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Virus-engineered resistance: Concepts, efficacy, and stability

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Abstract

This paper reviews four strategies being used in research on virus resistance through genetic engineering in terms of their concepts, efficacy, and stability. The most recent strategy is the use of ribozymes, which involves using catalytic RNAs to cause the cleavage of viral RNA molecules. Reports of in vitro experiments have documented cleavage of viral RNAs but there are no reports of in vivo experiments. The second strategy involves an attempt to block the translation of viral RNAs by expressing genes that encode sequences complementary to viral genes. Only a few cases have been reported, and little success achieved. The third strategy is to block virus replication, including the use of competitor sequences, subgenomic sequences, and the expression of satellite RNA. This strategy has been applied with some success in the field. The fourth and most promising strategy involves integrating a gene encoding viral coat protein (CP) into the plant genome. An increasing number of examples of the use of this strategy have been reported, and resistance specificity and efficacy have been evaluated for viruses belonging to 10 groups; furthermore, several successful field experiments have been conducted. The paper summarizes CP-mediated resistance and puts forward hypotheses on the possible mechanisms of resistance.

The recent development of gene transfer technologies has made it possible to transfer useful traits to a number of crop plants. Among the various possibilities, conferring of resistance to viruses has probably been the most successful application of plant genetic engineering. When used to complement current breeding programmes, these technologies can help control plant viruses and reduce their impact on crop productivity. Over the past 5 years, a variety of molecular strategies have been used in attempts to control viruses. Some of these are still under investigation in laboratories; others have reached the field testing stage.

This paper reviews these various strategies, discussing the state of development, the efficacy, and the stability of each of them. Particular emphasis is placed on coat protein (CP) mediated resistance because of the success of this strategy and the large amount of data and examples now available.

Ribozyme Strategy

A new approach to achieving virus resistance is the use of autocatalytic RNA cleaving molecules, known as 'ribozymes' (Cech 1986; Kim and Cech 1987). In viroid RNAs, such as avocado sunblotch viroid, and satellite RNAs, such as the satellite of tobacco ring spot virus (TobRSV), there is the possibility of self-cleavage during replication (Buzayan et al. 1986; Hutchins et al. 1986; Prody et al. 1986; Forster and Symons 1987). The sites of cleavage are intramolecular and presumably occur when the RNA molecule is in the correct configuration, thereby activating the cleavage reactions. Cleavage is effective on the positive and negative strand of the RNA; it is highly specific and is associated with conserved sequence domains. Several studies have been conducted to determine the optimal in vitro conditions of cleavage (Haseloff and Gerlach 1988; Gerlach 1989). Genes encoding sequences bearing specific virus cleavage sites have been integrated into transgenic plants and should generate sequence specific endonuclease activities. Constructs have been made to inactivate various viruses, including tobacco mosaic virus (TMV) and barley yellow dwarf virus (BYDV) (Gerlach 1989) but, to date, no in vivo results have been published.

Translation Strategy

Translation strategy involves the integration into 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 reduce the accumulation of gene products in both procaryotes and eukaryotes (Ecker and Davis 1986; Green et al. 1986; Rothstein et al. 1987). It is likely that antisense RNAs anneal with sense RNAs to form a double strand complex, which is rapidly degraded or which inhibits translation of the RNA. Several viral CP antisense constructs, including TMV, cucumber mosaic virus (CuMV), and potato virus X (PVX), have been integrated into plants and the plants tested for resistance to infection (Cuozzo et al. 1988; Hemenway et al. 1988; Powell et al. 1989). In all cases, resistance has been reported against infection by the homologous virus but only at low virus inoculum concentrations. In addition to CP antisense sequences, other genes have been tested with the antisense strategy, but to date little or no resistance to virus infection has been reported (Beachy et al. 1987; Rezaian et al. 1988).

Replication Strategy

One of the first steps in virus multiplication is the replication of the viral genome and it seems logical that blocking this phase should be an efficient way to protect plants. By

replication strategy we mean the blockage of the replication of the virus. Two approaches to blocking a virus infection have been considered, the antisense and the sense approaches.

Antisense approach

The replication strategy using the antisense approach attempts to block the replication of a 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 very preliminary stage of investigation but is presented because of promising in vitro results and the possibility that it may be applicable when other approaches fail. In transient assays with protoplasts of wheat, the antisense sequence of the first 250 nucleotides of the replicase gene of the geminivirus wheat dwarf mosaic virus (WDMV) completely inhibited virus replication (Gronenborg 1990). A second example involved the geminivirus tomato golden mosaic virus (TGMV). The complete antisense sequence of the replicase gene of the TGMV was integrated into the tobacco genome and several lines were reported to exhibit a level of resistance when challenged with varying concentrations of TGMV (Lichtenstein and Buck 1990). This approach is an interesting alternative but must be further tested before it can be considered as a useful and practical strategy. A final example concerns turnip yellow mosaic virus (TYMV), where antisense sequences corresponding to the tRNA-like structure of the 3' extremity of the TYMV RNA have been shown to strongly inhibit replicase activity (Cellier et al. 1990). Transgenic plants that produce such sequences are under evaluation.

Sense approach

The second approach to reduce replication involves the expression of "sense" viral sequences.

Competitor RNA

Sense sequences comprising the above-mentioned tRNA-like structure of TYMV have been used to compete with similar viral sequences and thus reduce virus replication activity. In vitro studies have demonstrated such competition (Morch et al. 1987; Cellier et al. 1990;) and in vivo experiments are currently in progress to confirm these results. In contrast, a similar approach used with 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 (Frischmuth et al. 1990; Stanley et al. 1990). Symptom amelioration is associated with a general reduction in the level of viral DNA, including B DNA which is responsible for symptoms, while the

subgenomic DNA is specifically amplified. This type of resistance is specific to ACMV; other geminiviruses are unable to amplify the subgenomic of ACMV.

Satellite RNA

Another approach to conferring protection against viruses is to induce 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 CuMV-infected tobacco reduces disease symptoms (Mossop and Francki 1979; Collmer et al. 1983). Similarly, in tobacco plants, infection with a mixture of TobRSV and SAT TobRSV led to an amelioration of symptoms (Gerlach et al. 1986). Transgenic plants that express these satellite sequences were tested for disease development and viral replication with the corresponding virus. Amelioration of symptoms and reduced virus replication was the result in both cases (Gerlach et al. 1987; Harrison et al. 1987; Jacquemond et al. 1988). However, when the CuMV/SAT RNA expressing plants are infected with a related but different cucumovirus, there is symptom amelioration but no reduction in virus replication.

For both systems, some systemically infected plants developed symptoms although they were less severe than those in the control plants. Recently, this strategy has been applied to tomato (Tien et al. 1990; Tousch et al. 1990), and has proved to be efficient for the reduction of symptoms in both greenhouse and field experiments (Tien et al. 1990). Not all satellite sequences provide symptom attenuation and sometimes they can cause necrosis; the sequences responsible for severe symptoms are reduced to a few nucleotides (Devic et al. 1990; Jaegle et al. 1990). 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 use of the SAT RNA strategy unless further studies demonstrate a high degree of stability in the system.

TMV replicase strategy

Recently, a new source of genetically engineered resistance has been reported, involving the transformation of plants with non-structural 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 µg/mL) of the strain U1 (Golemboski et al. 1990). This immunity is extremely specific to the strain U1, or a mutant YS1/1, of TMV and susceptible to the other strains, including the related U2 strain.

Coat Protein Strategy

Among the different strategies for controlling viruses by genetic engineering, the CP strategy currently appears to be the most promising. Many examples have been published and a number of experiments are in progress. Efficiency in terms of protection, stability, and specificity has been evaluated for several cases. The type of resistance and the mechanisms of action of the CP-mediated resistance have been investigated and there is now a large amount of information available. Laboratory experiments and field tests with various crops have been carried out and the first commercial use of this type of resistance has been forecast for 1995.

Definition, concept, and production of coat protein-mediated resistance

CP-mediated resistance refers to the resistance to virus infection caused by the expression of a CP gene in transgenic plants. The expression of a CP gene confers resistance to the virus from which the CP gene was derived and to related viruses. Resistance is not due to somaclonal variation caused by the transformation event or the tissue culture procedure, and it is stably inherited by subsequent generations.

The principle of 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 induce the expression of the CP gene at sufficient levels and with sufficient tissue specificity to produce disease resistance. Several different transcriptional promoters have been used and the one that has proved 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, pE35S, which is produced by duplication of an upstream regulatory sequence (Kay et al. 1987) causes even higher levels of gene expression. 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 used to increase the translation of any mRNA, following the consensus sequence rules described by others (Kozak 1988). These and other changes can increase the amount of CP produced in transgenic plants. 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 preferable 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 (Powell et al. 1986, 1990; Nelson et al. 1988).

Assessing disease resistance involves the inoculation of plants with viruses that express the CP gene (CP+) and those that do not (CP-). A comparison of numbers of sites of infection, disease incidence, development of disease symptoms, and accumulation of virus is 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 inoculating seedlings with a virus, the segregation of the introduced gene is usually determined by an immunological reaction to detect the CP or by following the expression of a gene that is introduced with the CP gene.

Efficacy of coat protein-mediated resistance

The efficacy of CP-mediated resistance is indicated by the number of examples where resistance has been achieved using this technique, by the spectrum of specificity of protection, and by the type of resistance achieved.

Specificity of resistance

Since 1986, the date of the first publication describing the CP strategy (Powell et al. 1986), there have been reports of CP-mediated resistance involving a variety of viruses and host plants. A list of published and unpublished reports is presented in Table 1. It includes viruses belonging to 10 virus groups and hosts that include members of the Chenopodiaceae, Leguminosae, and Solanaceae. A detailed review of CP-mediated resistance has been recently published (Beachy et al. 1990).

Spectrum of resistance

A study on the spectrum of resistance of transgenic plants expressing a CP gene was carried out on tobamoviruses (Anderson et al. 1989; Nejidat and Beachy 1990). In these studies, CP(+) tobacco lines that expressed the U1 TMV CP gene were inoculated with other tobamoviruses. Based upon comparisons of amino acid sequences and/or amino acid composition of virus 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) was inhibited by 95-98%, by 80-95% for ondontoglossum ringspot virus (ORSV), and by 40-60% for RMV and sunnhemp mosaic virus (SHMV) Nejidat and Beachy 1990). On the basis of these studies, it was concluded that viruses that are related to TMV on the basis of CP by greater than 50-60% are less able to infect resistant lines than are less closely related tobamoviruses. TMV CP(+) plant lines were also inoculated with members of different virus groups including CuMV, alfalfa mosaic virus (AlMV), PVX, and potato virus Y (PVY). There was no resistance against infection by any of the viruses on inoculated leaves of CP(+) plants; however, there were somewhat reduced rates of systemic spread of CuMV, PVX, and PVY in CP(+) compared with CP(-) plants (Anderson et al. 1989).

Experiments on CP protection against potyviruses, which are particularly important because many economically important plant viruses belong to this virus group, have shown that expression of the CP gene of soybean mosaic virus protected tobacco plants from infection by tobacco etch virus (TEV) and potato virus Y (PVY) (Stark and Beachy 1989). The CP gene sequences of these potyviruses are about 55-60% homologous. In the case of tobraviruses, heterologous protection is also effective for viruses having about 60% homology (van Dun and Bol 1988). The specificity of CP-mediated resistance is restricted to members of the same virus group and within a group. Results to date suggest the need for more than 60% amino acid sequence homology of CPs to result in heterologous protection in several of the virus groups tested.

Multiple manifestations of resistance

Resistance to inoculation

In each of the examples of CP-mediated resistance described above, resistance was manifested in several features. First, there was a reduction in the numbers of sites of infection on inoculated leaves. Fewer starch lesions were produced after inoculation with

Table 1 Examples of coat protein (CP) 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 and Beachy 1990
	"	"	TMGMV	"
	"	"	ORSV	"
	"	tomato	TMV	Nelson et al. 1988
	"	"	ToMV	"
	ToMV	tomato	ToMV	Sanders et al. (in press)
Tobravirus	TRV	tobacco	TRV	van Dun and Bol 1988
	"	"	PEBV	"
Carlavirus	PVM	potato	PVM	Wefels et al. 1990
	PVS	tobacco	PVS	McKenzie and Tremaine 1990
Potexvirus	PVX	tobacco	PVX	Hemenway et al. 1988
	"	potato	PVX	Lawson et al. 1990
Potyvirus	PVY	potato	PVY	"
	SMV	tobacco	PVY	Stark and Beachy 1989
	"	"	TEV	"
	ZYMV	tobacco	ZYMV	Namba et al. 1990
	WMV II	tobacco	WMV II	"
	PRSV	tobacco	PRSV	Ling et al. 1990
	"	"	TEV	"
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
	"	alfalfa	"	Hill et al. 1991
Cucumovirus	CuMV	tobacco	CuMV	Cuozzo et al. 1988
	"	"	"	Nakayama et al. 1990
	"	tomato	CuMV	Cuozzo et al. 1988
Ilarvirus	TSV	tobacco	TSV	van Dun et al. 1988
Luteovirus	PLRV	potato	PLRV	Tumer et al. 1990
	"	"	"	Kawchuk et al. 1990

Note: a AlMV = alfalfa mosaic virus; BNYVV = beet necrotic yellow vein virus; CuMV = cucumber mosaic virus; ORSV = ondontoglossum 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; 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; WMV II = watermelon mosaic virus II; ZYMV = zucchini yellow mosaic virus.

PVX on CP(+) tobacco plants than on CP(-) plants (Hemenway et al. 1988), and there were fewer chlorotic lesions caused by TMV infection on tobacco plants that expressed the TMV CP gene than on those that did not (Powell et al. 1986). Similarly, the numbers of necrotic

local lesions caused by TMV infection on CP(+) Xanthi *nc* tobacco local lesion were between 95 and 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.

Resistance to virus spread within the plant

The second manifestation of resistance in 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 tobacco, have demonstrated that the CP(+) segment prevented the virus from moving to the upper part of the grafted plant. Thus CP may play a role in the long distance movement of a 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 all examples of CP(-) mediated resistance, 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 systemically infected when inoculated with high levels of virus (Hemenway et al. 1988). Similar results have been reported for CP-mediated resistance against CuMV (Cuozzo et al. 1988), TMV (Powell et al. 1986), and other viruses.

Resistance to virus multiplication

A last manifestation of resistance is lower accumulation of virus in CP(+) compared with CP(-) plant lines. The enzyme-linked immunosorbent assay (ELISA) and semi-quantitative western or immuno dot-blots have been used to quantify virus accumulation in inoculated leaves and other plant parts in most examples of CP-mediated resistance (Powell et al. 1986; Nelson et al. 1987; Cuozzo et al. 1988; Hemenway et al. 1988; Lawson et al. 1990). In some cases of resistance, plants accumulated little or no virus after inoculation (Hemenway et al. 1988; Lawson et al. 1990), and could be considered to be immune to infection under the conditions of the tests.

Manifestations of CP-mediated resistance can usually, but not always, be overcome by inoculating with relatively high concentrations of virus. A virus concentration of 10 µg/mL of TMV almost breaks the CP-mediated resistance to TMV in a system where 0.01 µg/mL causes disease in CP plants (Powell et al. 1986); 50 µg/mL is needed to overcome CP resistance to AlMV, PVX, PVY, and TEV (Tumer et al. 1987; Hemenway et al. 1988; Stark and Beachy 1989; Lawson et al. 1990). In many cases, resistance is largely overcome by inoculation with RNA rather than virions, with the exception of PVX CP(+) lines of tobacco and potato (Hemenway et al. 1988; Lawson et al. 1990).

Most CP-mediated resistance has been assessed by mechanical inoculation with the virus, but the most important criterion for virus resistance is its effectiveness under natural modes of contamination (that is, in vegetative propagation) and via natural vectors. Information regarding these points is limited but significant. In the case of dually engineered resistance against PVX and PVY in potato (Lawson et al. 1990), it has not been possible to recover either of the viruses in the tubers. At least one line of potato has shown a good level of resistance against aphid inoculation of PVY. Potato leafroll virus (PLRV), a member of the luteovirus group, is non-mechanically transmissible and all CP-engineered potato plants challenged by using aphid inoculation (Kawchuk et al. 1990; Tumer et al. 1990) have demonstrated some degree of resistance.

Stability of resistance

CP-mediated resistance is, in most cases, monogenic and is inherited by the subsequent generations, as with 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 under natural conditions. One may argue that a single point mutation can change a vital amino acid in the expressed CP and consequently alter the resistance, but the probability of this occurring will not be greater than for any other monogenic resistance gene. Furthermore, we know that CP-mediated resistance is effective for viruses differing by up to 40% in their CP sequences. In order to test the stability of the system, TMV CP(+) lines of tomato have been inoculated 15 times, successively, 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. comm.).

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; 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 (K.J. Krahn, pers. comm.). CP(+) plants developed disease more slowly or not at all compared to 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 infection.

The first field test with TMV-resistant tomato plants (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. Only 5% of the CP(+) plants developed disease symptoms by fruit harvest, while 99% of the inoculated CP(-) plants developed symptoms. Lack of visual symptoms was associated with lack of virus accumulation. Fruit yields of infected plants decreased 26-35% compared to healthy plants, whereas yields from the CP(+) line equalled those of uninoculated CP(-) 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, USA. In Florida, progeny

that were homozygous for the TMV CP gene and VF36 CP(-) plants were challenged with a Florida isolate of ToMV (Naples C). The field test in Illinois was conducted to determine whether expression of the TMV CP gene in tomato would protect 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., in press).

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 recently 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, it produces the severe disease 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 Russet Burbank CP(-) 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 both PVX and PVY, as predicted from growth chamber tests. Tuber yields at maturity in non-inoculated plots were the same for all 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.

Conclusion

In the past few years a profusion of techniques to control viruses by genetic engineering have emerged. Most of them have demonstrated potential for conferring resistance in plants and provide hope for future crop improvement. Several techniques, such as the satellite and CP strategies, have already been applied in field experiments, and their potential to provide a source of resistance to viruses has been confirmed.

The ribozyme strategy is interesting because of its potential applicability to all viruses and its high specificity, but it needs to be demonstrated under in vivo conditions. The satellite strategy, although very effective, is strictly limited to viruses having satellites, which are very few in the plant virus world; its usage is questionable because of the risk that would accompany widespread use. The CP-mediated resistance for controlling viruses is the most safe, efficient, and widely documented type of engineered resistance. Several field experiments have been conducted with various crops, and CP-mediated protection has been shown to be effective in all cases. The specificity of CP-mediated resistance is broader than any other type of virus resistance, as plants can be protected against viruses of the same group sharing up to 60% in their CP sequence. CP transgenic plants are resistant to high

concentrations of virus inoculum, and in one case even RNA inoculum. Resistance against vector inoculation and inoculation via vegetative propagation have also been demonstrated. The resistance generated by the CP strategy is of a multiple type, reducing the number of infection sites, symptoms, virus multiplication, and long-distance spread through plants. All these manifestations could be the result of a single mechanism or multiple effects of the CP on different targets. Despite the number of studies conducted to understand the mechanisms of action of the CP strategy, the question of how the CP confers resistance to engineered plants remains unanswered.

Discussion

ENE-OBONG: The major insect pest of cassava in West Africa is probably *Zonocerus variegatus*. Most plants are known to possess certain secondary compounds that protect them from insects. Obviously, cassava is not resistant to this insect, which means that *Z. variegatus* contains enough enzymes to break down the HCN released by linamarin bioconversion. Protection of cassava against this insect should be a good topic for study.

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