheat Conference. June10-14, 1996 Ankara, Turkey. P: 120.

D'Arcy CJ, (1995). Symptomatology and host range of Barley yellow dwarf. (In Barley yellow dwarf 40 years of progress Edited by CJ D'Arcy and PA Burnett). P: 9-28. APS Press, St Paul, MN. USA.

D'Arcy CJ and Burnett PA, (1995). Barley yellow dwarf: A brief introduction. (In Barley yellow dwarf 40 years of progress Edited by CJ D'Arcy and PA Burnett). P: 1-5. APS Press, St Paul MN, USA.

Deb M and Anderson JM, (2007). Development of a multiplexed PCR method for barley and cereal *yellow dwarf viruses*, Wheat spindle streak virus, Wheat streake mosaic virus and Soil-borne wheat mosaic virus. Journal of Virological Methods 148:17-24.

Du ZQ, Li L, Wang XF and Zhou G, (2007). Evaluation of aphid transmission abilities and vector Pathology 89(2): 251-259.

Falke KC, Friedt W and Ordon F, (2000). Nachweis der expression von Bci-4 und Lox:2 Hv1 in Gerste (*Hordeum vulgare* L.) nach DCINA Applkation, (Diplomarbeit), Justus Liebig Universitaet Planzenbau und Pflanzenzüchtung.

Garret KA and Dendy SP, (2004). *Barley yellow dwarf* diseases in natural populations of dominant tallgrass prairie species in Kansas. Department of Plant Pathology, Kansas State University, Manhattan-Kansas, USA. P: 574.

Guncan A, (2010). Weeds and weed control. Selçuk University, Teknik Bilimler MYO, T.B.Yayım Atelyesi. Konya, Turkey. 278 pp. (In Turkish).

Halbert S and Voegtlin D, (1995). Biology and Taxonomy of vectors of *Barley yellow dwarf viruses*. (In Barley Yellow Dwarf. 40 years of progress Edited by CJ D'Arcy and PA Burnett). P: 217-258. APS Press, St Paul MN USA.

Ilbagi H, (2003). Identification of viruses as causal agents of yield loosing infections on some cereal crops in Trakya Region of Turkey. Ph.D. Thesis. Ege University, Graduate School of Natural and Applied Sciences. 136 pp.

Ilbagi H, Pocsai E, Citir A, Muranyi I, Vida G and Korkut KZ, (2003). Results of two years study on Incidence of *Barley yellow dwarf viruses*, *Cereal yellow dwarf virus*-RPV and *Wheat dwarf* virus. Debrecen-Hungary. S. Book p: 53-63.

Ilbagi H, Citir A and Yorgancı U, (2005). Occurence of virus infections on cereal crops and their identifications in the Trakya region of Turkey. J. Plant Diseases and Protection 112 (4): 313-320.

Ilbagi H, (2006). Common reed *(Phragmites communis*) Is a natural host of important cereal viruses in The Trakya Region of Turkey. Phytoparasitica 34(5): 441-448.

Ilbagi H, Rabenstein F, Habekuss A, Ordon F, Citir A, Cebeci O and Budak H, (2008). Molecular,Serological and Transmission Electron Microscopic Analysis of the *Barley yellow dwarf virus*-PAV and *Cereal yellow dwarf virus*-RPV in Canary Seed (*Phalaris canariensis* L.). Cereal Research Communications 36(2): 225-234.

Kinaci E and Yakar K, (1984). Situation reports. Turkey. Page 196 in: *Barley yellow dwarf*, a Proceedings of the Workshop. PA Burnett ed. CIMMYT, Mexico DF, Mexico. 209 pp.

King AMQ, Adams MJ, Carstens EB and Lefkowitz EJ, (2011). Virus Taxonomy. Classification and Nomenclature of Viruses. Ninth Report of the International Committee on Taxonomy of Viruses. Elsevier, Academic Press, 1327 pages.

Lister RM and Ranieri R, (1995). Distribution and economic importance of *barley yellow dwarf*. (In *Barley yellow dwarf* 40 years of progress Edited by C. J. D'Arcy and P.A. Burnett). P: 29- 53 APS Press, St Paul MN, USA.

Mastari J, Lapierre H and Dessens JT, (1998). Assymmetrical distributions of *Barley yellow dwarf virus*-PAV variants between host plant species. Phytopathology 88:818-821.

Ozder N and Toros S, (1999). Tekirdağ İlinde buğdaylarda zarar yapan yaprak biti türlerinin saptanması üzerinde araştırmalar. Türkiye Entomology Dergisi 23: 101-110.

Pakdel A, Afsharifar A, Niazi A, Almasi R and Izadpanah K, (2010). Distribution of cereal luteoviruses and molecular diversity of BYDV-PAV isolates in Central and Southern Iran: Proposal of a new species in the genus Luteovirus. Journal of Phytopathology 158: 357-364.

Pocsai E, İlbagi H, Citir A, Muranyi I, Vida G and Korkut KZ, (2003). Incedence of *Barley yellow dwarf viruses, Cereal yellow dwarf virus* and *Wheat dwarf virus* in Cereal Growing Areas of Turkey. Agriculture 49: 583-591.

Pokorny R, (2006). Occurrence of viruses of the family Luteoviridae on maize and some annual weed Grasses in the Czech Republic. Cereal Research Communications 34(2-3): 1087-1092.

Power AG and Gray SM, (1995). Aphid transmission of *Barley yellow dwarf viruses*: Interactions between viruses, vectors and host plants. (In Barley Yellow Dwarf 40 years of progress by CJD'Arcy and PA Burnett.). APS Press, St Paul MN. USA. P: 259-292.

Rastgou M, Khatabi B, Kvarnheden A and Izadpanat K, (2005). Relationships of *Barley yellow dwarf virus*-PAV and *Yellow dwarf virus* Cereal-RPV from Iran with the viruses of the Family Luteoviridae. European J. Plant Pathology 113: 321-326.

Robertson NL, French R and Gray SM, (1991). Use of group-specific primers and the polymerase chain reaction for the detection and identification of Luteoviruses. Journal of General Virology 72:1473-1477.

Rochow WF and Muller L, (1971). A fifth variant of *Barley yellow dwarf virus* in New York. Plant Diseases Rep. 55:874-877.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S, (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol. 28:2731-2739.