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Current Opinion in Virology

Prospects for engineering and improvement of cross-protective virus strains

Heiko Ziebell[1] and Robin MacDiarmid[2]

Mild strain cross-protection is currently an important method for the production of high quality plant products; despite challenge from severe virus isolates the initial protecting strain precludes symptom development. The mechanism of cross-protection is not yet resolved as RNA silencing does not sufficiently explain the phenomenon. Six requirements have been put forward to ensure long-lasting protection. We propose two additional requirements for effective and durable mild strain cross-protection; mild strains based on knowledge of the mechanism and consideration of impacts to consumers. Future research on predicting phenotype from genotype and understanding virus–plant and virus–vector interactions will enable improvement of cross-protective strains. Shared international databases of whole ecosystem interactions across a wide range of virus patho- and symbiotic-systems will form the basis for making step-change advances towards our collective ability to engineer and improve mild strain cross-protection.

Addresses
[1] Institute for Epidemiology and Pathogen Diagnostics, Julius Kühn-Institut, Messeweg 11-12, 38104 Braunschweig, Germany
[2] New Zealand Institute for Plant and Food Research Limited, Private Bag 92169, Auckland, New Zealand

Corresponding author: Ziebell, Heiko (heiko.ziebell@julius-kuehn.de)

Current Opinion in Virology 2017, **26**:8–14

This review comes from a themed issue on **Engineering for viral resistance**

Edited by **John Carr** and **Peter Palukaitis**

For a complete overview see the Issue and the Editorial

Available online 22nd July 2017

http://dx.doi.org/10.1016/j.coviro.2017.06.010

Introduction

Global agriculture and horticulture are under increasing pressure to provide food in sufficient qualities and quantities for an ever increasing world population [1••]. Major changes in agricultural practice, the acquisition of novel agricultural land, the devolvement of novel fertilisers and more efficient crop cultivars led to higher food availability during the last century but despite these efforts the proportion of undernourished and malnourished humans remains worrying high [1••]. In addition to the direct influences of changing climatic conditions on crop plants themselves such as extreme weather conditions, crop pests and diseases are also directly and indirectly influenced by climate change [2–4].

Plant viruses are one of the major classes of emerging plant diseases that are also greatly influenced by changes in climatic conditions [5]. All major crops are deeply affected by major economic crop losses due to viral infections [6]. As plant viruses are dependent on vectors for their dispersal, changes in global climatic conditions can favour the spread and establishment of these vectors in new geographic areas or can favour their proliferation even at the beginning of the new growing season [7]. Additionally, virus vectors such as insects can invade new ecological niches due to these climatic changes and carry viruses into new geographic areas and/or cropping systems thus posing biosecurity threats [8]. Problems with new incursions are further enhanced by political decisions to reduce the use of pesticides for insect controls and to limit the availability of licensed substances. As there are no cures available, prevention of virus infection is the major option available. Conventional breeding of virus-resistant cultivars is a lengthy and cost-intensive process, particularly for perennial crops such as fruit trees or grapevines. Furthermore, natural sources of virus resistance are not often available or cannot be transferred from closely related plant species to crop plants. Although quite successful in some cases, genetic modification to develop resistance to viruses or other pests and diseases in crop plants has been met with popular scepticism and is not politically desired in many countries around the globe [9–13].

Some time ago we envisaged the revival of cross-protection as an 'underused plant protection method' [14]. Despite examples of new applications, the use of cross-protection is restricted by the lack of a satisfactory explanation of its mechanism as this knowledge gap limits its predictability and acceptance.

Applied cross-protection — a success story?

'Cross-protection', infection with a mild or attenuated virus strain preventing secondary infection with more severe strains of the same virus, is also termed 'superinfection exclusion' in the context of basic research [14–16]. The phenomenon, first described almost one hundred years ago [17], has since been demonstrated for numerous combinations of plant viruses and different virus isolates but also for viroids

and satellite viruses, although strictly speaking this latter effect cannot be classified as cross-protection. For a detailed review on the history of cross-protection and a (non-exclusive) list of viruses, viroids, satellite RNAs and satellite viruses for which cross-protection has been described, the reader is referred to the review of H Ziebell and JP Carr [14].

The successful application of cross-protection to prevent yield losses on a field scale has been described only for a few virus/crop combinations. Zucchini yellow mosaic virus affecting global cucurbit production, papaya ringspot virus diminishing papayas in particular in Hawaii and other papaya-producing countries, cocoa swollen shoot virus damaging cocoa plants in Africa and citrus tristeza virus (CTV) affecting citrus production world-wide are a few examples where detailed trials for the search of mild strains and their ability to protect against most severe strains have been carried out and successfully applied to crops [14,16,18–33]. The most recent development is the approval of a mild pepino mosaic virus (PepMV) strain as biological plant protection product in the European Union (http://www.pmv-01.com/; accessed 20.05.2017). Despite being identified in Peruvian pepino (*Solanum muricatum*) plants in the 1970s [34], this virus lived in the shadows until 1999 when it was found in Dutch glasshouse tomatoes [35]. Since then, PepMV has become endemic in most European countries in which tomatoes are produced; PepMV has also been reported from numerous other countries in the world ([36] and references within). As this *Potexvirus* is easily transmitted mechanically, it is not surprising that high density production facilities of tomatoes became easy victims of PepMV [37–40]. The economic damage is caused by marbled fruits having a lower market value or not being sellable; discoloration and 'open fruits' have an equally negative impact on market yield [36,41]. With at least five different genetic PepMV groups [42,43] there have been shifts within the population and the geographic distribution of strains; the occurrence of mixed infections can lead to diverse symptoms which are difficult to control [36,42,44–47]. Due to the lack of PepMV-resistant tomato varieties, growers embraced cross-protection as a potential management strategy, with varying success [48–50]. A trial in 2010 identified a mild strain (named '1906', CH2 group) that provided cross-protection against a more aggressive CH2 strain whereas pre-inoculation with a second mild strain (LP group) had the opposite effect of enhancing symptoms induced by the aggressive CH2-strain [48]. Subsequent research led to the development and recent approval of PepMV-1906 as a 'no risk for humans' biological plant protection product in the European Union, and the European Food Safety Authority has already assessed a second PepMV mild strain [51,52]. If these officially approved protection products are successful, one can envisage a broader range of plant viruses becoming available to use for cross-protection.

Cross-protection — RNA silencing, cellular exclusion or what else?

Despite intensive research over the last century and many different hypothesis being put forward, the mechanism(s) underlying cross-protection are still not satisfactory explained [14]. As cross-protection only appears to work between very closely related strains of the same virus, it was hypothesised that antiviral RNA silencing might be the driving mechanism [53,54]. Although some studies support the idea of the involvement of RNA silencing in cross-protection, there is strong evidence that (at least with cucumber mosaic virus, CMV) RNA silencing is not solely responsible for the cross-protection phenomenon. Using a CMV deletion mutant which could not express the 2b viral suppressor of RNA silencing, Ziebell et al. [55] showed that this symptomless mutant was indeed able to protect against the more severe parental strain Fny-CMV. However, the protection extended to a less closely related strain, TC-CMV, which belongs to a different CMV subgroup and only shares little genetic homology with the mutant. In this instance, the mutant did not induce a strong systemic silencing signal and more importantly, the mutant provided protection in *Arabidopsis thaliana* plants that were compromised in the RNA silencing pathway [56]. Therefore, DICER-like enzymes in *A. thaliana* are not sufficient for successful cross-protection of CMV; a different mechanism must be operating here.

Cross-protection experiments with turnip crinkle virus (TCV) established in silencing-deficient plants also cast doubt on whether RNA silencing is indeed required or at least sufficient for cross-protection [57]. In addition, no correlation could be drawn between the exclusion of CTV strains and the induction of viral small RNAs [58]. A key research question yet to be addressed is whether RNAi is generally sufficient to explain cross-protection or whether the CMV, TCV, and CTV stories are anomalies. Therefore, there is a need for trials using a range of viruses on a catalogue of RNAi compromised lines across a number of plant species. Such trials could analyse whether cross-protection is effective and determine whether cellular exclusion is also occurring by tracking the location of virus variants.

An additional explanation of cross-protection might include the following cellular exclusion scenario whereby cells are re-programmed upon initial infection. Thereby, re-programmed cells behind the infection front can no longer be infected by a related strain due to the transient, virus-induced cellular structures being disassembled/assembled, for example, transient viral replication complexes that are sites of PVA replication [59••]. These cells may have switched from viral RNA replication to translation and movement, which perhaps precludes a secondary related virus the spatio-temporal opportunity