Viruses in Plants — Fascinating but Treacherous©

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INTRODUCTION

Plant viruses are fascinating pathogens that can induce beautiful symptoms on their hosts but also cause serious damage to commercial crops. To prevent damage from viral diseases it is important to understand and control virus spread. Recent research has given new insights into the mechanisms of plant defense against virus infection. This has enabled researchers to generate resistant plants. In addition, studies of viral defense reactions have contributed to the understanding of gene regulation.

PLANT VIRUS IMPACTS ON HUMAN LIFE

Plant viruses have affected humans in many ways. The first written evidence of this is found in a poem from 752 by Japanese empress Koken, who describes the yellowing of the leaves of pye-wye weed as follows: "For the plant I saw in the field of summer the color of the leaves were yellowing, looking like the effect of frosting during summer." The virus causing these symptoms is named eupatorium yellow-vein virus (Saunders et al., 2003).

Later in the years of tulipomania, which occurred from 1593 to 1638, the European upper class was fascinated by the flower-color-breaking in tulips caused by the potyvirus tulip-breaking virus (TBV) (Lesnaw and Ghabrial, 2000). At the peak of this madness, the price of 12 bulbs reached 13,000 Dutch guilders (equal to 1,500 dollars or the price of a big house). However, the market for these rarities collapsed when it was realized that grafting readily transferred the trait. In the same period, the flower-breaking symptoms inspired many artists, who painted still lives of vases with diseased tulips. Though Rembrandt is not famous for his tulip paintings, Rembrandt has lent his name to tulip cultivars with flower-color-breaking sold today. The slogan "recreate tulipomania in your garden" is even used by the Dutch company Breck's in their advertisement of an improved Rembrandt tulip mix. The flower-breaking of these cultivars is not caused by TBV but by mutations.

Viruses also cause severe damage to plants, causing wilting of trees, failure of grafting, discoloring of fruits, and necrotic lesions on leaves. In developing countries, viral diseases threaten income and food production. Examples include cocoa swollen shoot virus, which has killed many cocoa trees in Central Africa, and cassava mosaic virus and cassava brown-streak virus, both of which affect cassava production.

CHARACTERISTICS OF PLANT VIRUSES

Viruses are different from fungal and bacterial pathogens that affect plants. Both fungi and bacteria are cellular pathogens that are visible under the microscope. To understand what a virus is, it is necessary to look at what goes on inside the cell. In a eukaryotic cell like the plant cell, the chromosomes are located in the nucleus. The chromosomes carry the genes that hold the information for all the processes necessary for proper cell function and coordination of developmental processes.

The genes are controlled by transcription of the information into messenger RNA (mRNA). The mRNAs are transported from the nucleus to the cytoplasm where the information on the mRNAs is translated into protein. The proteins in turn conduct various processes necessary for growth, development, and reproduction. Most plant viruses are composed of an mRNA-like molecule (the viral genome) that is surrounded by protein. This complex of RNA and protein can exploit the cell to produce virus-specific proteins and to make new replicas of the virus RNA genome. In this process normal cell functions are compromised, and as a result, growth is reduced and disease symptoms develop.

TRANSMISSION AND CONTROL

In order to protect plants against virus infection, it is important to know how they spread from plant to plant. As indicated above, plant viruses depend on living plant cells, and therefore viruses have developed different strategies to be transmitted from one plant to another. In the plant, the virus spreads from cell to cell through plasmodesmata, but passage from one plant to another requires traversal of the plant cell wall.

Some viruses are transmitted through wounds that are generated by plants rubbing against each other, by the activity of humans or animals, or by handling during production and harvest. Collectively this type of transmission is known as mechanical transmission.

The particles of mechanically transmitted viruses are often very stable, and the most important control measure against these viruses is to develop work routines that prevent wounding and contact between plants.

Other viruses have specialized in transmission by vectors, which take up the virus during feeding. Insects are the most important viral vectors, but in addition mites, nematodes, and zoospores of fungal-like organisms in the soil can transmit plant viruses. Some viruses are retained in the feeding organs of the vectors and can be released shortly after acquisition. Other viruses must continue into the intestine and pass through the hemocoel to the salivary glands before they again can be released. The two types of association between virus and vector are referred to as stylet-borne and circulative, respectively. Tomato spotted wilt virus (TSWV) is an example of a virus that is transmitted in a circulative manner by thrips (Whitfield et al., 2005). The TSWV is taken up by thrips in the larval stage and persists in the insect for the rest of its lifetime. To control TSWV it is therefore important to detect thrips early using insect traps. It is also possible to monitor for the appearance of the virus by placing indicator plants in the greenhouse. Indicator plants are species that react with strong and characteristic symptoms. Petunia or faba bean are used as indicators to monitor TSWV because they display distinct symptoms shortly after infection.

Nematode-transmitted viruses can persist in the vector for up to a year, and zoospore-transmitted viruses for up to 10 years. This long persistence and the presence of the vector in the soil make control of these viruses difficult. Control is further complicated if the virus has a broad host range, which allows the virus to survive in wild species during crop rotations.

Several of the nematode-transmitted viruses cause severe diseases, and plants for propagation must be under strict control to ensure they are virus-free. One example is tomato ringspot virus (TomRSV), which causes apple union necrosis in

apples and prunus stem pitting in stone fruits. Symptoms appear as infected trees reach bearing age. Bud break is often delayed in the spring, and leaves are small and sparse. Terminal shoot growth is reduced, with shortened internodes. Infected trees flower heavily and set large numbers of small, highly colored fruit. The cause of the symptoms is found at the graft union, which turns dark as a result of a defense reaction in the scion towards virus spreading from the rootstock. This disease is only a problem on grafted trees where the fruiting variety is resistant to TomRSV and the rootstock is tolerant.

PLANT DEFENSE REACTIONS

While the defense reaction against TomRSV becomes the main cause of disease, the plant defense reactions and resistance to viruses are usually beneficial to plant production. To understand how plants combat virus disease it is necessary to outline what goes on inside a virus-infected plant cell. As indicated above most plant viruses are composed of protein and RNA that can replicate in the plant cell. Both viral protein synthesis and viral RNA replication require participation of host factors. When a virus particle enters the cell, the viral RNA is liberated and initially the virus exploits the host translation system to produce virus proteins. Then the virus proteins recruit host proteins to replicate new virus RNA. Initially, a strand is synthesized, which is complementary to the viral genomic RNA. This in turn serves as a template for production of new copies of the virus genome. The new viral genomes are either encapsidated to form virus particles or they move to the neighboring cell through plasmodesmata—a process that also requires host proteins.

It appears that plants use two strategies in defense. One is to initiate defense reactions upon recognition of viral proteins or double-stranded forms of viral RNA that are produced during replication (Soosar et al., 2005). In many cases the plant can recognize virus proteins and induce a defense reaction involving programmed cell death, which prevents further progress of the virus. If this reaction is quick it will prevent the virus from spreading from the first infected cell. This type of resistance is usually inherited as a dominant character. The other strategy is to eliminate or alter a host protein the virus requires at some stage of infection. An example is the modifications of translation initiation factors, which result in recessively inherited resistance (Robaglia and Caranta, 2006). The role of plant translation initiation factors in virus infection is still not clear, but experimental evidence suggests functions in translation and replication as well as cell-to-cell movement.

The plant can also recognize structures in the viral RNA, and initially this strategy was considered as specific towards viruses but now appears to be used against other pathogens as well. The defense relies on recognizing and targeting double-stranded RNA (dsRNA) for degradation. Depending on how efficient the degradation process is, the plant will be more or less resistant. The defense is initiated by an enzyme called DICER that recognizes and cleaves dsRNA into smaller fragments known as small interfering RNA (siRNA). The siRNA associates with an enzyme complex called RNA-induced silencing complex (RISC), and the siRNA guides RISC to cleave single-stranded RNA with complementarity to the siRNA. In this way both single-stranded viral RNA genomes and dsRNA generated during replication are disarmed (Voinnet, 2005). This RNA mediated defense is known as virus-induced gene silencing (VIGS) if a virus induces the RNA degradation. The term RNA interference (RNAi) is used if other types of dsRNA induce degradation.

GENERATING RESISTANT PLANTS

Even before the VIGS mechanism was fully elucidated, researchers started to speculate if this defense could be enforced to completely prevent virus infection, for example by generating plants with preformed RISC ready to target the single-stranded virus genome for degradation before it starts replicating. Early it became evident that transforming plants with only a fragment of the virus genome was sufficient to induce virus-specific RISC, and later it was realized that the fragment was most efficient when constructed as an inverted repeat, which base pairs to form dsRNA (Waterhouse et al., 2001).

The best described application of this strategy is development of papaya resistant to papaya ring spot virus in Hawaii (Gonsalves, 1998). Around 1990 papaya ringspot was becoming a serious problem, and at this time Dennis Gonsalves and coworkers generated the first resistant papaya plants based on transformation with a piece of the virus. After testing to secure consumer and environmental safety, resistant papayas were released for commercial use in 1998. Virus-resistant transgenic papayas are sold as Rainbow and SunUp papaya.

PLANT VIRUSES AFFECT PLANT DEVELOPMENT

The RNA-mediated resistance in transgenic plants is efficient because the virus is targeted for degradation more efficiently than in unmodified plants. In unmodified plants, viruses can suppress the defense reaction by expression of proteins known as silencing suppressors. These proteins act by interfering with the function of DICER or RISC or by binding siRNAs. In the last decade it has become clear that probably all viruses encode proteins that suppress RNA-mediated defense. Also the study of these proteins begins to reveal why some viruses induce spectacular symptoms like those induced by TBV.

The formation of dsRNA is a signal to the cell to degrade the RNA by the action of DICER and RISC. In addition to virus defense this RNA degradation mechanism is used to regulate gene expression and control developmental processes. The dsRNA can be generated by base pairing of a mRNA with a small RNA known as a microRNA (miRNA). DICER generates miRNA from larger precursors (pre-miRNA) containing imperfect inverted repeats. Similar to siRNAs, miRNAs act by guiding RISC to mRNA with complementarity to the miRNA strand. RISC either cleaves the mRNA or it prevents translation of the mRNA. The processing and function of miRNAs thus have striking similarities to the siRNAs during viral defense. This explained why virus infection sometimes alters developmental processes because the viral silencing suppressors may also interfere with the function of the miRNA pathway (Voinnet, 2005).

UNDERSTANDING HOW VIRUSES INDUCE SYPMTOMS

With the knowledge that viral silencing suppressors affect plant development it is tempting to speculate that flower-breaking in tulips is caused by the action of a silencing suppressor expressed by TBV. While this has not been demonstrated experimentally, similar color-breaking on the seed coat of virus-infected soybean has been shown to result from the activity of silencing suppressors expressed by soybean mosaic virus (SMV) or cucumber mosaic virus (CMV) (Senda et al., 2004). Most cultivated soybean varieties have yellow seeds because chalcone synthase is silenced in the seed coat. In black-seeded cultivars the activity of chalcone synthase

lead to production of anthocyanin and proanthocyanindin pigments. The silencing in yellow-seeded cultivars is due to a duplication of the gene encoding chalcone synthase. The gene duplication gives rise to transcripts that form dsRNA, which through formation of siRNAs targets the chalcone synthase mRNA for degradation. When yellow-seeded soybeans are virus infected, the silencing suppressors from SMV or CMV interfere with silencing and chalcone synthase is again expressed in sections of the seed coat. This gives rise to the dark colored areas on the seed coat.

As the example above indicates, basic research in plant virology has contributed much to the understanding of RNA-mediated gene regulation in plants. In addition virus vectors can be applied to the study of gene function by targeting mRNA sequences of specific genes for silencing (Watson et al., 2005).

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