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Emerging Viral Diseases and Recent Advances in Resistance Implementation in *Glycine max* (Soybean)

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ABSTRACT

One of the most common crop plants in the world that is a legume is Soybean (Glycine max L. Merr.), the crop is a major source of oil and protein in food consumed by human beings and animals. In recent years, viral diseases have re-emerged as a major limiting factor to the production of soybean affecting yield and quality of plants in other continents. Soybean mosaic virus (SMV), Bean pod mottle virus (BPMV), Soybean yellow mottle mosaic virus (SYMMV) and Cowpea mild mottle virus (CPMMV) are examples of viruses that have been increasing in occurrence as a result of the changing climate and the change in agronomy. High-throughput sequencing and similar technologies of RNA silencing, CRISPR-Cas gene editing, and transgenic expression of resistance genes have made it easier and faster to understand and control these pathogens. This mini review will overview viral pathogens of soybean that are important, the mode of resistance, and current advancements in the field of molecular breeding and biotechnological methodologies to attain long-term control of the virus. An integration of conventional breeding with genome-assisted and synthetic biology solutions is one of the keys to the attainment of permanent virus resistance in G. max.

Keywords: Glycine max, soybean, viral diseases, SMV, BPMV, RNA silencing, CRISPR-Cas, resistance breeding

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Introduction

Soybean (*Glycine max* L.) is a highly valued crop in the entire world due to its high protein content (about 40) and oil content (about 20) which maintain both the food and industrial needs around the world (1). Although economically and nutritionally important, viral diseases have emerged as significant limitations to soybean productivity as these diseases have been implicated in over 80% of the epidemics and yield losses of products (in some areas) in severe epidemics. The reason why soybean is susceptible to various viral attacks is its complexity of interaction with the host, which is affected by various biological and environmental factors.

There are more than 30 viruses that have been reported to attack soybean across the world but only a number of these cause serious economic losses. The active appearance of new species of viruses and variants of a strain, which are disclosed in the framework of the development of molecular virology and genomic studies, present serious threats to the available sources of resistance(2). The evolution of viruses and recombination processes resulting in virulence adaptation in the evolution of these viruses have rendered many traditional resistance genes ineffective. Therefore, current and sustainable production of soybean will require combination of genomic techniques, molecular diagnostics and contemporary breeding strategies of resistance to withstand viral diversity and environmental stress in order to create cultivars that can survive viral diversity and environmental stress(3).

Fig. 1: Normal and symptomatic leaves of Soyabean.

2. Major Viral Diseases of Soybean

There are a number of economically significant viral pathogens that impact soybean (Glycine max L.) and cause a considerable decrease in yield and quality. General viruses that cause soybean disease complexes are Soybean mosaic virus (SMV), Bean pod mottle virus (BPMV), Soybean yellow mottle mosaic virus (SYMMV), Soybean vein necrosis virus (SVNV) and Cowpea mild mottle virus (CPMMV)(4). Taxonomically, these viruses vary in their mode of transmission, pathogenic mechanisms and pose a serious limitation to the soybean productivity in large growing areas globally. The most common and well-defined soybean virus is SMV which is a genus Potyvirus of the family *Potyviridae(5).* It is spread in a non-persistent (also by several species of aphids) way and by infected seeds. Mosaics, distortion of leaves, stunting, and significant losses in yield are the results of SMV infection. Genetic differences among SMV strains also play a role in divergences in the virulence and host resistance mechanisms. BPMV belonging to the genus *Comovirus* (family *Secoviridae*) is a beetle-borne pathogen of the order Coleoptera whose transmission has been mechanical(6). It causes typical foliar symptoms of green mottle, leaf puckering and wrinkling which is usually followed by premature senescence and pod deformation. BPMV infections are frequent and mixed with SMV, which increases the severity of the symptoms and the reduction.

Table 1: Major soybean viral pathogens and their characteristics

Virus	Family/Genus	Transmission	Symptoms	Geographic Distribution
Soybean mosaic virus (SMV)	*Potyviridae / Potyvirus*	Aphids, seed	Mosaic, curling, stunting	Global
Bean pod mottle virus (BPMV)	*Secoviridae / Comovirus*	Beetles	Mottling, pod deformation	Americas, Asia
Soybean vein necrosis virus (SVNV)	*Tospoviridae / Tospovirus*	Thrips	Vein necrosis, chlorosis	USA, China
Cowpea mild mottle virus (CPMMV)	*Betaflexiviridae / Carlavirus*	Whiteflies	Leaf yellowing, reduction in seed size	Africa, Asia
Soybean yellow mottle mosaic virus (SYMMV)	*Tymoviridae / Tymovirus*	Unknown	Yellow mosaic, chlorosis	East Asia

SYMMV which is a virus of genus *Sobemovirus* causes yellowing of leaves, mosaic mottling and systemic chlorosis. It is mainly transmitted via seeds and has been observed in various countries in Asia and it has been reported to have high levels of synergistic interactions with other viruses that infect soybean. Thrips vectors are known to transmit SVNV which is an emerging virus belonging to the genus *Tospovirus* (family *Tospoviridae*)(7). It was first detectable in the United States in 2008 and since then it has permeated North America. Plants become infected showing the symptoms of vein clearing, vein necrotic lesions, chlorotic rings, patchy necrosis, which may develop to massive tissue destruction in acceptable conditions. CPMMV is an agent of transmission of the genus *Carlavirus* (family *Betaflexiviridae*), which can be transmitted by whiteflies (Bemisia tabaci) or by infected seeds(8). The virus causes mild chlorotic mottling, curling of leaves and hindered growth of pods. Co-infection with SMV or BPMV although mild per se leads to synergistic interactions increasing the disease expression and general plant debilitation.

Multi-soybean virus's co-infection is a significant issue in the epidemiology and control of disease. Such mixed infections disrupt the antiviral defensive mechanisms of the host and lead to increased expression of symptoms, amplified viral replication and loss of yield, as opposed to single infections. It is necessary to comprehend the molecular interactions, the transmission dynamics, and the host responses of these viruses in order to come up with long term resistance strategies and sustainable management practices in the soybean farming business.

Table 2: Resistance resources & tools for soybean viral disease management

Resource / Approach	Purpose	Implementation status
Rsv loci (Rsv1/3/4/5)	Strain-specific resistance to SMV	Widely studied; used in breeding programs.
SRC cluster (atypical R genes)	Broad-spectrum antiviral activity	Functionally validated in research; promising for breeding/pyramiding.
VIGS (TRV-based)	Rapid functional validation of candidate genes	Protocols optimized for soybean; used in research labs.
GWAS & genomic prediction	Map resistance loci and build prediction models	Multiple recent GWAS studies identify markers for breeding.
HTS / virome surveillance	Virus discovery and diagnostics at scale	Increasingly deployed in research & diagnostic networks.

3. Mechanisms of Resistance in Soybean

Soybean has a multilayered and complicated defense mechanism against viral pathogens which are regulated at the genetic, molecular and biochemical levels, and coordinate or work in co-ordination. It is the defense via R-gene-mediated defense, RNA silencing, and hormone-regulated signaling cascades that are the major mechanisms of antiviral resistance to the plant adaptive immune response(9). The classical resistance (R) genes are at the center of stage during specific recognition-based defense against viral infection. The loci Rsv1, Rsv3 and Rsv4 are some of them, and they have been well characterized as having a role in providing strain specific resistance to Soybean mosaic virus (SMV)(10). Such genes encode effector-triggered immunity (ETI) proteins, in which pathogen-derived viral avirulence factors are recognized by the host immune receptors, which trigger localized hypersensitive reactions and limit the dissemination of the virus. The differences in R-gene alleles are the determinants of the range of resistance or susceptibility between the various soybean genotypes.

RNA silencing (RNA interference, RNAi) is a highly conserved diversified antiviral response in plants. It depends on the generation of the double-stranded RNA (dsRNA), which is broken by the Dicer-like enzymes into small interfering RNAs (siRNAs). These siRNAs target Argonaute (AGO)-containing silencing complexes (RISCs) to complementary viral RNA targets leading to post-transcriptional gene silencing(11). This mechanism has sequence-specific and heritable resistance to a wide repertoire of RNA viruses and offers lasting protection in excess of the effect afforded by R-gene. Simultaneously, salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) signaling pathways in plants are essential in the regulation of defense gene expression and systemic acquired resistance (SAR). These hormones are synergistic/antagonist in their effects on the virus host interaction context, hence balancing defence and growth. The recent development of genome editing systems and specifically the CRISPR-Cas systems has made it possible to make specific and precise manipulations in host susceptibility genes to boost resistance to soybean viruses. Host factor editing (editing factors of translation initiation factors (eIF4E), Argonaute proteins (GmAGO2) and RNA-dependent RNA polymerases (GmRDR6)) can, in turn, suppress viral replication and movement throughout the body(12). These molecular strategies can provide robust tools to create soybean cultivars with extensive viral resistance that can be related to the conventional breeding methods.

4. Recent Implementation of Resistance Strategies

4.1 Transgenic Approaches

Transgenic strategies have been a revolution in the production of soybean varieties that are resistant to viral diseases. The RNAi lines expressing hairpin constructs containing essential areas of viral genomes like the SMV coat protein or the BPMV replicase sequences have shown strong and general resistance during field and controlled conditions. These transgenic plants are characterized by high level of reduction in the viral load and in the severity of symptoms and in most cases, they offer immunity to a wide range of various virus strains using a single genotype.

Systemic acquired resistance (SAR) responses to viruses and other pathogens induced by the overexpression of host defense-related genes, like GmSAMT1 and GmPR1, have also been associated with this(13). The process of regulating the expression of these genes boosts both defensive signaling and systemic protection of the plant, which enhances the performance of the field (reduced incidence disease, and better field performance). Combined, RNAi-mediated virus gene silencing and intelligent overexpression of major resistance genes represent an innovative approach to transgenic breeding of soybean, which allows the production of cultivars containing prolonged resistance and with few changes in nutritional or agronomic status.

4.2. Genome Editing and Marker-Assisted Breeding

CRISPR-Cas9 genome editing has emerged as one of the very useful tools in engineering virus resistance in soybean through targeting key host susceptibility genes, in particular, genes in the eIF4E gene family(14). The factors of translation initiation are vital to viral RNA replication and targeted inhibition by CRISPR-Cas9 has provided general resistance to numerous plant viruses with no appreciable yield loss or negative agronomic characteristics. This method is capable of producing non-transgenic and virus resistant cultivars that are productive and can adapt to the changing virus populations.

Simultaneously, genetic selection (GS) and marker-assisted selection (MAS) are finding more applications in breeding programs to effectively incorporate several resistance loci into high-performance soybean breeds(15). The rationale behind these molecular breeding tools is that they take advantage of the DNA markers associated with resistance genes and holistic genomic forecasts thereby hastening the process of introgression of durable resistance traits and enhancing the likelihood of swift cultivar development that would be applicable in different agro-ecological conditions.

4.3 Nanobiotechnology and Synthetic Applications

Recent biotechnology developments have emphasized the use of nanoparticle-mediated delivery of the type of molecules in the soybean and other crop diseases such as transient and efficient protection against viruses using the method of delivery, which involves the use of nanoparticles to deliver the segments of the molecules into the host cells. Nanocarrier systems (layered double hydroxides, chitosan, and other peptide/polymer nanoparticles) can stabilize, improve cellular uptake, and long-distance delivery of the dsRNA, increasing the duration and effectiveness of RNA interference-based antiviral responses in comparison to the use of naked dsRNA(16). These systems have been shown to have the potential of suppressing viral infection over a few weeks in the field and controlled conditions and without resulting in phytotoxicity or environmental risks. Also, inducible and tightly regulated resistance can be developed through the evolution of synthetic promoters that activate defense genes in response to pathogen challenge, reducing the risk of yield penalties or fitness costs of constitutive defense activation. Both inducible expression of defense genes and nanoparticle-mediated delivery of dsRNA are novel and environmentally friendly alternatives or supplements to traditional breeding and chemical control, which facilitates a reduction in the use of pesticides and enhanced protection of crops(17).

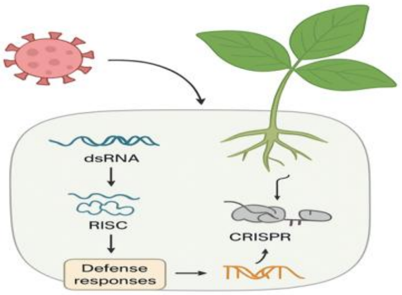

Figure 2: Overview of virus–host interaction and resistance mechanisms in soybean. Arrows indicate RNA silencing pathways, CRISPR-mediated editing sites, and defense activation routes.

5. Future Prospects

The combination of various omics technologies, such as transcriptomics, proteomics, and metabolomics, with precision breeding technologies is transforming the identification of new resistance genes and regulatory factors in soybean(18). The techniques will permit a global view of the multidimensional networks of complex molecular processes that dictate biotic stress responses, which will aid breeders to identify key genes and pathways linked with viral resistance. These integrated omics studies can be useful in identifying candidate markers and functional genes to be used to specifically enhance soybean cultivars(19). In addition, machine learning models and artificial intelligence (AI) can be used to predict breeding (using multi-omics data) to predict patterns of virus evolution and determine the stability of resistance loci(20). Such prediction technologies can streamline breeding plans to give rise to resistant cultivars that are efficient in the long run against the new viral strains.

International cooperative work in monitoring of viruses and exchange of germplasm cannot be ignored since it plays a crucial role in the early identification of new viral threats and expansion of the genetic pool of resistance. This type of international collaboration will help in the sustainable control of soybean viral outbreaks through the swift introduction of resistant germplasm that suits a wide variety of agro-ecologies(21). Combined, the multi-omics and AI-led analytics, precision breeding, and worldwide cooperation is a groundbreaking platform to boost soybean resistance to viral infections and protect the future yield massively.

6. Conclusions

Viruses have been a significant hazard to the world soybean production causing a lot of effects in terms of yield and economic value. Traditionally, the world has suffered huge losses in yield by diseases affecting soybean with estimates showing that the world has lost up to 27% of the total production to diseases, including viral pathogens, by 2006. In more recent times, diseases are still causing millions of metric tons of loss in production every year through major production countries like the United States, Brazil, China, and other countries. Some of the most common viruses include Soybean mosaic virus (SMV), and this has seen serious yield losses in several areas. In spite of these difficulties, there are encouraging prospects of biotechnology and breeding. The use of natural genetic resistance loci, but exploitation, coupled with the application of RNA interference and CRISPR-Cas9 gene editing, and nanoparticles to deliver antiviral dsRNA, are leading to the development of sustainable and long-term strategies to control viruses. Other complementary technologies, like multi-omics, complementary breeding and artificial intelligence based predictive model are improving the discovery of resistance genes and their use, which leads to the resilience of soybean cultivars.

The management will be sustainable not only based on technological innovations, but also on active surveillance of the virus on a global level, the exchange of germplasm, and the use of both the pest and crop management practices. The need of the future research should be to design and implement multi-gene resistance and environmentally friendly genetic interventions to protect the yield potential of Glycine max and secure the world food and industrial stability. This combined strategy is the basis of dealing with soybean viral diseases in dynamic agricultural environment of 21st century.

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