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Molecular Breeding Strategies for Begomovirus Resistance in Vegetable Crops

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Introduction

Geminiviruses are rapidly becoming major plant pathogens in tropical and subtropical countries affecting an increasing number of crops with a disastrous impact on productivity. On the basis of differences in genome organization, host range and insect vectors of geminiviruses, four different genera, namely Mastrevirus, Curtovirus, Begomovirus and Topocuvirus, are recognize. The genus Begomovirus comprises 117 of the 133 officially recognized geminiviruses. Begomoviruses pose a global threat to economically significant vegetable crops, prevalent in tropical, subtropical, and temperate regions. They cause diseases in various vegetables, including cucurbits, solanaceous, malvaceous, and legume crops (Tripathi *et al.*, 2017). These viruses possess a single-stranded DNA genome, either bipartite or monopartite. Geminiviruses, within the begomovirus genus, rely on the whitefly (Bemisia tabaci) for circulative persistent transmission. Once inside plant cells, they undergo DNA replication, accumulation, assembly, and spread. Begomovirus-encoded proteins fall into categories like DNA replication (Rep and REn), host gene regulation/silencing suppression (TrAP and/or AC4), structural (CP), and movement (MP, NSP). The first three groups are encoded by the DNA A component, and the coding sequences for movement associated proteins are found on the DNA B component. The size of each component is around 2.6-2.8 kb The size of both DNA-A and DNA-B of bipartite begomoviruses is same except common region. The common region includes a loopy structure with TAATATTAC sequenced nucleotide and many other regulatory elements.

Molecular breeding strategies for begomovirus resistance in vegetable crops

Breeding genetic resistance in the host is a reliable method for efficient and long-lasting management against any disease or pathogen as it can benefit quantitatively as well as qualitatively. Breeding genetic resistance in the host (use of virus-resistant cultivars) is a reliable method for efficient and long-lasting management against any disease or pathogen.

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Marker assisted selection for virus resistance

The use of DNA markers that are tightly linked to the R gene, facilitating identification of the target gene in the population.The construction of a genetic map is a common approach to detect quantitative trait loci (QTLs) for virus resistance in crops. MAS are widely used in the introgression of oligogenes and quantitative trait loci (QTLs) for disease resistance through backcross-breeding. Marker assisted gene pyramiding entails stacking multiple genes leading to the simultaneous expression of more than one gene in a variety to develop durable resistance expression.

Example: Molecular markers linked to genes (Ty-1, Ty-2, Ty-3, Ty-4, ty5, and ty-6) nable marker-assisted selection (MAS) Ty genes, both individually and in combined forms, were introduced to IARI varieties (Pusa Rohini, Pusa Ruby, Pusa Sadabahar, and Pusa Sheetal) through hybridization.

Resistance through pathogen genes / pathogen derived resistance (PDR)

The particular concept of induction of resistance against the begomoviruses was first introduced in 1980 by Hamilton when he proposed the idea of transformation of genes derived from pathogen itself. In 1985, the hypothetical concept was taken to next levels by Sanford and Johnson. Coat protein, replicase protein and movement protein play crucial role in virus replication and infection by the virus. Targeting of these proteins helps to reduce the virus accumulation and symptoms.

Coat protein mediated resistance

Achieving resistance through CP gene involves creation of transgenic plants via expression of virus coat protein genes. Expression of a mutated non-functional CP for vector interaction could therefore potentially impede the virus spread amongst its vectors in begomovirus infected fields. Blockage of initial uncoating or re-encapsidation of the challenging viral RNA by free CP of the protecting strain, there is competition between the protecting strain and the challenge virus for a factor present in the host cell, such as a replicase component. And it is involving the interference of CP towards un-coating the virions thus inhibiting the virus spread from cell to cell leading to minimization of virus accumulation.

Example: Viral coat protein against Tomato mosaic virus (ToMV) in Tomato and against Cucumber mosaic virus (CMV) in Cucumber (Ria et al., 2016)

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Replicase protein mediated resistance

The geminivirus Rep protein plays a pivotal role in transcription regulation and initiation of rolling circle replication. It interacts with cellular proteins and the REn protein, inducing genes crucial for geminivirus DNA accumulation (Settlage et al., 2005). Targeting Rep with mutant proteins disrupts its functions, hindering viral replicase enzyme complex assembly and significantly inhibiting viral replication. This interference likely involves binding to host or viral factors that regulate replication and gene expression.

Movement protein mediated resistance

The B component of bipartite Gemini viruses encodes two proteins, NSP and MP, which are required for cell-to-cell and long-distance movement, respectively. Partially defective or incompatible MPs have been shown to disrupt the systemic spread of RNA viruses. Mutants of movement protein are thought to bind to plasmodesmata and interfere with native MP-mediated plasmodesmatal trafficking of viral DNA/RNA.The use of mutated MPs could, therefore, lead to transgenic plants that efficiently inhibit the local and systemic spread of many different viruses.

RNA mediated resistance

Increased resistance by Post transcriptional gene silencing/ RNA interference

RNA interference (RNAi) is initiated by 'artificial' or viral double-stranded RNAs (dsRNAs) produced through replication intermediates or intramolecular RNA folding (hairpin structures). This is achieved by fusing corresponding regions in sense and anti-sense orientation within one transcript. Antiviral RNA silencing is activated when Dicer-like (DCL) enzymes process viral dsRNAs into virus-derived small RNAs (vsRNAs). These vsRNAs guide Argonaute (AGO) proteins within the RNA-induced silencing complex (RISC), leading to viral RNA degradation or translational arrest. In plants, RNA silencing predominantly involves cleavage, resulting in post-transcriptional gene silencing (PTGS). This mechanism suppresses gene expression through sequence-specific mRNA degradation.

Transcriptional gene silencing

Targeting viral promoters with dsRNAs

TGS of a transgene can be induced by infection with a virus carrying a sequence homologous to the promoter of the transgene. Double small RNA-directed methylation of begomovirus bidirectional promoters may down-regulate the transcription of viral genes,

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resulting in inefficient virus replication. Triggering TGS of begomovirus promoters by pre-expression or induced expression of specific dsRNAs may therefore constitute a promising strategy to interfere with virus replication (Vanderschuren *et al*.,2007).

dsRNAs homologous to viral coding sequences

Manipulation of this balance by pre-expression of dsRNAs or by interference with the viral counteraction should constitute a promising approach to increase plant resistance (Vanderschuren *et al*.,2007). Any hairpin dsRNAs homologous to viral coding sequences may enter both known RNAi pathways**.** On the one hand, they may act in TGS complexes as sequence-specific mediators for the methylation of homologous viral DNA sequences in the nucleus. siRNAdirected methylation may also affect coding regions and thereby cause reduced transcription. Methylation can spread from the primary dsRNA-targeted genomic DNA sequence in the 5′ and 3′ directions. The appearance of siRNA against a region within the Rep coding sequence may inhibit transcription of virus.

Satellite DNA

Some strains of virus possess satellite DNA which acts as parasite of the virus and depend on the host or helper virus for replication, movement within the plant, encapsicidation and transmission. The presence of satellite depresses symptoms as well as accumulation of helper virus in the host. This phenomenon has been utilized to engineer virus resistance in plants by introducing partial copies of satellite DNA.

Example: Satellite RNA against Cucumber mosaic virus (CMV) in Tomato

Antisense RNA (As-RNA)

Antisense RNA technology is effective for managing virus diseases in plants (Islam and Wu, 2017). Utilizing pathogen-derived resistance, antisense RNA, complementary to the viral genome, shows potential for safeguarding plants from systemic virus infections. Antisense RNAs are small molecules that negatively control the interaction of target RNA with other nucleic acids or proteins by pairing with the target RNA sequence. RNase H enhances the degradation of double-stranded RNA, operating entirely on homology with the target sequence.

Example: Antisense RNA of rep gene against Tomato leaf curl virus (ToLCV) in Tomato

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Non-viral Sources of Transgenic Virus Resistance

- Ribosomal inactivating proteins: RIPS are RNA glycosides that specifically remove purine 28s rRNA resulting in separation of the 3' end of the substrate RNA, r RNA becomes incapable of participating in the translation.
- Anti-viral plant bodies: Control of plant viral diseases can be done through the expression of antibodies.
- Protease inhibitors: Plant possessing cystein protease which is inhibitor replication of viruses and this strategy has been utilized to engineer resistance to these viruses.

Genome editing

Engineering ZFN- or TALEN-based resistance against viruses

In the last decade, genome editing, particularly CRISPR/Cas, has revolutionized genetic improvement for plant virus resistance, expediting resistance breeding (Zhao et al., 2020). ZFNs and TALENs, utilizing chimeric proteins, involve a DNA-binding domain (DBD) fused with FokI enzyme's non-specific cleavage domain (Zhao *et al.*, 2020). The DBD ensures specific nucleotide recognition in the DNA target, leading to double-strand breaks (DSBs) in the viral genome. Repair mechanisms, either imprecise non-homologous end-joining (NHEJ) or precise homology-directed repair, induce mutations in the viral genome. ZFN technology, applied to begomoviruses, targets the Rep gene. TALEs are engineered to target conserved motifs among begomoviruses.

CRISPR-Cas9 technology

CRISPR/Cas genome editing systems consist of an endonuclease SpCas protein and a single-guide RNA (sgRNA) which directs the Cas protein to the DNA. Designing a sgRNAs to target the Rep or CP loci, and significantly reduced or abolished disease symptoms of several begomoviruses. Destroying an essential host factor by CRISPR/Cas9 system is an effective way to generate virus-resistant crops. CRISPR/Cas9 machinery targeting non-coding sequences of different Gemini viruses is effective approach (Zhao *et al.*, 2020).

Example: Tashkandi *et al*., 2018 targeted CP and Rep genes using CRISPR-SpCas9 technology against Begomovirus (ssDNA)- Tomato yellow leaf curl virus in Tomato

Conclusion

Urgent action is required for breeding begomovirus-resistant crops. Marker-assisted breeding accelerates the development of virus-resistant varieties. Biotechnological insights,

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including protein and RNA-mediated resistance techniques, offer promising tools to combat begomovirus diseases in vegetables. Genome editing techniques enhance resistance by integrating, deleting, or mutating target genes. Multiplexing these strategies is crucial to engineer broad-spectrum virus-resistant vegetable crops. Molecular breeding, encompassing marker-assisted methods, genetic engineering, and genome editing, provides a rapid pathway for developing resistance against begomoviruses in vegetable crops.

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