Biological and Serological Identification of Barley Yellow Dwarf Virus (BYDV) and Its Distribution in Iraq

Rakib A. Al-Ani, Mustafa A. Adhab, Muthannah A. El-Muadhidi & Maadh A. Al-Fahad
Plant Protection Department, College of Agriculture, University of Baghdad, Iraq
E-mail: maa_adhab@hotmail.com

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Abstract

Barley yellow dwarf virus (BYDV) was identified by means of symptoms on indicator plants, transmission by aphids, and serological characteristics. Symptoms of yellowing and stunting were appeared on leaf tips of *Hordium vulgare*, *Triticum aestivum*, and *Avena fatua* accompanied by stem stunting. Four species of aphids, *Rhopalosiphum maidis*, *R. padi*, *Macrosiphum avenae*, and *Schizaphis graminum* were found vectoring the virus. Extracts from wheat and barley plants showing yellowing and stunting were reacted positively with polyclonal anti-BYDV antiserum in DAS-ELISA. Most of samples that gave positive reactions by ELISA gave positive reactions with tissue blot immunoassay (TBIA). Higher incidence of BYDV was found in the northern parts of Iraq. Several economical and weed plants, collected from wheat and barley fields and vicinity sites have been found to harbor the virus. These hosts may acts as virus reservoir transmitting to wheat and barley plants by aphids.

Keywords: BYDV, *Triticum aestivum*, *Hordium vulgare*, TBIA, DAS-ELISA, Aphid transmission

1. Introduction

Barley yellow dwarf virus (BYDV), a species of luteovirus group is the most widely distributed and most destructive virus on cereal crops in the world (Lister and Ranieri, 1995; Miller *et al.,* 2002; Kennedy and Connery, 2005).

Symptoms in *Triticum aestivum* were not always clear and characterized by stunting with yield losses (Irwin and Thresh 1990). On oat the symptoms yellowish green blotches near the leaf tip. These blotches enlarge, merge, and turn to red, purple, brown, or yellow-orange. The yellowish-green area extend to lower parts of the leaf and the leaves may curl inward (D'Arcy 1995). In *Hordium vulgare* the most characteristic symptoms are dwarfing with brilliant yellow color on the leaves which extend from the tip toward the basal parts (Rochow *et al.,* 1996). BYDV cause dwarfing with yellowing or reddening on corn (Rochow *et al.,* 1996).

The virus is transmitted by several species of aphids in a persistent manner, including *Rhopalosiphum padi*, which is the most efficient. Other species of aphids such as *Schizaphis graminum*, *Macrosiphum avenae*, *R. maidis* were also reported vectoring the virus. The aphids can acquire the virus by feeding on a diseased plant for a minimum of 30 min and the viruliferous aphids are capable of transmitting the virus, after an incubation period of 12 hrs to 4 days. The virus is not transmitted by eggs, and does not replicate in the vector. BYDV strains have been differentiated according to their aphid vectors (Halbert *et al.,* 1992; Olser *et al.,* 1992; Sadeghi *et al.,* 1997; Lucio-Zavaleta *et al.,* 2001; Miller *et al.,* 2002).

Different kinds of techniques have been used to detect BYDV. Double antibody sandwich-Enzyme linked immunosorbent assay (DAS-ELISA) is the most widely adopted using polyclonal antibodies (El-Zoubi *et al.,* 1992; Klein and Lister, 1992; Makkouk and Comeau, 1994; Makkouk *et al.,* 1994).

The objective of this study was to identify Barley yellow dwarf virus on *T. aestivum* and *H. vulgare*, determine its distribution at different sites in Iraq and test the ability of some aphid species to vector the virus.

2. Materials and Methods

Virus identification: The virus was identified biologically on test plants, serologically by Tissue blot immunoassay (TBIA) and double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA), and aphid transmission.

Virus source: Plants of *T. aestivum* and *H. vulgare* showing symptoms of yellowing and dwarfing suspected to be of Barley yellow dwarf virus (BYDV) were transplanted into plastic pots (20 × 30 cm), containing mix soil, from different fields of *T. aestivum* and *H. vulgare* in Iraq during 2007-2008 and 2008-2009 growing seasons. The plants were maintained in insect protected cages (36 × 27 × 53 cm) in insect-proof glasshouse and used as virus source.

Data analysis: Samples of wheat and barley plants showing yellowing and stunting were randomize collected from different sites of Iraq. The virus was characterized by means of symptoms on indicator plants, serological techniques (DAS-ELISA and TBIA), and aphid transmission. The distribution of the virus in wheat and barley fields and its secondary hosts were also determined.

Test plants: Seeds of *Hordeum vulgare*, *Avena fatua*, *T. aestivum*, *Zea mays*, and *Lolium temulentum* test plants for BYDV (Brunt *et al.,* 1996), were sown in pots (20 × 30 cm) containing mix soil and peatmoss (1:1) in a glasshouse (12-25 ºC). The seedlings, at 2-4 leaves stage, were transplanted to other pots (10 × 12 cm) containing the same mix soil and sprayed with Benlate at 1 g/L and Confidor at 1 g/L to prevent fungi and insects.

Inoculation: Apterous of *M. avenae*, identified at Plant Protection Department, College of Agriculture, University of Baghdad, Iraq, were collected from *H. vulgare* and *T. aestivum* fields. The aphids were reared on *H. vulgare* healthy plants in protective cages, maintained in insect-proof glasshouse and routinely used in virus transmission. Groups of reared aphids were transferred onto infected plants (*T. aestivum* and *H. vulgare*) (10 aphids/plant) for 24 hrs. The viruliferous aphids were placed on the test plants at 4 leaves stage for 48 hrs. The plants were then sprayed by an insecticide to eliminate the aphids. The symptoms were evaluated after 4 weeks of inoculation. *R. padi*, *R. maidis*, and *S. graminum*, were also collected and tested for their ability to transmit the virus.

Serological assays:

Tissue blot immunoassay (TBIA): TBIA procedure was done according to Abouzid *et al.,* (2002) with slight modification. A cross-section was made in the stem of infected and healthy plants with razor blade. The cut surface was firmly pressed on to the surface of nitrocellulose membrane (0.45 µm) for several seconds. Blotted membranes were allowed to air dry and incubated in blocking buffer, phosphate buffer saline (PBS)(15 mM KH_2PO_4, 20 mM Na_2HPO_4, 150 mM NaCl and 0.05% Tween-20), containing 5% powdered Skim milk for 15 min at room temperature in Petri dishes. The membranes were washed three times in PBS buffer containing 0.025 % Tween-20 (PBST) and incubated with polyclonal antiserum to BYDV (1:500) for 1 hr at room temperature. Membranes were then washed three times as before and incubated in alkaline phosphatase conjugated goat anti-rabbit IgG (1:2000) provided with Dr. Safa Kumari (Virology lab., ICARDA, Aleppo) at room temperature for 1 hr. The membranes were washed three times as previously and the conjugate was visualized by incubation in a substrate buffer (phosphate buffer) containing nitro blue tetrazolium (NBT) (750 µg/ml) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP) (500 µg/ml) for 15 min at room temperature with continuous shaking. The reaction was stopped by washing the membranes by sterile distilled water. The immunological reactions were detected by naked eye or by dissecting microscope; violet to purple color indicates positive reaction.

Virus purification: *T. aestivum* and *H. vulgare* plants showing BYDV symptoms approximately, 250 g, were ground by mortar and pestle in 0.1 M phosphate buffer, pH 6.0 containing 2% celluclast, 0.1% 2-mercaptoethanol, and 0.02% NaN_3, 1:3 (g/ml). The extract was filtered through two layers of filter paper. Triton X-100 was added to the filtrate at 1% with agitation at room temperature for 3 hrs, then a mixture of chloform-butanol (1:1) was added at 6:1 (v:v) with agitation for 10 min at room temperature. The extract was centrifuged at 5000 rpm for 30 min in (Sor) Hettich-Universal II. The virus was precipitated by 8% polyethylene glycol, 8000 MW, and 1% NaCl, and recuperated by centrifugation at 5000 rpm for 30 min. Virus pellets were suspended in 0.1 M phosphate buffer pH 6.0 containing 0.1% Triton X-100. The virus was separated by centrifugation in sucrose gradient 15-55% with reverse gradient of ammonium sulfate 55-15% at 5000 rpm for 3 hrs. The band correspond to virus was carefully withdrawn from the gradient, diluted fivefold with phosphate buffer 0.1 M pH 7.5 and centrifuged at 30000 rpm for 3 hrs in Beckman Ultracentrifuge – 35, using rotor – 30. The purified virus was resuspended in small volume of phosphate buffer and used for rabbit immunization.

Immunization: Antibody to BYDV was prepared by 5 adminstrations, 1 ml of the virus at 1 mg/ml weekly. The first two injections were intramuscularly emulsified with an equal volume of complete Freund's adjuvant, followed by three injections in the ear marginal vein without adjuvant. The blood was collected after 12 days of the last injection through the ear marginal vein in a beaker. The blood was held at room temperature for 2 hrs for

agglutination, and the serum was centrifuged at 5000 rpm for 15 min. The antiserum was purified and conjugated with alkaline phosphatase according to Clark and Adams (1977).

Virus survey: A survey was effectuated to 58 locations of *T. aestivum* and *H. vulgare* fields at the North, middle, and south of Iraq. Approximately 26150 plants from 520 fields were randomly collected, by walking a transect of the fields four times, at booting stage (from 41-49) according to Zadoks scale (Zadoks *et al.,* 1974) during February to April of 2007-2008 and 2008-2009 growing seasons.

BYDV detection: BYDV was detected by DAS-ELISA and TBIA protocols.

DAS-ELISA: Young leaves were ground with a mortar and pestle in phosphatase buffer saline (PBS) pH 7.0, 1:10 (g/ml). The extracts were centrifuged at 5000 rpm for 10 min and the supernatant was collected.

Anti-BYDV antiserum (200 µl) diluted to 1:1000 with coating buffer (35 mM $NaHCO_3$, 15 mM Na_2CO_3, 0.2% bovine serum albumin (BSA), pH 9.6), was loaded in each well of polystyrene ELISA plates. The plates were incubated at 37 ºC for 4 hrs and the wells were washed three times with phosphate buffer saline containing 0.05% Triton X-100. To each well, 200 µl of plant extracts previously prepared was added and the plates were maintained at 4 ºC overnight. The wells were washed three times as before and 200 µl of anti-BYDV IgG conjugated with alkaline phosphatase (1:1000) in Enzyme-labeled buffer, pH 7.0, was added to each well. After incubation at 37 ºC for 4 hrs and washed three times, 200 µl of substrate (p-nitrophenyl phosphate) at 1 mg/ml in 10% diethanolamine, pH 9.8 was added to each well. The plates were maintained at room temperature for 1 hr and the absorbances at 405 nm were recorded in ELISA-reader.

3. Results

Symptoms: The virus induced yellow-golden areas on leaves tips of *Hordium vulgare* 7 days after inoculation by *M. avenae*. These areas were extended toward the basal tissue, then to the entire plant accompanied by stunted growth. Similar symptoms were reported by Comeau *et al.,* (1992) concerning BYDV on *H. vulgare*.

Faint light green-yellowish spots were developed on leaves tips of *Avena fatua* after 14 days of inoculation by *M. avenae*, extended gradually to other parts of the leaves which turned finally to yellow-orange, sometimes accompanied by leaf curling inward. Identical symptoms recorded following the methods of Comeau (1987), and time of symptoms development by Gill (1967) on oat plants inoculated by BYDV.

Yellowing symptoms were observed on *T. aestivum* after 7 days of virus inoculation accompanied by stem stunting after 14 days. These symptoms are similar to those reported by Reporton (1989) on triticum plants inoculated by BYDV.

No visible symptoms on leaves of *Z. mays* and *L. temulentum* were developed after 21 days of inoculation, but the virus was detected in the inoculated plants by serological TBIA test.

Insect transmission: Four species of aphids *R. padi*, *M. avenae*, *R. maidis*, and *S. gramineum* were found in *T. aestivum* and *H. vulgare* field capable of transmitting the virus as proved by development of typical yellowing on the inoculated *T. aestivum* and *H. vulgare* plants, which indicates that the virus is vectored by these species of aphids. The inoculated *T. aestivum* plants developed typical symptoms of BYDV after 9, 7, 16, 17 days of inoculations by the four species above with percentages of infection 73.4, 50.0, 26.6, and 43.5% respectively, while the symptoms took 6 ,6, 12, and 14 days to develop on *H. vulgare* with percentages of infections 100, 60, 36, and 50% respectively (Table 1). These results indicate that *R. padi* is more efficient in vectoring the virus, followed by *M. avenae*, and *H. vulgare* is more susceptible host to the virus than *T. aestivum*.

Serological assays: Extracts from *T. aestivum* and *H. vulgare* plants showing yellowing and stunting collected from fields at different locations in Iraq, reacted positively with polyclonal anti-BYDV antiserum in DAS-ELISA. Most samples that gave positive reactions by ELISA, gave positive reaction with Tissue blot immunoassay (TBIA) as shown by intense purple staining on the nitrocellulose membrane blotted by stem cut surfaces. No reaction with extracts from healthy plants was observed (Fig. 1).

Virus survey: Remarkable differences in BYDV incidence between the different locations used for virus survey in Iraq were observed. The highest distribution of the disease were noted in the northern region of Ta'amim, Sulaimaniya, Erbil, Nineveh, and Dohuk provinces with disease incidences of 4, 3, 4.7, 11.5, and 5% on *T. aestivum*, 3.7, 4.8, 6.8, 14.4, and 8.3% on *H. vulgare* respectively. This was followed by middle region in Baghdad, Dayalah, Anbar, and Salah El-din provinces, with disease incidences of 4.4, 2.8, 0.0, and 3.4% on *T. aestivum*, 5.2, 5.2, 1.4, and 3.7% on *H. vulgare* respectively. The lowest disease incidence were found in southern region namely Basra, Missan, Thi-Qar, Wasit, Al-Muthanna, Al-Qadissiya, Babylon, Najaf, and

Karbala provinces, with disease incidences of 0.0, 0.0, 0.0, 1.6, 0.0, 0.0, 1.7, 1.6, and 2.0% on *T. aestivum*, 0.0, 0.0, 0.0, 2.3, 0.0, 0.0, 1.8, 2.4, and 2.5% on *H. vulgare* respectively in 2007-2008 growing season (Table 2).

Considerable increase in disease incidence values in 2008-2009 growing season was observed, 6.4, 6.2, 10.5, 17.5, and 12.3% on *T. aestivum*, 6.3, 7.2, 11.8, 24, and 13.7% on *H. vulgare* in North provinces, 8.5, 6.5, 2.0, and 5.4% on *T. aestivum*, 9.8, 7.2, 4.3, and 6.8% on *H. vulgare* in middle provinces, 0.0, 0.0, 0.0, 3.4, 0.0, 3.0, 4.0, 5.2, and 4% on *T. aestivum*, 0.0, 0.4, 0.4, 4.6, 0.0, 3.5, 4.9, 5.7, and 4.8% on *H. vulgare* in South provinces, for the same provinces above respectively (Table 3).

Variations in disease incidence between sites in the same province were also observed, with highest values in Nineveh provinces, 11.5 and 17.5% on *T. aestivum*, 14.4 and 24% on *H. vulgare* in northern region, followed by Baghdad province, 4.4 and 8.2% on *T. aestivum*, 5.2 and 9.8% on *H. vulgare* in middle region, and Najaf province, 1.6 and 5.2% on *T. aestivum*, 2.4 and 5.7% on *H. vulgare* in southern region in 2007-2008 and 2008-2009 growing seasons respectively.

Secondary hosts of BYDV: Positive reaction between extracts from economic and weed plants, collected from *T. aestivum* and *H. vulgare* fields and from vicinity sites, and polyclonal anti-BYDV by TBIA protocol was developed. The percentage of infection among these hosts ranged from 26.7% in *Phalaris minor* to 66.7% in *A. fatua* and *Z. mays*. Some of these plants were found to harbor the virus with unclear symptoms. These results indicate that these plants may serve as reservoirs for the virus (Table 4).

4. Discussion

The virus causing yellowing and dwarfing on *T. aestivum* and *H. vulgare* plants was considered as Barley yellow dwarf virus (BYDV) according to the symptoms shown on the indicator plants, the transmissibility by different species of aphids, and the positive reaction with serological TBIA, as shown by intense purple staining in the vascular bundles on nitrocellulose-membrane blotted by stem cut surface, using polyclonal anti-BYDV-PVA. No visible symptoms on *Z. mays* and *L. temulentum* were developed after 21 days of inoculation, although the virus was present in the plants as proved by TBIA. These results are in agreement with Osler *et al.,* (1985), Brunt *et al.,* (1996) in that BYDV infect maize plants without visible symptoms. Our results agreed also with Leather and Dixon (1981), and Fargetti *et al.,* (1982), in that Lolium plants harbor the virus but showed no visible symptoms. These hosts may serve as virus reservoirs.

Several species of aphids were found able to transmit the virus, more efficiently by *R. padi* and *M. avenae*, less efficiently by *R. maidis*, and *S. graminum*. These results may indicate the existence of more than one isolate for the virus. Some previous studies reported that BYDV strains can be vectored with varying efficiency by different species of aphids (Sadighi *et al.,* 1997; Lucio-Zavaleta *et al.,* 2001).

The results of virus survey provide information about the distribution and epidemiology of BYDV at different locations in Iraq. The results showed that the virus exist in most of the fields used for the survey with higher incidence in northern parts (Nineveh). The variation in disease incidence between the locations may be due to; sowing date, field expansion, prevailing and movement of aphid population during the season, weather conditions, alternative and alternate hosts, *T. aestivum* and *H. vulgare* fields nearby. The report of Lucio-Zavaleta *et al.,* (2001) indicated that the period September-October (sowing date of *T. aestivum* and *H. vulgare*) provide suitable environment for the alate of *P. padi* to acquire the virus from infected corn and migrating to emerging *T. aestivum* and *H. vulgare* plants. Other studies reported that the spread of BYDV early in the fall was due to the presence of corn plants and weed grass which act as virus source transmitted to *T. aestivum* and *H. vulgare* plants by alate aphids (El-Yamani, 1992; Irwin and Thresh, 1992; Van Reissen *et al.,* 1998).

Results of serological assays showed that TBIA is more reliable and easy to carry out than ELISA in the field for detecting BYDV in *T. aestivum* and *H. vulgare* tissues. We found that TBIA is also sensitive as ELISA but unlike ELISA, it does not require sample extraction or sophisticated tools. It was shown that tissue-specificity varied among crops for reliable tissue in detecting viruses by TBIA (Jonson *et al.,* 2007). In this study we have found that stem tissue was more efficient than other tissue in detecting BYDV. It was noted that some sample extracts that gave positive reaction with ELISA, failed to give reaction with TBIA, using anti-BYDV-PVA antiserum especially those from *H. vulgare* plants inoculated by *S. gramineum* that transmit BYDV-SGA (Irwin and Thresh, 1992). This finding support the conclusion mentioned above in this study that more than one strain may exist for BYDV in Iraq.

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References

Abouzid A.M., Freitas-Astua J., Purcifull D.E., Polson J.E., Beckham K.A., Crawford W.E., Peterson M.A., Peyser B., Patte C., & Hiebert E. (2002). Serological studies using polyclonal antisera prepared against the viral coat protein of four begomoviruses expressed in *Escherichia coli*. *Plant Disease,* 86, 1109-1114. http://dx.doi.org/10.1094/PDIS.2002.86.10.1109

Brunt A., Dallwitz M., Grabbs A., Watson L., & Zurcher E. (1996). *Plant virus-BYDV description lists from the ViDE database*. Version 20th August. URL.

Clark M.F., & Adams A.N. (1977). Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of Plant viruses. *Journal of General Virology*, 34, 475-483. http://dx.doi.org/10.1099/0022-1317-34-3-475

Comeau A. (1987). Effects of BYDV inoculation at antivirus dates for oats, barley, *Triticum aestivum*, rye and tritical. *Phytoprotection*, 68, 97-109.

Comeau A., Conin J., & Cheour F. (1992). BYDV symptoms and Elisa data in relation to biomass and yield loss. In: *BYDV in west Asia and North Africa*. Comeau, A. and K. Makkouk(eds.). Sayce Publishing, Exeter, UK. P, 155-168.

D'Arcy C.J. (1995). Symptomatology and host range of barley yellow dwarf. *Journal of Plant Pathology*, 4, 9-28.

El-Yamani M., Makkouk K., Hafidi B., & El-Kassemi K. (1992). Contribution to the study of BYDV in the Souss-massa region of Morocco. *Phytopathologia Mediterranea*, 31, 41-45.

El-Zoubi M., Almosa A., & Skaria M. (1992). Studies on BYDV in cereal crop in Jordan. In: *BYDV in west Asia and North Africa*. Comeau, A. and K. Makkouk (eds.). Sayce Publishing, Exeter, UK. P, 91-102.

Fargette D., Lister R., & Hood E. (1982). Grasses as a reservoir of BYDV in Indiana. *Plant Disease*, 66, 1041-1045. http://dx.doi.org/10.1094/PD-66-1041

Gill, C. (1967). Transmission of Barley yellow dwarf virus in isolate from Manitoba by five species of aphids. *Phytopathology*, 1, 713-718.

Halberd S.E., Connelly B.J., Lister R.M., Klein R.E., & Bishop G.W. (1992). Vector specificity of Barley yellow dwarf virus serotypes variants in southwestern Idaho. *Ann. Appl. Biol.,* 121, 123-132. http://dx.doi.org/10.1111/j.1744-7348.1992.tb03992.x

Irwin M.E., & Thresh J.M. (1992). BYDV epidemiology: A study in ecological complexity. In: *BYDV in west Asia and North Africa*. Comeau, A. and K. Makkouk(eds.). Sayce Publishing, Exeter, UK. P, 3-30.

Irwin M.E., & Thresh J.M. (1990). Epidemiology of barley yellow dwarf: A study of ecological complexity. Ann. Rev. *Phytopathology,* 28, 393-424. http://dx.doi.org/10.1146/annurev.py.28.090190.002141

Jonson G., Park J.C., Kim Y.K., Kim M.J., Lee M.J., Hyun J.N., & Kim J.G. (2007). Direct stem blot immunoassay (DSBIA): a rapid reliable and economical detection technique suitable for testing large number of barley materials for field monitoring and resistance screening to *Barley mild mosaic virus* and *Barley yellow mosaic virus*. *Plant Pathology Journal,* 23, 260-265. http://dx.doi.org/10.5423/PPJ.2007.23.4.260

Kennedy T.F., & Connery J. (2005). Grain yield reduction in spring barley due to Barley yellow dwarf virus and aphid feeding. *Irish Journal of Agricultural and Food Research*, 44, 111-128.

Klein R., & Lister R. (1992). Advantages and limitation of some methods for BYDV detection and diagnosis. In: *BYDV in west Asia and North Africa*. Comeau, A. and K. Makkouk (eds.). Sayce Publishing, Exeter, UK. P, 169-176.

Leather S., & Dixon A. (1981). The effect of cereal crop stage and feeding size on the reproductive activity of the bird cherry-out aphid, *Rhopalosiphum padi*. *Ann. Appl. Biol.*, 97, 135-141. http://dx.doi.org/10.1111/j.1744-7348.1981.tb03006.x

Lister R.M., & Ranieri R. (1995). *Distribution and economic importance of barley yellow dwarf*. pp. 29-53. In: Barley yellow dwarf (40 years in progress). D'Arcy, C.J. and P.A. Bumeft (eds.). The American Phytopathological Society. St. Paul. MN.

Lucio-Zavaleta E., Smith D.M., & Gray S.M. (2001). Variation in transmission efficiency among *Barley yellow dwarf virus*-RMV isolates and clones of the normally inefficient aphid vector *Rhopalosiphum padi*. *Phytopathogy,* 91, 792-796. http://dx.doi.org/10.1094/PHYTO.2001.91.8.792

Makkouk K., Comeau A., & Pierre S.t. (1994). Screening for BYDV luteovirus resistance in barley on the basis of virus movement. *J. Phytopathology*, 141, 165-172. http://dx.doi.org/10.1111/j.1439-0434.1994.tb01458.x

Makkouk K., & Comeau A. (1994). Evaluation of various methods for the detection of BYDV by the tissue-blot immunoassay and its use for virus detection in cereals inoculated at different growth stages. European *J. of Plant Pathology,* 100, 71-80. http://dx.doi.org/10.1007/BF01871967

Miller W.A., Liu S., & Beckelt R. (2002). Barley yellow dwarf virus; Luteoviriae or Tombusviridae? *Molecular Plant Pathology*, 3(4), 177-188. http://dx.doi.org/10.1046/j.1364-3703.2002.00112.x

Osler R., Loi N., Lorenzeni C., Snidaro M., & ReRafti E. (1985). BYDV infection in maize in brods and hybrids in northern Italy. *Maydica*, 30, 285-290.

Osler R., Loi N., & Pertot I. (1992). Characterization of a new RMV-like strain of BYDV by aphid transmission. *Riv. Pathol. Veg.*, 2, 33-38.

Reporton, C. (1989). BYDV disease of small grains. University of Illinois. *Report on Plant Disease.* RPD. 101.

Rochow W.F., Sward R.J., & Waterhouse P.M. (1996). Barley yellow dwarf luteoviruses. *Journal General Virology*, 72, 1473-1477.

Sadeghi E., Dedryver C.H., Riault G., & Gauthier J.P. (1997). Variation in transmission of two BYDV-MAV isolates by multiple clones of *Rhopalosiphum padi* L. *European Journal of Plant Pathology,* 103, 513-519. http://dx.doi.org/10.1023/A:1008673926685

Van Riessen H., Mask P., Fladrek K., Murphy J., & Gazawy W. (1998). BYDV in small grains. *Extension Plant Pathologist.* Ups 7m23, ANR-1082.

Zadoks J., Chang T., & Kozak C. (1974). A decimal code for growth stages of cereals. *Weed research,* 14, 415-421. http://dx.doi.org/10.1111/j.1365-3180.1974.tb01084.x

Table 1. Ability of aphid species to transmit BYDV on *T. aestivum* and *H. vulgare*

The crop	Aphid species	Percentage of infected plants to inoculated one	Infection percentage	Inoculation period
T. aestivum	*Schizaphis gramineum*	13 of 30	43.5 %	17
	Rhopalosiphum padi	22 of 30	73.4 %	9
	Rhopalosiphum maidis	8 of 30	26.6 %	16
	Macrosiphum avenae	15 of 30	50.0 %	7
H. vulgare	*Schizaphis gramineum*	15 of 30	50.0 %	14
	Rhopalosiphum padi	30 of 30	100.0 %	6
	Rhopalosiphum maidis	11 of 30	36.7 %	12
	Macrosiphum avenae	18 of 30	60.0 %	6

Table 2. Distribution percentage of BYDV in northern, middle, and southern Iraqi provinces for 2007-2008 grown season

Region	Province	Number of studied fields		Number of total samples		Number of infected samples		Percentage of infection	
		T. aestivum	*H. vulgare*	*T. aestivum*	*H. vulgare*	*T. aestivum*	*H. vulgare*	*T. aestivum*	*H. vulgare*
Southern provinces	**Basra**	5	6	250	300	0	0	**0**	**0**
	Missan	5	6	250	300	0	0	**0**	**0**
	Thi-Qar	5	6	250	300	0	0	**0**	**0**
	Wasit	5	8	250	400	7	13	**1.6**	**2.3**
	Al-Muthanna	3	4	150	200	0	0	**0**	**0**
	Al-Qadissiya	3	4	150	200	0	0	**0**	**0**
	Babylon	6	8	300	400	9	12	**1.7**	**1.8**
	Najaf	5	6	250	300	10	14	**1.6**	**2.4**
	Karbala	3	4	150	200	4	9	**2**	**2.5**
Middle provinces	**Baghdad**	6	7	300	350	19	29	**4.4**	**5.2**
	Dayalah	5	7	250	350	8	15	**2.8**	**5.2**
	Anbar	5	6	250	300	3	8	**0**	**1.4**
	Salah El-din	6	7	300	350	9	12	**3.4**	**3.7**
Northern provinces	**Ta'amim**	5	6	250	300	13	21	**4**	**3.7**
	Sulaimaniya	12	13	600	650	18	29	**3**	**4.8**
	Erbil	12	15	600	750	44	71	**4.7**	**6.8**
	Nineveh	16	20	800	1000	87	189	**11.5**	**14.4**
	Dohuk	10	14	500	700	34	65	**5**	**8.3**

Table 3. Distribution percentage of BYDV in northern, middle, and southern Iraqi provinces for 2008-2009 grown season

Region	Province	Number of studied fields		Number of total samples		Number of infected samples		Percentage of infection	
		T. aestivum	*H. vulgare*	*T. aestivum*	*H. vulgare*	*T. aestivum*	*H. vulgare*	*T. aestivum*	*H. vulgare*
Southern provinces	**Basra**	3	4	150	200	0	0	**0**	**0**
	Missan	4	6	200	300	0	0	**0**	**0**
	Thi-Qar	5	6	250	300	0	0	**0**	**0**
	Wasit	6	7	300	350	10	16	**3.4**	**4.6**
	Al-Muthanna	3	4	150	200	0	0	**0**	**0**
	Al-Qadissiya	2	4	100	200	3	7	**3**	**3.5**
	Babylon	5	7	250	350	10	17	**4**	**4.9**
	Najaf	5	7	250	350	13	20	**5.2**	**5.7**
	Karbala	4	5	200	250	8	12	**4**	**4.8**
Middle provinces	**Baghdad**	7	9	350	450	30	44	**8.5**	**9.8**
	Dayalah	4	7	200	350	13	25	**6.5**	**7.2**
	Anbar	4	8	200	400	4	17	**2**	**4.3**
	Salah El-din	6	8	300	400	16	26	**5.4**	**6.8**
Northern provinces	**Ta'amim**	6	8	300	400	19	25	**6.4**	**6.3**
	Sulaimaniya	10	12	500	600	31	43	**6.2**	**7.2**
	Erbil	10	15	500	750	52	88	**10.5**	**11.8**
	Nineveh	14	21	700	1050	122	252	**17.5**	**24**
	Dohuk	9	12	450	600	55	52	**12.3**	**13.7**

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Table 4. Detection of secondary hosts for BYDV using TBIA test

Name of the host	Number of total samples	Number of positive reacted samples	Percentage of infection
Phragmites communis	35	19	54.3
Avena fatua	63	42	66.7
Lolium rigidum	59	29	49.2
L. temulentum	50	27	54
Cynodon dactylon	32	10	31.3
Phalaris minor	30	8	26.7
Slibium marianum	20	0	0
Cyperus rotundus	25	10	40
Beta Vulgaris	30	0	0
Raphanus raphanis	30	0	0
Malva parrioflora	30	0	0
Militotus indicus	30	0	0
Lipidium draba	29	0	0
Carthamus oxycath	25	0	0
Sinapsis arvensis	23	0	0
Aegilops Lorenti	40	12	30
Hordeum glaucum	37	21	56.8
Convolvulus arvensis	33	0	0
Cephalaria syriaca	25	0	0
Ammi majus	25	0	0
Zea mays	27	18	66.7
Sorghum halepense	32	15	46.9
Solanum nigrum	25	0	0
Vicia angustifolia	29	0	0
Polypogon monspeliensis	32	0	0
Rumex dentatus	25	0	0
Glycyrrhiza glabra	21	0	0
Amarantnus blitoides	30	0	0
Logonychium farctum	29	0	0
Cynanchum acutum	35	0	0
Bromus tectorum	38	14	36.8

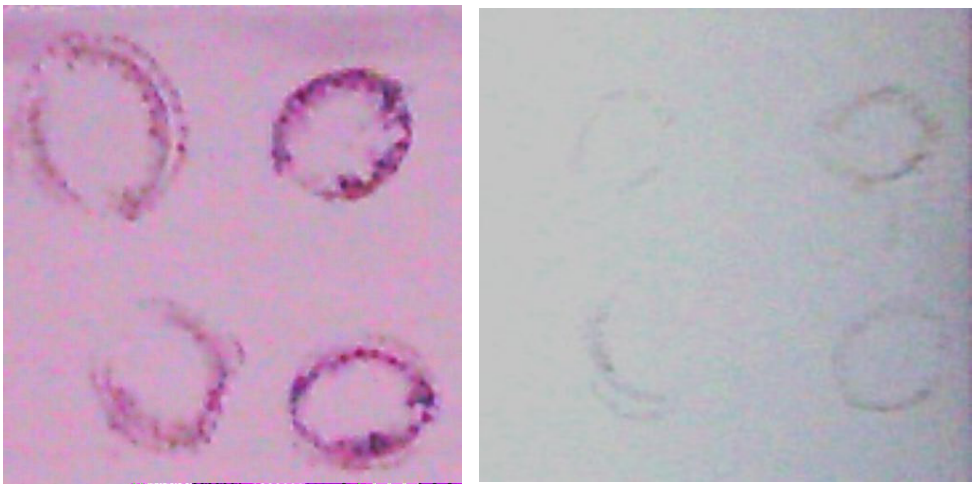

Figure 1. Tissue blot of cross-section of wheat (right) and barley (left) stem infected with Barley yellow dwarf virus (BYDV), on nitrocellulose membrane reacted with anti-BYDV polyclonal antibodies. The reactions were visualized by alkaline phosphatase conjugated to goat anti-rabbit immunoglobulins-G antibodies

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