ISSN 0095-4527, Cytology and Genetics, 2018, Vol. 52, No. 3, pp. 213–221. © Allerton Press, Inc., 2018.
Original Ukrainian Text © A.M. Kyrychenko, O.G. Kovalenko, 2018, published in Tsitologiya i Genetika, 2018, Vol. 52, No. 3, pp. 61–71.

Basic Engineering Strategies for Virus-Resistant Plants

A. M. Kyrychenko[a], * and O. G. Kovalenko[a]

[a]*Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, 03680 Ukraine*

**e-mail: kirangel.07@meta.ua*

Received August 31, 2017

Abstract—Viral diseases of plants remain the main causes of significant damage in agricultural production. Various epidemiological measures have been taken to avoid viral diseases, including phytosanitary measures, using virus-free planting material, the fight against viral vectors, and the introduction of resistant or tolerant varieties. In recent years, the efforts of researchers around the world have been aimed at investigating the factors related to both natural and induced virus resistance of plants as well as the development and introduction of varieties created by genetic manipulation (bioengineering methods). The development of new antivirus strategies requires deep understanding of the molecular mechanisms underlying the natural resistance and biochemical processes occurring in virus infected plants. The review provides a brief description of genetic modification methods and considers the main genetic engineering approaches used to develop virus-resistant plants.

Keywords: virus resistance, genetic engineering, plant protection

DOI: 10.3103/S0095452718030076

INTRODUCTION

Viral diseases of plants are one of the main problems of crop farming and agricultural production in general. Currently, there are no effective therapeutic agents for the control of viral diseases. The main strategy for the control is the prevention of diseases, in particular through the introduction of varieties that have a high natural resistance to pathogens. Despite the fact that "classical breeding" represents a powerful reserve for the production of resistant plants, it is characterized by high cost and longevity, and the harvest quality and quantity of such resistant varieties are significantly lower compared to "parental forms." In contrast, genetic manipulations are a relatively quick way of introducing plant resistance genes, which can be especially useful in combating viral diseases that occur unexpectedly, and genetic resistance is the most effective guarantee of the successful protection of plants against viruses. A number of researchers [1] repeatedly demonstrated the effectiveness of this approach. Difficulties arising during the production of resistant plants by genetic engineering methods are usually due to a limited number of available natural sources of resistance as well as a relatively high level of mutation of viral genomes leading to a rapid loss of breeding resistance under field conditions [2]. Therefore, traditional crop selection methods are often combined and supplemented by genetic engineering approaches. These approaches differ in the degree of efficiency and universalism, and their number is constantly increasing (Fig. 1).

A comparative analysis of new approaches to plant breeding for viral resistance can be found in a number of reviews [3–5]. In our opinion, the most successful classification of resistance mechanisms and approaches used to create genetic engineering resistance is the classification proposed by Goldbach [4]. Its main postulates are as follows.

(1) Pathogen-derived resistance (PDR) is a resistance arising from the transformation of host plants by viral genes or some genomic sequences of the virus, and it is associated with the blocking of the individual stages of the propagation or distribution of the virus by the transgenic products.

(2) Posttranscriptional gene silencing (PTGS) is the resistance occurring due to the insertion of viral genome fragments with specific orientation into a plant genome. The restriction of the viral RNA molecules occurs as a result of viral infection of a transgenic plant obtained by this method. This type of resistance can be considered as a type of PDR; however, in this case, the resistance occurs due to the activation of plant protection system–PTGS reacting to the appearance of replication products (double-stranded RNA) of the virus.

(3) Transformation of plants by dominant genes of plant resistance (use of natural resistance genes or genes–components of protective signaling systems).

(4) The insertion of heterologous "exotic" genes derived from nonrelated organisms with their own specific ways of suppressing viral infection into the

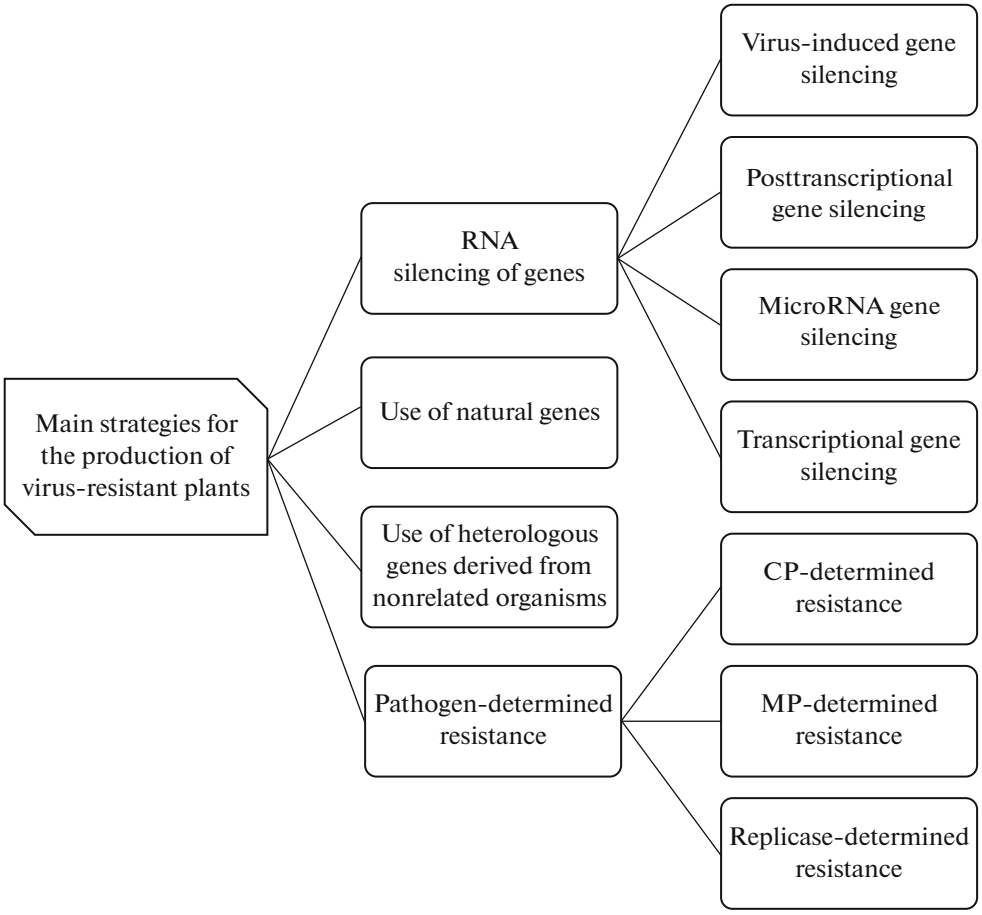

Fig. 1. Main strategies used for the production of virus-resistant plants.

plant genome that are untypical for the host plants of viruses.

Transgenic Virus Resistance of Plants Based on Dominant Resistance Genes

In general, most viruses have a limited (natural) range of host plants, and the number of virus-resistant plant species far exceeds the number of susceptible species. During the process of infecting host plants, viruses are exposed to various protection mechanisms. Some of these mechanisms are effective against all viruses and their response is a part of the congenital immune system, while other responses are virus-specific and require the presence of appropriate resistance genes in the genome. Upon activation of the latter, rapid tissue necrotisation in the places of penetration of the virus, which prevents further spread of infection, occurs. In some cases, the presence of dominant resistance genes does not guarantee the absolute resistance of plants, and only limited replication of the virus takes places in plant cells. Such genes are called "partial resistance genes."

For the first time, the N-gene conferring resistance to ToMV from *Nicotiana glutinosa* was transferred into the tobacco culture in the 1930s [6], and this method of preventing ToMV-infection is widely used as the basis of genetic engineering of virus resistance under field conditions. In the early 1990s, the gene was cloned, and the mechanism of resistance mediated by the gene was studied [7]. Recently, a number of other genes of plant resistance were cloned and products of their expression were analyzed. Scientists made attempts to develop a general theory of mechanisms of resistance or susceptibility and coevolution of plant pathogens and their hosts. The main attention was paid to monogenic dominant resistance to fungal and bacterial pathogens. However, clear evidence was obtained that protective mechanisms effective against bacteria and fungi also act against viral infection, e.g., these mechanisms are nonspecific.

Despite the increasing interest of scientists and breeders in the functioning of the dominant resistance genes (R-genes) activated in response to infection with the virus, only some of them (22 of the 200 known) are currently cloned. Other studies of the function of genes are limited to the Rx gene controlling resistance

to *Potato virus X* (PVX) and N gene controlling resistance to the tobacco mosaic virus (ToMV) [8]. Two dominant resistance genes—Tsw and Sw-5, used in selection of tomato resistant to *Tomato spotted wilt virus* (TSWV)—were studied. The first of these two genes was identified in *Capsicum chinense* Jacq. pepper and introgressed in the crop for the production of resistant bell peppers varieties (*Capsicum annuum*) [9, 10]. The Sw-5 gene from *Solanum peruvianum* plants was introgressed in tomato, and the resistance determined by this gene was sustainable [11]. It was shown that Sw-5 gene is a cluster of five genes (from Sw-5a to Sw-5e) [12, 13]. In this cluster, only the "b" copy was found to be [13, 14] functionally active and determined resistance not only to TSWV but also to more distant tospoviruses: *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV), and *Impatiens Necrotic Spot Virus* (INSV). Such a wide range of resistance is very unique for dominant resistance genes, and the use of the Sw-5 gene may be promising for the production of toxoplasma resistant varieties. Recently, the transport protein of the tomato spotted wilt virus was identified, which functions as a determinant of avirulence under resistance conditions caused by the Sw-5b dominant resistance gene belonging to the class of SD-CC-NB-LRR (*Solanaceae domain-coiled coil-nucleotide-binding-leucine-rich repeat*, SD-CNL). Temporal expression of this protein in tomatoes and transgenic *Nicotiana benthamiana* plants (with the Sw-5b gene) triggers the mechanism of an ultrasensitive reaction [15].

Tomato mosaic virus (ToMV) resistance is known to be controlled by the Tm-1, Tm-2, and Tm-2^2 genes. The stability determined by Tm-2^2 proved to be quite stable [16]; susceptible tomato plants transformed by the Tm-2^2 gene exhibited resistance to ToMV, which remained in all transgenic lines. The only dominant Ctv-R gene present in *Poncirus trifoliata* was cloned [17], and it was demonstrated that this gene determines a wide range of resistance to the *Citrus tristeza virus* (CTV), the main causative agent of citrus plants.

It should be noted that the resistance determined by dominant genes is usually short-termed, since the interaction between the factors of plant resistance and viral aviulent factors [18] is quite easily damaged as a result of viral mutations, although the resistance persists for a long time in some cases [19]. An example of this can be the resistance of the *snap bean*, determined by the dominant gene I that is derived from *Phaseolus vulgaris* plants resistant to BCMV and a number of other viruses [20]. In breeding programs, researchers, however, prefer using the resistance based on dominant genes, since it focuses on certain pairs of host and virus genes [21], which significantly simplifies the selection of plants.

Thus, an understanding of the structure and functions of R-genes opens up broad prospects for the production of pathogen-resistant plant varieties. Unfortunately, the functions of many proteins and other molecules interacting with R-genes during the course of protective reactions remain unknown. Therefore, using molecular biological and biochemical approaches and methods, researchers perform cloning and study of the functions of genes activated during the immune response of plants [22].

Pathogen-Derived Resistance

In the case where virus resistance is not associated with the expression of resistance genes, another approach is used for the production of transgenic plants, in particular the impairment of the life cycle of the virus. This is achieved by the introgression of certain genes of the viral genome able to control the synthesis of capsid proteins, untranslated viral RNA, antisense RNA, and viral replicase genes in the chromosomes of the host plant. The concept underlying this genetic engineering method is called pathogen-derived resistance (PDR) and is quite simple to understand: the expression of the viral genes encoding structural and nonstructural proteins in the plant genome prevents the development of the pathogen [23].

Three types of viral genes—capsid proteins (CP), replicase, and transport protein gene—characteristic for all (+) RNA viruses are most commonly used for the production of resistant plants based on the PDR strategy. Other approaches depend on the features characteristic for some viruses, in particular the presence of satellite or defective viral sequences or the synthesis of molecules, which do not occur in natural environment of plant viruses (specific toxins and antibodies).

Resistance determined by the expression of the structural viral capsid protein. One of the most successful genetic approaches for the production of resistant plants is the expression of the viral capsid protein gene. This resistance is characterized by the fact that transgenic plants expressing the CP gene are not infected with the same or homologous virus. The level of resistance may vary depending on immunity level (absence of any traits of a viral infection) to the delay and the reduction of the symptoms of infection as well as reduced level of the accumulation of the virus (as compared to control) in both inoculated and systemically infected leaves. Transgenic plants constitutionally expressing capsid protein genes acquire "cross-protection" against this virus, its various strains and isolates, and, in some cases, protection against close viruses from the same taxonomic group as related antigens.

The gene of the structural protein of the ToMV membrane was one of the first viral genes transferred into the tobacco plants. Thus, the fact that transgenic tobacco plants with the CP ToMV gene become resistant to subsequent infection with this virus was discovered for the first time in 1986 [24]. In 1988, Hemen-

way et al. [25] showed a direct correlation between the level of CP expression and the resistance level. Now, transgenic resistance based on the CP gene is known for over 35 viruses from 15 different taxonomic groups, including tobamo-, potex-, cucumo-, tobra-, carla-, poty-, luteo-, and alfamo-viruses.

It should be noted that this mechanism of stability requires the mandatory transcription and translation of CP-transgenes [26], e.g., this genetic engineering resistance operates on the protein level only. In the case of inoculation of plants with viral RNA, resistance does not develop (except for PVX). The resistance determined by CP genes is not absolute and may be affected by a very high concentration of the viral inoculum. Thus, transgenic tobacco plants are infected with ToMV at significantly higher concentrations of inoculum (by 10000 times) in comparison with control ones. Although the details of the mechanism of ToMV-mediated resistance are not known at present, the available evidence suggests a possible blockage of the dissociation of capsid protein of the ToMV virions, the process required for the release the viral RNA and initiation of the infectious process [27, 28].

Resistance determined by the expression of the replicase gene (replicase-mediated resistance). The first report about the production of resistant plants transformed with the replicase gene appeared in the United States in the early 1990s [29]. Replicase was the first nonstructural viral protein used to obtain virus-resistant plants. Despite the fact that transgenic plants with the protein of the replicase gene were found to be highly resistant to the virus, the direct correlation between protein expression and viral resistance was not observed in most cases [28]. In fact, the results of some studies convincingly indicate the involvement of the RNA sequences of the replicase gene, and not its product (protein) in the resistance [30, 31]. Therefore, the assumption about the coexistence of two types of resistance in the transgenic plants—protein-mediated and RNA-mediated—was made [32, 33]. One mechanism of resistance limits the replication of the virus at the level of one cell [34], while the other restricts the systemic [35] or intercellular distribution of the virus [36]. It was later found that replicase-mediated resistance is a characteristic feature of RNA silencing [37]. Therefore, it can be concluded that replicase gene-based transgenes can serve as a powerful source of resistance, despite the fact that the role of replicase proteins (modified) or their transcripts is still unclear.

Resistance determined by the expression of the movement protein gene (movement protein-mediated resistance). Transgenic plants resistant to viruses were also obtained by genetic engineering methods using mutant forms of viral genes of a dominant nature. Thus, constructions with a gene encoding a movement protein (MP) necessary for the intercellular and systemic distribution of virus in a plant were produced [38, 39]. Virus resistance was observed only in transgenic plants expressing defective MP: normally functioning wild-type MP genes did not affect the viral infection or increased the susceptibility of plants to viral infection [40]. A possible mechanism of movement protein-mediated resistance may be the competition between mutant MP and wild-type MP for binding sites on plasmodesmas [41]. The feature of this resistance type is its relative nonspecificity. Thus, the expression of mutant MP ToMV leads to the plant resistance to potex-, cucumo-, tobra-viruses, and, of course, to ToMV [42].

Despite numerous studies, the molecular mechanisms underlying protein-mediated resistance are not fully understood. It is evident that they are quite diverse and probably relate to several stages of the infectious process, and the virus transgenic plant has its own specific features in each pair. Moreover, in some cases, the resistance considered as protein-mediated PDR did not depend on the expression of the corresponding viral proteins, and a majority of PDR features operate via RNA-mediated mechanisms. One of the possible mechanisms of resistance may be the suppression of the replication of the virus as a result of the competition between transgenic protein and viral replicase for binding sites with host factors or viral proteins regulating the replication and expression of the viral genes.

Another mechanism underlying the resistance to viruses is the mechanism dependent on the presence of RNA.

Transgenic Viral Resistance of Plants Based on RNA-Silencing

RNA silencing is considered the most conservative mechanism of protection of cellular RNA from alien information in the form of nucleic acids of viruses, transposons, transgenes, etc. [43]. The basis of the RNA-silencing mechanism is the recognition by the cellular proteins (by Dicer enzyme with RNase III domains) of a two-chain RNA of exogenous or endogenous origin (the so-called "dsRNA," pre-dsRNA) and short-term (21–26 nucleotides) fragments known as short interfering RNA (siRNA) and microRNA (miRNA). The biogenesis of siRNA and miRNA proceeds virtually by an identical mechanism and is controlled by common genes. Both siRNA and miRNA in RICS-complexes (RNA-induced silencing complexes, RISC) formed by Argonaute (AGO) and other related proteins, which provided the complementarity of nucleotide sequence and small RNAs, are capable of inducing posttranscriptional endonuclease cleavage of the target mRNA [44].

The blocking of a single-stranded sense, and to a lesser extent antisense viral sequences, became a common strategy for preactivation of the RNA-silencing mechanism and obtaining plant resistance to homolo-

gous viruses from which a "transgenic" sequence was produced [21]. Transgenic plants are produced using various antisense constructs where a cDNA-containing copy of the viral RNA is expressed and the RNA-silencing mechanism of the viral or host genes required for viral replication is initiated [45, 46].

There are several mechanisms of RNA silencing:

(1) Post Transcriptional Gene Silencing, (PTGS) or RNA interference (RNAi).

(2) Transcriptional Gene Silencing (TGS).

(3) Virus-Induce Gene Silencing (VIGS).

(4) MicroRNA Gene silencing (miRNA).

Most studies of genetic engineering resistance based on RNA silencing have been conducted using the model of single-stranded +RNA viruses, but the use of such viral transgenes was also effective against other viruses, in particular tospoviruses [47], the genome of which is represented by single-stranded RNA with a negative polarity (–RNA) or geminiviruses with a single-stranded DNA genome [48, 49]. Genomes of RNA viruses are associated with nucleoproteins throughout their replication cycle and, therefore, are less susceptible to "targeted" degradation of RNA, whereas genomes of +RNA viruses are relatively readily available. However, viral mRNAs are not encapsulated and, therefore, represent a convenient target for sequencing-specific degradation, as was shown for TSWV. This strategy has been successfully used to produce transgenic plants resistant to *Pepper mild mottle virus* (PMMoV) and *Plum pox virus* (PPV) [50].

Despite the fact that viruses with the DNA genome are less susceptible to RNA silencing [51], this antiviral strategy can be quite effective against geminiviruses [52]. Despite the fact that the replication of these viruses occurs in the nucleus, their mRNA is synthesized in the cytoplasm. The "silencing" of viral mRNAs will arrest the translation of proteins required for the life cycle of viruses (replication proteins and transport proteins), and, thus, the reproduction and distribution processes of the virus will be arrested.

Although we already know many examples of genetically engineered virus-resistant plants based on RNA silencing, there are many limitations that affect the widespread use of this strategy. Thus, this resistance is the result of the interaction of many factors and it depends on the similarity of the sequences, the selected target, the pathogen titer and the temperature of the environment [53]; therefore, it is difficult to accurately predict the effectiveness of this resistance. Since most of the positive results were obtained in greenhouses, further field research wherein genetically modified plants will contact and confront mixed infections are required. In the future, the main tasks are the identification of factors affecting the resistance determined by the RNA silencing phenomenon in each case and the study of the stability of genetically modified plants under field conditions.

Introduction of Heterologous Genes into the Plant Genome

Another approach for the production of transgenic virus-resistant plants is based on the introduction of transgenes expressing monoclonal antibodies against viral proteins in the cells. Using this method, the effective protection of plants against *Artichoke mottle crinkle virus* (AMCV) virus was obtained. The first successful application of antibodies was made in 1993 by Tavladoraki et al. [54]. The researchers expressed antibodies reducing susceptibility to AMCV using single-stranded variable fragments (ScFv) directed against CP of the virus. Transgenic plants contained antibodies (0.1% of the total protein content) in their cells and were resistant to AMCV infection but not to CMV, indicating the specific nature of the resistance [55].

The expression of antibodies against major viral proteins in plants can be supplemented with an alternative approach providing genetic engineering resistance to the virus. A similar approach was used for the production of transgenic tobacco lines expressing antibodies capable of recognizing CP of ToMV [56] and conservative for most of the tospoviruses epitopes of the glycoprotein G1 of tomato spotted wilt virus (TSWV) [57].

Several methods were also developed for the production of transgenic plants with a wide range of resistance that are not associated with the transfer of viral genes. Thus, in the cellular membranes of *Phytolacca americana*, PAP (pokeweed antiviral protein) that is capable of inhibiting the ribosomal function was identified. Tobacco and potato plants transformed with cDNAs of RAP were resistant to not only VTM, PVX, PVY, and PLRV but also to *Rhizoctonia solani* fungi [58]. The main problem with the use of such genes may be the toxic effects of synthesized proteins on transformed plants [59].

The use of a resistance gene of animal origin in phyto-engineering can be important for the protection of plants against viral infection. Thus, the introduction of the beta-interferon gene or the gene encoding 2,5A-synthetase into plant cells increased the resistance to viral infection. Approaches are being developed that use genes that encode antiviral proteins of plant origin as well as genes that encode specific antibodies that recognize viral proteins [60].

Recently, studies have appeared that contain data about the production of transformants using ribozymes, such as *hammerhead*—small RNA with conservative sequences capable of cutting the RNA target. Ribozymes were detected in some viroids and some plant viruses associated with the satellite RNA [61]. Recently, transgenic plants were obtained that are able to express the ribozyme gene of the *hammerhead* type, the transcripts of which catalyze sequence-specific folding of phosphodiester bonds in RNA molecules. Examples of such plants include potato plants resistant to potato spindle viroid, tomato plants resistant to

Citrus exocortis viroid [62], tobacco plants resistant to ToMV [63], rice plants resistant to *Rice dwarf virus* [64], and melons resistant to *Watermelon mosaic virus* and *Zucchini yellow mosaic virus* [65]. Despite the fact that ribozymes are specific to any alien gene and can be introduced into the plant gene, the use of this method remains limited.

Over the past 80 years, scientists have made significant progress in understanding the mechanisms of the functioning of the virus resistance of plants, and genetic engineering has opened new opportunities for the practical use of this knowledge. Thus, in recent decades, in addition to dominant resistance genes, a large number of recessive resistance genes have been cloned, various pathways of interference of viral RNA have been investigated, and alternative approaches to the production of virus-resistant plants using heterologous genes derived from nonrelated organisms have been used. The search for new promising genes is constantly ongoing, and, as a result, the range of transgenes used for plant transformation is increasing. Despite these achievements, plant viruses quickly acquire the ability to overcome the resistances created by breeders. This is the reason why the most important problem in the control of viral infections in the coming years will be the development of effective and long-term resistance that can withstand extreme genetic plasticity of viral pathogens. Since viruses use a variety of mechanisms to overcome resistance based on RNA silencing, one of the tasks for scientists is to find RNA silencing suppressors (RSS), the lack or inactivation of which leads to a "cure" for plants [66]. Since RSSs play an important role in the development of intracellular and/or intercellular RNA silencing, they can serve as important tools for the detailed study of these mechanisms as well as for the development of new approaches in the fight against viruses. An especially important approach is the enhancement of plant viral resistance and gene expression associated with "molecular farming" in transgenic plants [67]. In recent years, the amount of evidence on activation of specific protection in plants against such suppressors by the pathogens themselves has increased and it convincingly illustrates the existence of a continuous "molecular weapons race" between plant pathogens and their hosts [68, 69].

The development of molecular genetics and associated technologies, in particular marker assisted selection (MAS), has led to the appearance of new approaches to plant gene pyramiding. Gene pyramiding allows the simultaneous expression of several genes, increasing the efficiency of plant breeding and obtaining longer-lasting resistance. The introduction of innovative research tools, such as DNA microchips and microprobes, as well as molecular genetic markers, in particular single nucleotide polymorphism (SNP), allows the rapid assessment of the effectiveness of genes. The power and effectiveness of genotyping is continuously increasing due to new molecular genetic approaches, and, in the long run, pyramiding genes will be used along with traditional plant breeding programs [70, 71].

Alternative approaches to increased resistance of agricultural crops include the use of protective potentials of plants themselves. With this approach, the increase of plant resistance can be achieved by the increase in the level of expression of its own genes involved in the protective reactions and the transgenesis of genes encoding proteins and peptides of other virus-resistant plant species.

In the future, the combination of modern bioengineering technologies and a deeper understanding of the "dialogue" between plants and viruses will lead to a new "green revolution" in agriculture and will be successfully used in programs for improvement of the productivity and commercial value of crops.

REFERENCES

1. Sahu, P.P. and Prasad, M., Application of molecular antiviral compounds: novel approach for durable resistance against geminiviruses, *Mol. Biol. Rep*., 2015, vol. 42, no. 7, pp. 1157–1162.
2. Whitham, S.A. and Hajimorad, M.R., Plant genetic resistance to viruses, in *Current Research Topics in Plant Virology*, Wang, A. and Zhou, X., Eds., Springer, 2016, pp. 87–111.
3. Baulcombe, D.C., Mechanisms of pathogen-derived resistance to viruses in transgenic plants, *Plant Cell*, 1996, vol. 8, no. 10, pp. 1833–1844.
4. Goldbach, R., Bucher, E., and Prins, M., Resistance mechanisms to plant viruses: an overview, *Virus Res*., 2003, vol. 92, no. 2, pp. 207–212.
5. Prins, M., Broad virus resistance in transgenic plants, *Trends Biotechnol.*, 2003, vol. 21, no. 9, pp. 373–375.
6. Holmes, F.O., Inheritance of resistance to tobacco mosaic virus in tobacco, *Phytopathology*, 1938, vol. 28, pp. 553–561.
7. Dinesh-Kumar, S.P. and Baker, B.J., Alternatively spliced n resistance gene transcripts: their possible role in tobacco mosaic virus resistance, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, vol. 97, no. 4, pp. 1908–1913.
8. de Ronde, D., Butterbach, P., and Kormelink, R., Dominant resistance against plant viruses, *Front. Plant Sci.*, 2014, vol. 5, art. 307.
9. Boiteux, L.S., Allelic relationships between genes for resistance to tomato spotted wilt tospovirus in *Capsicum chinense, Theor. Appl. Genet.*, 1995, vol. 90, no. 1, pp. 146–149.
10. Jahn, M., Paran, I., Hoffmann, K., Radwanski, E.R., Livingstone, K.D., Grube, R.C., Aftergoot, E., Lapidot, M., and Moyer, J., Genetic mapping of the *Tsw* locus for resistance to the Tospovirus Tomato spotted wilt virus in *Capsicum* spp. and its relationship to the Sw-5 gene for resistance to the same pathogen in tomato, *Mol. Plant Microbe Interact.*, 2000, vol. 13, no. 6, pp. 673–682.
11. Rosello, S., Diez, M.J., and Nuez, F., Genetics of tomato spotted wilt virus resistance coming from *Lycop-*

ersicon peruvianum, Eur. J. Plant Pathol., 1998, vol. 104, no. 5, pp. 499–509.

12. Brommonschenkel, S.H., Frary, A., Frary, A., and Tanksley, S.D., The broad-spectrum tospovirus resistance gene *sw-5* of tomato is a homolog of the root-knot nematode resistance gene *Mi, Mol. Plant Microbe Interact*, 2000, vol. 13, no. 10, pp. 1130–1138.
13. Spassova, M.I., Prins, T.W., Folkertsma, R.T., Klein-Lankhorst, R.M., Hille, J., Goldbach, R.W., and Prins, M., The tomato gene *Sw5* is a member of the coiled coil, nucleotide binding, leucine-rich repeat class of plant resistance genes and confers resistance to TSWV in tobacco, *Mol. Breed.*, 2001, vol. 7, no. 2, pp. 151–161.
14. Hallwass, M., de Oliveira, A.S., de Campos, DianeseE., Lohuis, D., Boiteux, L.S., Inoue-Nagata, A.K., Resende, R.O., and Kormelink, R., The tomato spotted wilt virus cell-to-cell movement protein (NSM) triggers a hypersensitive response in Sw-5-containing resistant tomato lines and in *Nicotiana benthamiana* transformed with the functional Sw-5b resistance gene copy, *Mol. Plant Pathol.*, 2014, vol. 15, no. 9, pp. 871–880.
15. De Oliveira, A.S., Koolhaas, I., Boiteux, L.S., Caldararu, O.F., Petrescu, A.J., Oliveira, ResendeR., and Kormelink, R., . Cell death triggering and effector recognition by Sw-5 SD-CNL proteins from resistant and susceptible tomato isolines to Tomato spotted wilt virus, *Mol. Plant Pathol.*, 2016, vol. 17, no. 9, pp. 1442–1454.
16. Hall, T.J., Resistance at the *Tm*-2 locus in the tomato to tomato mosaic virus, *Euphytica*, 1980, vol. 29, no. 1, pp. 189–197.
17. Rai, M., Refinement of the *Citrus tristeza* virus resistance gene (*Ctv*) positional map in *Poncirus trifoliate* and generation of transgenic grapefruit (*Citrus paradisi*) plant lines with candidate resistance genes in this region, *Plant. Mol. Biol.*, 2006, vol. 61, no. 3, pp. 399–414.
18. Lecoq, H., Moury, B., Desbiez, C., Palloix, A., and Pitrat, M., Durable viral resistance in plants through conventional approaches: a challenge, *Virus Res.*, 2004, vol. 100, no. 1, pp. 31–39.
19. Kang, B.C., Yeam, I., and Jahn, M.M., Genetics of plant virus resistance, *Annu. Rev. Phytopathol.*, 2005, vol. 43, pp. 581–621.
20. Keller, K.E., Johansen, I.E., Hampton, R.O., and Martin, R.R., A recessive plant resistance gene governs the action of a putative potyvirus replication genome, *Phytopathology*, 1996, vol. 86, p. S18.
21. Ritzenthaler, C., Resistance to plant viruses: old issue, news answers?, *Curr. Opin. Plant Biothechnol.*, 2005, vol. 16, no. 2, pp. 118–122.
22. Kyrychenko, A.M. and Kovalenko, O.G., Genetic bases and functioning of plant signal transduction at virus resistance, *Cytol. Genet.*, 2011, vol. 45, no. 4, pp. 55–66.
23. Sanford, J.C. and Johnston, S.A., The concept of parasite derived resistance. Deriving resistance genes from the parasite's own genome, *J. Theor. Biol.*, 1985, vol. 113, no. 2, pp. 395–405.
24. Abel, P.P., Nelson, R.S., De, B., Hoffmann, N., Rogers, S.G., Fraley, R.T., and Beachy, R.N., Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene, *Science*, 1986, vol. 232, no. 4751, pp. 738–743.
25. Hemenway, C., Fang, R.X., Kaniewski, W.K., Chua, N.H., and Turner, N.E., Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA, *EMBO J.*, 1988, vol. 7, no. 5, pp. 1273–1280.
26. Mundembe, R., Matibiri, A., and Sithole-Niang, I., Transgenic plants expressing the coat protein gene of cowpea aphid-borne mosaic potyvirus predominantly convey the delayed symptom development phenotype, *Afr. J. Biotechnol.*, 2009, vol. 8, no. 12, pp. 2682–2690.
27. Asurmendi, S., Bergi, R.H., Koo, J.C., and Beachy, R.N., Coat protein regulates formation of replication complex during tobacco mosaic virus infection, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, vol. 101, no. 5, pp. 1415–1420.
28. *Plant Viruses As Molecular Pathogens*, Khan, J.A. and Dijkstra, J., Eds., New York: Food Products Press, Inc., 2002.
29. Golemboski, D.B., Lomonossoff, G.P., and Zaitlin, M., Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, vol. 87, no. 16, pp. 6311–6315.
30. Rubino, L. and Russo, M., Characterization of resistance to cymbidium ringspot virus in transgenic plants expressing a full-length viral replicase gene, *Virology*, 1995, vol. 212, no. 1, pp. 240–243.
31. Mueller, E., Gilbert, J., Davenport, G., Brigneti, G., and Baulcombe, D.C., Homology-dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing, *Plant J.*, 1995, vol. 7, no. 6, pp. 1001–1013.
32. Tenllado, F., García-Luque, I., Serra, M.T., and Díaz-Ruíz, J.R., *Nicotiana benthamiana* plants transformed with the 54-kDa region of the pepper mild mottle tobamovirus replicase gene exhibit two types of resistance responses against viral infection, *Virology*, 1995, vol. 211, no. 1, pp. 170–183.
33. Tenllado, F., García-Luque, I., Serra, M.T., and Díaz-Ruíz, J.R., Resistance to pepper mild mottle tobamovirus conferred by the 54-kDa gene sequence in transgenic plants does not require expression of the wild-type 54-kDa protein, *Virology*, 1996, vol. 219, no. 1, pp. 330–335.
34. Carr, J.P., Gal-On, A., Palukaitis, P., and Zaitlin, M., Replicase-mediated resistance to cucumber mosaic virus in transgenic plants involves suppression of both virus replication in the inoculated leaves and longdistance movement, *Virology*, 1994, vol. 199, no. 2, pp. 439–447.
35. Wintermantel, W.M., Banerjee, N., Oliver, J.C., Paolillo, D.J., and Zaitlin, M., Cucumber mosaic virus is restricted from entering minor veins in transgenic tobacco exhibiting replicase-mediated resistance, *Virology*, 1997, vol. 231, no. 2, pp. 248–257.
36. Nguyen, L., Lucas, W.J., Ding, B., and Zaitlin, M., Viral RNA trafficking is inhibited in replicase-medi-

ated resistant transgenic tobacco plants, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, vol. 93, no. 22, pp. 12643–12647.

37. Marano, M.R. and Baulcombe, D., Pathogen-derived resistance targeted against the negative-strand RNA of tobacco mosaic virus: RNA strand-specific gene silencing?, *Plant J.*, 1998, vol. 13, no. 4, pp. 537–546.
38. Deom, C.M., Schubert, K.R., Wolf, S., Holt, C.A., Lucas, W.J., and Beachy, R.N., Molecular characterization and biological function of the movement protein of tobacco mosaic virus in transgenic plants, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, vol. 87, no. 9, pp. 3284–3288.
39. Holt, C.A. and Beachy, R.N., In vivo complementation of infectious transcripts from mutant tobacco mosaic virus cDNAs in transgenic plants, *Virology*, 1991, vol. 181, no. 1, pp. 109–117.
40. Ziegler-Graff, V., Guilford, P.J., and Baulcombe, D.C., Tobacco rattle virus RNA-1 29K gene product potentiates viral movement and also affects symptom induction in tobacco, *Virology*, 1991, vol. 182, no. 1, pp. 14–155.
41. Lapidot, M., Gafny, R., Ding, E., Wolf, S., Lucas, W.J., and Beachy, R.N., A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants, *Plant J.*, 1993, vol. 4, no. 6, pp. 959–970.
42. Cooper, B., Lapidot, M., Heick, J.A., Dodds, J.A., and Beachy, R.N., A defective movement protein of TMV in transgenic plants confers resistance to multiple viruses whereas the functional analog increases susceptibility, *Virology*, 1995, vol. 206, pp. 307–313.
43. Matzke, M.A., Aufsatz, W., Kanno, T., Mette, M.F., and Matzke, A.J., Homology-dependent gene silencing and host defense in plants, *Adv. Genet.*, 2002, vol. 46, pp. 235–275.
44. Koochakpour, Z. and Fakheri, B.A., RNA silencing and plant viral diseases, *Int. J. Farm. Allied Sci.*, 2014, vol. 3, no. 8, pp. 946–951.
45. Pruss, G.J., Lawrence, C.B., Bass, T., Li, Q.Q., Bowman, L.H., and Vance, V., The potyviral suppressor of RNA silencing confers enhancer resistance to multiple pathogens, *Virology*, 2004, vol. 320, no. 1, pp. 107–120.
46. Andika, I.B., Kondo, H., and Tamada, T., Evidence that rna silencing-mediated resistance to beet necrotic yellow vein virus is less effective in roots than in leaves, *Mol. Plant Microbe Interact.*, 2005, vol. 18, no. 3, pp. 194–204.
47. Prins, M., Kikkert, M., Ismayadi, C., de Graauw, W., de Haan, P., and Goldbach, R., Characterization of RNA-mediated resistance to tomate spotted wilt virus in transgenic tobacco plants expressing *NSM* gene sequences, *Plant. Mol. Biol.*, 1997, vol. 33, no. 2, pp. 235–243.
48. Bonfim, K., Faria, J.C., Nogueira, E., Mendes, E.A., and Aragao, F.J., RNAi-mediated resistance to bean golden mosaic virus in genetically engineered common bean (*Phaseolus vulgaris*), *Mol. Plant Microbe Interact.*, 2007, vol. 20, no. 6, pp. 717–726.
49. Fuentes, A., Ramos, P.L., Fiallo, E., Callard, D., Sanchez, Y., Peral, R., Rodriguez, R., and Pujol, M., Intron-hairpin RNA derived from replication associated protein *C1* gene confers immunity to tomato yellow leaf curl virus infection in transgenic tomato plants, *Transgenic Res.*, 2006, vol. 15, no. 3, pp. 291–304.
50. Tenllado, F., Martínez-Garcia, B., Vargas, M., and Díaz-Ruíz, J.R., Crude extracts of bacterially expressed dsRNA can be used to protect plants against virus infections, *BMC Biotechnol.*, 2003, vol. 3, pp. 1–11.
51. Ribeiro, S.G., Lohuis, H., Goldbach, R., and Prins, M., Tomato chlorotic mottle virus is a target of RNA silencing but the presence of specific short interfering RNAs rantee resistance in transgenic plants, *J. Virol.*, 2007, vol. 81, no. 4, pp. 1563–1573.
52. Ammara, Ue., Mansoor, S., Saeed, M., Amin, I., Briddon, R.W., and Al-Sadi, A.M., RNA interference-based resistance in transgenic tomato plants against tomato yellow leaf curl virus-Oman (TYLCV-OM) and its associated betasatellite, *Virol. J.*, 2015, vol. 12, no. 1, p. 38.
53. Szittya, G., Silhavy, D., Molnar, A., Havelda, Z., Lovas, A., Lakatos, L., Banfalvi, Z., and Burgyan, J., Low temperature inhibits rna silencing-mediated defense by the control of siRNA generation, *EMBO J.*, 2003, vol. 22, no. 3, pp. 633–640.
54. Tavladoraki, P., Benvenuto, E., Trinca, S., De Martinis, D., Cattaneo, A., and Galeffi, P., Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack, *Nature*, 1993, vol. 366, no. 6454, pp. 469–472.
55. Binz, H.K. and Pluckthun, A., Engineered proteins as specific binding reagents, *Curr. Opin. Biotechnol.*, 2005, vol. 16, no. 4, pp. 459–469.
56. Sherwood, J.L., On efforts to improve detection of selected groundnut viruses and expression of tomato spotted wilt virus antibodies in plants, in *Working Together on Groundnut Virus Diseases*, Hyderabad, Indeia, 1994, pp. 48–49.
57. Franconi, R., Roggero, P., Pirazzi, P., Arias, F.J., Desiderio, A., Bitti, O., Pashkoulov, D., Mattei, B., Bracci, L., Masenga, V., Milne, R.G., and Benvenuto, E., Functional expression in bacteria and plants of an scFv antibody fragment against tospoviruses, *Immunotechnology*, 1999, vol. 4, nos 3-4, pp. 189–201.
58. Lodge, J.K., Kaniewski, W.K., and Tumer, N.E., Broad spectrum resistance in transgenic plants expressing pokeweed antiviral protein, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, vol. 90, no. 15, pp. 7089–7093.
59. Cao, B., Lei, J., Chen, G., Cao, P., Liu, X., Chen, Q., and Wei, X., Testing of disease-resistance of pokeweed antiviral protein gene (PacPAP) in transgenic cucumber (*Cucumis sativus*), *Afr. J. Biotechnol.*, 2011, vol. 10, no. 36, pp. 6883–6890.
60. Truve, E., Studies on specific and broad spectrum virus resistance in transgenic plants, *Thesis (Doctoral)*, Tartu, 1996.
61. Forster, A.C. and Symons, R.H., Self-cleavage of plus and minus RNAs of a virusoid and a structural model for the active sites, *Cell*, 1987, vol. 49, no. 2, pp. 211–220.
62. Atkins, D., Young, M., Uzzell, S., Kelly, L., Fillatti, J., and Gerlach, W.L., The expression of antisense and ribozyme genes targeting *Citrus exocortis* viroid in trans-

genic plants, *J. Gen. Virol.*, 1995, vol. 76, pp. 1781–1790.

63. de Feyter, R., Young, M., Schroeder, K., Dennis, E.S., and Gerlach, W., A ribozyme gene and an antisense gene are equally effective in conferring resistance to tobacco mosaic virus on transgenic tobacco, *Mol. Gen. Genet.*, 1996, vol. 250, no. 3, pp. 329–338.
64. Han, S., Wu, Z., Yang, H., Wang, R., Yie, Y., Xie, L., and Tien, P., Ribozyme-mediated resistance to rice dwarf virus and the transgene silencing in the progeny of transgenic rice plants, *Transgenic Res.*, 2000, vol. 9, no. 3, pp. 195–203.
65. Huttner, E., Tucker, W., Vermeulen, A., Ignart, F., Sawyer, B., and Birch, R., Ribozyme genes protecting transgenic melon plants against potyviruses, *Curr. Issues Mol. Biol.*, 2001, vol. 3, no. 2, pp. 27–34.
66. Ziebell, H., Payne, T., Berry, J.O., Walsh, J.A., and Carr, J.P., A cucumber mosaic virus mutant lacking the 2b counter-defense protein gene provides protection against wild-type strains, *J. Gen. Virol.*, 2007, vol. 88, pp. 2862–2871.
67. Qu, J., Ye, J., and Fang, R., Artificial microRNA-mediated virus resistance in plants, *J. Virol.*, 2007, vol. 81, no. 12, pp. 6690–6699.
68. Pumplin, N. and Voinnet, O., Rna silencing suppression by plant pathogens: defence, counter-defense and counter-counter-defense, *Nat. Rev. Microbiol.*, 2013, vol. 11, pp. 745–760.
69. Sansregret, R., Dufour, V., Langlois, M., Daayf, F., Dunoyer, P., Voinnet, O., and Bouarab, K., Extreme resistance as a host counter-counter defense against viral suppression of RNA silencing, *PLoS Pathog.*, 2013, vol. 9, no. 6, e1003435.
70. Fuchs, M., Pyramiding resistance-conferring gene sequences in crops, *Curr. Opin. Virol.*, 2017, vol. 26, pp. 36–42.
71. Joshi, R.K. and Nayak, S., Gene pyramiding—a broad spectrum technique for developing durable stress resistance in crops, *Biotechnol. Mol. Biol. Rev.*, 2010, vol. 5, no. 3, pp. 51–60.

Translated by V. Mittova