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CHAPTER 2

Diagnosis and Control of Cereal Viruses in the Middle East

Aboul-Ata E. Aboul-Ata,* Hamed Mazyad,* Ahmad Kamal El-Attar,* Ahmed Mohamed Soliman,* Ghandi Anfoka,† Muhammad Zeidan,‡ Rena Gorovits,§ Iris Sobol,§ and Henryk Czosnek§

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Abstract

Middle Eastern countries are major consumers of small grain cereals. Egypt is the biggest bread wheat producer with 7.4 million tons (MT) in 2007, but at the same time, it had to import 5.9 MT. Jordan and Israel import almost all the grains they consume. Viruses are the major pathogens that impair grain production in the Middle East, infecting in some years more than 80% of the crop. They are transmitted in nonpersistent, semipersistent, and persistent manners by insects (aphids, leafhoppers, and mites), and through soil and seeds. Hence, cereal viruses have to be controlled, not only in the field but also through the collaborative efforts of the plant quarantine services inland and at the borders, involving all the Middle Eastern countries. Diagnosis of cereal viruses may include symptom observation, immunological technologies such as ELISA using polyclonal and monoclonal antibodies raised against virus coat protein expressed in bacteria, and molecular techniques such as PCR, microarrays, and deep sequencing. In this chapter, we explore the different diagnoses, typing, and detection techniques of cereal viruses available to the Middle Eastern countries. We highlight the plant quarantine service and the prevention methods. Finally, we review the breeding efforts for virus resistance, based on conventional selection and genetic engineering.

I. INTRODUCTION

The Middle East is the cradle of most wild cereal species that were domesticated during the past 8000 years (Salamini et al., 2002). The wild progenitors of modern cereal species are all found in the Middle East, in particular, in the Fertile Crescent. These species include wild wheat (Triticum urartu, Triticum boeoticum, and Triticum dicoccoides), wild barley (Hordeum spontaneum), and wild rye (Secale vavilovii). Humans who settled in the Middle East harvested the wild forms of cereals from their natural habitats before domestication (Zohary and Hopf, 2000). Our mission, as scientists from the region, is to protect not only the crop in the field but also the priceless cereal genetic resources.

Cereals constitute the basic food stuff and a major commodity for many Middle Eastern countries. However, because of insufficient production, they need to rely on importations to face the needs of fast-growing populations. As for wheat, for instance, in 2007, Egypt produced 7.4 million tons (MT) and imported 5.9 MT. Israel produced 0.16 MT and imported 1.2 MT. Jordan imported 0.8 MT, almost all its needs. More information on cereal production can be found in the FAO website (for production, http://faostat.fao.org/site/339/default.aspx; for importation, http://faostat.fao.org/site/342/default.aspx).
As most agricultural crops, cereals are prone to viral diseases. Several virus outbreaks have been recorded in the Middle East (Ammar et al., 1989, 2008) as well as in European and Asian countries (Jones, 2009). Virus outbreaks can have dramatic effects. During the summer of 1989 and 1999, Maize yellow stripe tenui-like virus (MYSV) caused 80–90% yield loss in Egyptian wheat and maize (Ammar et al., 2008) and 70% in sweet corn (Aboul-Ata and Ammar, 1988). Many of the corn fields had to be eradicated because of large-scale severe MYSV infection.

Management of cereal viruses includes diagnosis, protection of cultures, and breeding for virus-resistant cultivars. The variable epidemiological behavior of the multiple virus genera and families that affect cereals, together with the genetic diversity of virus isolates, greatly complicates control efforts. Moving the date of sowing and/or controlling insect vectors with pesticides can significantly contribute to the reduction of virus infection. Nonetheless, introduction of genetic resistance in the major cultivars is the most efficient approach to reduce the economic losses caused by viruses (Kosová et al., 2008). Sources of natural virus resistance in cereals have been identified in some grasses and introduced into cereal genotypes via crossing (reviewed by Ordon et al., 2009). Unfortunately, the Middle Eastern countries never developed significant breeding programs for virus resistance. For instance, Egypt has a strong cereal breeding program but does not have a program for virus resistance.

This chapter discusses methodologies for cereal virus detection, presents routines for plant protection and quarantine, and explores breeding programs for virus resistance. In the frame of the USAID CDR Middle East Research and Cooperation Program (MERC), Egypt, Israel, and Jordan have joined efforts to standardize detection procedures and plant protection routines. We wish to convince decision makers in the Middle East to consider virus control as high priority, to encourage research institutes to develop improved detection means, and to persuade producers in the Middle East to breed virus-resistant varieties that farmers will be willing to use.

II. DIAGNOSIS, DETECTION, AND TYING OF CEREAL VIRUSES

A. Major viruses infecting cereals in the Middle East

Sixty-six viruses are able to infect grasses and cereal crops (Lapierre and Signoret, 2004); only a few of them cause economically important yield losses. The major cereal viruses found in the Middle East are listed in Table I. Symptoms caused by some of these viruses, and their insect vectors, are shown in Fig. 1A and B. The six cereal viruses with the highest
### TABLE I  Viruses infecting maize, sorghum, wheat, and barley in Middle Eastern countries (Ammar et al., 2008)

<table>
<thead>
<tr>
<th>Virus</th>
<th>Genus</th>
<th>Family</th>
<th>Countries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viruses of maize and sorghum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cynedon chlorotic streak virus CCSV</td>
<td>Nucleorhabdovirus</td>
<td>Rhabdoviridae</td>
<td>Jordan</td>
</tr>
<tr>
<td>Maize dwarf mosaic virus MDMV</td>
<td>Potyvirus</td>
<td>Potyviridae</td>
<td>Egypt, Israel, Yemen</td>
</tr>
<tr>
<td>Maize mosaic virus MMV</td>
<td>Nucleorhabdovirus</td>
<td>Rhabdoviridae</td>
<td>Sudan, Yemen</td>
</tr>
<tr>
<td>Maize rough dwarf virus MRDV</td>
<td>Fijivirus</td>
<td>Reoviridae</td>
<td>Israel</td>
</tr>
<tr>
<td>Maize streak virus MSV</td>
<td>Mastrevirus</td>
<td>Geminiviridae</td>
<td>Egypt, Sudan, Yemen</td>
</tr>
<tr>
<td>Maize yellow stripe virus MYSV</td>
<td>Tenui virus</td>
<td>Tenuiviridae-like?</td>
<td>Egypt, Sudan</td>
</tr>
<tr>
<td>Sugarcane streak virus SCMV</td>
<td>Mastrevirus</td>
<td>Geminiviridae</td>
<td>Egypt</td>
</tr>
<tr>
<td>Zea mosaic virus ZMV</td>
<td>Potyvirus</td>
<td>Potyviridae</td>
<td>Israel</td>
</tr>
<tr>
<td>Viruses of wheat and barley</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Barley yellow dwarf-MAV BYDV</td>
<td>Luteovirus</td>
<td>Luteoviridae</td>
<td>Egypt, Yemen</td>
</tr>
<tr>
<td>Barley yellow dwarf-PAV BYDV</td>
<td>Luteovirus</td>
<td>Luteoviridae</td>
<td>Egypt, Yemen</td>
</tr>
<tr>
<td>Barley stripe mosaic virus BSMV</td>
<td>Hordeivirus</td>
<td>Not assigned</td>
<td>Egypt</td>
</tr>
<tr>
<td>Wheat dwarf virus WDV</td>
<td>Mastrevirus</td>
<td>Geminiviridae</td>
<td>Egypt</td>
</tr>
<tr>
<td>Wheat streak mosaic virus WSMV</td>
<td>Rymovirus</td>
<td>Potyviridae</td>
<td>Jordan</td>
</tr>
</tbody>
</table>

?, MYSV grouping is not confirmed as tenui virus.
impact in the Middle East are Wheat dwarf virus WDV (genus Geminivirus), Barley stripe mosaic virus BSMV (genus Hordeivirus) which is seed-borne, Wheat streak mosaic virus WSMV (genus Rymovirus), Barley yellow dwarf BYDV—with PAV, MAV, RMV, and SGV strains—(genus Luteovirus), Cereal yellow dwarf virus CYDV-RPV (genus Plerovirus), and the leafhopper-borne Maize yellow stripe tenui-like virus MYSV (genus Tenui-like; Aboul-Ata et al., 1992; Ammar et al., 2008). WDV is a single-stranded DNA (ssDNA) virus; MYSV, BSMV, WSMV, BYDVs, and CYDVs are ssRNA viruses. Cereals can be infected by economically less important viruses such as Agropyron mosaic virus and Ryegrass mosaic virus (both
FIGURE 1 (A) Viruses infecting maize in the Middle East: symptoms and vectors. (1–3) Symptoms of *Maize yellow stripe tenui-like virus* MYSV. Three stages of MYSV symptoms: (1) fine stripe, (2) course stripe, (3) chlorotic stunt. (4–9) Different MYSV symptoms: (4) fine stripe and stunting, (5) fine stripe, (6) curving of apical stem, (7) less roots formation and reddening, (8 and 9) severe dwarfing and yellowing. (10–14) MYSV-leafhopper vector *Cicadulina chinai*: (10) embryo with brown eye, (11) nymph, (12) dorsal view, (13) ventral view of male, (14) ventral view of female. All pictures are from A. E. Aboul-Ata, unpublished data. (B) Viruses infecting wheat and barley in the Middle East: symptoms and vectors. (1) Symptoms of barley in the field inoculated with seed-borne *Barley stripe mosaic hordeivirus* BSMV, (2 and 4) symptoms of *Barley yellow dwarf luteovirus*. (3) BYDV-aphid vector * Macrosiphum avene*, (5) BYDV-aphid vector *Rhopalosiphum padi*. (6) BYDV-aphid vector *Rhopalosiphum maidis*. (7) Symptoms of *Wheat streak mosaic virus* (WSMV). Pictures 1, 2, and 4 are from A.E. Aboul-Ata, unpublished data; pictures 3, 5, and 6 are after Cullen (2010), picture 7 is from G. Anfoka presentation at annual meeting of M027-063 MERC-funded project, Cyprus 2009.
belonging to the genus *Rymovirus*, *Rice stripe necrosis virus* (genus *Furovirus*), *Maize streak virus* MSV (genus *Geminivirus*), *Rice yellow mottle virus* (genus *Sobemovirus*), *Maize rough dwarf virus* (genus *Fijivirus*), and *Brome mosaic virus* BMV (genus *Bromovirus*; Lapierre and Signoret, 2004).

B. BYDV, a major virus infecting cereals

BYDV is the most widely distributed virus infecting cereals. BYDV was common on barley and wheat as early as the 1940–1950s in Egypt. At that time, the main method for controlling the disease was to spray insecticides against the aphid vector. Since then, BYDV has been recognized in the developing countries as a serious constraint to the production of wheat, barley, oats, and triticale, resulting in the loss of substantial amounts of important food grains. The virus also infects at least 100 other grass species, including maize and rice. It produces symptoms on wheat, barley, and other grain cereals characterized by small spikes, yellowing of leaves starting from the leaf tip and expanding to the base, and stunting (Fig. 1B). BYDV is transmitted by aphids in a persistent, circulative manner, but not mechanically and not through seeds (D’Arcy and Burnett, 1995). Aphids acquire and transmit BYDV while feeding on the phloem sieve tube elements of host plants (Power and Gray, 1995). Transmission depends on specific interactions between the virus and insect proteins (Andret-Link and Fuchs, 2005; Li et al., 2001).

Technological advances have allowed distinguishing five BYDV strains and their principal vectors (in parenthesis): BYDV-RPV (*Rhopalosiphum padi*), BYDV-RMV (*Rhopalosiphum maidis*), BYDV-MAV (*Sitobion avenae*), BYDV-SGV (*Schizaphis graminum*), and BYDV-PAV (*R. padi*, *S. avenae*, and others). These strains can be discriminated using antibodies and DNA-based techniques. BYDV-PAV is the major strain infecting wheat in Egypt (Aboul-Ata et al., 1992; Lister et al., 1994) and other countries in the Middle East (Syria and Jordan) and the Maghreb (Tunisia, Algeria, and Morocco). The International Committee on the Taxonomy of Viruses has divided the BYDVs into two distinct virus groups: BYDV (PAV, RMV, SGV, and MAV) and *Cereal yellow dwarf polerovirus* CYDV (RPV) (D’Arcy et al., 2000). This nomenclature system has been adopted by all BYDV researchers.

C. Detection of BYDV and other cereal viruses

1. Serology

Direct and indirect ELISAs are routinely used in the Middle Eastern countries to detect cereal viruses (Aboul-Ata et al., 1992). Usually, polyclonal antibodies are produced in rabbits injected with purified virus particles. Accordingly, the five BYDV isolates from Egypt (PAV, MAV,
RPV, RMV, and SGV) have been serotyped in field-collected cereal samples (Lister et al., 1994). Tissue blots have been used as targets for BYDV immunodetection during seasonal field surveys in Egypt (Makkouk and Comeau, 1994). Monoclonal antibodies have been used to discriminate between BYDV-PAV and BYDV-MAV serotypes (Mastarie et al., 1998; Miller et al., 2002). Different epitopes could differentiate between BYDV-PAV variants, CpA and CpB (Table II). The coat protein (CP) of two distinct groups of BYDV-PAV (PAVcpA and PAVcpB) has been sequenced (Mastarie et al., 1998; Miller et al., 2002). BYDV-PAV cpA was diagnosed in Australia, Japan, France, Egypt, Morocco, and USA, while BYDV-PAV cpB was detected in Egypt, France, and Morocco. Mixed infections of BYDV-PAV cpA and cpB were found in Egypt and France. Two severe (lethal, FHv1 and FHv2) and two mild cpA isolates differed by eight amino acid substitutions. Monoclonal antibodies were also used to detect infection by BYDV and CYDV (Belkahla and Lapierre, 1999; Mastarie and Lapierre, 1999). TAS-ELISA was used with specific monoclonal antibodies to diagnose CYDV-RPV infection (Belkahla and Lapierre, 1999; Mastarie et al., 1998).

In the frame of the MERC research program, we are mass producing the CP of the major cereal viruses (Fig. 2). The virus CP genes are overexpressed in *Escherichia coli* under an inducible promoter and the proteins are purified by affinity chromatography (Akad et al., 2004). Expressed CP constitutes a large source of antigens for polyclonal antibody production. The CP epitopes can be manipulated by modifying the sequence of the CP gene in order to increase the ability to detect a given virus strain. The antibodies will be distributed to other Middle East countries to standardize cereal virus detection by national plant quarantine and extension services.

| TABLE II | Characterization of BYDV-PAV subserotypes PAVcpA and PAVcpB from different hosts |
|-----------|-----------------|-----------------|-----------------|
| BYDV-PAV criteria | cpA | cpB | Host |
| Isolate$^a$ | EW1 | EW2 | Wheat |
| Symptoms | Severe | Mild | Barley |
| Epitope P6 | + | + | Maize |
| Epitope P14 | + | – | Maize |
| Fresh weight$^b$ | 0.04–0.5 | 0.9–1.6 | Barley |
| Tillering | No | Yes | Barley |

$^a$ EW1, Egypt wheat isolate 1; EW2, Egypt wheat isolate 2.

$^b$ Not infected control, fresh weight 1.7.
Molecular hybridization with DNA probes has been used to detect BYDV (Habili et al., 1987). In Egypt, wheat genotypes infected with BYDV-PAV show different degrees of severity. This biological diversity was paralleled with polymorphism in the genome sequence, which can be revealed by single strand confirmation polymorphism. The samples tested were found to be infected with either PAVcpA or PAVcpB, and some with the two variants (Mastarie et al., 1998).

Today PCR and reverse-transcription PCR (RT-PCR) are routine for plant virus detection (Webster et al., 2004). PCR is one of the most accurate detection means of cereal viruses, provided the sequence of the virus is known in order to design specific primers (Robertson et al., 1991). In a field survey in Hungary, PCR and ELISA were compared. While 46% of the tested wheat plants proved to be infected by BYDV-PAV in ELISA, using PCR, BYDV-PAV was found in 58% of the same samples, indicating that BYDV-PAV detection by PCR was more sensitive than ELISA (Áy et al., 2008). BYDV-PAV was identified in single aphids by RT-PCR (Canning et al., 1996) indicating that this method could be used to study the epidemiology of virus–plant–insect interactions.

Several viruses infecting a single cereal plant have been detected by multiplex PCR (Áy et al., 2008; Malmstrom and Shu, 2004). Using the primers previously described to detect BYDV and CYDV (Table III; Deb and Anderson, 2008), we have used single and multiplex PCR to detect several cereal viruses: BYDV strains (MAV, PAV, and SGV, but not RMV) on wheat in Jordan (Fig. 3A), Maize dwarf mosaic virus MDMV on maize in Jordan and Egypt (Fig. 3B), and BYDV-PAV on barley in Egypt (Fig. 3C). Restriction fragment length polymorphism (RFLP) of PCR amplicons was used to discriminate between BYDV species (Kundu et al., 2009) and to fingerprint CpA and CpB BYDV-PAV variants (Mastarie et al., 1998).
<table>
<thead>
<tr>
<th>Target virus (accession no.)</th>
<th>Primer</th>
<th>Sequence</th>
<th>5' Position</th>
<th>Amplicon size (BP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BYDV-PAV (D11032)</td>
<td>PAVL1</td>
<td>AGAGGAGGGCAATCCTGT</td>
<td>2999</td>
<td>295</td>
</tr>
<tr>
<td></td>
<td>PAVR1</td>
<td>ATTGTGAAGGAATTAATGTA</td>
<td>3272</td>
<td></td>
</tr>
<tr>
<td>BYDV-MAV (D11028)</td>
<td>MAVL1</td>
<td>CAACGCTTAACGCAGATGAA</td>
<td>896</td>
<td>295</td>
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<tr>
<td></td>
<td>MAVR1</td>
<td>AGGACTCTGCAGACCACATCT</td>
<td>1071</td>
<td>175</td>
</tr>
<tr>
<td>BYDV-SGV (AY541039.1)</td>
<td>SGVL2</td>
<td>ACCAGATCTTAGCCGGGTTT</td>
<td>631</td>
<td>237</td>
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<tr>
<td></td>
<td>SGVR2</td>
<td>CTGGACGTGCGACATTTC</td>
<td>911</td>
<td></td>
</tr>
<tr>
<td>BYDV-RMV (L12757.1)</td>
<td>RMVL1</td>
<td>GACGAGGACGACGAACGAATGA</td>
<td>41</td>
<td>365</td>
</tr>
<tr>
<td></td>
<td>RMVR1</td>
<td>GCCATACCTCACCTCCGATT</td>
<td>357</td>
<td></td>
</tr>
<tr>
<td>CYDV-RPV (AF235168.2)</td>
<td>RPVL</td>
<td>ATGTGTTACCGCTTGTGCAC</td>
<td>3275</td>
<td>400</td>
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<tr>
<td></td>
<td>RPVR</td>
<td>GCGAACCATGCAGCATTC</td>
<td>3655</td>
<td></td>
</tr>
<tr>
<td>WSSMV (X73883)</td>
<td>WSSMVL1</td>
<td>GCAACCTTAGCGAGTGCA</td>
<td>4059</td>
<td>154</td>
</tr>
<tr>
<td></td>
<td>WSSMVR1</td>
<td>GAGCCTGGCTGTCTCATAGC</td>
<td>4213</td>
<td></td>
</tr>
<tr>
<td>WSMV (NC_001886)</td>
<td>WSMVL2</td>
<td>CGACAAATCGCAAGCACCA</td>
<td>5444</td>
<td>193</td>
</tr>
<tr>
<td></td>
<td>WSMVR2</td>
<td>TGAGGATCGCTGTGTCAG</td>
<td>5622</td>
<td></td>
</tr>
<tr>
<td>SBWMV (NC_002042)</td>
<td>SBMVL2</td>
<td>CCTATGCGTCCTAACGTTG</td>
<td>2584</td>
<td>219</td>
</tr>
<tr>
<td></td>
<td>SBMVR2</td>
<td>CACATCTGCAGGAAGACGA</td>
<td>2803</td>
<td></td>
</tr>
</tbody>
</table>
3. Microarrays

DNA microarrays, which were introduced to measure the levels of expression of multiple genes in a high-throughput mode (Schena et al., 1995), have become a major tool for the detection of human pathogens such as HIV, influenza, and SARS (Uttamchandani et al., 2007, and references herein). In the past decade, this technology has been applied for the detection of pathogens of agricultural crops (Boonham et al., 2007) such as potato (Agindotan and Perry, 2008), tomato (Tiberini et al., 2010), cucurbits (Lee et al., 2003), and stone fruits (Pasquini et al., 2008). This technology has also been used to identify other plant pathogens such as nematodes (François et al., 2006) and Fusarium species (Nicolaisen et al., 2005). With the improvement in the technologies, arrays have been designed that allow to detect viruses belonging to a broad range of genera and infecting plants from many families. For example, a platform has been recently designed that allows detecting 52 different virus species (Nicolaisen, 2011).

The microarray, platforms used today, involve the design of a series of 40–70-mer oligonucleotides (or oligos) with a sequence derived from that of the pathogens to be detected (Boonham et al., 2007). Sometimes shorter

FIGURE 3  PCR-based detection of cereal viruses in Jordan and Egypt. (A) Singleplex detection of BYDV-MAV (MAV), BYDV-PAV (PAV), and BYDV-SGV (SGV), and multiplex (Mlx) detection of the three viruses in Jordan. (B) Detection of MDMV in Jordan. (C) Detection of BYDV-PAV in Egypt. The primers used are shown in Table III. M, molecular-weight marker, 1 kbp ladder; 0, noninfected plant.
oligos are used (Wei et al., 2009). For each pathogen, three to five oligos covering the entire genome are sufficient for adequate detection. The oligos (termed the probes) are amino-labeled at their 3’ end in order to ensure stable binding to the glass slide on which they are printed using a microarray printer. A single glass slide may contain more than 50,000 oligos. Hence, a single slide may contain probes representing thousands of pathogens allowing detection of individual plant virus or combinations of many plant viruses and/or virus-like pathogens. Usually the pathogen is identified by hybridization of fluorescently labeled DNA (usually with Cy3 dye) derived from RNA of infected plants that has been reverse-transcribed (or DNA when the pathogen has a DNA genome) and amplified by PCR using random primers. The hybridizations on the glass slide are analyzed using an ad hoc scanner and the reactive virus-derived oligos are identified using adequate software, leading to identification of the virus (see, e.g., Tiberini et al., 2010 for technical details). In case the expensive hardware necessary for printing and scanning glass slides is not available, cDNAs or oligos can be deposited on a nylon membrane (then called macroarray). In a recent study, 200–500-bp fragments of viral cDNAs amplified by PCR and printed on a membrane have been used to simultaneously detect several potato viruses (Maoka et al., 2010).

The Middle Eastern countries participating in the MERC project have microarray capacities in their laboratories (Israel, Jordan) or in central facilities (Egypt). In the frame of MERC program, we have designed a 70-mer oligonucleotide microarray based on the sequence of the major cereal viruses present in the Middle East (Table IV). Between two and three oligonucleotides were designed to specifically represent each virus. The samples were printed in Israel in a double spot, double array design. This microarray has been proven to be useful and has allowed detecting several cereal viruses. For example, Fig. 4 shows the microarray-based detection of MYSV. The chip was hybridized with reverse-transcribed RNA from MYSV-infected maize plants from Egypt; the results show specific hybridizations with targets of MYSV segment 2 and 3.

4. Deep sequencing of plant DNA, RNA (cDNA), and small-interfering RNA (siRNA)

Deep sequencing (also referred as high-throughput sequencing) is based on new and powerful technologies, which allow sequencing billions of nucleotide in a single run. Two main technologies are in use: 454 (www.454.com), generating reads of 200–300 nucleotides, and Illumina (http://www.illumina.com), providing reads of 20–50 nucleotides. These technologies have been instrumental in sequencing full-length prokaryotic and eukaryotic genomes as well as entire transcriptomes. The application of high-throughput sequencing in phytopathology has been reviewed recently (Studholme et al., 2011). Viruses can be discovered
### TABLE IV  Cereal viruses used to design the diagnostic oligonucleotide microarray

<table>
<thead>
<tr>
<th>Virus</th>
<th>GenBank accession no.</th>
<th>Coordinates of coat protein (CP)</th>
<th>Number of oligonucleotides in microarray</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Barley stripe mosaic virus</em> BSMV</td>
<td>U35772</td>
<td>90–686</td>
<td>–</td>
</tr>
<tr>
<td><em>Barley yellow dwarf virus</em> BYDV-MAV</td>
<td>D11028</td>
<td>2858–3460</td>
<td>3</td>
</tr>
<tr>
<td><em>Barley yellow dwarf virus</em> BYDV-PAV</td>
<td>X07653</td>
<td>2858–3460</td>
<td>1</td>
</tr>
<tr>
<td><em>Bermuda grass etched-line virus</em> BGEV</td>
<td>AY040531</td>
<td>377–964</td>
<td>3</td>
</tr>
<tr>
<td><em>Cereal yellow dwarf virus</em> CYDV</td>
<td>EF521827</td>
<td>3745–4359</td>
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<tr>
<td><em>Maize dwarf mosaic virus</em> MDMV</td>
<td>AJ001691</td>
<td>8390–9262</td>
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<tr>
<td><em>Maize mosaic virus</em> MMV</td>
<td>AY618418</td>
<td>–</td>
<td>3</td>
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<tr>
<td><em>Maize streak virus</em> MSV</td>
<td>Y00514</td>
<td>315–1049</td>
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<td><em>Maize yellow stripe virus</em> MYSV</td>
<td>AJ969412</td>
<td>–</td>
<td>3</td>
</tr>
<tr>
<td><em>Sugarcane mosaic virus</em> SMV</td>
<td>AJ297628</td>
<td>8400–9338</td>
<td>3</td>
</tr>
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<td><em>Wheat dwarf virus</em> WDV</td>
<td>AJ311031</td>
<td>415–1197</td>
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<tr>
<td><em>Wheat streak mosaic virus</em> WSMV</td>
<td>NC_001886</td>
<td>8189–9235</td>
<td>3</td>
</tr>
<tr>
<td><em>Zea mosaic virus</em> ZMV</td>
<td>AF228693</td>
<td>145–1056</td>
<td>2</td>
</tr>
</tbody>
</table>

**FIGURE 4**  Microarray-based detection of MYSV in infected maize samples from Egypt. The chip was hybridized with reverse-transcribed RNA from MYSV-infected maize leaves labeled with Cy3 (red) and Cy5 (green). The labeled cDNA targets hybridized with the MYSV segment 3 (left panels) and segment 2 (right panels) probes.
from plant samples without the need for laborious and costly purification, cloning, and screening techniques. For example, the sequence of a cDNA pool prepared from plant tissues can be compared to the sequences of the host posted in public databases using sophisticated bioinformatics tools. Many of the nonhost sequences represent pathogens, some known and others unknown. All the viruses discovered in an infected plant using deep sequencing have been termed “virome.” These technologies have allowed analyzing viruses-infected tomato (Adams et al., 2009) and grapevine (Coetzee et al., 2010).

Plant-pathogenic viruses can also be detected indirectly. In response to infection by RNA/DNA viruses, the host plant generates specific 21–24 nucleotides in length RNA molecules called short-interfering RNAs (siRNA). RNA silencing (RNAi) is a cytoplasmic cell surveillance system to recognize double-stranded RNA (dsRNA) and specifically destroy ssRNA and dsRNA molecules homologous to the inducer, using siRNA as a guide (see review by Mlotshwa et al., 2008). Viruses are both inducers and targets of RNA interference (RNAi) that constitutes a fundamental antiviral defense mechanism in eukaryotic organisms. Plant posttranscriptional gene silencing (PTGS) enzymes catalyze the digestion of viral RNAs and the generation of siRNAs that mediate antivirus immunity during infections. In brief, plant Dicer-like (DCL) RNases process viral dsRNAs or viral ssRNAs with fold-back structures, to ds-small RNAs of mainly 21–24 bp in length. Plant Argonaute proteins then select and incorporate one strand (the guiding strand) of the DCL product to form the so-called RNA-induced silencing complex (RISC). RISC uses the guiding strand siRNA to seek viral ssRNAs (including mRNAs) based on complementary homology and then either cleaves or interferes with the viral ssRNA.

Deep sequencing of siRNAs offers new opportunities to identify viruses-infecting plants, even at extremely low titers symptomless infection, and including previously unknown viruses. Deep sequencing can provide thousands to millions of siRNA sequences from virus-infected plant materials. When virus-derived (v)siRNAs are abundant enough, virus genome fragments can be assembled (Kreuze et al., 2009). This approach requires relatively sophisticated bioinformatics. Since the vsiRNAs are 21–24 nt in length, their sequences can be employed directly as primer sequences to amplify viral fragments by PCR or RT-PCR. This approach has been used to reveal unrecognized CYDV (genus Polerovirus, family Poleroviridae) infections in a wild population of Dactylis glomerata (cocksfoot grass or orchard grass; Pallett et al., 2010).

Deep sequencing is a relatively new technology in the Middle Eastern countries. Illumina and 454 sequencers are fully functional in Israel but have not been used yet for the diagnosis and discovery of plant viruses. It is expected that, in the near future, deep sequencing will be routine either in situ or outsourced.
III. ACTIVITIES OF NATIONAL PLANT PROTECTION SERVICES FOR SUSTAINED PRODUCTION OF CEREAL GERMLASM

The risk of new pests, being introduced into our region, is increasing due to the opening of the international agriculture produce markets. Hence, as stated by the International Plant Protection Conventions, the purpose of National Plant Protection and Inspection Services (in agreement with international agreements, national laws, and directives) is the protection of plant life and health from the introduction and spread of pests.

Well-orchestrated preventive measures are pivotal to reduce the likelihood of entry and establishment of new and harmful pests. For example, in Israel, the Plant Protection and Inspection Services (PPIS) are investing large efforts to maintain cereal germplasm free of harmful viruses and virus-like pathogens. Primarily, the import of seeds is restricted to research purposes and needs to fully comply with Post Entry Quarantine (PEQ) regimens. To fulfill this purpose, the PPIS operates through several services and departments that include (1) seed and nursery stock certification service, (2) quarantine service, (3) quality assurance systems, (4) plant pest diagnostics service, (5) pest risk analysis department, (6) quarantine service for the introduction of new varieties, and (7) import and export control units.

The missions of the PPIS are carried out by PPIS inspectors and they include (a) official sampling of seed and plant materials; (b) supplying samples to the diagnostic laboratories under controlled storage conditions; (c) issuing certificates, labels, and phytosanitation documents and granting licenses for trading with plant materials; and (d) monitoring the importation of nonregistered varieties. To carry out these missions, quarantine inspectors are posted at all entrance ports of the country (harbors, airports, and land terminals). The health status of every imported shipment of plant material is verified. Every shipment needs to comply with all the predetermined importation terms, the proper documentation, visual examination, and sampling for laboratory analysis.

The growing demand for new cereal varieties and the need to encourage research and development may conflict with the demands of the PEQ services. To alleviate this conflict, the PEQ unit offers Containment Facilities (CFs) and safeguards the use of importers and researchers. Several CFs are currently operating for cereal crops: they include net houses, quarantine greenhouses, and field trial plots (Fig. 5). Phytopathologists and containment experts carefully review each case, and according to the possible level of containment needed, one of the PEQ facilities listed above is allocated.

In order to carefully test the cereal germplasm, the sites of the CFs are in a secluded location, at a distance of at least 1 km from agricultural and natural conservation areas (Fig. 5C). In addition, potential host plants and disease vectors are excluded from the surrounding area and the climate should favor the development of quarantine plant pathogens. Moreover,
the CF location is separated from other buildings, surrounded by a buffer area without vegetation and has a security fence to restrict access to authorized staff only. In the CF, each imported line of cereal seeds is sown in a separated subplot labeled with tags to facilitate sampling for diagnosis of virus and virus-like pathogens. Currently, viruses and virus-like pathogens are monitored using ELISA and multiplex RT-PCR (Fig. 5D). The demand for the increasing numbers of diagnosis tests should be fulfilled by developing the microarray detection methodology which is presently validated by the MERC-funded Project.

IV. BREEDING CEREALS RESISTANT TO VIRUSES

A. Genetics of resistance to viruses common in the Middle East

Breeding for resistance is the environmentally most sound and also most cost-effective approach to prevent losses caused by viruses. In the past few years, several quantitative trait loci (QTL) linked with resistance to cereal
viruses have been identified in cereal varieties and their wild relatives (reviewed by Ordon et al., 2009). These loci contain candidate genes for virus resistance in cereals. Expression profiling techniques have allowed identification of genes expressed differently between BYDV-resistant and susceptible lines of wheat (Gao et al., 2009). The isolation of genes involved in resistance will allow transferring these genes to susceptible varieties by genetic engineering and developing markers based on their sequences to speed up breeding. Knowledge on synteny between the sequenced rice genome and barley and wheat will lead to an enhanced isolation of virus-resistance genes and a deeper understanding of pathogenesis.

1. Barley yellow mosaic virus and Barley mild mosaic virus
Resistance to Barley yellow mosaic virus (BaMMV) and Barley mild mosaic virus (BaYMV) is quite frequent within the barley gene pool (Konishi et al., 1997). Different recessive resistance genes have been identified by genetic analyses. Using molecular techniques, they were mapped on chromosomes 1H, 3H, 4H, 5H, and 6H and tagged with PCR-based markers (Ordon et al., 2005). In addition, dominant resistance genes derived from Hordeum bulbosum have been mapped on chromosome 2H and 6H (Ruge-Wehling et al., 2006).

2. Barley yellow dwarf (BYDV/CYDV)
Breeding of cereals for resistance to BYDV is usually associated with breeding for resistance to other viral diseases, especially diseases caused by the WDV and by the soil-borne BaYMV complex. The most important BYDV-resistance gene is Ryd2 (Yd2), which comes from Ethiopian spring barley lines and is widely used in breeding programs in barley. It has been located on chromosome 3HL. In addition, several QTL for tolerance against BYDV-MAV and BYDV-PAV have been mapped on chromosomes 7H, 4H, and 1H. QTL for BYDV tolerance have been detected in wheat by the analysis of two different populations (Ayala et al., 2002). The Bdv2 gene, which originates from the intermediate wheatgrass Thinopyrum intermedium, has been introduced into some wheat cultivars (Stoutjesdijk et al., 2001). Breeders struggle to introduce these genes into BYDV highly susceptible barley and wheat cultivars. Wheat resistance to BYDV has been detected in several Thinopyrum species. Resistance has been transferred to wheat and markers specific for resistance have been developed (Ayala et al., 2001). Three wheat varieties with BYDV resistance from T. intermedium were developed and released in Australia and China, respectively (Zhang et al., 2009).

3. Wheat dwarf virus
Resistance or tolerance to WDV is rather scarce. In winter wheat, only very few genotypes were identified that show a slightly reduced virus concentration or lesser yield reductions (Lindblad and Waern, 2002;
Even worse is the situation in barley. So far, tolerance to WDV has been observed in a single cultivar only.

4. Wheat streak mosaic virus
The level of resistance in *Triticum* sp. is generally low. Resistance to WSMV and the virus vector has been identified and transferred into wheat from rye, *Aegilops*, and several grass species (Li et al., 2004). In addition, two temperature-sensitive resistances to WSMV have been described (Seifers et al., 2007).

5. Maize yellow stripe virus
All the locally produced maize hybrids and many exotic genotypes from different countries have been tested in Egypt against MYSV infection. All tested materials were susceptible at different degrees; hence, sources for resistance have not been found yet (A.E. Aboul-Ata et al., in collaboration with the Department of Maize Breeding, FCRI, ARC, unpublished data).

B. Status of conventional breeding for resistance to those viruses that infect cereals in the Middle East

The Middle Eastern countries have no established breeding program for virus resistance. For instance, Egypt has a strong cereal breeding program but does not have a program for virus resistance. Some work has been done as collaborative research, for example, between the Nile Valley and Red Sea project directed by the International Center for Agricultural Research in the Dry Areas (ICARDA) aimed at identifying germplasm resistant to BYDV (http://www.icarda.org/Publications/AnnualReport/96/Annual96.pdf). The detection and exploitation of tolerance to cereal viruses depends on efficient and reliable inoculation systems in the field and in the laboratory. *In vivo* and *in vitro* BYDV inoculation systems have been developed in Egypt, as described below.

1. Insect-mediated inoculation
The BYDV-PAV aphid vector *R. padi* and BYDV-PAV-infected barley are kept at the Department of Plant Virus and Phytoplasma Research, Plant Pathology Research Institute, ARC, Egypt. Aphids are mass-reared on wheat seedlings grown in light- and temperature-controlled rooms; they are checked daily to avoid emergence of winged insects. For inoculation tests, insects are caged with infected barley for an 18-h acquisition period, and then the viruliferous insects are transferred to wheat seedlings at their three-leaf stage grown in a small double-door insect-proof net house (Fig. 6). After 2 days, the leaves with high aphid density are harvested in plastic bags and transferred onto tested wheat seedlings grown in a double-door insect-proof net house (20 m long, 3 m height, and 10 m wide,
BYDV-PAV resistance/tolerance in infected and noninfected wheat and barley plants were evaluated 45 days after inoculation for the appearance and severity of symptoms, agronomic traits, and yield components (Ayala et al., 2002; Cheour et al., 1989; Scheurer et al., 2001). A symptom scale of BYDV symptom severity from 0 to 9 was used (Qualset, 1992). Virus occurrence was tested by tissue blot immune assay (Cameau et al., 1992). Only one BYDV-PAV-resistant barley genotype and two BYDV-PAV-resistant wheat genotypes, both from ICARDA, were identified (unpublished data). BYDV tolerance was found in both wheat and barley genotypes from CIMMYT, ICARDA, local parents, and other trials of A, B, and D. One barley and 27 wheat genotypes were tolerant to BYDV-PAV. In local commercial materials, infection of 82–95%, degree of severity of 2.5–3.6, and yield losses of 0.6–9.3% were observed (unpublished data).

2. Vascular puncture inoculation

This technique is aimed at replacing insect vectors in the selection process of resistant germplasm. It consists in injecting virus-infected crude sap, purified virus, or viral DNA into embryos (recently reviewed by Weiland and Edwards, 2011). Vascular puncture inoculation (VPI) presents several
advantages (Redinbaugh et al., 2004). It is easy to use and is cheap. It avoids the need to raise insect vectors and helps avoid virus dissemination by insects. Only little space is required. Purified viral genome could be used for inoculation and a few microliters may serve as inoculum. Persistent insect-borne viruses such as MSV and MRFV can be inoculated successfully (Madriz-Ordeñana et al., 2000). VPI was used to screen for resistance to different insect-borne maize viruses (Jones et al., 2004; Louie and Abt, 2004). The seed-borne High Plain Virus and 13 more viruses in maize were evaluated for resistance using VPI (Redinbaugh et al., 2004).

In Egypt, six wheat varieties (Gemmaza 7, 9, Giza 168, Sakha 69, 39, and Sids 7) and four barley varieties (Giza 123, 124, 125, and 126) were evaluated for resistance to seed-borne BSMV using VPI. Infected barley leaves were homogenized and debris discarded by centrifugation. Seeds of wheat and barley varieties were soaked in distilled water for 4 h at 27°C. Seeds with embryo side up were inoculated with 5–7 μl of sap diluted 10⁻¹ to 10⁻⁵, using a single pin engraving tool (Fig. 7). The inoculated kernels were placed in a glass dish covered by a plastic sheet to keep humidity, and incubated for 2 days at 30°C. Each germinated kernel was sown in pots kept in an insect-proof glass house for 2–3 weeks. All wheat and barley varieties were susceptible to BSMV. VPI was 10–1000 times more efficient than rubbing inoculation (unpublished data).

C. Transgenesis for viral disease resistance

1. Expression of viral genes

Apart from the natural sources of resistance, modern techniques of transgenesis have been employed in the improvement of cereal resistance to BYDV. However, their practical use in cereal breeding is limited due to EU legislation and public acceptance. In the past two decades, transgenesis has been used to produce lines of virus-resistant cereals. In general,
resistance was achieved by expressing the virus replicase and/or CP gene in the transgenic plants, delivering the DNA by bombarding actively growing embryogenic calli with DNA-coated particles. Transgenic maize plants expressing MDMV strain b CP were resistant to mixed infections of MDMV and Maize chlorotic mottle virus (Murry et al., 1993). Resistance to WSMV has been achieved by expressing the viral replicase (Sivamani et al., 2000) and the viral CP (Sivamani et al., 2002) in transgenic wheat. The expression of a MSV-truncated Rep gene conferred MSV resistance to the transgenic maize (Shepherd et al., 2007). Coexpression of genes from several viruses aimed at obtaining multivirus resistance has been envisioned (Jan et al., 2000).

2. Resistance to cereal viruses mediated by transgenic RNA interference

RNA silencing plays an important role as a natural antiviral response in plants. It is a PTGS phenomenon induced by dsRNA (Vance and Vaucheret, 2001). When dsRNA occurs in eukaryotic cells, Dicer, an RNase III-like ribonuclease, specifically cleaves dsRNA into siRNAs of 21–25 nucleotides. siRNAs act as a guide to recognize complementary RNAs for their degradation to suppress gene expression, which was a manifestation of PTGS induced by dsRNA, named as RNAi (Fire et al., 1998). Successful virus infection requires evasion or suppression of RNA silencing (Voinnet et al., 1999). Indeed, many plant viral proteins have been identified as suppressors of RNA silencing (Andrew, 2000; Guo and Ding, 2002).

RNA silencing can be triggered by dsRNA produced during RNA virus replication, which induced the viral RNAs degradation. In this case, dsRNA is delivered by stably transforming plants with transgenes that express a self-complementary RNA. The resulting transcript hybridizes with itself to form a hairpin structure (hpRNA) that contains a single-stranded loop region and a base-paired stem, which mimics the dsRNA structure that induces viral gene silencing (Fig. 8). The efficacy of various hpRNAs constructs in gene silencing has been discussed (Stoutjesdijk et al., 2002).

In recent years, RNAi triggered by self-complementary hpRNA (Fig. 8) has been proved to be efficient to generate virus resistance in cereals. In general, immature embryos are cocultured with recombinant Agrobacterium vectors containing hpRNAs; transgenic plantlets are regenerated from calli (Tingay et al., 1997). Barley lines transformed with an hpRNA-containing BYDV-PAV polymerase sequences under the control of a maize ubiquitin promoter provided immunity to this virus (Wang et al., 2000). Maize plants were rendered resistant to Sugarcane mosaic virus (SCMV) expressed hpRNA-containing fragments of the SCMV polymerase NiLb gene (Bai et al., 2008). Similarly, maize resistant to MDMV was obtained by expressing hpRNA targeting the MDMV CP gene (spaced by an intron of maize actin gene). A longer hpRNA expression construct was
more efficient than a shorter one (Zhang et al., 2011). These results indicate that RNAi will be robust in the field and is potentially useful in minimizing losses in cereal production worldwide.

PTGS technologies have been successfully implemented by the MERC partners against tomato (Rezk et al., 2006) and potato (Soliman et al., 2008) viruses, but not yet with cereal viruses. Replicase and movement protein genes are commonly used as PTGS targets (Wani et al., 2010). Silencing constructs have been delivered by Agrobacterium (Abhary et al., 2006; Rezk et al., 2006; Soliman et al., 2008), delivery of DNA-coated microparticle (gene gun; Rezk et al., 2006), and laser beam, nanosecond-pulsed laser-induced stress wave (ns-p LISW; Tang, et al., 2006).

3. Silencing of cereal genes using vectors based on cereal viruses  
(virus-induced gene silencing)

Development of virus-induced gene silencing (VIGS) vectors for grasses is at an early stage. Several viruses have been shown to have the potential for VIGS in cereals (see review by Scofield and Nelson, 2009). BSMV-based vectors were the first to be used to silence genes in barley (Hordeum vulgare), allowing for functional genomics research in the grass species. Silencing of the Phytoene desaturase (PDS) gene endogenous from barley, oats, wheat, and maize was achieved using BSMV-silencing vectors containing sequences of part of the PDS cDNA cloned downstream of the 35S promoter, the selection gene bar (conferring resistance to the herbicide Basta) under the control of the ubiquitin Ubil promoter, the T-DNA octopine (Ocs) and nopaline (Nos) synthase genes (after Gasparis et al., 2011). Another VIGS system based on BMV was developed for rice (Oryza sativa), maize (Zea mays), and barley (Ding et al., 2006). Similar to BSMV, the genome of BMV is also tripartite and the
three RNAs, designated RNAs 1, 2, and 3, have been cloned in a similar manner as for BSMV, so that infectious RNAs can be produced by *in vitro* transcription. *PDS* genes in maize and barley have been silenced using BSMV-based vectors (Scofield and Nelson, 2009).

**V. CONCLUSION**

A coordinated regional effort is needed to curb the deleterious effects of viruses affecting cereal production in the Middle East. Several technologies for virus diagnosis and typing are available, each with its advantages and disadvantages. Some are based on serology, others on nucleic acid sequences. The production of viral CP by overexpressing the cloned gene (or a synthetic polypeptide) in bacteria provides unlimited amounts of antigen and allows fine-tuning the epitope by modeling *in silico* the CP in order to fit the particularities of the local viral pathogens. PCR, RT-PCR, and RFLP-PCR may provide easy ways to diagnose a given virus and even one of its strains. Microarray techniques offer a platform where all important viruses (up to 100 species and strains) can be represented and be diagnosed in a single operation. This technology has been proven to be effective for the diagnosis of plant pathogens (Boonham et al., 2007) and it is thought to be applicable for counteracting agricultural bioterrorism (Uttamchandani et al., 2007). Deep sequencing constitutes already an exquisite complement for the discovery of new viruses and their diagnosis (Kreuze et al., 2009). Although still expensive compared to ELISA, for example, the costs of microarray (design and handling) and deep sequencing are decreasing rapidly. These methods allow discriminating all pathogens infecting a given crop in a single experiment, while many ELISA tests and many different antibodies will need to be tested one by one to identify the culprit pathogens, all this with less accuracy.

Virus diagnostic routines need to be coordinated and standardized at the regional level to comply with the highest quality control. This could be done only if protocols are shared, tested, and implemented by the various laboratories and plant protection services. The tests should be authorized by the national ministries of agriculture and specialized laboratories should be licensed to perform the tests provided regular inspection. Once protocols are established and agreed upon, surveys of infected plants and carrying vectors could be performed on a regular basis according to the epidemiology of each virus and variant. The cooperation of the growers is essential in such an endeavor. A close cooperation between the grower and the laboratory is a must, including educational presentations, instructions, Web site for extension specialists, and publications of leaflets. MERC partners can use the project Web site to post technical sheets explaining processes for virus detection and diagnosis. Extreme awareness of the grower will allow a
rapid passage of information from the field to the laboratory in order to take rapid countermeasures on a local, regional, national, and international basis. A public database under construction (http://merc-cerealviruses.com/home) will be the repository of any relevant information, from symptoms, epidemiology, diagnosis to worldwide database. The web should help exchange communication and information.

Besides efforts toward more precise, rapid, and cheap detection means, breeding and release of virus-resistant material is a must and efforts should be encouraged by decision makers at the highest governmental levels, by public funding and by private companies. Plant extension and protection service can assist large and small farms in technology transfer. Trials have been made to identify resistant and susceptible wheat and barley parents for breeding program aimed at controlling cereal viruses, that is, BYDV-PAV. Both aphids and VP1 have been used to inoculate insect-borne viruses. Countries in the Middle East, as well as in the developing world, need to master efficient, nonexpensive, and easy inoculation technique to minimize breeding program costs.

Genetic engineering offers large panoply of means that could be used in rendering cereals resistant to viruses. Gene silencing technologies could be implemented to control cereal viruses (Miller et al., 2001). These authors have experience with successful silencing of Tomato yellow leaf curl virus (TYLCV), a whitefly-transmitted geminivirus, rendering tomato plants TYLCV resistant (Abhary et al., 2006; Rezk et al., 2006). Expressing various viral and insect genes has also led to resistance to TYLCV in tomato (Akad et al., 2007; Kunik et al., 1994). For now, genetically modified cereals are not accepted by the public in the Middle East and elsewhere.

Regional cooperation among plant quarantine services is essential to prevent cereal viruses from spreading over borders. A regional effort that is aimed at providing answers to the above concerns comprises researchers from Egypt, Israel, and Jordan.

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