International Problems in Tropical Plant Pathology

JOHN E. BOWMAN, PepsiCo Foods International, Dallas, TX; Chairman, Tropical Plant Pathology Committee, 1986–1987

The theme of the 1986 annual meeting of The American Phytopathological Society was international cooperation. The objective of the Tropical Plant Pathology and Postharvest Pathology and Mycotoxicology committees in cosponsoring this symposium was to expose a wider APS audience to tropical plant disease problems. These papers emphasize how control strategies in the tropics often require a blending of international insight and technology with local insight and hands-on experience.

Cross-Protection Techniques for Control of Plant Virus Diseases in the Tropics

DENNIS GONSALVES, Department of Plant Pathology, Cornell University, New York State Agricultural Experiment Station, Geneva 14456, and STEPHEN M. GARNSEY, USDA-ARS, Horticultural Research Laboratory, 2120 Camden Road, Orlando, FL 32803

Control of many plant virus diseases in the tropics is difficult for several reasons. Virus-resistant or virus-tolerant cultivars are not available for many tropical crops, disease cycles are not broken by killing winter temperatures, virus and insect vector reservoirs are present throughout the year, and, typically, crops are grown in numerous small plots over a wide area with little isolation. We believe that cross protection is a strategy that can be used to good advantage to selectively control virus diseases of tropical crops.

It is not our purpose to justify the use of cross protection. Basic criteria for selection of cross protection as a disease control strategy are well known, and we assume these have already been considered (4). Two principal factors favoring the use of cross protection are the prospect of severe economic loss (usually chronic) and the lack of other control options. The main thrust of this paper is on the various technical aspects that must be considered when developing and implementing a cross-protection program. We will use examples from some of our current work on papaya ringspot and citrus tristeza viruses to illustrate specific points.

Papaya ringspot virus (PRV) is a flexuous rod-shaped virus, about 750–850 nm long, that belongs to the potyvirus group and is nonpersistently transmitted by numerous aphid species (12). It is prevalent in nearly every papaya-growing region and causes the most destructive disease of papaya on a worldwide basis. Our work on PRV was started in anticipation of the virus eventually spreading into Hawaii's largest papaya-growing area, the Puna district on the island of Hawaii, despite the use of quarantine and eradication measures to prevent its spread (16,17).

Citrus tristeza virus (CTV) is also a flexuous rod-shaped virus but is about 2,000 nm long, belongs to the closterovirus group, and is transmitted semipersistently by several species of aphids (1). It has caused tremendous damage to citrus in many areas and is still of major concern in every citrus-growing region. Current cross-protection work with CTV in Florida is in response to increasing losses from CTV-induced decline on sour orange rooted trees (6,18). Florida growers are reluctant to abandon sour orange as a rootstock because of its cold tolerance and resistance to other diseases. Cross protection is being used extensively in Brazil to protect certain scion cultivars against damage by severe isolates of CTV (9). In Brazil, work was initiated after severe economic losses had occurred and no other control was found.

Cross Protection Defined

Our working definition of cross protection is "the use of a mild virus isolate to protect plants against economic damage caused by infection with a severe challenge strain(s) of the same virus." Three important points are evident in this definition: 1) The foremost requirement is the availability of a mild strain, 2) the effectiveness of the mild strain is largely evaluated by the economic benefit derived by cross protection with this strain, and 3) our definition does not imply knowledge of the mechanism(s) of cross protection. Although it is possible, and indeed likely, that cross protection would not restore crop production to the level of virus-free plants, cross protection is considered to be effective if the grower gains significant economic benefit. Ultimately, the farmer decides whether cross protection is useful in a given situation.

Ideally, cross-protection control strategies should be developed before the specific virus involved becomes a severe problem in the region of concern. Time is needed to identify and test selected virus strains for mildness and protective ability and to test the necessary parameters for use of these strains. This is especially true with perennial crops, in which yield effects may take several years to determine.

A cross-protection program can be divided into several interrelated elements: 1) selection, 2) preliminary evaluation, 3) pilot tests, 4) field evaluation of mild strains, and 5) integration of cross protection into crop management systems. Development of a cross-protection research program follows a logical sequence of steps. Each step is a continuation of an experimental process built on results obtained from the previous step. Thus, objective and realistic evaluation of each step is critical. For example, one would not want to make preliminary evaluations so stringent that strains that may be useful under field conditions are unnecessarily discarded. On the other hand, the test must be stringent enough to indicate effectiveness of the mild strain under field conditions.

Selection of Mild Strains

This is perhaps the most crucial and difficult step to accomplish in a cross-

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protection program. Mildness of a strain is relative and must be evaluated for each target crop or crops to be exposed. Mild strains can be obtained by: 1) selection from natural viral populations (by choosing existing natural mild strains under field conditions or by analyzing local lesion populations), 2) induced mutation of natural populations followed by selection, or 3) passage through selective hosts or vectors. In method 2, mutations are induced with mutagenic agents such as nitrous acid, then screened for desirable isolates. All three strategies have been used successfully for PRV and CTV.

PRV. We did not succeed in isolating naturally occurring mild isolates of PRV (16); several promising isolates did not maintain a mild reaction after subpropagation. We also tried unsuccessfully to select mild strains by evaluating single lesion isolates from inoculated *Chenopodium quinoa* Willd., a local lesion host for PRV. We therefore focused on selecting chemically induced mutants. With nitrous acid, a mutagen widely used for obtaining viral mutants, we obtained two promising mutants—PRV HA 5-1 and HA 6-1—from a severe PRV strain originating in Hawaii. In the screening process, inoculated papaya seedlings with severe symptoms were discarded and plants with no or only mild symptoms were tested for infection by enzyme-linked immunosorbent assay (ELISA) (2). ELISA-positive plants were selected for further tests. With this straightforward method we obtained mild mutants within 9 mo. Two important requirements for using this technique efficiently are the availablility of a local lesion host and mechanical transmissiblity of the virus.

CTV. Disease symptoms caused by CTV can be categorized as stem pitting on the woody cylinders of infected plants or decline of trees on sour orange rootstocks. Useful mild isolates of CTV that protect against the stem pitting syndrome of CTV have been selected by isolation from vigorous trees in areas severely affected by the disease (9). This approach was chosen because mild isolates of CTV are relatively common, mechanical transmission is relatively difficult, and there are no local lesion hosts. Recently, other selection methods, such as inoculation of strain-differentiating hosts, have been used to isolate mild CTV variants. However, several problems have been encountered: 1) Many naturally occurring mild isolates are not highly protective, and extensive testing may be needed to find an effective isolate. For example, mild isolates of CTV that do not cause a decline in citrus trees grown on sour orange rootstocks are common in Florida (6), but none has been highly protective against decline of challenged trees with sour orange rootstock. 2) Isolates that are mild in some hosts may be severe in others. For example, a Hassaku dwarf isolate from Japan (8) that protected sweet on sour orange trees caused severe stem pitting in other cultivars. 3) Some field sources are actually mixed infections that do not subpropagate consistently. 4) Experimentally selected mild variants from severe sources often are not stable.

Preliminary Evaluation of Mild Strains

Factors to be considered in the initial evaluation of a mild strain are the purpose for which the mild strain will be used (i.e., to ameliorate symptoms or to increase fruit production and quality), the complexity of the evaluation scheme, and the relation of the evaluation scheme to the final practical implementation. The PRV and CTV systems illustrate how evaluation schemes differ according to the virus and also the target crop.

PRV. The methods for evaluating the cross-protectiveness of PRV under greenhouse conditions were more straightforward and less complex than those used for CTV. Our basic aims were to determine the effects of the mild PRV strains on the growth of papaya and cucurbits and to determine their ability to cross-protect against the severe (Hawaiian) parent strain.

Cross-protection tests (16) with PRV nitrous acid mutants (HA 5-1 and 6-1) were designed to determine: 1) the minimal time after mild strain inoculation for plants to become effectively cross-protected, 2) the effect of challenge inoculation to different numbers and locations of leaves, and 3) the effect of continuous challenge inoculations on test plants. These tests were also selected because they mimicked events we felt would occur under field conditions. Our tests showed that the mild strains did cross-protect papaya against the severe parent strain.

CTV. Preliminary tests of candidate mild strains of CTV are initiated by evaluating the types of symptoms caused in the major reactive hosts (6,8,9,14,18). To evaluate for stem pitting, isolates are graft-inoculated to several cultivars, including Mexican lime, grapefruit, and sweet orange. Inoculations are also made to grafted combinations of sweet orange on sour orange rootstock to evaluate decline responses. Experimental replicates and the use of appropriate controls are essential. Usually 6 mo or more are required to make this evaluation.

Selected mild CTV isolates are then tested for their protective ability against one or more severe isolates. Challenge may be applied by aphid inoculation (to mimic the challenge inoculation under field conditions) or by graft inoculation. Graft inoculation is usually considered a severe form of challenge because the receptor plant is under continuous exposure to virus being generated in the inoculum tissue. Therefore, the donor tissue is often excised several weeks after grafting to reduce this effect. Sometimes the donor tissue is left on the test plant to reduce the time required to evaluate the degree of protection. Under these conditions, protection may not be complete, but the mild strain may still provide a significant delay in expression of symptoms by the severe challenge isolate. The basic objective of this graft inoculation test is to identify sources that would stand up under severe challenge pressure.

Pilot Tests of Mild Strains

The purpose of pilot tests is to further evaluate the mild strain under controlled field conditions. These tests are on a small scale and usually under complete control of the investigator. Normally, protected plants are prepared under controlled (greenhouse) conditions, then planted in an area with suitable environmental conditions for plant growth, an appropriate reservoir of severe-challenge isolates, and a favorable vector population. In some cases, pilot tests cannot be done in the area for proposed future use of cross protection because severe strains either are not yet present or are not widespread enough to create a suitable challenge.

PRV. In Hawaii, PRV is epidemic on the island of Oahu and is the major limiting factor for commercial papaya production on that island. PRV has not been found on the island of Hawaii in the Puna district, the state's major papaya production area. Thus, the nursery trials established on Oahu did not pose any economic danger to the papaya industry on the island of Hawaii.

Two main objectives were pursued in the Hawaiian nursery trials. First, we wanted to compare tree growth and fruit characteristics of healthy plants and those infected with the mild strain of the virus in the absence of severe virus infection. Trees of the major commercial cultivar infected with the mild strain grew well and fruit were of normal size, shape, and sugar content. A number of fruit from infected trees did have ring spots, however, but these became less obvious as the fruit matured and turned yellow. Second, we wanted to determine the effectiveness of the mild strain in protecting papaya against challenge inoculations by aphids under field conditions. A small trial was set up at the University of Hawaii experimental farm at Waimanalo. Rows of healthy plants and plants infected with the mild strain were established in an area adjacent to PRV-infected papaya plants. All uninoculated test plants showed severe symptoms of PRV, but cross-protected plants grew well. Limited space and manpower precluded further trials, but the results gave us impetus to pursue the cross-protection program with PRV.

CTV. The work on CTV is an example of pilot tests that were not done in the region (Florida) where cross protection might be used commercially. Although it was possible to do cross-protection nursery trials in Florida with endemic strains, we did not want to import more severe isolates or the efficient vector (*Toxoptera citricidus* Kirk.). Nevertheless, it was important to determine whether mild Florida isolates already widespread in commercial plantings would be effective against severe strains that could be introduced accidentally in the future. Establishing pilot tests in Hawaii provided a nearly ideal experimental condition. Previous observations had established that Hawaii has severe CTV strains similar to those observed in the Far East and Latin America and also has abundant populations of the vector, *T. citricidus*. Hawaii does not have a large commercial citrus industry, so importation of mild isolates from Florida posed little risk.

In initial CTV nursery trials in Hawaii, the mild strain from Florida protected against the much more severe Hawaiian strains. Protection did not last long under the severe challenge conditions in which the trial was done, however. These observations gave us the first evidence that cross protection with CTV would work best if used as one virus control component in an integrated crop management approach.

Field Evaluation of Mild Strains

Field trials should be set up so that cross-protection effectiveness of the mild strain can be determined under various disease pressures and under conditions that at least approximate those on commercial farms. The primary goal of these trials is to obtain a realistic assessment of the effectiveness and practicality of cross protection. If all tests are too severe and unrealistic, one may conclude that the mild strain is not effective, whereas a practical level of cross protection may be achieved if the mild strain is used under conditions of lower challenge inoculation pressure. Furthermore, one needs to evaluate the economic feasibility of the cross-protection program. After all, if cross protection is to be used as a practical control measure, it must be cost-effective.

Ideally, field trials should be done on a commercial farm and designed so that the research objectives can be investigated and quantitated while the plants are cared for by the farmer. Unfortunately, farmers usually do not want to undertake such risky experiments if they are not suffering catastrophic losses from the disease. Thus, this all-important step often is not carried out properly because of circumstances beyond the researcher's control.

PRV. Some of our recent work with PRV in Taiwan illustrates the basic points in designing and conducting field evaluation trials (15,17). Certain circumstances made Taiwan a good alternative site to Hawaii for field trials with PRV. PRV was discovered in Taiwan in 1974 and spread in epidemic proportions until it became the major limiting factor for papaya production. The government of Taiwan welcomed cross protection as a potential control method.

Before the field evaluation in Taiwan, efforts were made to develop an efficient mass inoculation procedure, to test the mild strains for their protective ability on Taiwan's major papaya cultivar, and to determine their effect on cucurbits widely grown in Taiwan. Mass inoculation of papaya seedlings by means of a high-pressure spray was successful. Also, the mild strains, HA 5-1 and 6-1, did not cause obvious damage to cucurbits. These mild strains gave good protection against Taiwan's PRV strains, although protection was more effective against the parental Hawaiian strain. The overall conclusion was that the mild strains were effective enough against PRV strains from Taiwan to warrant field evaluation trials.

The initial field trials for PRV control by cross protection were conducted in three different locations in the southern part of Taiwan where most of the papaya crop is grown. Two of the trials were in areas with high incidences of disease, at Feng-Shan and Kao-Shu. One plot was adjacent to an infected papaya orchard and the other was within 50 m of a papaya orchard. These two field trials had healthy rows of papaya alternating with rows of plants infected with the mild strain, or individual healthy plants interspersed randomly with plants infected with HA 5-1 or HA 6-1. The third trial, at Ta-Liao, was designed to simulate, to a degree, commercial conditions and was located in an area with a lower disease incidence. This trial involved a square block of about 400 plants divided into four equal quadrants. Two quadrants, situated diagonally from each other, consisted of healthy plants and two consisted of plants infected with HA 6-1. The block was bordered by sugarcane and rice paddies, and the nearest papaya plants were about 500 m away. The orchard was managed by the farmer, and data on cross-protection breakdown (i.e., test plants showing severe symptoms), fruit yield and quality, and income earned by the farmer were taken by research workers. All plots were established in November, the period when most Taiwanese farmers establish new papaya orchards.

Results from the Feng-Shan and Kao-Shu trials indicated that cross protection would not be economical in Taiwan under heavy disease pressure. About 80% of the unprotected plants showed severe disease reactions within 3–5 mo after being transplanted. Although the occurrence of severe infection was measurably delayed in protected plants, the delay was not long enough to give economic benefit. Two points seemed clear from these two trials. First, cross protection would not work under severe disease pressure with healthy plants interspersed among protected plants, i.e., it would not be economical for a farmer to plant cross-protected plants within or close to an infected orchard. Second, plants that show breakdown in cross protection before the flowering stage would generally have too few fruit for economic purposes.

The trial at Ta-Liao, in which solid blocks of protected plants were grown under lower disease pressure, showed much more promise. At 6 mo, more than 90% of the unprotected plants, but only 25% of the cross-protected plants, had severe infection. Also, the rate of breakdown of cross protection was much lower at the Ta-Liao trial than at the Feng-Shan and Kao-Shu trials. For example, the Feng-Shan and Kao-Shu trials showed a 50% cross-protection breakdown within 5 mo, whereas a 50% breakdown at the Ta-Liao trial took 10 mo. Of more importance, cross-protection breakdown at the time of flowering was about 15% at Ta-Liao and 70% at Feng-Shan and Kao-Shu. Severely infected plants at the Ta-Liao trial were rogued once during the initial flowering period to reduce disease pressure within the experimental field. Such a procedure had not been contemplated before the trials, but as the data came in on the first two trials, the practice seemed a prudent one to follow.

The Ta-Liao trial was economically beneficial to the farmer. Total fruit yield from the two cross-protected blocks was twice that of the unprotected blocks. Moreover, the weight of symptomless fruit and ring-spotted but not deformed fruit was almost 200-fold and twofold greater, respectively, than that of fruit from unprotected trees.

These examples of cross-protection tests for PRV in Taiwan show the importance of having balanced experiments. If only the trials at Feng-Shan and Kao-Shu had been done, we could easily have concluded that cross protection with strains HA 5-1 and 6-1 does not work in Taiwan. Furthermore, these trials did not simulate commercial practices (it is unlikely that farmers would interplant healthy and protected trees in the same orchard). The Ta-Liao trial more closely simulated commercial practice and also showed that PRV cross protection could work under conditions that minimized challenge inoculum pressure. The Ta-Liao trial also gave us indications that an "integrated" approach would be the most successful way to implement cross protection in

general for controlling PRV disease of papaya.

Subsequently, other cross-protection trials with HA 5-1 were done in Taiwan using 44,000 plants over 22 ha. Farms were selected and entire orchards of cross-protected or healthy plants were established. Plants were put out in May 1984, the beginning of the rainy season and the time when aphid populations are relatively low. From the standpoint of providing cross protection and minimizing inoculum pressure, this was an ideal time to plant papaya. The rate of appearance of severe disease symptoms was much lower in protected than in unprotected orchards. This lower rate of disease development probably was associated with lower numbers of aphids carrying severe strains of PRV in the cross-protected orchards. Establishing the plants in May was not entirely suitable, however, because fruit matured in December, the time of the year when fruit quality (sugar content) is lowest and growing conditions are poor because of cold, dry weather. Thus, most farmers are not willing to start orchards during May. The point here is that a cross-protection program also should consider horticultural and market aspects of the target crop.

Larger scale trials of cross protection (200,000 papaya seedlings) were started in Taiwan in the fall of 1984. Government workers raised the mild strain in a cucurbit (*Cucumis metuliferus* (Naud.) Mey.) and mass-inoculated papaya seedlings. Cross-protected plants were then distributed to selected farmers who were willing to follow guidelines put forth by the government. Data were taken primarily on disease incidence because detailed evaluations of these large field trials were difficult to do. The positive responses from farmers provided a strong impetus to the government of Taiwan to expand cross protection as a control measure of PRV.

Since 1983, the PRV cross-protection program has expanded in Taiwan, and about 800,000 cross-protected papaya seedlings were released in 1985. We therefore can regard the cross-protection program in Taiwan as being done on a commercial scale.

CTV. Field tests with mild isolates of CTV have been conducted in different ways. Most commonly, trees infected with the mild strain are planted in areas where severe strains are present and the efficient vector, *T. citricidus*, is abundant. Mild isolates from Florida have been established in field plots in Hawaii, Brazil, and South Africa, areas where challenge pressure is severe. In Hawaii, additional test sites have been selected where challenge pressure is expected to be low because of isolation or environmental conditions that limit natural spread of the aphid vector.

Field tests in Florida, where challenge pressure is thought to be much less severe, have also been established. Protected plants are exposed to natural challenge and also are challenged experimentally with aphids previously fed on endemic severely diseased plants.

Results of cross protection against tristeza (decline) are of two types. In areas with very severe challenge (such as Hawaii), protection has been noticeable but not of economic level. In areas with less severe challenge, protection apparently may occur in terms of reducing the total rate of decline, but individual trees will still decline when heavily challenged. There has not been adequate time to fully evaluate the extent of cross protection in the latter case, however.

Results against stem-pitting symptoms of CTV are more promising and clear-cut. Cross-protected trees have markedly outperformed unprotected trees. Cross protection does eventually break down under severe infection pressure, but these results can be used to predict the performance of mild strains in areas with less infection pressure. A rapid test can be performed under the most severe conditions and the results utilized for more typical and milder commercial conditions.

Integrating Cross Protection into Crop Management Systems

Our data on cross protection with PRV and CTV show that cross protection alone will not give a high level of control of the disease throughout the life of the crop. One problem is that in different field situations there may not be sufficient homology between mild and severe strains to achieve a high level of protection. Our information with PRV HA 5-1 and HA 6-1 strains indicates that cross protection holds up best with homologous strains (those from which the mild mutants were derived, as in Hawaii) and not as well with heterologous strains (such as those from Taiwan). We also have recently made similar observations with cross-protection trials in Thailand. The PRV cross protection being done in Florida and Mexico should provide more information on this aspect. A second problem is that challenge pressure may be too extreme in some situations to achieve economic protection. Furthermore, elimination of severe sources of inoculum and/or reduction of vector populations may not be feasible. Third, environmental conditions may adversely affect results of cross protection. Long periods of high temperature may cause at least localized thermotherapy of the mild strains and leave these areas of the plant more vulnerable to infection by challenge strains.

For these reasons, use of other virus management practices along with cross protection is often desirable (17,19). The type of crop management system used in relation to cross protection will naturally depend on the crop itself, on the characteristics of the virus, and on the environment in which the crop is being grown.

It is clear that the longer protected plants can be shielded from severe challenge, the more likely it is that long-term protection can be achieved. This shield period will vary with the length of the crop life. For example, it may be a matter of a few weeks for an annual crop, a few months for a medium-term crop like papaya, and several years for citrus. Accurate knowledge of disease epidemiology may permit adjustment of planting dates to avoid high vector populations in young plantings. For example, oil sprays to reduce vector transmission in young plants have been used for some nonpersistently transmitted viruses (19).

Isolation of the protected crop from severe sources of inoculum can greatly reduce challenge pressure. Isolation can be achieved in various ways and need not be complete to be useful. Windbreaks or positioning crops upwind from severe inoculum sources may reduce transmission by vectors. Roguing infected plants from nearby plantings or use of alternate crops to increase distance between protected crops and inoculum sources also are useful. Roguing of primary infections of severe strains within protected plantings may also reduce secondary spread.

Combining cross protection with virus-tolerant cultivars seems to be an excellent approach to virus control. Control of PRV by this approach is very promising. The fruit of currently available tolerant cultivars is horticulturally inferior to that of most commercial cultivars, however. This control strategy for PRV should become more widespread as the fruit quality of tolerant cultivars improves.

In summary, a number of management practices that delay infection or reduce virus disease severity in crops can be reexamined for potential integration with cross protection in a total disease management program. For example, planting entire orchards with protected plants is preferred over mixing healthy and protected trees. With papaya, all diseased trees should be removed from an orchard before protected trees are planted. Protective effects also may be extended by roguing severely infected plants that appear early in the life of the crop. This can greatly reduce losses caused by secondary spread within the orchard.

If cross protection has been used successfully in a location for several years, periodic planting of unprotected trees may be possible, since (presumably) populations of the mild strain may eventually replace the severe strain.

Producing and Applying Mild Virus Strains

Once cross-protective virus strains are isolated and an appropriate strategy developed to use them, a permanent source of contamination-free inoculum must be developed. Viruses that are readily transmitted mechanically can usually be stored for long periods as freeze-dried infected tissue. By preparing many aliquots, one can assure access to a consistent nonmutating source of inoculum. For viruses such as CTV that are not easily transmitted mechanically, the mild isolate is usually stored in a symptomless host that must be maintained free from contamination by isolation.

When large amounts of inoculum are needed, the primary source is increased in a suitable host plant, which then serves as the inoculum source to infect the plants to be protected. In some cases, the primary inoculum is used to infect a small number of plants that are used as an intermediate inoculum source to infect a larger number of plants serving as the final inoculum. This would be the case, for example, when thousands of papaya seedlings need to be inoculated in a short time. To avoid contamination, the number of transfers between the primary and the final inoculum source must be kept to a minimum. Furthermore, the primary inoculum source should again be used when a new generation of plants is to be protected. The important point is that one should use the primary inoculum as a base whenever possible and not rely on inoculum that has been transferred repeatedly. Lastly, the primary inoculum should be evaluated periodically to ensure that it gives the expected mild reaction, preferably on a host that will reveal possible contaminations.

Plants are usually inoculated with protecting viruses in one of two ways: 1) Plants that are grown from seed and are to be protected with a mechanically transmitted virus are usually inoculated as soon as possible after germination. In the case of PRV, young plants in seedling flats are spray-inoculated before transplanting. Infection should be verified either visually or by an indexing test such as ELISA. 2) Clonally propagated plants are normally protected by inoculating a healthy propagating source plant with the mild strain, then propagating from this plant after it becomes systemically infected. The South African Citrus Budwood Improvement Program illustrates the basic steps (14). Cultivars are selected for horticultural characteristics and are freed from existing CTV infection by shoot-tip grafting; the resulting plants are inoculated with a mild protecting isolate of CTV. A foundation planting of trees protected by the mild strain is maintained free from contamination and provides budwood for increase in commercial nurseries. Budwood from the increase nursery (which is maintained for only a short while) is used to bud-graft commercial trees. In this fashion, many uniformly cross-protected trees are produced from a single inoculation. In South Africa, consistent results are not achieved if growers or nurserymen propagate new trees directly from old commercial trees. The likely reason is that many of these trees are infected with severe forms of CTV and other viruses, even if they appear healthy. The key points in the inoculation process are that it be rapid, efficient, and economical.

Genetically Engineered Cross Protection

Recently, exciting experimental data have shown that transgenic plants expressing tobacco mosaic virus (TMV) coat protein are "cross protected" against challenge inoculation with TMV (11). The same phenomenon of engineered cross protection has also been observed for cucumber mosaic, potato virus X, and alfalfa mosaic viruses (3,5,7,13). Some obvious advantages of engineered cross protection over "classical" cross protection (the type we have been discussing) are that protected plants do not contain infectious virions, the protective effect (coat-protein expressing trait) is transferable to progenies, and the possibility of mild strains mutating to severe strains is eliminated in engineered cross protection.

The work with TMV has been extended to greenhouse and field conditions, and the results show great potential for the use of engineered cross protection to minimize losses caused by viruses under commercial conditions (10). Tomato plants expressing the coat protein gene to the U1 strain of TMV were highly resistant to the U1 strain and completely or partially resistant to the L, 2, and 2^2 strains of tomato mosaic virus (ToMV), a tobamovirus closely related to TMV. The latter two viral strains, 2 and 2^2, can overcome the TMV resistance in plants with the Tm-2 and Tm-2^2 genes, respectively. Tm-2 and Tm-2^2 are alleles of the TMV-resistant gene Tm-2, that is, tomato with the Tm-2 gene is susceptible to the ToMV-2 strain but resistant to the ToMV-2^2 strain, and vice versa. In one test, 20 and 60% of coat-protein expressing plants inoculated with ToMV-2^2 and ToMV-2 strains, respectively, produced symptoms 14 days after inoculation. In contrast, all of the susceptible control plants showed symptoms 7 days after inoculation by these strains. In field tests, fruit yield was not decreased in coat-protein expressing transgenic plants inoculated with the U1 strain, whereas similarly inoculated susceptible plants showed a 20% decrease in fruit yield. Furthermore, greenhouse tests showed that in the absence of ToMV, leaf and stem dry weight accumulations of transgenic plants were similar to those of nontransgenic plants.

The TMV data, at least, suggest that "genetically engineered" cross protection is somewhat analogous to classical cross protection in that protection is largely overcome at high inoculum doses of virions and inoculation by viral RNA. Also, the degree of effectiveness depends on the strain of challenge virus used. Thus, the approaches and techniques for using classical cross protection under practical conditions would be largely applicable to engineered cross protection.

Engineered cross protection holds much promise for controlling virus diseases. The near future will tell us more about the practicality of this approach as it is used with different viruses under various field and vector conditions. Thus, cross protection, both classical and engineered, should become a more widely used method for controlling viral diseases in the tropics and throughout the world.

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