Molecular characterization of barley yellow dwarf virus coat protein gene in wheat and aphids

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ABSTRACT

Barley yellow dwarf viruses (BYDVs) are members of the luteovirus group transmitted only by aphids. The five serotypes (PAV, RMV, RPV, MAV and SGV) were reported. In Egypt, BYDV is common with PAV serotype being dominant. In the current report, total RNA was purified from infected wheat leaf plants and Aphids. RT-PCR technique was used to amplify and identify the coat protein gene sequence of BYDV- PAV and RMV serotypes in wheat using specific primers designed by ABI primer express software. Expected PCR products were sequenced and aligned together with related gene bank sequences and revealed the high similarity up to 93%. RT- Real Time PCR technique was used to detect and quantify BYDV. The results indicate that the infection ratio of Giza 164 samples are higher than the infection ratio of Sids 7 based on Ct value, and virus concentration in aphids are higher than in wheat for both serotypes. In addition, the sensitivity of RT- Real Time PCR is 3 to 5 fold higher than conventional PCR for detecting virus infection.

Key words: *Wheat, BYDV, molecular characterization, coat protein, Real Time PCR, sequencing.*

INTRODUCTION

Every year, barley yellow dwarf viruses (BYDVs) cause substantial losses throughout the world wherever their hosts, mainly wheat, barley, and oats, occasionally rice and maize, are grown. The barley yellow dwarf (BYD) disease was first identified by Oswald and Houston (1951). Barley yellow dwarf viruses (BYDVs) are members of the luteovirus group and are phloem limited transmitted in a persistent manner by several species of aphids (over 100) species of cultivated and wild grasses (Lister and Sward, 1988). Differences among BYDV isolates were first characterized by Rochow (1969). These isolates are distinguishable serologically (Waterhouse *et al.,* 1988). Symptoms induced by luteoviruses are often difficult to distinguish from symptoms caused by other pathogens, nutritional deficiencies, or cold weather (D'Arcy, 1995). The symptoms in wheat are not always obvious; often they are limited to stunting resulting in a substantial yield loss, while remaining undetected (Irwin and Thresh, 1990). In contrast, BYDV causes yellowing and stunting in barley, and yellowing, reddening, leaf stiffness, reduced tillering and heading, and numerous sterile florets in oats (D'Arcy, 1995). BYDV (PAV-129) isolates vary greatly in symptom severity (Chay *et al,* 1996). In Egypt, BYDV is common and was first recorded on wheat (Abdel-Hak and Ghobrial, 1984). The virus was isolated and identified (Aboul-Ata *et al.*, 1992). The five serotypes (PAV, RMV, RPV, MAV and SGV) were found in Egypt, with PAV being dominant (Lister and Sward, 1988). In addition, BYDV-PAV fluctuated from year to year (Aboul-Ata *et al.*, 2001). Much progress has been made recently in elucidating the roles of the proteins most conserved among all luteoviruses, those encoded by ORFs 3, 4, and 5 (Hussien, 1996). Besides its obvious function in forming virions, the coat protein (encoded by ORF 3) may have roles in virus movement and replication in plants (Shen and Miller, 2004). The coat protein is required for aphid transmission and it may confer aphid vector-specificity. Young *et al.*, (1994) found that a portion of the CP itself harbors the vector-specificity determinant. Real-time RT-PCR is a highly sensitive technique enabling amplification and quantification of a specific nucleic acid sequence with detection of the PCR product in real time. Quantification of DNA, cDNA, or RNA targets can be easily achieved by determination of the cycle, when the PCR product could be first detected (Fabre *et al.* 2003). Real Time PCR is based on TaqMan technology, detects and quantifies from 10^2 to 10^8 BYDV-PAV RNA copies. This test is 10^1 and 10^3 times more sensitive than the standard RT-PCR and ELISA assays published previously for BYDV-PAV detection and significantly improves virus detection in single aphids (Sijun *et al,* 2006).

In this investigation, the molecular characterization of the Egyptian BYDV the coat protein genes of PAV and RMV serotypes in aphids and infected wheat plants was investigated. The results showed that the infection ratio for wheat Giza 164 samples are higher than the infection ratio of wheat Sids 7 and the virus concentration in aphids are higher than in wheat for both serotypes. In addition, the coat protein gene aligned sequence for these serotypes showed highest similarity.

MATERIALS AND METHODS

Extraction and purification of total RNA from infected wheat leaf plants and aphids

Infected wheat leaf plants (Giza 164 and Sids 7) were collected from The Agricultural Research Center (ARC) open field, according to BYDV symptoms. Total RNAs which include BYDV ssRNA were extracted using RNeasy Plant Mini Kit from Qiagen (Germany) according to the manufacturers protocol. Total RNA from aphids was extracted by grinding two to three aphids in 200 ul of lysis buffer using RNA Microprep Kit from Stratagene (USA) according to the manufacturers protocol.

Sequence alignment, RT- PCR and Real Time RT-PCR conditions

The data search for Luteovirus family coat protein nucleotide sequence were performed using NIH GeneBank. The nucleotide sequence for Luteovirus coat protein gene with accession number L10356 was employed (Cheng *et al.,* 1997). Forward and reverse RT-PCR primers, Real Time RT-PCR primers and TaqMan probes for Luteovirus were designed to amplify Luteovirus coat protein gene using ABI primer express software. Also, three coding sequences for each coat protein gene of BYDV-PAV and RMV serotypes from NIH GeneBank with accession numbers AY879231 (Malmstrom and Shu, 2005), AJ563414 (Bisnieks *et al.*,2004) and AY450454 (Rastgou *et al.*, 2005) for PAV serotype and with accession number L12757(Geske *et al.*, 1996), L12758 (Geske *et al.*, 1993) and Z14123 (Domier *et al.*,1994) for RMV serotype were used in a multiple alignment and consensus of these coding sequences were carried out using DNA Man software. The sequence, used to design coat protein gene RT- PCR primers, Real Time

RT-PCR primers and TaqMan probes by ABI primer express software were used to detect and confirm infection with these serotypes.

Diagnosis and detection of BYDV coat protein gene

Reverse transcription and PCR were carried out sequentially in the same tube using one step RT-PCR kit from Qiagen (Germany) according to the manufacturers protocol using RT- PCR primers designed by ABI primer express software. This part of work was done in Virology Department, Plant Pathology Institute, Agricultural Research Center (ARC). A gel-free real-time one-step reverse transcription polymerase chain reaction protocol was done using MX3000p Real Time PCR system from Stratagene (USA) and Quantitect Probe RT-PCR kit from Qiagen (Germany) according to manufacturers protocol. For specific detection and quantitation of Luteovirus families, BYDV-PAV and RMV coat protein gene serotypes using primers and TaqMan probes designed for each serotype by ABI primer express software to confirm diagnosis and infection detection in infected leaf wheat plants (Giza 164). This part of work was done in Virology Department, National Cancer Institute, Cairo University.

Sequencing of the coat protein gene

ABI prism 310 Genetic Analyzer automated sequencer with Big Dye terminator chemistry from Applied Biosystems (USA) was used to sequence the forward strand of each purified PCR product of BYDV-PAV and RMV coat protein genes. This analysis was done in Bayer Reference Laboratory in Erany-France.

RESULTS AND DISCUSSION

Sequence alignment and computer analysis for coat protein gene

The data base analysis gives the research the ability to study the investigated case before starting the experimental steps. In addition to determination the best way to tackle this problem, multiple alignment of the coding sequences were carried out using DNA Man software. The sequence alignment analysis data released the consensus sequence which was used to design the coat protein gene RT-PCR primers, are listed in (Table 1) and The Real Time RT-PCR primers and TaqMan probes in (Table 2).

Table (1): PCR primer sequences designed and used to amplify coat protein gene for luteovirus, BYDV PAV and RMV serotypes.

Primer Code	Sequence	Length	Start	PCR Product size (bp)
Luteo-F	Aaaggtttccgacccacatt	20	581	400 bp
Luteo-R	Aagctcctcgcaccagttt	19	980	
PAV- F	Aggacctagacgcgcaaa	18	21	520 bp
PAV-R	Ggttccattggccttgtaga	20	540	
RMV-F	Cgtgaatgaatacgggaggt	20	43	600 bp
RMV-R	Cctatttggggttttgaaca	20	642	

Table (2): Real Time PCR primer and TaqMan probe sequences that used to amplify coat protein gene for luteovirus, BYDV PAV and RMV serotypes.

Primer Code	Sequence	Length	Start	PCR Product size (bp)
Luteo-F	Gatacaacccggaatgtggt	20	257	170 bp
Luteo-R	tgtgtccggctagttttgtg	20	426	
Luteo TaqMan probe	FAM tgcgctcaacttcaaaagtg TAMRA	20	352	
PAV- F	Aaattcggccccagtctatc	20	238	179 bp
PAV-R	Tagctacccagggctgattg	20	416	
PAV TaqMan probe	FAM gcgctttcagacggaatact TAMRA	20	268	
RMV-F	Cgtgaatgaatacgggaggt	20	43	172 bp
RMV-R	Cgtctgactccaggtcttcc	20	214	
RMV TaqMan probe	FAM acgcgttcgcaataataacc TAMRA	20	92	

Diagnosis and detection of coat protein gene for BYDV PAV and RMV serotypes

The primers designed were used for diagnosing and detecting the infection of luteovisus families (BYDV PAV and RMV) from wheat (Giza 164 and Sids 7) by RT-PCR. After different optimizations for RT-PCR reactions, the minimum RNA concentration as a template for RT-PCR reaction for the Giza 164 was 250 ng. The best primer concentration used for 50 ul total reaction volume was 60 pmol for all primers designed by ABI primer express software. The best primer annealing temperature for Luteovirus coat protein gene primer is 48 °C that produced amplified product of 480 bp (Fig. 1).The best primer annealing temperature for BYDVPAV coat protein gene primer was 52 °C that produced amplified product of 520 bp (Fig. 2). The best primer annealing temperature for BYDV-RMV coat protein gene primer was 54 °C that produced amplified product of 600 bp (Fig. 3).

For wheat (Sids 7), infection could not be detected by BYDV-PAV serotype using RT- PCR primers designed for coat protein gene by ABI primer express software, although was used up to 2 ug template RNA . This may be due to low viral RNA concentrations or may be due to Sids 7 being resistant to BYDV- PAV infection (Fig. 2) (Aboul-Ata *et al.*, 2001).The minimum RNA concentration used as a template for RT-PCR reaction was 850 ng that to detect BYDV infection for RMV serotype. The high RNA template amount indicated that low virus copy numbers were in samples or may be due to Sids 7 being resistant to BYDV- RMV infection (Aboul-Ata *et al.*, 2001). The best primer concentration used for 50 ul total reaction volume is 120 pmol for all primers designed. The best primer annealing temperature for BYDV RMV coat protein gene primer was 54 °C that produced amplified product of 600 bp (Fig. 3) (Hussien, 1996).

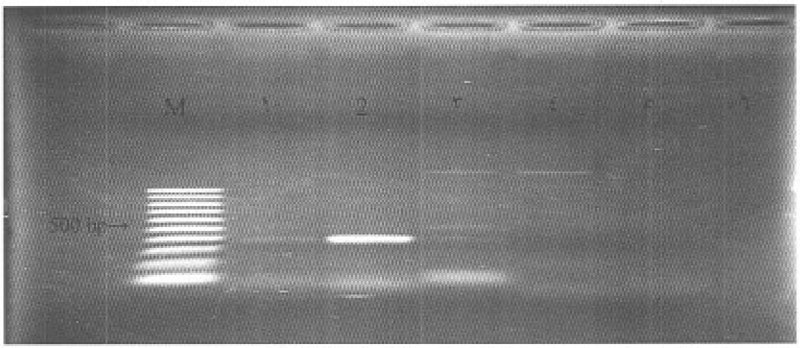

Fig.(1): Optimizing primer annealing temperature, concentrations and template RNA concentrations for Luteovirus coat protein gene from infected leaf wheat plants (Giza 164 and Sids 7). (M): PCR marker. (Lanes 1,2,3): Giza 164.(Lanes 4,5,6) Sids 7.

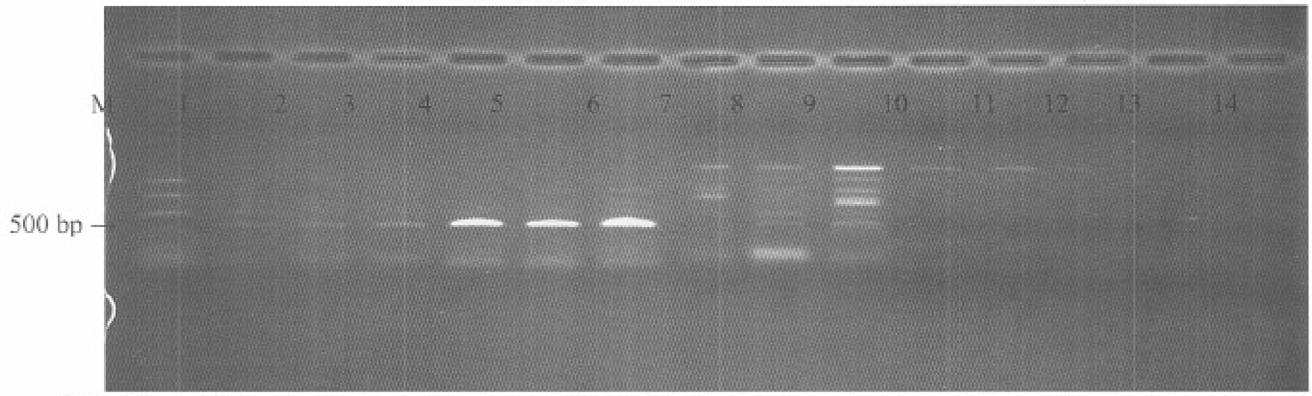

Fig.(2): Optimizing primer annealing temperature, concentrations and template RNA concentrations for BYDV-PAV coat protein gene serotype from infected leaf wheat plants (Giza 164 and Sids 7). (M): PCR marker.(Lanes 1,2,3,4,5,6): Giza 164.(Lanes 7,8,9,10,11,12,13,14): Sids 7.

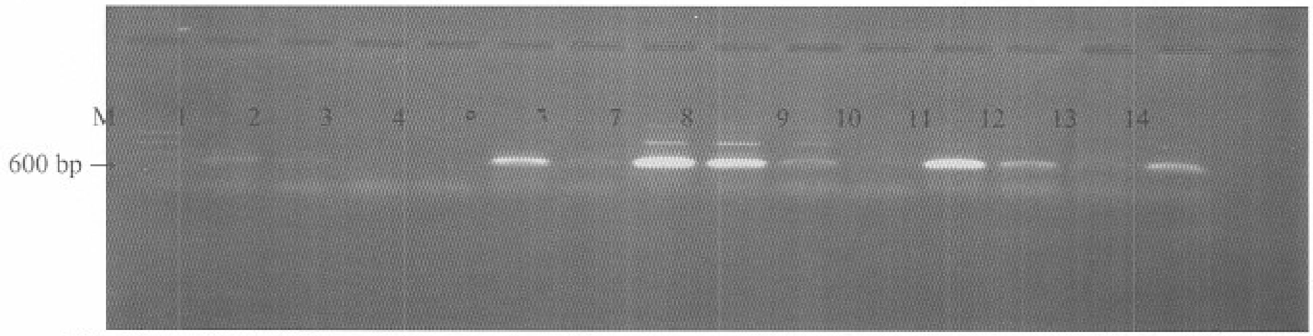

Fig.(3): Optimizing primer annealing temperature, concentrations and template RNA concentrations for BYDV-RMV coat protein gene serotype from infected leaf wheat plants (Giza 164 and Sids 7). (M): PCR marker. (Lanes 1,2,3,4,5,6,7,8): Giza 164. (Lanes 9,10,11,12,13,14) : Sids 7.

Purification of amplified RT-PCR coat protein gene from BYDV-PAV and -RMV serotypes in wheat

Several PCR reactions were done and in each case the purification of the amplified product was essential for the subsequent steps (Geske *et al.,* 1996). Qiaquick PCR purification kit from Qiagen (Germany) was used for purification of PCR products according to manufacturers protocol. Amplified PCR products of BYDV-PAV and RMV coat protein genes were obtained through RT-PCR from infected wheat (Giza 164) (Fig. 4). The purified products were sequenced (Fig. 5 and 6) (Bisnieks *et al.,* 2004).

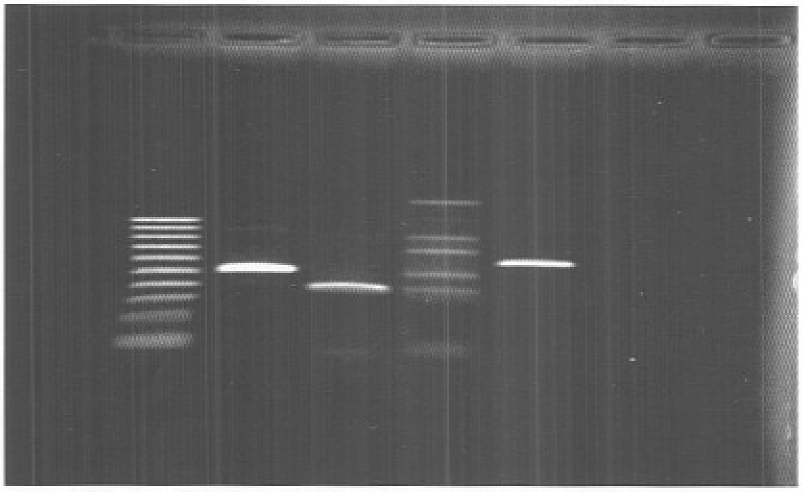

Fig. (4):Purified amplified RT-PCR products for coat protein genes from infected leaf of wheat plants (Giza 164).(M): PCR marker.Lane (1): BYDV-PAV CP gene- 520 bp.Lane (2): Luteovirus CP gene- 400 bp.Lane (3): BYDV-RMV CP gene- 600 bp.

Sequencing and alignment analysis of purified RT-PCR coat protein gene of BYDV PAV and RMV serotypes

The forward strand of each purified PCR product of BYDV-PAV (Fig. 5) and RMV (Fig. 6) coat protein genes were sequenced using forward primer used in RT-PCR technique from infected wheat (Giza 164).

Using DNA MAN software version 4.0, the sequence obtained from BYDV- PAV coat protein gene was aligned to the sequence obtained from BYDV-RMV coat protein and found sequence similarity of 93% (Fig. 7). The data confirmed that the coat protein for both PAV and RMV of BYDV are very close to each other with high relationship for both of them (Geske *et al.,* 1996, Bisnieks *et al.,* 2004).

Another alignment was done between BYDV- PAV coat protein gene and two other BYDV- PAV coat protein genes obtained from NIH GeneBank with accession numbers AY 879231 (Malmstrom and Shu, 2005) and AJ 563414 (Bisnieks *et al.,*2004). Also, sequence alignment made between BYDV- RMV coat protein gene with two other BYDV- RMV coat protein genes obtained from NIH GeneBank with accession numbers L 12757(Geske *et al.,* 1996) and Z 14123 (Domier *et al.,*1994). These alignments showed sequence similarity reaching 89% (Fig. 8) and 91% (Fig. 9) respectively. However, this slight difference may be due to mutational events or different aphid vector species (Bisnieks *et al.,* 2004).

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1GGGCGCGGNA ANTTTGNTTT TCGAAGGACT CTCTCCGGGC AATGCCTCCG GGAAGCTCAC CTTCGGGGCG  TCTCTATCAG  80
81AGTGCGCAGC ATTCAGTAGT GGAATTCTCA AGGCCTACCA TGAGTATAAG ATCTCAAAGG TCACTTTGGA GTTCATCTCC 160
161GAGGCCTCTT CCCAATCTGA AGGCTCCATC GCTTATGAGC TTGATCCACA CAACAAGCTC TCTAGCNCTC TCTTCCACCA240
241TCAACAAATT CTCAATCGTC AAANGGTGGT AAGAGAACCT TTACGTTCAA TCAAATCGGA GGTGGAATTT GGCGAGANCT320
321TCNCCATAAG ATTNAATCCG CTCATCTTNC TTACAAGGGT ANNGGAAAAT CCNTTCNTCN TNGNTNTCCN NCTCCCNTCC400
401TCTTGANNGT TTTNANNCAT ANAACCCCNT AAAGNCCCGA NNANCNNCTN CGCCGGNANN TCCNTTTTTT TCCTTCCTTC480
481ACCNNANATG CCCTNCNNNC NCNNTCCNNC NCCTNAGANC GTCNAGTATN TC                             540
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Fig. (5): BYDV-PAV coat protein gene sequence by automated florescence sequencer.

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1TTNTNTTGAC TAGACACGGA GCCGCTCGCG TTCNNCTGCA GTCGCACCCA CCAGTGGTTG TGGTCGCGGC  AGGTCAGCGT  80
81CGACGCCGCC CCAGAAGACG AGGACGACGA ACTGGAAACA CTCCAGGAGG ATCTGGAATC CGAAGAGGGT CGCGGGAAAC 160
161ATTTGTATTT TCGAAGGACT CTCTCACGGG CAATGCCTCC GGGAAGCTCA CCTTCGGGGC GTCTCTATCA GAGTGCGCAG240
241CATTCAGTAG TGGAATTCTC AAGGCCTACC ATGAGTATAA GATCTCAAAG GTCACTTTGG AGTTCATCTC CGAGGCCTCT320
321TCCCAATCTG AAGGCTCCAT CGCTTATGAG CTTGATCCAC ACAACAAGCT CTCAGCCCTC TCTTCCACCA TCAACAAAT400
401CTCAATCGTC AAAGGTGNGT AAGAAGANCC TTTACGTCCA ATCAAATTCG GANGTGGTNT TTNGCTGAAT ACTTNAANAT480
481ANNATTCAAT TTTCCTTCTC CNTATNAGGN NNNATGGNTA ATNNCTCNNA NTTCNGNCTT NCNNTCCGCG NTTNCTCCTC560
561GATTTTTCTN ACCCAAAAAT TCCTTANTTG GTANNCATTC                                             640
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Fig. (6): BYDV-RMV coat protein gene sequence by automated florescence sequencer.

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Alignment of PAV-CP(upper line) and RMV-CP(lower line)          Identity=93%
1      GGCGCGGNAAN..TTTGN.TTTTCGAAGGACTCTCTC.CGGGCAATGCCTCCGGGAAGCT
       | |||||  ||    ||||   ||||.|||||||||||||||| ||||||||||||||||.|||
1      GTCGCGGGAAACATTTGTATTTTCGAAGGACTCTCTCACGGGCAATGCCTCCGGGAAGCT
61     CACCTTCGGGGCGTCTCTATCAGAGTGCGCAGCATTCAGTAGTGGAATTCTCAAGGCCTA
       |||||||||.||||||||||||||||||||||||||||||||||||||||||||||||||
61     CACCTTCGGGGCGTCTCTATCAGAGTGCGCAGCATTCAGTAGTGGAATTCTCAAGGCCTA
121    CCATGAGTATAAGATCTCAAAGGTCACTTTGGAGTTCATCTCCGAGGCCTCTTCCCAATC
       ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
121    CCATGAGTATAAGATCTCAAAGGTCACTTTGGAGTTCATCTCCGAGGCCTCTTCCCAATC
181    TGAAGGCTCCATCGCTTATGAGCTTGATCCACACAACAAGCTCTCTAGCNCTCTCTTCCA
       ||||||||.|||||||||||||||||||||||||||||||||||||| .||  ||||||||
181    TGAAGGCTCCATCGCTTATGAGCTTGATCCACACAACAAGCTCTC.AGCCCTCTCTTCCA
241    CCATCAACAAATTCTCAATCGTCAAANGGTGGTAAGAGAA.CCTTTACGTTCAATCAAAT
       ||||||||||||||||||||||||||| |    ||||||  | |||||||||  ||||||||
241    CCATCAACAAATTCTCAATCGTCAAAGGTGNGTAAGAAGANCCTTTACGTCCAATCAAAT
301    .CGGAGGTGGAATTTGGC.GAGANCTTCNCCATAAGATTNAATCCGCTCAT
        |||| ||||   ||| || ||     |||      |||   ||| |||     |
301    TCGGANGTGGTNTTTNGCTGAATACTTNAANATANNATTCAATTTTCCTTC
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Fig. (7): Sequence alignment between coat protein gene of BYDV-PAV and RMV.

Relative quantification of BYDV PAV and RMV serotypes

Fabre *et al,* (2003) used TaqMan probe Real Time RT-PCR technique to quantify the infection of BYDV PAV and RMV serotypes in wheat and aphids as a relative quantitative method based on Ct value obtained from the experiment to show the rate of infection. This new method is very sensitive for detecting very small concentration of viruses infected wheat. This protocol can be used as a new tool to determine the infection ratio of BYDV-PAV and RMV serotypes in wheat and aphids. A 200 ng template RNA extracted from *R. padi* aphid vector that transmit BYDV-PAV serotype and 200 ng template RNA extracted from *R. maidis* aphid vector that transmit BYDV-RMV serotype were used as a calibrator standard to use its Ct value to compare it with Ct value obtained from 200 ng RNA concentration extracted from different infected wheat leaf plants (Giza 164 and Sids 7) to determine the infection ratio based on Ct value for BYDV-PAV (Fig. 10) and RMV coat protein gene (Fig. 11). The results indicate that the infection ratio for Giza 164 samples are higher than the infection ratio for Sids 7 (Aboul-Ata *et al.*, 2001) and virus concentration in aphids are higher than in wheat for both serotypes (Sijun *et al.*, 2006). The final conclusion is that Wheat (Sids 7) quite resistance to both BYDV- PAV and

RMV serotype comparing to Wheat (Giza 164). In addition, sensitivity of RT- Real Time PCR is 3 to 5 fold higher than conventional PCR for detection of virus infection (Malmstrom and Shu, 2005).

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CLUSTAL multiple sequence alignment                     Identity 89%
AY879231 05     GGACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCA
AJ563414 04     GGACTGAGGTATTCGTATTCTCAGTTGACAACCTTAAAGCCAACTCCTCCGGGGCAATCA
PAV             GGGCGCGGNAANTTTGNTTTTCGAAGGACTCTCTCCGGGC-AATGCCTCCGGGAAGCTCA
                ** *   *  * *    ** **    ***   **    ** **  ********    ***

AY879231 05     AATTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACC
AJ563414 04     AATTCGGCCCCAGTCTATCGCAATGCCCAGCGCTTTCAGACGGAATACTCAAGTCCTACC
PAV             CCTTCGGGGCGTCTCTATCAGAGTGCGCAGCATTCAGTAGTGGAATTCTCAAGGCCTACC
                  *****  *   ******  * *** ****  *       ***** ****** ******

AY879231 05     ATCGTTACAAGATCACAAGTATCCGAGTTGAGTTTAAGTCACACGCGTCCGCCACTACGG
AJ563414 04     ATCGTTACAAGATCACAAGTATCCGAGTTGAGTTTAAGTCACACGCGTCCGCCACTACGG
PAV             ATGAGTATAAGATCTCAAAGGTCACTTTGGAGTTCATCTCCGAGGCCTCTTCCCAATCTG
                **   ** ****** ***   **    * ***** *  **  * ** **  **    * *

AY879231 05     CNGGCGCTATCTTTATTGANCTCGACACCGCGTGCAAGCAATC-AGCCCTGGGTAGCTAC
AJ563414 04     CCGGCGCTATCTTTATTGAACTCGACACCGCGTGCAAGCAATC-AGCCCTGGGTAGCTAC
PAV             AAGGCTCCATCGCTTATGAGCTTGATCCACACAACAAGCTCTCTAGCNCTCTCTTCCACC
                  *** * ***  *  *** ** **  *      *****  ** *** **   *  *  *

AY879231 05     ATTAATTCCTTCACCATCAGCAAG-ACCGCCTCCAAGGTCTTCAGGTCAGAGGCAATTAA
AJ563414 04     ATTAATTCCTTCACCATCAGCAAG-ACCGCCTCCAAGGTCTTCCGGTCAGAGGCAATTAA
PAV             ATCAACAAATTCTCAATCGTCAAANGGTGGTAAGAGAACCTTTACGTTCAATCAAATCGG
                ** **    *** * ***  ***     *     *    ***   **   *   ***

AY879231 05     CGGGAAGGAATTCCAGGAA--TCAACGATAGACCAATTCTGGATGCTCT---
AJ563414 04     CGGGAAGGAATTCCAGGAA--TCAACGATAGACCAATTTTGGATGCTCT---
PAV             AGGTGGAATTTGGCGAGANCTTCNCCATAAGATTNAATCCGCTCATCTTNCT
                 **       *  *  **   **  *   ***   * *  *       *
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Fig. (8): Sequence alignment between coat protein genes of BYDV-PAV and other PAV serotypes from GeneBank.

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CLUSTAL multiple sequence alignment                     Identity: 91%
RMV             GTGGTTGTGGTCGCGGCAGGTCAGCGTCGACGCCGCCCCAGAAGACGAGGACGACGAACT
L12757 99       GTGGTTGTGGTCGCGCAAACCCAGCGTAGACGCA---CCCGAAGACGAGGACGACCAAGT
Z14123 94       GTGGTTGTGGTCGCGGCAAATCCGCGTCGAGGACGCTCTCGAAGACGAAGACGATCAAGT
                ***************  *   * **** ** *     *  ******** *****  ** *

RMV             GGAAACACTCCAGGAGGATCTGGAATCCGAAGAGGGTCGCGGGAAACATTTGTATTTTCG
L12757 99       GGAGACACTTCAGGAGGACCTCGAGGGCGAGGAGGCTCCGGGGAGACTTTCGTATTTTCG
Z14123 94       GGAAACATTACAGGAAGACCTGGAGTCAGACGAGGCTCGCGGGAGACTTTTGTATTTTCA
                *** *** * ***** ** ** **    ** **** **  **** ** ** ********

RMV             AAGGACTCTCTCACGGGCAATGCCTCCGGGAAGCTCACCTTCGGGGCGTCTCTATCAGAG
L12757 99       AAGGACTCTATCGCGGGCAGTGC-TCCGGAAAGCTCACCTTCGGGGCGTCTCTTTCTGAG
Z14123 94       AAGGACTCTATCGCGGGCAACGCCTCCGGGAAAATCACCTTCGGACCGTCTTTATCAGAG
                ********* ** ******  ** ***** **  **********  ***** * ** ***

RMV             TGCGC-AGCATTCAGTAGTGGAATTCTCAAGGCCTACCATGAGTATAAGATCTCAAAGGT
L12757 99       TGCGCCAGCATTCTCTGGTGGAATTCTCAAGGCCTACCATGAGTATAAGATCACAAAAAT
Z14123 94       TGTGC-AGCATTCAGTGGCGGAATTCTCAAGGCCTACCATGAGTATAAGATCTCAAAGAT
                ** ** *******  * * ********************************* ****  *

RMV             CACTTTGGAGTTCATCTCCGAGGCCTCTTCCCAATCTGAAGGCTCCATCGCTTATGAGCT
L12757 99       CATACTGGAGTTCATCTCGGAGGCCTCTTCAACGCAGTCCGGTTCCATCGCTTATGAGCT
Z14123 94       CATACTGGAGTTCATCTCCGAGGCCGCTTCCACCGCCGAAGGTTCCATCGCTTATGAACT
                **   ************* ****** ****          ** ************** **

RMV             TGATCCACACAACAAGCTCTCAGCCCTCTCTTCCACCATCAACAAATTCTCAATCGTCAA
L12757 99       GGATCCCCACAACAAGCTCAGCACCCTCGCATCAACAATCAATCAAATCTCGATCGTCAA
Z14123 94       TGATTCACACAACAAGCTCTCAACCCTTGGCTCCACCATCAACAAATTCTCAATCGTCAA
                 *** * ************    ****    ** ** *****  ** **** ********
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Fig. (9):Sequence alignment between coat protein genes of BYDV-RMV and other RMV serotypes from GeneBank.

Amplification Plots

Relative Quantitative BYDV-PAV

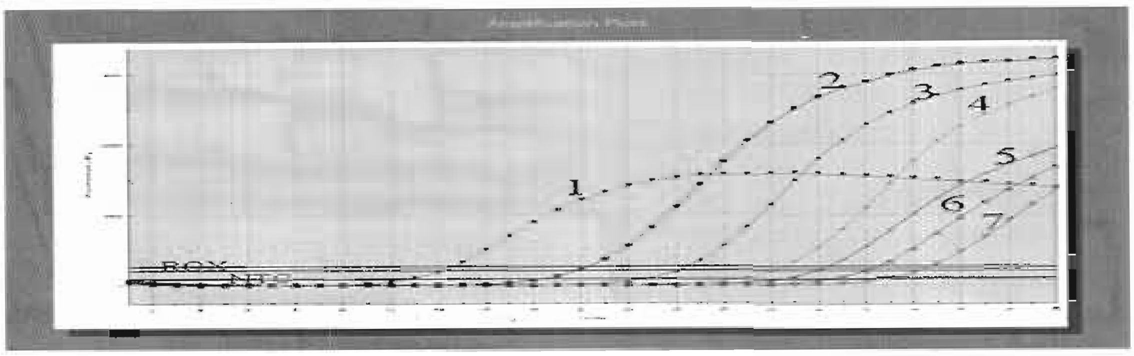

Fig. (10): Florescence amplification curve vs cycles for relative quantitation of amplified Real Time RT-PCR coat protein gene of BYDV-PAV serotype, using 200 ng template RNA extracted from R.padi aphid vector as a calibrator standard and 200 ng template RNA from different infected wheat leaf plants (Giza 164 and Sids 7).Curve (1):R.padi aphid vector.Curves (2,3,4): wheat Giza 164.Curves (5,6,7): Wheat Sids7. NTC (negative control), Rox (fluorescent normalization dye).

Amplification Plots

Relative Quantitative BYDV-RMV

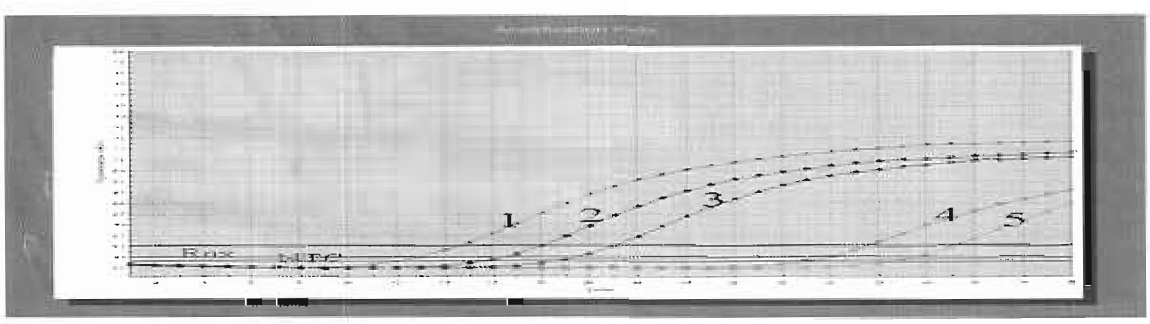

Fig. (11): Florescence amplification curve vs cycles for relative quantitation of amplified Real Time RT-PCR coat protein gene of BYDV-RMV serotype, using 200 ng template RNA extracted from R.maidis aphid vector as a calibrator standard and 200 ng template RNA from different infected wheat leaf plants (Giza 164 and Sids 7).Curve (1):R.maidis aphid vector.Curves (2,3): wheat Giza 164.Curves (4,5): wheat Sids 7.NTC (negative control), Rox (fluorescent normalization dye).

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الملخص العربي

التوصيف الجزيئى لجين الغلاف البروتينى لفيروس التقزم الاصفر فى القمح والمن

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يعتبر فيروس التقزم الاصفر(BYDV) من مجموعه الليتوفيرس ويتم انتقاله بواسطه المن فقط. ومن اكثر السلالات انتشارا فى مصر سلالة PAV وهى ضمن سلالات الفيروس الخمس(SGV PAV, RMV,RPV andMAV). تم استخدام تكنيكات البيولوجيا الجزيئية لتشخيص الفيروسات النباتية على مستوى الجينوم نتيجة لحساسيتها العالية فى الكشف عن التركيزات المنخفضة من الفيروس. وفى هذا البحث ، تم استخدام تكنيك النسخ العكسي لتفاعل البلمرة المتسلسل(RT-PCR) وجهاز تحليل التتابع النوتيدى الاوتوماتيكى (Automated Sequencer) لتكبيروتحديد التتابع النوتيدى لجين الغلاف البروتينى (CP) لفيروس التقزم الاصفر فى القمح من سلالتي PAV. RMV ومقارنm التتابع النوتيدى للسلالتين اثبتت النتائج درجة تشابه عالية تصل الى ٩٣% لكل من جين الغلاف البروتينى لسلالتى فيروس التقزم الاصفر فى القمح. وقد تم تحديد نسة الاصابة فى كل من القمح والمن بواسطة التحليل الكمى النسبى باستخدام تكنيك النسخ العكسى لتفاعل البلمره المتسلسل فى الوقت الحقيقى(RT- Real Time PCR). واوضحت النتائج ان معدل الاصابة فى صنف جيزه ١٦٤ اعلى من صنف سيدس– ٧ كما اظهرت النتائج أن نسبة تركيز الفيروس فى المن أعلى من القمح فى كل من السلالتين.