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First Report of Yellow Dwarf Viruses (YDVs) in the Rice Fields in the Trakya Region of Turkey

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ABSTRACT

Yellow dwarf viruses (YDVs) are responsible for the economically significant disease that affects cereal crops worldwide, reducing harvested yield and quality of grains. These virus diseases on cereals have been prevailed and caused yellowing, dwarfing, reddening, and the reduction of grain yield on cultivated cereals since 1999 in the Trakya region of Turkey. This study was conducted to investigate the presence of YDVs on rice and competitive weeds as barnyard grass *Echinochloa crusgalli* (L.) P. Beauv, Johnson grass *Sorghum halepense* (L.) Pers. and common reed *Phragmites australis* (Cav.) Trin ex. Steudel in the Trakya region of Turkey. For this purpose, 120 symptomatic rice leaves and 18 weed leaf samples were collected from the rice fields in Trakya. A total of 138 symptomatic leaf samples were tested by DAS-ELISA and RT-PCR methods for the diagnoses of *Barley yellow dwarf virus*-PAV (BYDV-PAV), *Barley yellow dwarf virus*-MAV (BYDV-MAV) and *Cereal yellow dwarf virus*-RPV (CYDV-RPV). The screening test results revealed that 26 out of 138 leaf samples had CYDV-RPV, 5 of them infected with BYDV-PAV, and 10 leaf samples were found infected with a mixture of CYDV-RPV+BYDV-PAV. Thus, 41 out of 138 leaf samples at the rate, 30% were found infected with BYDV-PAV and CYDV-RPV. None of the samples had BYDV-MAV. To our knowledge, this is the first report of BYDV-PAV and CYDV-RPV in the most important rice-growing areas of Turkey

Keywords: Rice, weed, YDVs, CYDV-RPV, BYDV-PAV

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops as a source of human nourishment. Peoples of Far-East and South-Eastern Asian countries consume rice as basic food. Among them, the People's Republic of China is an important rice producer worldwide, with 212 million tons of annual yield (Anonymous 2018a). Rice is grown as a semi-aquatic cereal field crop in Turkey, having an annual yield of 940000 tons, being 460726 tons of it, at the rate of 49.01% produced in Edirne, Kırklareli and Tekirdağ provinces in the Trakya region of Turkey (Anonymous 2018b). Although, rice is susceptible to 36 fungal, 12 bacterial, 18 viral, 2 phytoplasma diseases, and 6 nematode species have been found harmful to rice crops in the Tropical Far East, South East Asian, and African countries (Webster and Gunnell 1992). Among them, rice blast disease caused by *Pyricularia grisea* (Cooke) Sacc. (Syn. *Pyricularia oryzae* Cavara; teleomorph: *Magnaporthe grisea*) has been considered the most destructive fungal disease widespread worldwide in all those rice-growing countries and Turkey (Bonman, 1992). White tip nematode *Aphelenchoides besseyi* Christie occurred worldwide on rice and also was diagnosed by Öztürk and Enneli (1997) in the rice fields of the Trakya region in 1995. In European, rice-growing areas, Osler *et al*. (1980) reported that rice Giallume disease in Italy caused a strain of *Barley yellow dwarf virus* (BYDV), on its weed host *Leersia oryzoides*

was the over-seasoning host of BYDV. Following, Osler *et al*. (1984) observed severe yellow dwarf virus (YDV) infections caused by rice Giallume strain and their aphid vectors in rice fields of Italy. Similarly, Medina *et al*. (1986) identified 8 out of 30 aphid species as the vectors of YDVs at the important rice-growing regions of Valencia and La Albufera in Spain. Later, Jorda *et al*. (1987) identified BYDV to be a disease caused 'enjorat' disease in the rice areas in Spain and determined preventing by decreasing the effectiveness of *Rhopalosiphum padi.* Subsequently, Belli *et al*. (1990) determined the disease cycle, with their aphid vectors in Italy and the occurrence order of YDVs on cereal species, beginning with winter barley, spring wheat, rice, corn and oat in the annual disease cycle. YDV disease is classified in the genera *Luteovirus* and *Polerovirus*. *Barley yellow dwarf virus* (BYDV)-PAV, BYDV-PAS, BYDV-MAV, BYDV-GAV, BYDV-SGV is included in the genus *Luteovirus.* Members of the genus *Polerovirus* comprise *Cereal yellow dwarf virus* (CYDV)-RPV, CYDV-RPS, Wheat yellow dwarf virus-GPV (WYDV-GPV), and Maize yellow dwarf virus-RMV (MYDV-RMV) (Miller and Rasochová 1997; Chay *et al*.1996, Ueng *et al*. 1992; Jin *et al*.2004; Liu *et al*. 2007; Rochow and Miller 1971; Mayo 2002; Zhang *et al*. 2009; Kruger *et al*. 2013). YDVs have isometric particles of 25-30 nm in diameter and ss(+) RNA genome of approximately 5600 nucleotides (Vincent *et al*.1991). These viruses are phloem-limited and are transmitted in a persistent circulative manner by over 25 aphid vectors. (Smith and Plumb1981; Halbert and Voegtlin 1995). For the first time in Turkey, Bremer and Raatikainen (1975) observed and reported sporadic YDV diseases with their aphid vectors on cereals. After that, epidemic of YDV diseases and wheat dwarf infections on winter bread wheat (*Triticum aestivum* L.) and other cereals were observed in Trakya by İlbağı (2003) and İlbağı *et al*. (2005). Subsequently, İlbağı (2006) identified Common reed (*Phragmites communis* Trin) as an over-summering and over-wintering host of YDVs. As the most critical YDV species that BYDV-PAV was identified in maize fields individually and had the coinfection with *Maize dwarf mosaic virus* (MDMV), *Sugarcane mosaic virus* (SCMV) and *Johnson grass mosaic virus* (JGMV) species in the Trakya region (İlbağı *et al*. 2006). In addition to major small grains, canary seed (*Phalaris canariensis* L.) was also found to be an extremely susceptible host of YDVs in canary seed fields of Tekirdag province (İlbağı *et al*. 2008). Poaceae weed host species of YDVs and their aphid vectors were determined and identified in and surroundings of cereal fields in the Trakya Region (İlbağı *et al*. 2011; İlbağı *et al*. 2013; İlbağı *et al*. 2018). Up to now, another virus disease on rice as *Rice ragged stand virus* (RRSV) and *Rice yellow mottle virus* (RYMV) were identified in the rice fields of Edirne province by Köklü and Yilmaz (2004). Additionally, Moletti *et al*. (1990) determined the destructive effects of Rice giallum virus diseases on rice crop development stages, like yield loss and yield quality reductions on 11 Italian rice cultivars. *Rice ragged stand virus* (RRSV) causes epidemics on rice paddies in Far-East countries, transmitted by a brown planthopper *Nilaparvata lugens* (Stal) in which virus circulates and propagate. Another one is *Rice yellow mottle virus* (RYMV) that occurs in African countries and transmitted by a Chrysomelidae species *Sesselia pusilla* Gerstaecker none persistently (Hibino 1992).

This present investigation was aimed to determine the infections of YDVs on rice and some weeds as potential reservoir inoculum sources in the border of the rice fields, which cause yellowing, redness, stripe mosaic, browning of leaves, dwarfing and stunting symptoms.

Materials and Methods

Survey studies and sampling: Survey studies were carried out in 17 rice growing fields of 7 districts in Edirne, Kırklareli, and Tekirdağ provinces of the Trakya region. Characteristic YDV disease symptoms such as yellowing, dwarfing, and redness were observed in the rice fields. Similar symptoms were observed rarely on Barnyard grass (*Echinochloa crusgalli (*L.) P. Beauv), Johnson grass (*Sorghum halepense* L., Pers.) and Common reed (*Phragmites austrialis* (Cav.) Trin ex. Steudel) weed samples during the surveys. 63 symptomatic rice leaf samples from Edirne, 28 leaf samples from Kırklareli, 29 leaf samples from Tekirdag, totally 120 symptomatic rice leaf samples were collected as plant materials of this study. Separately, 18 weed leaf samples were collected from the rice-growing areas of Trakya. A total of 138 leaf samples composed of study plant materials for the identifications of YDVs.

Serological test: 138 leaf samples were tested with polyclonal antibodies (manufactured by AGDIA Inc.; Elkhart IN, USA) for the presence *Barley yellow dwarf virus*-PAV (BYDV-PAV), *Barley yellow* dwarf *virus*-MAV (BYDV-MAV) and *Cereal yellow dwarf virus*-RPV(CYDV-RPV) by employing Double Antibody Sandwich Enzyme-Linked Immunosorbent Assays (DAS-ELISA) as described by Clark and Adams (1977). Optical densities at 405 nm (OD_{405}) were measured with an ELISA reader (Thermo Fischer

Scientific Instruments Co Ltd.Waltham, MA USA) a positive reaction was recorded when the OD_{405} of a sample was twice that given by sample from a healthy control plant.

Nucleic acid isolation and cDNA synthesis: The symptomatic leaf samples gave a positive reaction with the Enzyme-Linked ImmunoSorbent Assay (ELISA) test were verified by Reverse Transcription Polymerase Chain Reaction (RT-PCR) test. These symptomatic leaf samples to investigate YDVs; BYDV-PAV, BYDV-MAV and CYDV-RPV were subjected to the isolation of the viral nucleic acids by employing the total nucleic acid extraction method described by Falke *et al*. (2000). First-strand cDNA was synthesized from total isolated RNA by using RevertAidTM First Strand cDNA Kit (Fermentas; Vilnius, Lithuania). In each reaction, 0.5 µg RNA sample and 20 pmol of reverse complementary primer pair of BYDV-PAV, BYDV-MAV and CYDV-RPV were used and processed according to the manufacturer's instructions.

RT-PCR amplifications: A total of 41 symptomatic samples were tested by RT-PCR. Lu1 and Lu4 specific primers of *Luteoviruses* (Robertson *et al*. 1991) were used for the identification of BYDV-PAV, while specific primers were used for BYDV-MAV and CYDV-RPV (Deb and Anderson 2007). The PCR reaction mixture contained 2 µl cDNA, 10 mM dNTPs, 10 µM each of forward and reverse primers, 10x PCR buffer, $MgCl_2$ (25 mM), 2.5 U of Taq DNA polymerase (Fermentas; Vilnius, Lithuania) and RNase free water in a 25 µl reaction volume. PCR conditions were optimized for each virus against a range of concentrations and annealing and extension temperatures. The PCR cycling conditions for BYDV-PAV consisted of an 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. The thermal cycling conditions for BYDV-MAV consisted of 40 cycles at 95°C for 30 sec, 55°C for 1 min, 72°C for 1 min and the final extension step at 72°C for 10 min. Cycling conditions of CYDV-RPV comprised an 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. The obtained PCR products were analyzed by electrophoresis in 1.5% agarose gel, stained with EtBr and viewed under UV illumination in a gel documentation system (Vilber Lourmet; Marne La Vallee Cedex 1, France).

Results and Discussion

In the rice-growing areas of the Trakya region are taken part of the large rice fields and small rice paddies side by side. Rice cultivation is a monoculture type without rotation with any other field crop. During the surveys in rice fields and paddies, virus infections have been observed as patches as well as the whole surface area of the rice fields. Moreover, infected rice plants exhibited color changes of leaves, stems from green to yellowing, and got into orange color. Furthermore, infected individual rice plants displayed erected stems and dwarfing, stripe mosaic, yellow main-vein, and necrotic browning leaves similar to Hibino's (1992) description. Also, İlbağı (2006) and İlbağı *et al*. (2013) reported that stripe mosaic, yellowing on the central vein and necrotic symptoms have been observed on common reed (*P. austrialis*), Johnson grass (*E. crusgalli*) and barnyard grass (*S. halepense*). These findings were the consensus on our results. Osler *et al*. (1984) cited that early infection of seedlings and the severe disease usually destroy rice plants. Because of YDVs infections, the reduced number of tillers, and the reduction of grain yield have occurred from 5% to 100%. The screening test results in this present study revealed that 23 out of 120 symptomatic rice leaf samples were infected with CYDV-RPV. Symptomatic 4 other samples had BYDV-PAV, and 9 of them were found infected with a mixture of CYDV-RPV+BYDV-PAV. The tested 18 symptomatic weed leaf samples revealed that 5 of them infected with the same YDVs. As a result, 26 out of 138 leaf samples were found infected with CYDV-RPV at the rate of 18.94%. Symptomatic 5 leaf samples at a rate of 3.72% had BYDV-PAV. The other 10 symptomatic leaf samples had coinfection with CYDV-RPV+BYDV-PAV at the rate of 7.34%. None of the tested leaf samples had BYDV-MAV. Thus, 41 out of 138 symptomatic leaf samples were found infected with YDVs at the rate of 30%, including BYDV-PAV and CYDV-RPV. Our results confirmed the previous reports of Osler *et al*. (1980), Osler *et al*. (1984) in Italy, as well as the reports of Medina *et al*. (1986) and Jorda *et al*. (1987) in Spain. By evaluating 119 articles about BYDVs, Miller and Rasochova (1997) reviewed that YDVs occasionally infect warm-season cereal species of rice and maize, and their epidemics occur rarely. Global climatic changes, however, cause a kind of variation on the population dynamics of vectors. Accordingly, YDVs may occur wherever their cool-season and warm-season cereal hosts and their Poaceae weed host are present. Otherwise, tropical rice diseases of Rice ragged stand virus (RRSV) and Rice yellow mottle virus (RYMV) defined by Hibino's (1992), however, occurrence in rice fields of Edirne in Turkey reported by Köklü

and Yilmaz (2004) could not be explained. Mainly, YDVs and their epidemic infections on cereals have been reported in the Trakya Region by İlbağı (2003) and İlbağı *et al*. (2005). Besides rice leaf samples, 3 out of 18 weed samples had CYDV-RPV, only one of them was positive with BYDV-PAV, and one weed sample was found infected with the mixture of both virus species of YDVs (İlbağı, 2013). Thus, efficient control measures of those YDV infections of winter bread wheat and other cereals were established by İlbağı (2016). Those control measures include the use of tolerant cultivars, usage of selective herbicides for weed hosts control of YDVs, usage of imidacloprid insecticide for seed dressing also suggested like Royer *et al*. (2005) before sowing and the timing of sowing date. YDVs cause infections on all of the cereal species, including wheat, barley, rye, maize, oat, triticale, bird seed in Turkey as reported by İlbağı (2003), İlbağı *et al*. (2005), İlbağı *et al*. (2006), İlbağı *et al*. (2008). The results of this study revealed that rice is a new cereal host of YDVs in Turkey. To our knowledge, this is the first report of YDVs for the present on rice in Turkey.

Table 1. The screening test results of YDVs on rice and three species of weeds in rice growing provinces of Trakya region.

Name of Host Plants	**Number of Plant Samples**	**Number of Virus Infected Samples**				**Total Number of Infected Samples**
		RPV	**PAV**	**MAV**	**RPV+PAV**	
Rice (*Oryza sativa*)	120	23	4	-	9	36
J. grass (*S. halepanse*)	10	2	-	-	-	2
B. grass (*E. crusgalli*)	2	1	-	-	-	1
C. reed (*P. austrialis*)	6	-	1	-	1	2
Total	**138**	**26**	**5**	**-**	**10**	**41**
Rate of infections (%)	**-**	18.84	3.62	-	7.25	29.71

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