Technical review

Protected Edibles

Pepino mosaic virus: strains, symptoms and cross-protection

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First described in tomato in 1999, *Pepino mosaic virus* (PepMV) is now one of the most economically important epidemic virus diseases of tomato worldwide. No varietal resistance is available. Efforts to exclude the virus from a nursery and prevent persistence between successive crops by strict hygiene measures have had limited success. Cross-protection as a new strategy for PepMV disease management was recently introduced in Belgium and the Netherlands. This short review examines the pros and cons of using cross-protection against a virus which shows high genetic diversity.

April 2014

Pepino mosaic virus cross-protection

What is cross-protection?

Cross protection is a phenomenon in which plants infected with one strain of a virus are protected from the effects of superinfection with other related strains. One of the most successful examples of cross-protection in vegetable production was the control of *Tomato mosaic virus* (ToMV) in tomato by the mild MII-16 protector isolate, widely used in the UK in the 1970s (and in some cherry tomato varieties in the 1980s) until resistant varieties became available. Another example was control of *Zucchini yellow mosaic virus* (ZYMV) in cucurbits by the mild wk protector isolate of ZYMV.

Like vaccination in humans or animals, cross-protection is generally specific to the virus used. Thus, inoculation of tomato plants with a mild isolate of PepMV may give protection against other isolates of PepMV, but it will not give protection against other tomato viruses such a ToMV, *Tomato yellow leaf curl virus* (TYLC) or *Tomato torrado virus* (TToV).

The mechanisms by which cross-protection works are varied and not fully understood. In some instances the phenomenon is dependent on the virus coat protein and in others on the virus genotype. With PepMV, cross-protection is dependent on genotype and operates through inhibition of RNA replication. A mild protector isolate of PepMV inhibits RNA replication of a challenge isolate only when the two isolates have close nucleotide sequence homology (i.e. are very closely related).

PepMV strains

Currently, four strains or genotypes of PepMV are identified. Each strain has a distinct evolutionary origin. PepMV was first described in pepino (*Solanum muricatum*) in Peru in 1974 where it was found to be the cause of yellow leaf mosaic in this host. An isolate obtained from *Lycopersicon peruvianum* at the time was very similar to the original pepino isolate; designated the LP strain, it causes no symptoms in tomato. The rate of evolution of PepMV, even more so than other RNA viruses, is high, and at some stage, probably in South America, the virus made a species jump from pepino to tomato (*Solanum lycopersicum*). PepMV was first reported in tomato in the Netherlands in 1999 and soon after in the UK and other European countries. Designated the EU strain, these first isolates from tomato were found to have a 95% RNA nucleotide sequence homology to the LP strain.

Subsequently another genotype (CH2) was isolated from tomato seeds from Chile. This genotype is rather different as it displays only 78-80% sequence homology with the EU and LP genotype groups. A fourth genotype (US1), described in the USA, shares 78% sequence homology with CH2 and 82% with the EU/LP genotypes. The US1 genotype was confirmed in the Canary Islands in 2007. During early epidemics, isolates of the EU strain (from England, France and Spain) were found to be genetically highly similar (99% sequence homology) and predominantly of the EU genotype. Since 2004, studies have shown that the prevalent PepMV genotype in Europe has shifted from predominantly EU to CH2. Experimental work has shown that CH2 is 'fitter' than the EU strain in tomato, showing more rapid accumulation, faster systemic movement within plants and greater transmission between plants. However, although CH2 isolates predominated, EU isolates were found to have persisted in mixed infections in some greenhouse tomato crops (reported in Belgium, Spain and Poland).

Cause of symptom variation

It was not until 2009 that different symptom expression by PepMV in greenhouse tomatoes was unambiguously attributed to PepMV isolate: the occurrence of a particular isolate in a crop can at least partially explain the huge variation in the level of damage caused by PepMV in commercial tomato production.

Variety, in particular, and greenhouse environment can also modify symptoms; for example, more severe symptoms often occur under low temperature and low light conditions. However, a minor difference in the genome sequence can cause significant differences in symptoms between two isolates that infect a crop under the same environmental conditions. Thus, in Poland it was shown that a single amino acid sequence change converts a mild isolate of the CH2 genotype to an aggressive isolate that causes severe leaf necrosis and even plant death. In Belgium, a mild and an aggressive isolate of the CH2 genotype showed 99.4% sequence similarity; while the mild isolate caused mild symptoms in tomato such as leaf distortion (Figure 1), the severe isolate on the same variety (Tricia) in the same environment caused obvious leaf bubbling, nettle-head symptoms, premature senescence, increased fruit marbling (Figure 2) and flaming, 'open' fruit (fruit that split shortly after setting so that seeds are visible) and sepal necrosis. The objective of cross-protection is to use a mild strain of PepMV to prevent occurrence of severe symptoms.

1. **Transient leaf distortion and nettle-head symptoms may be overlooked**

2. **Fruit marbling, caused by PepMV repression of carotenoid synthesis, is a major cause of economic loss**

Experience with PepMV cross-protection

To reduce economic losses caused by PepMV infection, some tomato growers have deliberately inoculated their plants with PepMV at the start of a growing season as it was suggested that early PepMV infections are less damaging than infections that occur later in the growing season. Subsequent detailed studies have shown that this is a risky strategy. Work in Belgium showed that the EU genotype does not provide cross-protection towards the CH2 genotype or vice-versa. In another study, neither a mild LP isolate nor a mild EU isolate provided cross-protection against an aggressive CH2 isolate, rather, they enhanced symptom severity. In particular, the incidence of fruit marbling increased considerably while plant vigour and leaf development were reduced, resulting in an overall yield reduction of 13% compared to control plants.

Efficient cross-protection against the aggressive CH2 isolate was only obtained in plants pre-inoculated with a mild CH2 isolate. The incidence of PepMV fruit symptoms was notably reduced. The nucleotide sequence homology of the EU and LP genotypes with the CH2 genotypes was as low as 79%, while the sequence homology of the mild and aggressive CH2 isolates used in the study was 99.4%. These results indicate that RNA sequence homology is a strong determining factor in PepMV cross-protection effectiveness in tomato. Similar results have been found for other RNA viruses.

Risk from mixed infections

Co-infection of tomato plants with different PepMV genotypes can lead to enhanced symptom severity. Enhanced symptom severity can arise as a result of synergism between different PepMV genotypes, or through new PepMV genotypes arising as a result of recombinations during viral replication. The US2 genotype of PepMV described in America is believed to be a natural recombinant of US1 and EU strains. Genetic recombination was found to occur frequently in mixed EU and CH2 infections under natural conditions in Belgium. Identical recombinants were detected at different time points in different plants, suggesting that the recombinant genotype was sufficiently viable to be transmitted from one plant to another. It is not known whether the enhanced symptom severity seen with the co-infection described above was due to the recombinant genotype or synergistic activities between the different PepMV genotypes. It should be noted that the opposite effect has also been observed, where infection by one PepMV genotype has suppressed symptoms developed by a different genotype. However, in the absence of knowledge about the genotypes commonly occurring in an area at a particular time, or the genotype of the intended protector strain, it must be appreciated that attempts to protect a tomato crop may increase rather than minimise PepMV damage.

Should cross-protection be used?

The four main disease characteristics required before considering cross-protection as a strategy for disease control are:

- Failure to control the disease by eradication.
- Rapid spread.
- Large enough production losses to make cross-protection the preferred alternative over its potential drawbacks.
- Availability of a mild strain that protects effectively without causing undue harm.

Additionally, where cross-protection is mediated by the viral RNA sequence, it is necessary that there is a homogenous population (one predominant genotype) in a particular region.

PepMV fulfils the first two criteria for consideration of cross-protection and in some varieties and in some years production losses in the UK are large enough to fulfil criterion 3 also (e.g. severe losses to poor quality fruit as well as lost production in Piccolo in 2013). Experience to date from the near continent indicates the fourth criterion is also fulfilled.

However, there are some additional potential drawbacks that need to be considered.

Firstly, is there sufficient information about the host range of the mild strain to be used e.g. is there evidence that it does not infect or detrimentally affect other major Solanaceous crops (i.e. potato, pepper)? PepMV is considered a potential threat to potato crops. It has been shown that following mechanical inoculation the virus can systemically infect several potato varieties, though no symptoms have been reported. In Spain, PepMV has been found to occur naturally in many weed hosts comprising a range of non-solanaceous plant families (e.g. *Sonchus oleraceus; Rumex* spp., *Taraxacum* spp.).

Secondly, is there a risk of synergism between a mild isolate of PepMV and other viruses that occur in tomato? The use of a mild isolate of ZYMV (ZYMV-wk) to protect squash against severe isolates of this virus was halted because of synergism with *Watermelon mosaic virus* (WMW). Currently the occurrence of viruses other than PepMV in UK tomato crops is rare, but there are occasional outbreaks of unrelated viruses (e.g. *Arabis mosaic virus, Tomato spotted wilt virus*), and the potential for some viruses emerging elsewhere in Europe to occur here (e.g. ToTV). In Poland, it has been reported that co-infection of tomato by PepMV and ToTV resulted in more severe symptoms than either virus alone.

An additional criterion before considering cross-protection is the requirement for a homogenous population in a given region. The current occurrence of different PepMV genotypes in Britain is not well known. A survey in late 2013 of 12 crops in the UK indicated six were infected with PepMV, predominantly with the CH2 genotype. Surprisingly however, the US1 genotype was found in crops on one nursery, alongside the CH2 genotype.

Next steps

A PepMV mild isolate used for the purpose of cross-protection is, by definition, a Plant Protection Product and as such requires authorisation from CRD before it can be used in Britain. At present, no products are authorised. At least one mild isolate has received 120 day emergency authorisation for use in Belgium and the Netherlands. Regulatory authorities here would, among other factors, need to assure themselves that any mild isolate offered for registration is stable and offers no significant risk to potato, other crops and the natural environment. Ideally, one would also like reassurances that cross-protection with the mild isolate works on a range of varieties and under different climatic conditions.

If a mild isolate is to be used in Britain, regular annual surveys would be required to ensure the mild isolate used was of the same sole or predominant genotype. Unlike in The Netherlands, UK tomato production is dispersed over a wide geographic area. Some UK tomato nurseries have remained unaffected by PepMV. If PepMV mild isolates are to be used here, should England be considered as one area for assessment of PepMV genotype diversity, or could smaller delimited areas be considered?

In the Netherlands, before using a mild isolate of PepMV for cross-protection it is a requirement that plants are adequately tested and found to be free of the virus before inoculation is permitted. The same requirement would apply here. After inoculation with a mild isolate of PepMV, strict hygiene practices to prevent entry of PepMV to the crop would still need to be maintained to minimise the risk of different PepMV genotypes co-infecting the crop. In addition to the risk of severe symptoms in tomato when two different genotypes co-infect, work in Spain indicated that co-infection by an EU and a CH2 isolate expanded the range of susceptible hosts.

At present, a management strategy based on cross-protection can only be successful in areas where one PepMV genotype is predominant, where the PepMV population is monitored intensively, and where very strict hygiene measures are taken during cultivation and between crops. The recent publication of a LAMP assay that distinguishes the main genotypes of PepMV could permit rapid on-site monitoring, if it is decided that mild strain cross-protection can be used in the UK.

Resistant varieties

In the longer term, it is to be hoped that suitable genetic resistance to PepMV will be incorporated into commercial tomato varieties.

Moderate resistance to the EU strain of PepMV has been found in some accessions of the wild species *S. chilense* and *S. peruvianum*, but some sources at least show strain-specific PepMV resistance. One promising accession from *S. habrochaites* is resistant to EU, CH2 and US1 strains of PepMV. However, the resistance appears to be polygenetic in nature and it would be difficult to introduce these traits into commercial varieties.

PepMV ‘factfile’

Virus features

PepMV belongs to a group of small RNA viruses in the potato virus X group (Genus: *Potexvirus*). It is a flexuous rod 508 nm long (0.0005 mm) consisting of a single strand of RNA encapsulated in a protein coat; the RNA strand comprises 6,400 nucleotides. Notable features are its high level of genetic diversity, its stability in plant tissues and the huge range of symptoms produced in tomato.

Host range

There are 41 known hosts of PepMV, primarily but not exclusively in the Solanaceae family. In addition to tomato and pepino, natural infection has been recorded in basil (*Ocimum basilicum*) and in various weeds (e.g. species of *Malva, Sonchus* and *Taraxacum*) in the vicinity of tomato glasshouses. Mechanical inoculation has shown that PepMV can infect aubergine (*Solanum melongea*), pepper (*Capsicum annuum*), potato (*Solanum tuberosum*) and black nightshade (*Solanum nigrum*). Tomato is currently the only host where it causes economic damage. Weed hosts could be a symptomless reservoir of the virus. Different isolates of PepMV can have different host ranges.

Transmission

In tomato, PepMV is very readily spread by contact, a reflection of its stability outside its host. It is also transmitted in seed which has resulted in rapid spread of the CH2 genotype around the world. With high density cropping, frequent plant handling and ready contact spread, even an extremely low level of seed infection can lead to an outbreak in tomato. Once the virus is present in a tomato crop, it is usual for all plants to become infected eventually. Where the isolate produces only mild symptoms, initial infection can be easily overlooked. In addition, PepMV is transmitted by whitefly (*Trialeurodes vaporariorum*), bumble bees, *Macrolophus caliginosus* (used for whitefly control), the zoosporic oomycete *Olpidium virulentus* which is common in hydroponic crops, and by hydroponic solution. At 20°C the virus remains infectious for up to 3 weeks in water. PepMV released from plant roots into nutrient solution can infect healthy plants through their roots, ultimately spreading to green parts. Within a tomato plant, PepMV moves systemically through the phloem in the vascular system.

Genotype shifts

Crop monitoring has revealed shifts in the predominant genotype on several occasions. In Europe the genotype switched from predominantly EU in 2004 to predominantly CH2 in 2008; in the USA there was a switch from EU in 2006/07 to CH2 in 2009/10; in Mexico there was a switch from CH2 in 2010 to US1 in 2012. These have probably occurred through the introduction of the new genotypes on infected seed. The new genotype becomes predominant if it is ‘fitter’ than the original; for example, greater temperature stability, faster multiplication rate, faster transmission. The original genotype is not necessarily displaced completely. Naturally mixed infections of EU and CH2 genotypes in tomato have been reported in Canada, USA, Mexico and Belgium; such mixed infections can result in more severe symptoms than either genotype alone. It was recently shown that PepMV incidence was high in populations of wild tomatoes in Southern Peru and a strain not yet reported in cultivated tomato was characterised. A molecular test (LAMP assay) has been developed to achieve rapid identification of the EU, US1 and CH2 genotypes.

Symptoms

PepMV can result in a huge range of symptoms (Figures 3–8), most commonly: nettle-head, stunting, leaf distortion, leaf bubbling, leaf mosaics, yellow rectangular leaf spots, chlorosis, and marbling and flaming of fruit. In the last few years new symptoms have been reported: necrotic spots on leaves, petioles and stems, premature leaf senescence, necrotic sepals, ‘open’ fruit (fruit split soon after setting and seeds are visible on ripe fruit), reduced yield and (occasionally) plant death.

Marbling of fruit is induced by local accumulation of PepMV virus particles during ripening resulting in repression of lycopene (red pigment) synthesis. Lycopene concentration was found to be 10x lower in the yellow than red areas of marbled fruit. There was also an increased concentration of alkaloid compounds associated with plant defence in the yellow sectors of affected fruits.

In addition to reducing leaf area through distorted leaves, inoculation of tomato with PepMV also represses photosynthesis.

It should be noted that use of grafted plants does not affect yield reduction caused by severe isolates of PepMV.

In contrast to the progressive development of many fungal diseases, virus disease symptoms are often transient as plants can recover from the initial infection shock, though symptoms may re-appear later in response to environmental changes (e.g. light, temperature).

The particular isolate of PepMV present in a tomato crop markedly influences symptom severity. Two isolates of the same genotype which differ in only a relatively small number of nucleotides in their RNA sequence (as little as 0.6%) can differ greatly in symptom severity. Currently it is unclear which regions of the PepMV genome are important for symptoms. There is no correlation between genotype strain and symptom severity.

In addition to PepMV isolate, symptoms are influenced by environment, tomato variety, crop age at time of infection, co-infection with other isolates and co-infection with other viruses.

Cross-protection

Cross-protection against PepMV in tomato only works when the protector and challenge isolates have close RNA sequence homology. The protector isolate inhibits replication of the challenge isolate. The challenge isolate is barely detected in a plant if cross-protection is established. Belgium experience with cross-protection in 2005 was bad and mild strain inoculation was then abandoned; it was subsequently shown that neither a mild EU isolate nor a mild LP isolate protected against a severe isolate of the CH2 genotype.

Later, a mild CH2 protector isolate was shown to produce only very mildew symptoms and to provide protection against two CH2 severe isolates. Successful cross-protection against PepMV has been demonstrated on both own-root and grafted plants.

Evolution

PepMV is present within tomato plants as a 'quasispecies' population with slightly differing but related RNA sequences. These differing sequences arise due to a high mutation rate during viral replication. The dominant members may vary during shifts between successive hosts. This level of genetic variability allows PepMV to quickly adapt to different hosts. The rate of evolution by PepMV was calculated to be 10x higher than similar RNA plant viruses.

In addition to natural mutation during replication, variation can also arise when a tomato plant is co-infected with two different isolates of PepMV and there is recombination of the RNA. Recombination of EU and CH2 genotypes under natural conditions has been confirmed. Recombinants have 'mosaic' genomes containing distinct nucleotide sequence regions which do not have a common evolutionary descent. The recombinant isolate may be less fit than the original isolates and be selected out, or have greater fitness and come to predominate. Recombinant PepMV isolates sufficiently fit to transmit to other plants in a tomato crop have been detected.

The emergence of at least four genotypes of PepMV in tomato over a relatively short period of time is indicative of its high rate of evolution.

3. Severe leaf distortion in the plant head

4. Yellow rectangular leaf spots are a common symptom

5. A striking leaf chlorosis caused by PepMV

6. Isolates of PepMV causing necrotic leaf spots and premature senescence, rarely seen in the UK to date, can be very damaging

7. Leaf necrosis associated with PepMV infection

8. Leaf yellowing and distortion due to PepMV

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