Review

Pepino Mosaic Virus: a serious threat to tomato crops worldwide

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Abstract

Tomato (*Solanum lycopersicum*) is one of the widely grown crops worldwide. It is consumed in various forms and has excellent nutritional values. Presently, this crop is facing a serious threat to its yield and survival because of a *potexvirus* infection. One of the *potexvirus* species hampering tomato productions worldwide is *Pepino mosaic virus* (PepMV). This emerging virus is one of the most destructive plant diseases destroying tomato crops globally. It has spread to many countries worldwide including France, Italy, the UK, Poland, Belgium, the USA, Canada and China. PepMV genome consists of a positive-sense, single-stranded RNA molecule, approximately 6.4 kb in length. The genomic RNA contains five open reading frames (ORFs) encoding for the coat protein (CP), the putative viral polymerase (RdRp) and the triple gene block (TGB) proteins. PepMV is efficiently transmitted mechanically. In other studies, seed transmission has been demonstrated. This article provides an overview of PepMV symptoms, transmission, different strains of PepMV, its genome organization and strategies employed for controlling it. The knowledge about the recent progress in the study of PepMV would help develop novel strategies for its control in agriculture.

Keywords: PepMV, Tomato, *potexvirus*, genome organization, transmission, control.

Résumé

La tomate (*Solanum lycopersicum*) est l'une des cultures largement cultivées dans le monde entier. Elle est consommée sous diverses formes et possède d'excellentes valeurs nutritionnelles. À l'heure actuelle, cette culture est confrontée à une grave menace pour son rendement et sa survie en raison de l'infection par les *potexvirus*. Un des *potexvirus* qui entravent la production des tomates dans le monde entier est le virus de la *mosaïque du pepino* (PepMV). Ce virus émergent est l'une des maladies des plantes les plus destructrices qui détruisent la culture de tomate à l'échelle mondiale. Le virus s'est propagé dans de nombreux pays, y compris la France, l'Italie, le Royaume-Uni, la Pologne, la Belgique, les États-Unis, le Canada et la Chine. Le génome du PepMV se compose d'une molécule d'ARN simple brin à polarité positive, d'environ 6,4 kb de longueur. L'ARN génomique contient cinq cadres ouverts de lecture (ORF) codant pour la protéine de la capside (CP), La protéine de la réplicase (RdRp) et la protéine de bloc génique triple (TGB). PepMV est transmis de manière efficace mécaniquement. Dans d'autres études, la transmission par les semences a été démontrée. Cet article donne un aperçu sur les symptômes, la transmission, les différentes souches du PepMV, son organisation et les stratégies utilisées pour contrôler le génome. Les connaissances des progrès récents dans l'étude du PepMV pourraient aider à développer de nouvelles stratégies pour son contrôle dans le secteur agricole.

Mots clés: PepMV, Tomate, *potexvirus*, génome, transmission, contrôle.

INTRODUCTION

Tomato (*Lycopersicon esculentum*) is a vegetable crop cultivated worldwide. The increase of tomato production is the result of technological innovations which include development of new varieties, new techniques of irrigation and the use of fertilizers and pesticides (Gliessman, 2002). However, because of its perennial growth on a large scale, tomato is susceptible to a number of pathogens including bacteria, fungi and viruses. Amongst these, *potexivirus* including *pepino mosaic virus* causes globally great loss in yield of tomato production. Losses are estimated to be 30% (Peters et *al.,* 2010) and the reduction in fruit quality may result in economic losses depending on differences in prices and in the market situation. In Spain, PepMV is associated with the collapse of tomato crops (Soler-Alexandre et *al.,* 2005). In the United Kingdom in 2006, the yield loss was estimated between 5 and 10% (Hanssen et *al.,* 2009). In Canada, the losses were usually estimated between 5 to 15% (Anonymous, 2005).

GEOGRAPHICAL DISTRIBUTION

PepMV was initially identified in Peru, in 1974, as the agent responsible for a previously uncharacterized disease affecting pepino *(Solanum muricatum*) (Jones et *al.,* 1980). The virus was first isolated from tomato in the Netherlands in 1999 (Wright and Mumford, 1999; van der Vlugt et *al.,* 2000). Since then, rapid spread of the virus occurred throughout tomato production areas worldwide, with official reports of PepMV incidence from Spain, France, Italy, the UK, Poland, Belgium, the USA and Canada (Soler et

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al., 2000; French et *al.*, 2001; Jorda et *al.*, 2001; Mumford and Metcalfe, 2001; Roggero et *al.*, 2001; Cotillon et *al.*, 2002; Pospieszny Borodynko, 2006; Hanssen et *al.*, 2008). In 2001, the PepMV becomes part of the EPPO Alert list (EPPO, 2003). In Denmark, it was detected in tomato seedbeds during 2001 and 2002 (EPPO, 2003). In Slovakia, the only outbreak reported in 2004 was eradicated (EPPO, 2004). In Finland, it was first detected in 2001 and reappeared in 2003 (EPPO, 2003). In France, different outbreaks have been reported between 2000 and 2003 (Anonymous, 2000; Cotillon et *al.,* 2002; EPPO, 2003). In Italy, the virus was found for the first time in Sardinia in 2001 (Roggero et *al.,* 2001) and in 2005 reappeared in Sicily (Davino et *al.,* 2006). The virus was also reported from Syria (Fakhro et *al.,* 2010). Subsequent outbreaks of the disease were also reported in other countries, i.e. China (Zhang et *al.,* 2003) and South Africa (Carmichael et *al.,* 2015).

DIFFERENT STRAINS OF PepMV

As PepMV was found in several locations, countries and species, a number of different strains were reported (Vander Vlugt and Stijger, 2008).

Since 2005, new genotypes sharing up to 80% nucleotide sequence identity with the European tomato strain have been identified, originating from tomato crops in the United States (US1 and US2) (Maroon-Lango et *al.,* 2005) and from tomato seed from Chile (CH1 and CH2) (Ling, 2007). Nucleotide sequence comparisons suggest that US2 is a recombinant of US1 and CH2. Currently, five main strains of PepMV are recognized (Hanssen et *al.,* 2009; van der Vlugt and Stijger, 2008; van der Vlugt, 2009): (1) the Peruvian (PE) strain, originally found on pepino (*S. muricatum*) and wild *Solanum spp*., (2) the EU-tomato (EU-tom) strain, (3) the US1/Ch1 strain, (4) the Chile-2 (Ch2) strain, and (5) the PES strain of PepMV recently isolated and described in wild tomato populations in Peru. After the initial dominance of the EU genotype in European tomato production, a population shift toward the CH2 genotype has been reported in several European countries (Gómez et *al*., 2009b; Hanssen et *al.*, 2008). In the United States and Canada, the EU genotype remains dominant (Ling et *al.*, 2008). Mixed infections of both genotypes are common and have been suggested to contribute to PepMV population dynamics (Gómez et *al.,* 2009 b). In addition, recombinants of both genotypes have been reported (Pagán et *al.,* 2006; Hanssen et *al.,* 2008).

The EU and LP genotypes share 96% of nucleotide sequence homology and cluster phylogenetics. The CH2 genotype is rather different as it displays only 78 to 80% sequence homology with the EU and LP genotype groups. The US1 genotype shares 78% sequence homology with CH2 and 82% with EU/LP genotypes.

As differences between genotypes at the nucleotide level are considerable, several molecular assays, including an RT-PCR-RFLP method, a TaqMan RT-qPCR method and a multiplex RT-PCR method combined with RFLP have been developed to discriminate these four PepMV genotypes (Hanssen et *al.,* 2008; Gutiérrez-Aguirre et *al.,* 2009; Alfaro-Fernández et *al.,* 2009).

However, the different genotypes cannot be distinguished based on biological characteristics considering that biological differences between isolates from the same genotype can be considerable (Córdoba-Sellés et *al.,* 2007; Hanssen et *al.,* 2009; Hasiów-Jaroszewska et *al.,* 2009). Mild, moderate and aggressive isolates are sharing over 99% sequence identity for both the EU and CH2 genotypes, indicating that minor differences at the viral genome level can account for considerable differences in symptomatology (Hanssen et *al.,* 2009; Hasiów-Jaroszewska et *al.,* 2009).

HOST RANGE

Determining the host range of PepMV has been an essential part of the work carried out by several research groups. PepMV causes a variety of symptoms in tomato (Van der Vlugt et *al.,* 2000; Hanssen et *al.,* 2009). PepMV has also been found to infect several other solanaceous crops and test plants like *Datura stramonium*, *Nicotiana benthamiana*, *Physalis floridana*, *Solanum melongena* (eggplant) and *Solanum tuberosum* (potato). PepMV is known to infect a relatively broad host range of plants representing different families, including both cultivated and wild hosts. Most host species are in the family of *Solanaceae*, but several solanaceous hosts do not support systemic infection (Jones et *al.,* 1980; Salomone and Roggero, 2002; Verhoeven et *al.,* 2003; Jordá et *al.,* 2001; Córdoba et *al.,* 2004).

MAIN SYMPTOMS

The disease severity of infected plants vary from minor to severe depending on the type of PepMV strain, age, vigor, variety of tomato plant, different geographic areas and the climatic conditions (Jordá et *al.,* 2001; Spence et *al.,* 2006). Stressful periods or situations that can cause stress to the plant during the cultivation seem to favor expression of the virus symptoms; whereas, symptoms seem to be related to environmental conditions and possibly the cultivar. During the fall and winter months, when light levels and temperatures are lower, several damages of PepMV occur (Jorda et *al.,* 2001). General symptoms in tomato included mosaic and yellowing of leaves, bubbling, necrosis, and alteration of the fruit color resulting in uneven ripening (Figure 1).

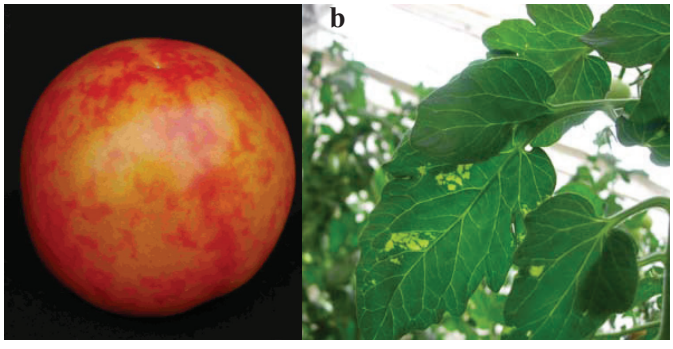

Figure 1: Tomato plant infected with PepMV: (a) Fruit marbling, (b) Isolated yellow spots scattered throughout the leaflet (Córdoba et *al.,* ***2007).***

TRANSMISSION

Mechanical transmission

The main way of transmission and natural dispersion of PepMV in the field is the mechanical transmission. According to the literature, it is transmitted very easier and faster than the *Potato virus X* (PVX) and *Tomato mosaic virus* (ToMV) (Wright and Mumford, 1999). The virus multiply in large amounts in the cells of host plants and it can be transmitted from one infected plant to another healthy one by rubbing between them, then the disease progresses along the rows in the greenhouse (Wright and Mumford, 1999). However, results in greenhouse experiments indicate that the rate of virus transmission due to friction with the contaminated clothing is less relevant than that due to cultural practices mainly during pruning and harvesting (Lacasa et *al.,* 2001).

Insect vectors

Aphid (*Myzus persicae*) and whitefly (*Trialeurodes vaporariorum*) are considered important vectors of tomato virus, but these insects have not be proven to transmit PepMV (Jones et *al.,* 1980; Loomans et *al.,* 2000); even if the virus has been detected in wild populations of different species of the genus *Lycopersicum sp.* which were kept isolated in Peru and not subjected to human manipulation. It was noticed a possible involvement of some unknown vectors in the diffusion of PepMV in these isolated populations (Soler et *al.,* 2002).

Several species of bumble bees used as pollinators [*Bombus. terrestris, B. canariensis* (Perez) and *B. impatiens* have also shown experimentally to be vector of the virus among tomato plants both in Spanish (Lacasa et *al.,* 2003) and Canadian greenhouses (Shipp et *al.,* 2008).

Seed transmission

Seeds are considered a source of transmission and persistence of many viruses. Seed transmission is an ideal starting point for the establishment of a disease in the field. First, the infection occurs in the early stages of seedling development and secondly the production of infected seeds in the field. So they constitute reservoirs of the virus which can be transmitted later mechanically or by vectors. Seed transmission can occur at low rates (less than 1%) when seed is not properly cleaned before sowing. PepMV is external, as it is found contaminating the seed coat and not in the embryo or endosperm (Krinkels, 2001). It has been suggested that isolates of the EU type might have dominance over the CH2 type in seed transmission, which may explain the earlier establishment of the former isolates in European countries (Hanssen et *al.,* 2010).

Vegetative propagation

PepMV can be transmitted by vegetative propagation from infected tuber potato accessions (Van der Vlugt, 2009). In the case of tomatoes, grafted seedling over resistant rootstocks represents high risk of transmission of viral diseases such as PepMV.

Transmission by the fungus *Olpidium virulentus*

Although the virus is easily detected in plant roots, it has not been detected in the water, not in the nutrient solutions in hydroponic systems (Cooke, 2000). Moreover, it has been found that PepMV can be transmitted to the tomato plants irrigated with water drainage from PepMV infected plants in the presence of the fungus *Olpidium virulentus* with an efficiency of 8% (Alfaro-Ferna´ndez et *al.,* 2010). The high density of zoospores from this fungus in the drainage water may increase PepMV transmission by irrigation or the recirculation of contaminated nutrient solution in a closed hydroponic system (Schwarz et *al.,* 2010).

MORPHOLOGY AND GENOME ORGANIZATION

PepMV belongs to the genus *Potexvirus* (family: *Flexiviridae*) and, like other members of this genus, has virions that are non-enveloped flexuous rods approximately 508 nm in length (Adams et *al.,* 2004; Jones et *al.,* 1980). The PepMV genome consists of a positive-sense, single-stranded RNA molecule, approximately 6.4 kb in length. The genomic RNA contains five open reading frames (ORFs) and two short untranslated sequences flank the coding regions and there is a poly (A) tail at the 3' end of the genomic RNA (Figure 2) (Mumford and Metcalfe, 2001; Aguilar et *al.,* 2002; Cotillon et *al.,* 2002). The genomic RNA contains five open reading frames (ORFs) and

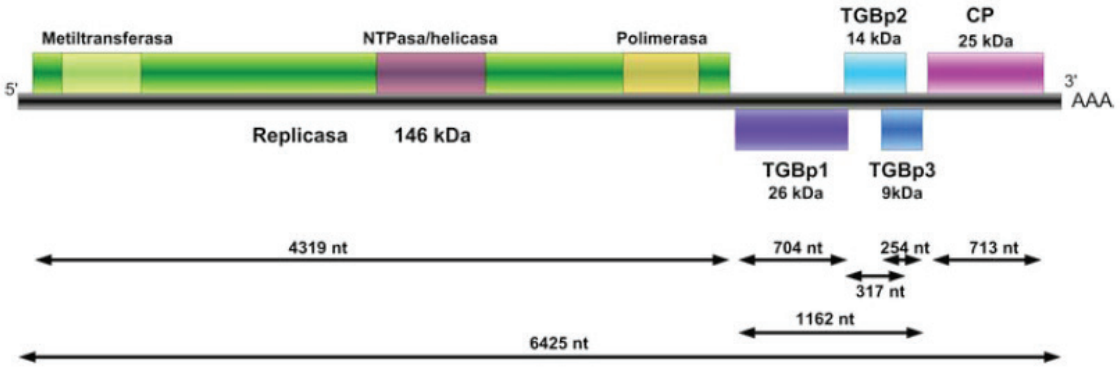

Figure 2: Genome organization of Pepino mosaic virus. The PepMV genome compromises a single, positive- sense, 6400-nt RNA strand containing five open reading frames encoding the putative viral polymerase (RdRp), the triple gene block proteins (TGBp1, TGBp2, and TGBp3), the coat protein (CP), and two short untranslated regions (UTRs) flanking the coding regions; there is a poly (A) tail at the 30 end of the genomic RNA. Genes are expressed from genomic and subgenomic RNAs (Verchot* et al., *1998; Aguilar* et al., *2002).

two small inter-cistronic regions. ORF1 encodes for the putative viral polymerase (RdRp) (Aguilar et *al.,* 2002). ORFs 2, 3 and 4 encode the triple gene block (TGB) proteins: TGBp1, TGBp2 and TGBp3, which are essential for virus movement (Morozov and Solovyev 2003; López et *al.,* 2005) and ORF5 encodes the coat protein gene (CP).

DETECTION TECHNIQUES

The diagnostic and detection of the virus in both the plant and seeds are based on the serological and molecular assays directly from the plant extract of tomato sample or after a process of extraction of viral RNA from the same extract.

Serological detection

Serological detection of PepMV by DAS-ELISA method (Double Antibody Sandwich Enzyme-Linked Immunosorbent Assay) is used routinely in laboratories for its simplicity, fastness and ability to analyze large numbers of samples as in the case of seed certification (Salomone and Roggero, 2002). Another serological technique is also available for sample analysis which is immunoprinting-ELISA (Jordá et *al.,* 2001). It involves performing a printing of the sample into a nitrocellulose membrane followed by a serological analysis with specific commercial antibodies to the virus. This technique has the advantages that it is not necessary to prepare extracts, as the fresh material is immobilized directly onto nitrocellulose membranes; it allows the simultaneous analysis of a large number of samples.

Molecular detection

There are different molecular procedures used for the identification of PepMV in plant samples and molecular variability studies. Generic primers and probes have been described for a conventional RT-PCR (Ling et *al.,* 2008) and a real-time RT- PCR (Ling et *al.,* 2007). Other molecular assays have also been shown to be suitable for strain differentiation of PepMV (Martínez-Culebras et *al.,* 2002; Hanssen et *al.,* 2008; Alfaro-Fernández et *al.,* 2009; Gutiérrez-Aguirre et *al.,* 2009).

PRESENT STATUS OF PEPMV SPREAD IN MOROCCO

The virus was detected in Morocco in 2007 (Córdoba et *al.,* 2007). Since its finding, the Moroccan Plant Protection Service made a disease survey on tomato products to evaluate the real situation and to find effective measures to control this emerging virus. Therefore, several methods have been developed for the detection of PepMV in plants. The majority of investigations conducted throughout the world to assess the presence of the pathogen have been based mainly on serological and molecular tests (Souiri et *al.,* 2013).

The double antibody sandwich (DAS-ELISA) test using monoclonal antibodies was used to identify *Pepino mosaic virus* (Souiri et *al.,* 2013). This study demonstrated the development and the characterization of two specific monoclonal antibodies named 1B11-G10 and 5A1-G5 against PepMV and their use in the diagnosis of plant infection. In another study, Souiri et *al.*, (2016) have demonstrated that the Moroccan population of PepMV shares a very high sequence identity with the CH2 strains by sequencing a part of RNA-dependant-RNA polymerase gene, triple gene block and the coat protein gene of twelve PepMV isolates collected from Moroccan areas of tomato production. Moreover, this study showed that the Moroccan isolates reveals a specific single nucleotide polymorphisms that lead to distinct variants and for a subset of isolates, a possible recombination with EU genotypes. The same results were reported in a study by Hanssen et *al.*, (2010) where they demonstrated the high sequence similarity of PepMV Moroccan isolates with the Chilean genotype. The cross protection of tomatoes production under greenhouse is a strategy adopted by Moroccan farmers to reduce the virus accumulation and to limit the severity of aggressive PepMV isolates (Hanssen et *al.,* 2010) by applying a new product which has been recently authorized in 2015 by the national phytosanitary organization. Trials were conducted in Morocco in 2014 and the samples from greenhouses treated with these mild strains revealed that no viral particles were detectable eight months after the application (ONSSA, 2015).

Such studies are a key step in obtaining methods that allow rapid and reliable detection of different viral strains present in the Morocco. They are therefore necessary studies to set up strategies for more effective and long term control.

CONTROL METHODS

Despite efforts in the affected countries, the PepMV is a very difficult virus to eradicate completely in semi-protected crops or open field, especially when plants are asymptomatic. The virus continues producing annual epidemics and every year, new detections are reported in other countries. In order to control PepMV, effective measures should be considered not only to reduce the initial inoculum, but also to prevent the establishment and spread of pathogen, and to decrease the susceptibility of the host against infection.

Preventive methods

Measures adopted to prevent the introduction and the movement of the virus are principally the use of healthy tomato seeds for sowing because "the virus is considered as a seed quarantine organism" and therefore, seeds must be inspected and certified. Whereas, disinfection of seed using treatments based on dry heating at 72 C for 48–72 h, or disinfection with 0.5-1.0% sodium hypochlorite or 10% trisodium phosphate will eradicate the virus without affecting seed germination (Córdoba-Sellés et *al.,* 2007; Ling, 2010).

The use of resistant or tolerant varieties to the virus is a preventive measure that has been successfully adopted for other viral diseases, however, in the case of PepMV, resistant cultivars are not yet available (Gómez et *al.,* 2009a).

Preventive actions

In order to avoid the introduction and/or diffusion of the virus, some preventive actions are taken, such:

• Hand washing of the operators with a solution of soapy hot water and disinfection of wet shoes before entering the greenhouse (Fletcher, 2000). Use of disposable protections for head, body, hands and feet (Jordá and Lacasa, 2002),

• Elimination of potential natural reservoirs of the virus inside the greenhouse: fallen fruits on the ground, remains of previous crops and control the weed flora,

• Control of the presence of *Olpidium virulentus* in substrates and hydroponic solutions by disinfectants treatments with surfactant Agral (Tomlinson and Thomas,1986), treatments with ultraviolet light (Campbell, 1996) or at temperatures greater than 70°C to kill spores of the fungus,

• Soil sterilization, avoiding contact with infected substrate and destruction of infected bags when artificial substrate is used,

• In greenhouses where diseased plants are detected, after removing the previous crop, and at least 3 weeks before planting, all greenhouse structures, tools and corridors, should be washed with water and then treated with disinfectants solutions such as sodium hydroxide (0.125%) 0.01% sodium hypochlorite, 10% trisodium phosphate or organic acids (1%).

CONCLUSION

Global trade of agricultural products is leading to the spread of many viruses including *Pepino mosaic virus*. Presently, PepMV is distributed globally and its center of diversity region is around Mediterranean basin including Italy, France, Spain and Morocco, where the spread of new viruses is taking place, creating a serious threat to tomato production.

The subsequent pandemic spread of PepMV was probably facilitated by the stability of the virus and therefore, its persistence in contaminated plant material, its transmission through seeds, and the global trade of tomato seeds and fruits. Once well-adapted tomato PepMV strains have entered a new region, successful epidemic infections are facilitated again by the stability of the virus and the seed transmission route, but also the efficient contact-based transmission and the inconspicuous symptoms, which prevent the prompt identification of infected plants. The genetic diversity of PepMV probably favored its emergence by promoting the jump from adapted to non adapted hosts and has subsequently favored the evolution of strains adapted to tomato. Interactions among PepMV strains add a further layer of complexity and also have a significant effect on PepMV evolutionary dynamics.

There is a perception that the emergence and reemergence of viral diseases are occurring with greater frequency compared to previous decades. The case of PepMV is a recent example but there are many others. In the context of global trade and climate change, there is an urgent need to establish global plant health systems for surveillance, diagnosis, integrated research, communication, and technology transfer to address these problems. In fact, it was recommended to disinfect tools and hands, then removing infected plants representing the source of the virus the use of healthy seeds for sowing as an appropriate method to avoid the primary PepMV source of infection and then, combining it with the cross protection with mild strains (CH2).

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