Status of Coat Protein-Mediated Resistance and its Potential Application for Banana Viruses

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Introduction

The recent development of gene transfer technologies to plants has made it possible to transfer useful traits to a number of crop plants. Among the different possibilities, virus resistance is probably one of the most successful applications of plant genetic engineering. When used to complement current breeding programs, these technologies have the potential to help control plant viruses and consequently to decrease the impact of plant viruses on crop productivity. During the past 6 years different molecular approaches have been developed to attempt to control viruses, some of which are still under investigation in laboratories, while others have reached the field-testing level. This paper briefly reviews these different approaches, discussing the state of development, the efficacy, and the stability of each concept for controlling viruses by genetic engineering. Particular emphasis is placed on coat protein-mediated resistance (CPMR) because of the success of this strategy and because of the large resource of data and examples now available derived from the use of this technique. The type of resistance obtained and the spectrum of protection produced is exposed, and the examples of viruses belonging to virus groups the members of which are infecting bananas are particularly detailed in order to evaluate the potential of application of the coat protein strategy for this crop.

Multiple Strategies for Controlling Viruses

With the increase of the knowledge of virus replication and virus genome organization, scientists are testing different approaches for controlling virus infection. Different viral genes or sequences, inserted into the plant genome, interfere with the virus replication in a beneficial way for the infected plant.

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Ribozyme strategy

A novel approach to achieve virus resistance is the use of autocatalytic RNA-cleaving molecules, also called "ribozymes" (Cech 1986). Viroid RNAs such as avocado sunblotch viroid and satellite RNAs such as the satellite of tobacco ring spot virus (TobRSV) have the possibility of self-cleavage during replication (Buzayan et al. 1986; Forster, Symons 1987; Hutchins et al. 1986; Prody et al. 1986). Cleavage is effective both on the positive and negative strand of the RNA and is highly specific and associated with conserved sequence domains. Several studies have been conducted to determine the optimal in-vitro conditions of cleavage (Gerlach 1989; Haseloff, Gerlach 1988). Replicase genes of tobacco mosaic virus (TMV) and barley yellow dwarf virus (BYDV) encoding sequences bearing specific virus cleavage sites have been integrated in transgenic plants and should generate sequence-specific endonuclease activities (Gerlach 1989), but no in-vivo results have been published yet.

Translation strategy

By translation strategy we refer to the strategies involving integration in the plant genome of sequences generating complementary sequences to viral RNA that interfere with the translation of viral genes by hybridization of the coding sequence. It has been reported that synthesis of complementary RNA (antisense RNA) can decrease the accumulation of gene products in both procaryotes and eukaryotes (Ecker, Davis 1986; Green et al. 1986). It is likely that antisense RNAs anneal with sense RNAs to form a double-strand complex which is rapidly degradated or which inhibits translation of the RNA. Several viral coat protein antisense constructs including TMV, cucumber mosaic virus (CMV), and potato virus X (PVX) have been integrated into plants and tested for resistance to infection (Cuozzo et al. 1988; Hemenway et al. 1988; Powell et al. 1989). In all these cases resistance has been reported against infection by the homologous virus but only at low virus inoculum concentrations. Recently, a similar study done with the potato leafroll virus (PLRV) (Kawchuk et al. 1991) proved to induce resistance to the virus inoculation of the same level as the CPMR.

Replication strategy

One of the first steps of the virus cycle is replication of the viral genome and it seems logical that any approach to block this phase should be an efficient way to protect plants. Two approaches have been considered to block a virus infection, the antisense and the sense approaches.

Antisense approach of the replication strategy

This strategy attempts to block the replication of virus by hybridization of complementary sequences to the replicase viral gene or to sequences recognized by the replicase during replication. This strategy is at a preliminary stage of investigation but is presented because of promising in-vitro results and the potential that it may be applicable when other approaches fail. The complete antisense sequence of the replicase gene of the tomato golden mosaic virus (TGMV) was integrated into tobacco genome and several lines

were reported to exhibit a high level of resistance when challenged with different concentrations of TGMV (Lichtenstein, Buck 1990). This approach may be an interesting alternative but must be further tested before being considered as a useful strategy for practical purposes. The last example concerns turnip yellow mosaic virus (TYMV) where antisense sequences corresponding to the tRNA-like structure of the 3' extremity of the TYMV RNA can strongly inhibit replicase activity (Cellier et al. 1990).

Sense approach of the replication strategy

Competitor RNA. A study using sense sequences comprising the tRNA-like structure of TYMV (see above) in order to compete with the similar viral sequences and thereby decrease virus replication activity has been conducted. In-vitro studies have shown such competition (Morch et al. 1987) and in-vivo experiments show some level of resistance (Cellier et al. 1991). In contrast a similar approach used with the TMV seemed not to induce any resistance (Powell et al. 1990).

Subgenomic DNA. Some viruses produce subgenomic molecules during virus infection; for example several geminiviruses produce subgenomic DNA molecules of the B component. Insertion of one copy of such DNA of the African cassava mosaic virus (ACMV) into the tobacco genome reduced disease symptoms when the plants were challenged with ACMV (Stanley et al. 1990). Symptom amelioration is associated with a reduction in the level of viral DNA, including B DNA which is responsible for the symptomatology, and the resistance is specific to ACMV.

Satellite RNA. Another approach taken to confer protection against viruses is to cause the expression of virus satellite (Sat) RNAs. Sat RNAs are associated with several viruses and are dependent upon a helper virus for their replication and spread in the infected plant. It has been reported that the presence of Sat RNAs in cucumber mosaic virus (CMV)-infected tobacco reduces disease symptoms (Mossop, Francki 1979). Similarly, tobacco plants infected with a mixture of tobacco ring spot virus (TobRSV) and ToBRV Sat caused amelioration of symptoms (Gerlach et al. 1986). Transgenic plants that express these satellite sequences were shown to decrease symptoms and virus replication (Gerlach et al. 1987; Harrison et al. 1987; Jacquemond et al. 1988). However, when the plants expressing CMV Sat RNA are infected with a related but different cucumovirus, there is symptom amelioration but no decrease in virus replication. Recently, this strategy has been applied to tomato (Tien et al. 1990; Tousch et al. 1990), and proven to be efficient for the reduction of symptoms both in greenhouse and field experiments (Tien et al. 1990). Not all satellite sequences provide symptom attenuation but sometimes they can also cause necrosis; the sequences responsible for severe symptoms are reduced to a few nucleotides (Devic et al. 1990; Jaegle et al., 1993). There is a risk that amplifying a satellite in transgenic plants may result in some of the molecule reverting to a necrotic form, causing dramatic symptoms when naturally infected by the helper virus. This possibility will greatly limit the utilization of the Sat RNA strategy unless further studies can demonstrate a great stability of the system.

TMV replicase strategy. Lately, a new source of genetically engineered resistance has been identified involving the transformation of plants with nonstructural viral genes.

Tobacco plants transformed with the TMV 54 kDa gene, which is derived from a portion of the replicase complex, are immune to extremely high concentrations of TMV virions or RNA (up to 500 $\mu g\ mL^{-1}$) of the strain U_1 (Golemboski et al. 1990). This immunity is highly specific to the strain U_1, or a mutant YS1/1 of TMV and susceptible to the other strains, including the related U_2 strain of TMV. This approach is undertaken with several viruses and preliminary results with the expression of the entire replicase gene of the potato virus X are producing a high level of resistance into transgenic tobacco plants (Braun, pers. com.) and portions of the replicase gene of CMV as well (Anderson, pers. com.).

Status of the Coat Protein Strategy

Among the different strategies for controlling viruses by genetic engineering, the coat protein strategy is currently the most promising. Many examples have been published and the efficiency in terms of protection, stability, and specificity has been evaluated for several viruses. The type of resistance and the mechanisms of action of the CPMR have been investigated and much information is available. Finally, both laboratory experiments and field tests with different crops have been conducted and the first commercial use of this type of resistance is scheduled for 1995.

Definition, concept, and production of coat protein-mediated resistance (CPMR)

CPMR refers to the resistance to virus infection caused by expression of a coat protein (CP) gene in transgenic plants. The expression of a CP gene confers resistance to the virus from which the CP gene was derived. Resistance is associated to the expression of the CP gene, and is stably inherited to subsequent generations.

The CP strategy is the expression by the plant of the viral CP gene integrated into the plant genome. Construction of the chimaeric gene should include the selection of an appropriate transcriptional promoter to cause the expression of the CP gene at sufficient levels to produce disease resistance. Several different transcriptional promoters have been used and the promoter that has proven most effective is the CaMV 35S. This promoter leads to high levels of mRNA and protein in most of the plants in which it has been tested. Furthermore, an enhanced 35S promoter, e35S, which is produced by duplication of an upstream regulatory sequence (Kay et al. 1987), causes higher levels of gene expression and is now widely used. The coding region used for the gene is obtained by deriving double-stranded DNA from the virus genome. When necessary, specific mutations of the gene may be needed to increase the translation of any mRNA following the consensus sequence rules described by others (Kozak 1988). The third part of the chimaeric genes is a sequence to confer transcript termination and polyadenylation. Little evidence has been published to date to indicate that a specific 3' end is preferred in transgenic plants. The 3' ends used for most chimaeric genes expressed in plants have

been taken from the T-DNA region of the Ti plasmid (Nelson et al. 1988; Powell et al. 1986; Powell et al. 1990).

Assessing disease resistance involves inoculation with virus plants that express the CP gene (CP+) and those that do not (CP-). There is also increasing evidence that nontranslated messengers might interfere with virus replication and, consequently, transgenic plants expressing the CP messengers without CP production should be used as control. A precise estimation of the number of inserted copies of the chimaeric gene, the level of messenger expression, and the amount of CP produced are required for a valuable evaluation of the CPMR. A comparison in the numbers of sites of infection, the disease incidence, the development of disease symptoms, and the accumulation of virus are generally used to evaluate resistance.

In order to use populations of plants that are identical in age, growth rate, and size, R1 or successive generations of plants are used. Prior to the inoculation of seedlings with virus, the segregation of the introduced gene is determined generally by an immunological reaction to detect the CP or by following the expression of a gene that is co-introduced with the CP gene.

Efficacy of coat protein-mediated resistance

The efficacy of CPMR is demonstrated by the number of examples where resistance has been achieved, by the spectrum of specificity of protection, and also by the type of resistance achieved.

Specificity of coat protein-mediated resistance

Since 1986, the date of the first publication describing the coat-protein strategy (Powell et al. 1986), there have been a number of reports of CPMR involving a variety of different viruses and host plants (Beachy et al. 1990). A list of published and unpublished reports is presented in Table 1. It includes viruses belonging to 13 different virus groups and hosts that belong to dicotyledons and monocotyledons. Most of the examples are from nonenveloped ssRNA(+) viruses, but it seems that the morphology of the virus, the fact that the viral genome is divided or not, and the type of viral genome organization does not matter for the obtention of resistance. There are positive examples of CPMR for two groups of enveloped ssRNA(-) viruses: Tenuivirus and Tospovirus, but there is some question about the mode of action of the strategy because the CP mRNA could also act as antisense of the complementary strand of the CP. As for the DNA viruses, we have information only about geminiviruses where the CPMR has been achieved: the tomato yellow leaf curl virus from Thailand (TYLCV-Th) and the African cassava mosaic virus (ACMV); but in these cases the CP levels were extremely low and the protection limited (Rochester, pers. com.). There is only one example of the use of CPMR in monocotyledons where the CP expression is very high and the resistance to the virus evaluated by insect transmission is also very high (Hayakawa et al. 1991). It is anticipated that more examples of CPMR for different virus groups will be achieved in the very near future, which will provide substantial information about the strategy.

Spectrum of coat protein-mediated resistance

The best TMV CP(+) tobacco lines were inoculated with members of different virus groups including CMV, AlMV, PVX, and PVY (see Table 1), but there was no noticeable resistance against infection by any of the tested viruses (Anderson et al. 1989). Though there is no protection for viruses belonging to other virus groups, there is growing evidence that the CPMR has a large spectrum of specificity within the group and that a particular CP can provide resistance to more than the homologous virus.

A complete study on the spectrum of resistance of transgenic plants expressing a CP gene was carried out on tobamoviruses (Nejidat, Beachy 1990); CP(+) tobacco plant lines that expressed the U_1 TMV CP gene were inoculated with other tobamoviruses. Based upon comparisons of amino acid sequences of CPs (Gibbs 1986), tobamoviruses have degrees of relatedness to TMV ranging from 85 to 39%. Infection by TMV, ToMV, pepper mild mosaic virus (PMMV), and tomato mild green mosaic virus (TMGMV) were inhibited by 95-98%, *Odotonglossum* ring spot virus (ORSV) by 80-95%, ribgrass mosaic virus (RMV), and sunn hemp mosaic virus (SHMV) by 40-60% (Nejidat, Beachy 1990). Similarly, transgenic tomato plants expressing the CP of TMGMV were found resistant to TMV and ToMV (Sturtevant et al. 1991). On the basis of these studies it was concluded that viruses that are related to the CP of TMV by more than 60% are less able to infect the resistant lines than are less related tobamoviruses.

Results of experiments of CPMR in the Potyvirus group, particularly important since many economically important plant viruses belong to this virus group, demonstrated that expression of a particular CP gene can induce protection for several other potyviruses. The soybean mosaic virus (SMV) CP protected tobacco plants from infection by tobacco etch virus (TEV) and potato virus Y (PVY) (Stark, Beachy 1989). Transgenic tobacco plants expressing the CP of papaya ringspot virus (PRSV) were found resistant to PVY, PeMV and TEV (Ling et al. 1991). The CP gene sequences of these potyviruses are homologous to the level of 65%.

In the case of tobraviruses the heterologous protection is also effective for viruses having about 60% homology (van Dun, Bol 1988). For cucumoviruses it has been proven for several cases that the CPMR is extended to several strains among and across the two subgroups of the Cucumovirus group (see below).

Multiple manifestations of coat protein-mediated resistance

Resistance to inoculation. In each of the examples of CPMR described to date, resistance is manifested by several features. First, there is a reduction in the numbers of sites where infection occurs on inoculated leaves. Fewer starch lesions were produced after inoculation with PVX on CP(+) tobacco plants than on CP(-) plants (Hemenway et al. 1988), and there are fewer chlorotic lesions caused by TMV infection on tobacco plants that express the TMV CP gene than on those that did not (Powell et al. 1986). Likewise, the numbers of necrotic local lesions caused by TMV infection on CP(+) Xanthi *nc* tobacco local lesion were 95-98% lower than on CP(-) plants (Nelson et al. 1987). These experiments indicate that the expression of a CP gene causes a reduction in the number of sites where infection is established upon inoculation.

Table 1. Examples of coat protein-mediated resistance in transgenic plants.

Virus group	CP gene	Transgenic plant	Virus resistance	Reference
Tobamovirus	TMV	tobacco	TMV	(Powell et al. 1986)
	"	"	ToMV	(Nelson et al. 1988)
	"	"	PMMV	(Nejidat, Beachy 1990).
	"	"	TMGMV	"
	"	"	HRSV	"
	"	"	ORSV	"
	"	tomato	TMV	(Nelson et al. 1988)
	"	"	ToMV	"
	ToMV	tomato	ToMV	(Sanders et al. 1990)
	TMGMV	tobacco	TMGMV	(Sturtevant et al. 1991)
	"	tomato	TMV	"
	"	"	ToMV	"
Tobravirus	TRV	tobacco	TRV	(van Dun, Bol 1988)
	"	"	PEBV	"
Carlavirus	PVM	potato	PVM	(Wefels et al. 1990)
	PVS	tobacco	PVS	(MacKenzie, Tremaine 1990)
		potato	PVS	(MacKenzie et al. 1991)
Potexvirus	PVX	tobacco	PVX	(Hemenway et al. 1988)
	"	potato	PVX	(Lawson et al. 1990)
	CCMV	tobacco	CCMV	(Fauquet et al. 1991)
Potyvirus	PVY	potato	PVY	(Lawson et al. 1990)
	SMV	tobacco	PVY	(Stark, Beachy 1989)
	"	"	TEV	"
	ZYMV	tobacco	ZYMV	(Namba et al. 1990)
	WMV II	tobacco	WMV II	"
	PRSV	tobacco	PVY	(Ling et al. 1991)
	"	"	TEV	"
	"	"	PeMV	"
Furovirus	BNYVV	beet (protoplast)	BNYVV	(Kallerhoff et al. 1990)
AlMV group	AlMV	tobacco	AlMV	(Loesch-Fries et al. 1987)
	"	"	"	(Tumer et al. 1987)
	"	"	"	(van Dun et al. 1987)
	"	tomato	"	(Tumer et al. 1987)
Cucumovirus	CMV-D	tobacco	CMV-D	(Cuozzo et al. 1988)
	"	"	"	(Nakayama et al. 1990)
	"	tomato	CMV-D	(Cuozzo et al. 1988)
	CMV-C	tobacco	CMV-C	(Quemada et al. 1991)
			CMV-Chi	"
			CMV-WL	"
	CMV-WL	tobacco	CMV-WL	(Namba et al. 1991)
			CMV-C	"
			CMV-Chi	"
Ilarvirus	TSV	tobacco	TSV	(van Dun et al. 1988)
Luteovirus	PLRV	potato	PLRV	(Tumer et al. 1990)
	"	"	"	(Kawchuk et al. 1990)
Tenuivirus	RSV	rice	RSV	(Hayakawa et al. 1991)
Tospovirus	TSWV	tobacco	TSWV	(MacKenzie, Ellis 1992)
	"	"	"	(Gielen et al. 1991)

Abbreviations: AlMV, alfalfa mosaic virus; BNYVV, beet necrotic yellow vein virus; CCMV, cassava common mosaic virus; CMV, cucumber mosaic virus; ORSV, *Odotonglossum* ringspot virus; PEBV, pea early browning virus; PLRV, potato leafroll virus; PMMV, pepper mild mosaic virus; PRSV, papaya ringspot virus; PVM, potato virus M; PVS, potato virus S;.PVX, potato virus X; PVY, potato virus Y; RSV, rice stripe virus; SMV, soybean mosaic virus; TEV, tobacco etch virus; TMGMV, tobacco mild green mosaic virus; TMV, tobacco mosaic virus; ToMV, tomato mosaic virus; TRV, tobacco rattle virus; TSV, tobacco streak virus; TSWV, tomato spotted wilt virus; WMV II, water melon mosáic virus II; ZYMV, zucchini yellow mosaic virus.

Resistance to virus spread within the plant. The second manifestation of resistance of CP-engineered plants is a reduced rate of systemic disease development throughout the CP(+) plants. Thus, if inoculation results in infection on the inoculated leaves, the likelihood that the infection will become systemic is considerably lower in CP(+) plants than in CP(-) plants. Grafting studies in which a stem segment of a transgenic TMV (CP+) tobacco plant was inserted between the rootstock and apex of a wild-type tobacco, demonstrated that the (CP+) segment prevented the virus from moving to the upper part of the grafted plant. This experiment shows that the CP may play a role in the long-distance movement of the virus and, consequently, resistance has a component that affects systemic spread of the infection, at least in the TMV-tobacco system (Wisniewski et al. 1990).

Resistance to symptom expression. A third manifestation of resistance is a reduced rate of disease development on systemic hosts that are CP(+). In most of the examples of CPMR, CP(+) plant lines were less likely to develop systemic disease symptoms than those that were CP(-). Several plant lines that expressed the PVX CP gene did not become severely infected when inoculated with high levels of virus (Hemenway et al. 1988). Similar results were reported for CPMR against CMV (Cuozzo et al. 1988), TMV (Powell et al. 1986), PVY and TEV (Stark, Beachy 1989), and other viruses. On the contrary, in other cases the transgenic plants that are becoming infected showed similar symptoms to those of the control plants as, for example, the CMV in transgenic tobacco plants (Quemada et al. 1991) and the cassava common mosaic virus (CCMV) in transgenic *Nicotiana benthamiana* (Fauquet et al. 1991). In one transgenic line of tobacco with CMV the plants were asymptomatic but showed a normal level of virus replication (Quemada et al. 1991), suggesting that the CP might play a direct role in the suppression of symptoms in addition or not to suppression of virus replication.

Resistance to virus multiplication. Another manifestation of resistance is lower accumulation of virus in CP(+) compared with CP(-) plant lines. Elisa and semi-quantitative western blots have been used to quantify virus accumulation in inoculated leaves and other plant parts in most examples of CPMR (Cuozzo et al. 1988; Hemenway et al. 1988; Lawson et al. 1990; Nelson et al. 1987; Powell et al. 1986). In certain examples of resistance, plants accumulate no virus after inoculation (Hemenway et al. 1988; Lawson et al. 1990), and can therefore be considered to be immune to infection under the conditions of the tests. In other cases, the virus accumulation in the infected transgenic plants is normal and comparable to control plants (Fauquet et al. 1991; Quemada et al. 1991).

All resistance manifestations of CPMR can usually, but not always, be overcome by inoculating with relatively high concentrations of virus. A virus concentration of 10 μg mL^{-1} of TMV nearly breaks the CPMR to TMV in a system where 0.01 μg mL^{-1} causes disease in CP(-) plants (Powell et al. 1986). Fifty μg mL^{-1} are needed to overcome CP resistance to AlMV, PVX, PVY and TEV (see Table 1) (Hemenway et al. 1988; Lawson et al. 1990; Stark, Beachy 1989; Tumer et al. 1987) and 100 μg mL^{-1} of CCMV are required to break the CPMR in tobacco plants (Fauquet et al. 1991). Resistance is largely overcome by inoculation with RNA rather than virions in many cases except the PVX CP(+) lines of

tobacco and potato (Hemenway et al. 1988; Lawson et al. 1990), and the CCMV lines of tobacco (Fauquet et al. 1991); this feature, shared by two members of the potexvirus group may reflect a property specific to viruses of this group.

The majority of evaluation of CPMR has been assessed by mechanical inoculation of the virus and, of course, the most important criterion for virus resistance is its evaluation in natural modes of contamination, i.e., in vegetative propagation and with the natural vectors. Information related to these points is limited but significant. In the case of the dually engineered resistance against PVX and PVY in potatoes (Lawson et al. 1990), it has not been possible to recover in the potato tubers any of these two viruses. At least one line of potato showed a good level of resistance through aphid inoculation of PVY. PLRV, a member of the Luteovirus group, is nonmechanically transmissible and the CP-engineered potato plants have all been challenged by using aphid inoculation (Kawchuk et al. 1990; Tumer et al., 1991), and demonstrated some degree of resistance. CMV CP(+) transgenic tobacco plants have been challenged with viruliferous aphids and proven to be resistant as for mechanical inoculations (Quemada et al. 1991).

Stability of coat protein-mediated resistance

CPMR is, in most cases, monogenic and is inherited by the following generations, like any other genetic trait. Consequently the genetic stability of this resistance gene is expected to be the same as any other gene. The biological stability of such resistance can be questioned, but no answer can be provided until it is used in natural conditions. One may argue that a single-point mutation can change a vital amino acid in the expressed coat protein and consequently alter the resistance, but the probability will not be greater than for any other monogenic resistance gene. Furthermore, we know that CPMR is effective for viruses differing in up to 40% in their CP sequences. In order to test the stability of the system, TMV CP(+) lines of tomato have been inoculated successively 15 times with the same isolate of virus, with the idea of selecting TMV molecules able to overcome the protection; however, no resistance breaking strain has yet been identified (White and Beachy, pers. com.).

Field experiments with coat protein-mediated resistant plants

There have been several field tests of virus-engineered resistant plants. Tomato plants expressing the TMV and ToMV CP genes have been tested in the field for several years, and potato plants expressing the PVX and PVY CP genes have also been tested in the field.

Tobacco plants that expressed the CP gene of AlMV were field-tested in Wisconsin in 1988 (Krahn, pers. com.). CP(+) plants developed disease more slowly or not at all compared with CP(-) plants; symptom development was correlated with virus accumulation. At 85 days after inoculation only 9% of the CP(+) plants had developed a systemic infection, while 93% of the CP(-) plants had systemic infections.

The first field test with TMV-resistant tomato plants, from cultivar VF36, was conducted in 1987 (Nelson et al. 1988). CP(+) plants, mechanically inoculated with TMV, exhibited a delay in the development of disease symptoms or did not develop symptoms compared with the CP(-) plants. No more than 5% of the CP(+) plants developed disease

symptoms by fruit harvest, while 99% of the VF36 plants developed symptoms. Lack of visual symptoms was associated with a lack of virus accumulation. Fruit yields of the infected VF36 plants decreased 26-35% compared with healthy plants, whereas yields from the CP(+) line were equal to those of uninoculated VF36 plants.

To determine if the TMV CP gene conferred protection against infection by field isolates of ToMV, tests were conducted in 1988 in Florida and Illinois. Progeny that were homozygous for the TMV CP gene and VF36 CP(-) plants were challenged with a Florida isolate of ToMV, Naples C, in Florida. The field test in Illinois was conducted to determine if expression of the TMV CP gene in tomato would protect it against a number of different strains of TMV and ToMV. The TMV CP gene conferred resistance against ToMV-Naples C infection under Florida field conditions and against two strains of TMV under Illinois field conditions. Only weak protection was conferred against infection by the ToMV strains under Illinois field conditions (Sanders et al. 1990).

To enhance protection against ToMV, plants were produced that expressed a CP gene derived from ToMV-Naples C. These lines were evaluated under field conditions in Illinois along with the control tomato line UC82B, with lines expressing the TMV CP gene, and with lines expressing both TMV and ToMV CP genes. The TMV CP(+) lines were resistant to TMV infection, as shown in the earlier field test; however, they were less resistant to infection by ToMV-Naples C. The tomato lines expressing ToMV CP gene were highly resistant to infection by ToMV-Naples C. Plants that expressed both TMV and ToMV CP genes were equally well protected against TMV and ToMV.

Field tests were conducted with Russet Burbank potato plants expressing the CP genes of PVX and PVY (Lawson et al. 1990). PVY causes significant yield depression in potato and, in combination with PVX, PVY produces a severe disease called "rugose mosaic". To determine if expression of PVX and PVY CP genes would protect potato plants from the synergistic effects of PVX and PVY infection in the field, plants propagated from CP(-) Russet Burbank and from plant lines expressing both CP genes were inoculated with both PVX and PVY and transplanted into the field (Kaniewski et al. 1990). Plants from four CP(+) lines were significantly protected from infection by PVX. However, three of the lines were not protected from infection by PVY when simultaneously inoculated by both viruses. Plants of one line, however, were highly resistant to PVX and PVY, as predicted from growth-chamber tests. Tuber yields at maturity in uninoculated plots were the same for all the lines. In contrast, tuber yields of all inoculated lines were markedly reduced, except the line resistant to both viruses, which was unaffected by virus inoculation.

For the last 3 years, field trials of cucurbits transformed with the CMV CP gene have been conducted with success (Gonzalves et al. 1991). In 1991, four transgenic lines of the cv Poinsett 76 were compared with resistant cv Marketmore 76; infection was allowed to occur naturally by aphids using a low percentage of infected plants as initial virus sources. After 4 months of growth, the transgenic plants performed much better than the control plants; 8% of transgenic plants showed symptoms versus 98% for the control and 8% for the resistant line.

Potential Use of Coat Protein-Mediated Resistance for Banana Viruses

The choice of a particular strategy for controlling plant viruses by genetic engineering depends greatly on the types of virus chosen as a target; but, because of the apparent universality of the efficacy of CPMR and because of the strength of the resistance, coat protein strategy should be first attempted.

Viruses infecting bananas

Viruses infecting banana are limited in number and still insufficiently described; however, the following have been identified :

Banana bunchy top DNA-virus (Dietzgen, Thomas 1991; Harding et al. 1991; Thomas, Dietzgen 1991; Wu, Su 1990). This virus has been isolated only in Australia, and it is not known whether it is the causal agent of the banana bunchy top disease or a helper of the banana bunchy top luteovirus. This virus with a genome of multipartite single-stranded DNA most probably belongs to a new group of viruses. The genome comprises a large number (seven or more) of monogenic components. It is somewhat similar to the geminiviruses for which CPMR so far has not been extremely effective and, if the CP strategy was poorly efficient with this virus, another strategy, like the sense or antisense strategy for the replicase gene, should be considered.

Banana bunchy top luteovirus (Brunt et al. 1990). Luteoviruses are single-stranded RNA+ viruses with a monopartite genome. With the example of the PLRV, we know that CPMR is an efficient strategy to create resistance by biotechnology, provided that the CP is engineered and the gene expression increased. Resistance to the virus through insect-vector inoculation has been demonstrated in potato and tobacco, and should therefore be effective in banana with appropriate chimaeric gene constructs. If banana bunchy top disease results from the effect of two viruses, the usefulness of CPMR would have to be investigated using one or the other CP or a combination of the two CPs.

Cucumber mosaic cucumovirus (Brunt et al. 1990). This virus is one of the most commonly used for CPMR and we know that the CP strategy is extremely effective with a wide spectrum of protection (see below). In any project of banana transformation to control viruses through biotechnology, the CMV CP gene should be used because it is an important viral disease of banana and because it can be an internal marker for other viral genes. We know that if CPMR cannot be achieved with CMV, the unique banana environment should be taken into account to improve gene expression and efficacy of CPMR.

Banana bract mosaic potyvirus (Dale, pers. com.). This virus has been recently discovered in the Philippines where it is causing a lot of damage. There are many examples of CPMR for such viruses and the strategy could be applied directly to banana with the appropriate cloning of the CP gene for this potyvirus. There are also indications that different potyviruses in other places in the world infect banana: proper identification is needed, but, with the wide spectrum of protection effected by CPMR, an overall protection against banana potyviruses using one CP could be envisaged.

Banana streak badnavirus (Lockhart 1990). Badnaviruses are double-stranded DNA viruses for which we have not achieved any examples of CPMR. Studies with a virus of the same group are under way with the rice tungro bacilliform virus, and should provide valuable information to extrapolate to the banana streak virus. This virus does not seem to be very widespread in the world and, therefore, is not an important economic target.

Summary of coat protein-mediated resistance for PLRV

The CPMR for PLRV has been achieved by two different groups which obtained similar results (Kawchuk et al. 1991; Tumer et al. 1991). The CP of the PLRV is expressed at a very low level, at the limit of the detection (0.01%), but resistance to the virus replication was nevertheless effective. The transgenic plants were resistant to inoculation by the natural aphid vector, even in the case where large numbers of insects were used. There was no relation between the amount of CP detected in the transgenic lines and the level of resistance estimated in the same lines. Field trials have been successfully conducted, but the spectrum of specificity remains to be established. The CP expression has been improved by sequence protein engineering, removing the internal ORF, resynthesizing the CP coding sequence, and by expressing two chimaeric genes in the same transgenic plant. In these conditions up to 9% of the regenerated lines were highly resistant or immune (Cuozzo et al. 1988; Tumer et al. 1991). Furthermore, it has been proven that the CP antisense strategy is also effective against PLRV, providing an alternative to the CPMR (Kawchuk et al. 1991).

Summary of coat protein-mediated resistance for CMV

CPMR for CMV has been achieved by three different groups which obtained similar results in different plants (Ye et al. 1991; Cuozzo et al. 1988; Beachy, pers. com.). The CP of CMV was expressed at a very high level (0.6%), and resistance to the virus multiplication was effective. The transgenic plants were resistant to mechanical inoculation and/or inoculation with the natural aphid vector. There was no direct relation between the amount of CP detected in the transgenic lines and the level of resistance estimated in the same lines, though the highest expressors were among the most resistant lines, and no line was highly resistant without any CMV CP. Field trials of transgenic cucumbers have been conducted successfully for 3 years, where the best transgenic lines had less than 10% of contaminated plants after 4 months of survey. Studies have been conducted to evaluate the spectrum of specificity of the resistance, and it was shown that the plants were resistant to strains within one subgroup of CMV strains and across the two subgroups of the cucumovirus group.

Conclusion

The last few years have provided a profusion of techniques to control viruses by genetic engineering. Most have demonstrated potential for conferring resistance to plants and constitute future hopes for crop improvement. Several techniques have already been

applied in field experiments and have proven to be real sources of resistance to viruses, e.g., the satellite and the coat protein strategies. The ribozyme strategy is interesting because of its potential applicability to all viruses, and because of its high specificity, but it needs to be demonstrated under in-vivo conditions. The satellite strategy, though very effective, is strictly limited to viruses having satellites, which are very few in the plant virus world, and its use is limited by the risk that mutations would accompany widespread use.

CPMR for controlling viruses is the safest, most efficient, and widely documented type of engineered resistance. Several field experiments have been conducted with different crops and CPMR was shown effective in all cases. The spectrum of specificity of CPMR is broader than in any other type of virus resistance (including natural resistance), because plants can be protected against viruses of the same group sharing up to 60% in their coat protein sequence. Coat protein transgenic plants are resistant to high concentrations of virus inoculum, and even in some cases to RNA inoculum. Resistance against vector inoculations and vegetative propagation have also been demonstrated. The resistance generated by the coat protein strategy is of a multiple type, reducing the number of infection sites, the symptoms, the virus multiplication and the long-distance spread through plants. It seems that the banana is an excellent candidate for using biotechnology to control viruses. And because there is no natural source of resistance for any banana virus, because it is a vegetatively propagated crop, and because CPMR has been used to describe most of the viruses of banana described to date, CPMR is applicable and should be useful.

All the manifestations of CPMR can be the result of a single mechanism or of multiple effects of the coat protein on different targets. Nevertheless, despite the number of studies conducted to understand the mechanisms of the action of the coat protein strategy, the question of how the coat protein confers resistance to engineered plants remains unanswered.

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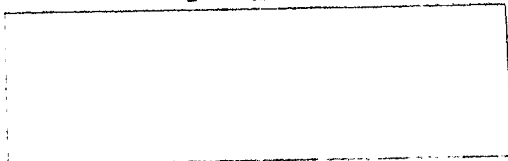

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