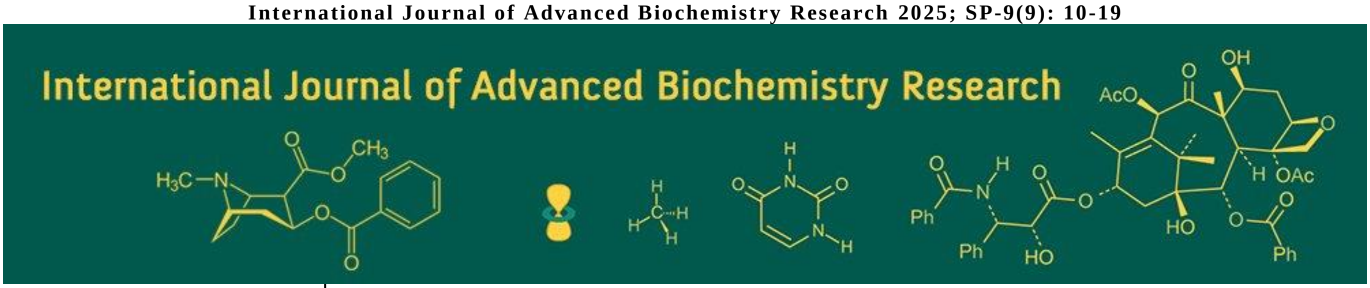

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Advanced breeding strategies for enhancing biotic stress resistance in cucurbits: A comprehensive review

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Abstract

Cucurbits, including cucumbers, melons, watermelons, pumpkins, and squashes, hold significant value as horticultural commodities with global economic significance. Their cultivation faces severe threats from diverse biotic stresses such as bacterial, fungal, viral pathogens, and insect pests, causing considerable reductions in productivity and quality. Conventional breeding methods have traditionally been essential in developing resistant cultivars but encounter challenges due to pathogen evolution and linkage drag. Recently, molecular breeding technologies such as marker-assisted selection, genomic selection, as well as genome editing tools like that CRISPR/Cas9 have transformed the process of breeding for resistance, enabling accurate and effective incorporation of genes. This review synthesizes current breeding strategies, significant advancements, and future prospects for sustainably enhancing biotic stress resistance in cucurbits.

Keywords: Cucurbits, biotic stress, powdery mildew, marker-assisted selection, genomic selection, CRISPR/Cas9, disease resistance

Introduction

Cucurbits including cucumbers melons, watermelons, pumpkins, and squashes are important crops grown extensively across the globe. However, their cultivation faces significant challenges from various biotic stresses such as viral, bacterial, and fungal diseases, along with infestations by insect pests. Key threats include DM (downy mildew), PM (powdery mildew), FW (Fusarium wilt), CMV (cucumber mosaic virus), ZYMV (zucchini yellow mosaic virus) and pests such as aphids and whiteflies.

To combat these issues, both classical breeding methods and modern biotechnology have been employed to develop resistant varieties. Traditional procedures, namely selection, hybridization, and backcrossing, have supported the advancement of transferring resistance attributes. Meanwhile, contemporary molecular breeding techniques including where genomic selection, marker-assisted selection and genome-editing platforms such as CRISPR/Cas9 have provided precision in incorporating resistance genes. This review examines these breeding strategies, their benefits, challenges, and future directions aimed at ensuring sustainable cucurbit cultivation.

The Cucurbitaceae family is diverse, consisting of two subfamilies, eight tribes, 118 genera, and roughly 825 species, about 20 of which are cultivated commercially (Jeffrey, 1980). These crops flourish mainly in warm climates but some temperate species survive through underground tubers or seed dormancy. They are found across tropical, subtropical, arid, and temperate zones, especially across tropical Africa and the neotropical region (Jeffrey, 1980). Cucurbitaceae family contribute substantially for nutrition and economy, consumed fresh or cooked in various forms—cucumbers and melons in salads, ash gourd and pointed gourd in culinary dishes, and bitter gourd for medicinal use. Advancements in seed quality, including hybrid vigor, along with improved production practices and supportive policies, have the potential to boost productivity and sustainability in cucurbit farming.

The developing cultivars resistant to biotic stresses through traditional breeding remains complex, largely due to the fast evolution of pathogens, which often outpace the effectiveness of resistance genes. Moreover, linkage drag-where undesirable traits are inherited alongside desired ones-further complicates breeding efforts.

Recent developments in molecular biology and genomics, however, have facilitated rapid progress with respect to cucurbit breeding. Respective discovery of resistance genes and quantitative trait loci associated with major diseases has enabled marker-assisted breeding programs, speeding up the development of resilient varieties. In recent decades, genetic engineering has been increasingly used to upgrade disease resistance, yield, and quality in cucurbits (Parmar *et al.*, 2017) [30]. Transgenic methods have introduced resistance genes from diverse sources into cucurbit genomes, while gene editing technologies like CRISPR/Cas9 have facilitated precise gene modifications, such as mutations, repression, activation, and epigenome editing, thereby advancing breeding strategies (Wang *et al.*, 2019) [41].

Looking ahead, the integration of traditional breeding with advanced genomics, transcriptomics, and genome editing is expected to shape the future of cucurbit improvement. Combining genomic selection, CRISPR technology, and biotechnological innovations promises to develop durable, high-producing and quality-enhanced cucurbit cultivars with enhanced resistance to evolving biotic stresses, supporting sustainable agricultural production.

Various Biotic Stresses Influencing Cucurbits

Diseases in Cucurbits

Vegetable diseases are primarily caused by biotic various pathogens such as fungi, bacteria, viruses, viroids, phytoplasmas, along with nematodes, leading to significant reductions in both production efficiency and quality attributes. Major challenges in vegetable production is disease pressure, which affects crops from the seedling stage to harvest, along with post-harvest losses due to microbial spoilage during transit, storage, and marketing. Due to their high water content and rapid metabolic activity, vegetables are particularly vulnerable to pathogen attacks. Among plant pathogens, fungi represent the most serious constraint to potential yields, particularly in tropical and subtropical developing regions. In cucurbits, the primary biotic stressors are fungal, bacterial, and viral diseases, with some also attributed to mycoplasmas. An overview among the key diseases impacting cucurbits is provided below.

Powdery Mildew in Cucurbits

It's causal organism *Podosphaera fusca*, is a highly destructive disease that results in substantial yield losses and reduces fruit quality. Various resistant germplasm to powdery mildew have been identified in cucumbers. In China, P. xanthii infection has been observed to cause white, talcum-like fungal growth symptoms. For more than forty years, the cucumber accession PI 197087 has demonstrated a high level of resistance to PMR (Block and Reitsma, 2005) [7]. Quantitative trait loci mapping has emerged as a very effective molecular technique for investigating PMR in cucumbers (Liu *et al.*, 2008) [22]. Sakata *et al.* (2006) [32] identified temperature-regulated QTLs associated along with powdery mildew (PM) resistance, while Shanmugasundaram (1971) [34] was the first to examine powdery mildew resistance in hypocotyls as well as leaves, identifying a recessive gene responsible for hypocotyl resistance.

The classical genetic analysis has linked powdery mildew resistance to the D locus on chromosome 5, which is also associated with dull fruit color in cucumber (Fanourakis and Simon, 1987) [11]. In the European cucumber accession S09, five quantitative trait loci associated with powdery mildew (PM) resistance were mapped across three linkage groups (Liu *et al.*, 2008) [22]. Similarly, Alfandi *et al.* (2009) [1] reported an ISSR marker (UBC809) tightly linked to PMR in the cucumber accession JIN5, positioned on chromosome 5 in proximity to the QTL *pm5.2*.

A comprehensive understanding of PMR gene control in cucumber remains incomplete. Variability in Quantitative Trait Loci locations as well as numbers across different chromosomes and linkage groups is likely due to differences in PMR sources, detection methods, and environmental conditions. He *et al.* (2013) [14] performed Quantitative Trait Loci mapping using Simple Sequence Repeat markers and identified six genomic regions associated with powdery mildew (PM) resistance (PMR) distributed across four cucumber chromosomes. Among these, two major QTLs, *pm5.1* and *pm5.2*, both located on chromosome 5, explained 21% and 74.5% of the phenotypic variation, respectively. In further investigations, also identified three PMR genes (*pm-1*, *pm-2*, and *pm-3*) along with an additional gene (*pm-h*) associated with hypocotyl resistance.

A candidate gene linked to powdery mildew resistance (PMR) was identified on chromosome 5, where the QTL *pm5.1* confers resistance through the loss of function of the susceptibility gene (*CsMLO1*) activity (Berg *et al.*, 2015) [5]. Nie *et al.* (2015b) [27] further demonstrated that a single base-pair insertion in *CsMLO1* contributed to PMR in the cucumber accession PI 197088. Single nucleotide polymorphisms (SNPs) have emerged as powerful molecular tools for genetic studies, and next-generation sequencing (NGS) technologies utilizing SNPs are now widely applied for functional genomic analyses across diverse species (Mokry *et al.*, 2011) [25].

Overview of Downy Mildew in Cucurbits

Downy mildew, which occurs due to *Pseudoperonospora cubensis*, is among the most destructive foliar diseases in cucumber. In the Indian cucumber accession PI 197087, resistance was initially attributed to a single recessive gene (*dm-1*) prior to 2004 (Van Vliet and Meysing, 1974) [39]. However, the emergence of new, virulent pathogen races has reduced the effectiveness of *dm-1*, which now confers only moderate resistance (Cohen *et al.*, 2015) [10]. New sources of resistance have been identified, including PI 197088 and PI 605996 both of Indian origin (Call *et al.*, 2012) [8]

The STAYGREEN (*CsSGR*) gene has been identified as a major genetic determinant of downy mildew resistance in cucumber (Wang *et al.*, 2019) [41]. Berg *et al.* (2020) [6] further mapped the *dm4.1* QTL on chromosome 4 and identified the receptor-like kinase gene (*CsLRK10L2*), which is upregulated upon pathogen infection, as a contributor to resistance. In addition, within the sub-QTL *dm4.1.3*, Berg (2020) [6] reported that a mutation in the amino acid permease gene (*CsAAP2A*), which encodes an amino acid transporter, also plays a role in conferring resistance to downy mildew.

Liu *et al.* (2020) [23] found 18 loci that contribute to downy mildew (PM) resistance in all the linkage groups and picked six loci with consistent effects on resistance. Some candidate genes, such as Csa1G575030, Csa2G06036 and Csa7G004020, have been found to be possible contributors to downy mildew (DM) resistance in cucumber.

Anthracnose in cucurbits

Anthracnose is a serious foliar disease in cucumber, capable of causing substantial yield losses under favorable environmental conditions. The earliest sources of anthracnose resistance were identified in cucumber genotypes PI 197087 and PI 234517. Subsequent research, demonstrated that resistance in PI 197087 is governed by specific major genes. Moreover, several cucumber accessions, including PI 175111, PI 175120, PI 179676, PI 183308, and PI 183445, have shown moderate levels of resistance to anthracnose (Barnes and Epps, 1954) [3].

Gy3 and Gy14 cucumber lines exhibited higher resistance to anthracnose (Wyszogrodzka, 1987) [44], while accession AR79-95 demonstrated resistance against race-2 of the disease (Goode and Browers, 1973) [13]. In the 19B cucumber line, anthracnose resistance was found to be governed by a single recessive gene (cla). A SCAR marker was later identified as being linked to A single recessive gene controls anthracnose resistance in cucumber line 66′.

Identified that resistance to anthracnose race 1 is also controlled by the cla gene in Gy14 and WI 2757, pinpointing it to a 32 kb region on chromosome 5 during fine mapping (Pan *et al.* 2018) [28]. They further identified three candidate genes associated along with resistance, with STAYGREEN (CsSGR) emerging as a strong candidate gene based on multiline evaluations. Understanding the inheritance of anthracnose resistance and identifying closely linked markers are essential for developing resistant cucumber varieties via marker-assisted selection and backcross breeding.

Overview of Gummy Stem Blight in Cucurbits

It’s causal organism *Phoma cucurbitacearum*, is a severe disease affecting various cucurbits crops such as muskmelon, cucumber, ridge gourd and bottle gourd. The disease is particularly aggressive in hybrid cultivars.

The first signs manifest as water-soaked lesions at the stem base near the soil line. As the infection progresses, translucent, gum-like exudates form over the affected part of plants. The disease is characterized by silvery grey shade to dark brown lesions at the stem base, leading to girdling, which ultimately causes plant death. Black dots resembling pycnidia can also be observed on the damaged bark. In some cases, rapid wilting and plant collapse occur, especially in bottle gourd and cucumber.

Cercospora Leaf Spot in cucurbits

Cercospora Leaf Spot is a destructive disease of cucumber, caused by *Cercospora citrullina*. It predominantly affects older foliage, beginning with small lesions that have pale to tan-brown centers. As the infection progresses, these spots enlarge, eventually covering significant portions of the leaf surface. The lesions often develop dark margins with surrounding chlorotic halos, and in advanced stages, the centers may dry out, turn brittle, as well as crack. The pathogen survives in crop residues and is disseminated mainly by wind and water splashes. This disease is many prevalent in tropical as well as subtropical regions, where warm, humid environments favor its spread.

Overview of Scab in Cucurbits

Scab disease in cucumbers is caused by *Cladosporium cucumerinum*, a fungal pathogen that thrives in cool, wet conditions. The disease manifests as angular, dark spots on the leaves, often restricted by small veins. Initially, the lesions appear water-soaked and pale green but later dry out, leading to the formation of holes in the affected leaves. In some cases, lesions may also develop on stems, petioles as well as fruits, causing significant damage. The fungus overwinters in plant debris present in the soil, with infection being most prevalent in humid conditions and temperatures below 21 °C. Proper field sanitation, crop rotation, and resistant varieties can help manage the disease effectively.

Overview of Verticillium Wilt in Cucurbits

Verticillium Wilt, caused by Verticillium dahliae, typically appears after fruit set, leading to chlorosis (yellowing) and necrotic (dead) regions on leaves, which eventually collapse. A distinctive feature of this disease is that symptoms often develop on only one side of the vine. Additionally, infected plants exhibit vascular tissue discoloration in the roots. The fungus can persist in the soil for extended periods, making it challenging to control. Cool to mild temperatures in spring favor disease development, allowing the pathogen to infect susceptible plants and cause significant yield losses.

Overview of cumber Mosaic virus (CMV) in Cucurbits

Cucumber Mosaic Virus is many severe viral diseases affecting cucumbers, transmitted primarily by aphids. Over 80 aphid species, including Aphis gossypii and Myzus persicae can spread the virus.

Symptoms of CMV include severe stunting, a prominent yellow mosaic pattern on the foliage, and downward curling of leaves, which become smaller than normal. Infected plants may also produce malformed flowers with green petals. The fruits are often deformed, undersized, and discolored, reducing marketability. CMV can infect a wide range of plant species, making it difficult to control. However, the virus does not survive well in extremely dry conditions, which can limit its spread in arid environments.

Cucumber Green Mottle Mosaic Virus in Cucurbits

This virus was first identified in the United States (US) on a melon seed production farm in California. It infects mostly all cucurbit species, symptoms include leaf mottling and mosaic patterns, along with fruit mottling and deformation. Young leaves exhibit vein clearing and distortion as early signs, while chlorosis and bleaching become evident in fully developed leaves. In severe cases, cotyledons may turn yellow, though symptoms typically become noticeable by the first or second leaf stage.

It looks like you're describing Cucurbit yellow stunting disorder virus or another similar cucurbit infecting virus. CYSDV transmitted by whiteflies and causes heavy yield losses in all cucurbit crops worldwide. The symptoms you mention, such as mottling, chlorosis, and deformation of leaves as fruits, are characteristic of many viral diseases in cucurbits.

Zucchini Yellow Mosaic Virus in Cucurbits

This viral disease, initially identified in Europe in 1981, primarily affects cucurbit crops. By 1983, it had also been detected in New York State and has since been reported in most southern and south western regions. Zucchini yellow mosaic virus (ZYMV), classified under the *Potyvirus* genus, induces characteristic foliar symptoms such as a pronounced yellow mosaic, tissue necrosis, green vein banding, and chlorotic spotting, blistering, leaf deformation, and overall stunted growth. The virus is transmitted by several aphid species, with *Aphis craccivora* and *Aphis gossypii* being the primary vectors.

Chlorotic Curly Stunt in Cucurbits

Infected plants exhibit severe stunting, with small, chlorotic, and slightly curled leaves. The disease is primarily transmitted by the whitefly (*Bemisia tabac*i) but is not spread through sap. Several cucurbit species, including *Cucumis sativus*, *Luffa acutangula*, and *Luffa cylindrica*, are susceptible to the virus. The disease was first reported in India between 2003 and 2006, where it appeared as chlorotic curly stunt disease (CCSD) in vegetable growing regions of Delhi and the adjacent state of Haryana.

Biotic Stress Tolerance in Cucurbits

Resistance to Cucurbits' Viral Diseases

Viral diseases pose significant threats to watermelon crop yield, severely restricting its productivity. The most critical viruses affecting watermelon include cucumber mosaic virus, squash mosaic virus, watermelon mosaic virus 1 (WMV-1), watermelon mosaic virus-2 , and zucchini yellow mosaic virus (ZYMV) are major viral pathogens affecting cucurbits. While SqMV is seedborne in melons and predominantly transmitted by beetles, the remaining viruses are spread by various aphid species in a non-persistent manner.

Most cucurbit crops lack inherent virus resistance, although some varieties exhibit resistance to specific viruses, and certain factors enhance disease response. Conventional breeding has had limited success in developing virus-resistant cucurbit crops due to incompatibility barriers between species, which restrict the transfer of resistance traits. In cucurbits, the efficiency of traditional breeding approaches depends on the genetic basis, specifically the number of dominant or recessive genes involved. For instance, CMV resistance in cucumber is influenced by three recessive genes (Kooistra, 1969) [18], while resistance to ZYMV in melon is controlled by three complementary dominant genes. To enhance virus resistance, transgenic cucumbers have been developed by introducing both single and multiple resistance genes. Among these, transgenic cucumbers carrying several genes derived from different viruses have demonstrated the best results achieved in the field trials.

Viruses such as Cucumber Mosaic Virus, Zucchini Yellow Mosaic Virus (ZYMV) and Yellow Mosaic Virus (YMV), pose significant threats to cucurbits. To enhance resistance against these viral diseases, the coat protein (CP) genes of these viruses have been introduced into plants using Agrobacterium-mediated transformation. The overexpression of these genes in the plant system has been shown to confer resistance by interfering with viral replication and movement.

Nishibayashi *et al.* (1996) successfully introduced the CMV-cp coat protein gene into cucumbers using Agrobacterium-mediated transformation, demonstrating a potential strategy for developing virus-resistant varieties. This approach has paved the way for advanced genetic engineering techniques aimed at improving viral resistance in cucurbit crops.

Sheng-Niao *et al.* (2005) engineered transgenic watermelon plants with resistance to three viral diseases by introducing the coat protein gene from Watermelon mosaic virus (WMV) and the replicase genes from Cucumber mosaic virus and Zucchini yellow mosaic virus. Virus resistance was assessed through greenhouse and field trials, where transgenic lines exhibited varying responses, including susceptibility, resistance, immunity, or recovery from infection at later growth stages. Notably, T3 plants of the BH1-7 line demonstrated a relatively high level of resistance. These findings suggest the potential for developing virus-resistant watermelon varieties through transgenic approaches.

Grafting is commonly practiced in cucurbitaceous crops such as watermelon, cucumber, and melons to enhance disease resistance through resistant rootstocks. Transgenic watermelon plants overexpressing viral coat protein genes exhibit superior virus tolerance. In a study by Park *et al.* (2005), gongdae transgenic lines, engineered to overexpress CGMMV coat protein (CGMMV-CP), were grafted onto susceptible watermelon rootstocks. The grafted wild-type watermelon displayed the same resistance, indicating that resistance was conferred through grafting. However, the gene products from transgenic watermelon rootstocks did not transfer to the non-transgenic watermelon scion. These findings are crucial for assessing the food safety of transgenic watermelon fruits intended for human consumption.

The origin of muskmelon has been a topic of debate. While it was initially believed to have originated in Africa, recent findings suggest otherwise, as a number of related wild species have been found in India and Australia (Sebastian *et al.*, 2010) [33]. This has led to a reassessment of its evolutionary history and domestication patterns.

Muskmelon is not only a widely cultivated fruit but also a powerhouse of nutrition. Rich in vitamins A and C, antioxidants, and essential minerals, it contributes to overall health and well-being. Traditionally, muskmelon has been valued for its medicinal properties, with historical records highlighting its role in hydration, digestion, and immune support. Its high water content makes it a refreshing and hydrating fruit, particularly beneficial during hot climates.

Cantaloupe seeds have been traditionally used in China to regulate fevers and aid digestion, while in other regions, they were ground into powder for tuberculosis treatment. Cantaloupes are particularly beneficial for heart health due to their high content of adenosine, a natural anticoagulant. They are also rich in potassium, which helps manage high blood pressure (HBP). Given a high water content, melons act as natural diuretics.

Commercial melons are categorized into three primary groups

Cantalupensis Group: True cantaloupes with rough, warty skin, orange flesh, and no netting, commonly cultivated in Europe.

Inodorous melon category: Melons with a smooth rind of white color enclosing sweet green flesh, lacking a musky aroma.

Reticulatus Group: The netted melon varieties exhibit a sweet aroma and distinct rind pattern, typical of the Reticulatus Group

The post-harvest life of melons is short, making them prone to viral pathogens. Consequently, transgenic crop development has focused on enhancing fruit quality, extending shelf life, and improving resistance to viral infections.

Bitter gourd, also known as bitter melon, is recognized as extensively grown across Asia, Africa, and the Middle East for its bitter-tasting, nutrient-rich green fruits, which are typically consumed, cooked as a vegetable. The fruit is also recognized for its medicinal properties (momordin, charantin, charantosides, goyaglycosides, momordicosides, charantin and good source of Vitamin A, Vit-C, carbohydrates, phosphorus and iron).

Bitter gourd was successfully transformed through Agrobacterium-mediated transformation employing immature cotyledonary nodes as explants. These cotyledonary nodes, derived from immature fruits, were imbibed and efficiently employed for transformation. A standardized protocol for transformation and regeneration was established using the GUS (β-glucuronidase) and NPT II (Neomycin Phosphotransferase II) genes, involving callus induction and shoot regeneration on a medium supplemented with thidiazuron and naphthaleneacetic acid (Sikdar *et al.*, 2005) [35].

Bitter gourd, also known as bitter melon, is extensively grown widely in Asia, Africa as well as the Middle East on account of its nutritious, bitter-tasting green fruits, which are commonly consumed as a cooked vegetable. The fruit is recognized for its medicinal properties and contains several bioactive compounds, including momordin, charantin, charantosides, goyaglycosides, and momordicosides. Among these, charantin has been shown to encourage hypoglycemia in diabetics. It is a good source of Vit-A, Vit-C, phosphorus, iron and carbohydrates. It also contains RIPs, which are known to prevent tumor cell development. Notably, the fruit has exceptionally high β-carotene content-nearly five times that of carrots.

Corynespora cassiicola, causal organism of target leaf spot in cucumber, is a major foliar disease that affects both seedlings as well as mature plants, leading to yield losses of 20-70% (Yang *et al.*, 2012) [45]. The genetic control of Target leaf spot resistance varies among cucumber lines. In the Royal Sluis 72502 line, resistance is determined by a single dominant gene.

Table 1: Different Genetic resources of cucurbits resistant/tolerant to various diseases

Crop	Disease/Insect pests	Resistance source
Watermelon	Fusarium wilt (FW)	All Sweet, Charleston Gray, Lousiana Queen, Citron, Calhoun Gray, Dixielle Crimson Sweet, Sornkylee and Summit
Musk melon	Fusarium wilt (FW)	*Indorus*, *chito*, Delicious-51, *Cucumis melo* var. *flexuosus* and *reticulatus*
	Watermelon mosaic virus (WMV)	Table Green and Sarinam
	Cecospora leaf spot	IC536594, IC550741, IC297489, IC567545, IC541223, IC279731
Bottle Gourd	Cucumber mosaic virus	Table Green, Chinese Long, Wisconsim, TMG-1 and Tokyo Long Green,
Cucumber	Cucumber green mottle mosaic virus (CGMMV)	*Cucumis anguria*
	Watermelon mosaic virus WMV)	Sarinam and Table Green
	Watermelon mosaic virus (WMV)	*Cucurbita ecuadorensis* and *Cucurbita foetidissima* against
Pumpkin & squash	Squash mosaic virus	*Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita moschata*

(Naik *et al.* 2013) [26]

Transgenic Approaches for Biotic Stress Management

Insect Pest in cucurbits

"Insect pests represent a major biotic constraint to cucurbit production. Beyond causing direct feeding damage, many pests act as vectors for a wide range of viral diseases. The following are insect pests that are very important to vegetable crops and cause production loss (Table: 2). In recent years, a shift in pest status has been observed, driven by changes in cropping practices, climate variability, and the adoption of high-yielding, input-intensive hybrid varieties. Consequently, many pests have expanded to new host plants, developed resistance to pesticides, and caused frequent secondary outbreaks. A brief overview of the most prevalent insect pests impacting cucurbit crops is provided below."

Table 2: Yield losses in cucurbits caused by major insect pests in India

Crop/Pest	Yield loss (%)
Bitter gourd	60-80
Cucumber	20-39
Sponge gourd	50
Musk melon	75-100
Ivy gourd	63
Snake gourd	63

(Ranjan, *et al.*, 2022) [31]

Overview of Red Pumpkin Beetle in Cucurbits

Both grubs and beetles cause significant damage to crops, with beetles being the more destructive pests. They feed on flowers and create holes in leaves, cotyledons, and foliage, leading to extensive damage. Almost all cucurbit crops are susceptible, with early-planted cucurbits suffering the most, often requiring re-sowing. The characteristic damage includes numerous holes on leaves due to beetle feeding. Meanwhile, grubs hatch and feed on plant roots below the soil surface, weakening the plants. Additionally, grubs bore through vines and consume fruits that come into contact with the soil, further reducing crop yield and quality.

Fruit Fly in cucurbits

Maggots exclusively damage mature fruits by burrowing into the flesh and feeding on the pulp, creating sores and riddling the fruit. Their feeding activity causes brown, resinous fluid to leak, leading to fruit deformation. The infestation often results in bacterial infections, accelerating fruit rot. Melons and bitter gourds are particularly vulnerable, suffering the most damage. Young developing fruits are also attacked, stunting their growth and preventing proper development. Infested fruits often drop prematurely, further reducing yield and quality.

In addition to major insect pests, cucurbits are susceptible to several minor pests, including:

1. **Stem gall fly (*Neolasioptera falcata*):** Induces gall formation on stems, resulting in stunted growth.
2. **Stem borer / Clear-winged moth (*Melittia eurytion*):** Larvae tunnel into stems, weakening plants and causing wilting.
3. **Stem-boring grey beetle (*Apomecyna saltator*):** Feeds on stems, creating tunnels that disrupt nutrient transport.
4. **Plume moth (*Sphenarches caffer*):** Larvae damage flowers and young fruits, reducing fruit set.
5. **Stink bug (*Aspongopus janus*):** Sucks sap from leaves and stems, causing deformation and reduced vigor.
6. **Flower feeder (*Mylabris pustulata*):** Consumes flowers, negatively impacting fruit set and yield.

The genetic resources of resistance genes are important for breeding programs

These genetic resources form the basis of breeding programs focused on enhancing cucurbit resistance to major biotic stresses. Cucurbit accessions reported by Naik *et al.* (2013) [26] with notable resistance traits include**:**

1. **Cucumber (*Cucumis sativus*):** Sources resistant to Downy mildew, Powdery mildew, and Fusarium wilt.
2. **Watermelon (*Citrullus lanatus*):** Accessions exhibiting resistance to Fusarium wilt, Gummy stem blight and Anthracnose.
3. **Bitter gourd (*Momordica charantia*):** Identified resistance against Powdery mildew, Cercospora leaf spot (*Cercospora citrullina*), and fruit flies.
4. **Muskmelon (*Cucumis melo*):** Resistant accessions for Downy mildew, Powdery mildew and Melon aphid.
5. **Pumpkin (*Cucurbita moschata, Cucurbita maxima*):** Resistance reported for viral pathogens including Zucchini yellow mosaic virus (ZYMV) and Squash mosaic virus (SqMV)

Transgenic Approaches for Biotic Stress Resistance

Resistance to Insect Infestation

Present, most crop plants lack inherent resistance to insect pests. Reliance on chemical treatments for pest control poses health risks to users and is environmentally unsustainable. From a producer's perspective, any genetic improvement that reduces the need for chemical applications would be highly advantageous. Fuchs *et al.* (2004) [12] examined the performance of transgenic squash expressing coat protein genes from three potyviruses: Zucchini yellow mosaic virus, Watermelon mosaic virus and Cucumber mosaic virus (Tricoli *et al.*, 1995) [37]. Plants carrying these genes exhibited tolerance to all three aphid-transmitted viruses. Across multiple field trials under heavy disease pressure, the transgenic squash and its hybrid progeny showed enhanced viral resistance, more vigorous growth, and higher fruit yield compared to non-transgenic and wild-type hybrid segregants.

Due to the low transformation efficiency in watermelon, only a few transgenic watermelons have been reported to exhibit viral resistance (Yu *et al.*, 2008) [46]. The primary approach for achieving virus resistance in watermelon has involved virus coat protein (CP) manipulation through RNA silencing, specifically siRNA-mediated gene suppression.

Recent advances in synthetic microRNA (miRNA) technology have enabled more efficient gene silencing, making it possible to generate antiviral plants through transgenic techniques. Previous studies demonstrated that transgenic tomatoes expressing artificial microRNA (amiRNA) targeting Cucumber mosaic virus (CMV) 2a/2b genes or the highly conserved 3′-untranslated region (UTR) exhibited effective resistance to CMV infection (Zhang *et al.*, 2011) [47]. Similarly**,** transgenic watermelon engineered to express amiRNA against the CMV 2a/2b gene demonstrated marked resistance to CMV infection.

Diseases Resistance in cucurbits

The major limitation affecting cucurbit crop productivity is the prevalence of various diseases caused by bacteria, fungi, and viruses. "The application of traditional breeding in cucurbit crops has been limited due to the unavailability of tolerant genes within their genetic system. A primary objective of crop genetic improvement is to enhance resilience and develop resistance against diverse pathogens. In cucurbits, gene-level modifications for disease tolerance have gained significant attention due to their effectiveness and cost-efficiency.

In recent years, the range of diseases affecting cucurbit crops has expanded to include Cucurbit yellow stunting disorder virus, Rhizopycnis root rot, Cucurbit leaf curl virus, Cucurbit leaf crumple virus, Acremonium collapse, cucumber root mat, bacterial blight and cucurbit yellow vine disease have been documented (Vasudevan *et al.*, 2007) [40]. Other major diseases of concern include powdery mildew in watermelon, vine decline, bacterial wilt, Phytophthora blight, Fusarium infections, and viral pathogens such as Melon necrotic spot carmovirus and various criniviruses (Clough and Hamm, 1995) [9].

To combat these diseases, several strategies are employed, such as crop rotation, soil fumigation, minimizing harvest-related injuries, post-harvest treatments like chlorine sprays or hot water, sanitation practices, drip irrigation, removing symptomatic fruits before storage, using pathogen-free seeds, applying plastic mulch or other soil barriers, deep ploughing, adjusting soil pH, and controlling weeds and insects. Additional methods include leveraging plant resistance, applying fungicides, soil solarization, managing greenhouse climates, improving soil drainage, using treated seeds, planting in optimal soil temperatures, removing infected plants, and ensuring proper storage conditions, including refrigeration. Furthermore, various transgenic cucurbit lines have been developed and field-tested by commercial entities (Azadi *et al.* 2011) [2].

Virus Resistance in cucurbits

Zucchini yellow mosaic virus, Watermelon mosaic virus II (WMV-II), Papaya ringspot virus, as well as other potyviruses are among the many prevalent viral pathogens affecting cucumbers (Ling *et al.*, 1991) [21]. Genetic engineering techniques, particularly those involving coat protein genes, have been employed to develop virus resistance. However, the resistance achieved is generally limited to viruses within the same group or those that are closely related.

Resistance varies from reducing symptom severity to delaying symptom onset or complete susceptibility, where no adverse effects are observed, and viral infections persist in the host for an extended period. Employed Agrobacterium-mediated transformation in muskmelon to introduce a conserved core region along with three variants of the ZYMV coat protein (CP) gene: an antisense version, a full-length gene, and a truncated form. Western and Northern blot analyses were used to confirm gene expression. The T1 progeny of transgenic plants expressing the full-length CP gene exhibited complete immunity to Zucchini yellow mosaic virus (ZYMV), showing no symptoms or detectable infection titers for at least three months. In contrast, plants expressing truncated or antisense versions of the CP gene displayed delayed symptom onset and reduced viral titers. This study highlights that genetic engineering can confer high levels of protection against viral infections in cucurbit crops.

Unlike Pathogen-Derived Resistance methods used to create virus-free plants, the application of ribozyme-linked genes offers an alternative approach to safeguarding plants against viral infections. While some plant virologists have raised valid concerns about the use of viral genes in transgenic plants, ribozyme genes hold potential for diverse applications in agricultural biotechnology. Ribozyme genes have been successfully employed to enhance resistance in melon plants against two potyviruses, namely Zucchini yellow mosaic virus and Watermelon mosaic virus 2 (WMV-2).

Except for Squash mosaic virus (SqMV), which is beetle-transmitted and seed-borne and primarily affects melon, most major cucurbit viruses are spread in a non-persistent manner by aphid species. Sheng-Niao *et al.* (2005) developed transgenic watermelons resistant to Cucumber mosaic virus (CMV), Watermelon mosaic virus, and Zucchini yellow mosaic virus using Agrobacterium-mediated transformation, achieving a transformation efficiency of 1.7%. The effectiveness of viral protection was assessed under both field and controlled greenhouse conditions. Transgenic watermelon plants exhibited varied responses during late growth stages, including resistant, sensitive, and immune phenotypes. Notably, T3 plants from the BH1-7 line demonstrated a high level of tolerance. These results indicate that transgenic technology offers significant potential for developing watermelon varieties resistant to viral diseases.

Through biotechnological approaches, the virus coat protein gene has been transferred to confer resistance to PRSV. Three transgenic lines previously identified as tolerant to CMVP1 virus also showed tolerance to CMVP0 virus. Watermelon productivity is significantly impacted globally by PRSV type W and ZYMV. Yu *et al.* (2011) successfully transformed three watermelon cultivars to achieve resistance against both PRSV and ZYMV. The RNA interference (RNAi) technique has proven effective in providing protection against various viral diseases in cucurbits. Transgenic studies conducted on different cucurbit crops have demonstrated resistance to multiple biotic stresses, as summarized in Tables 1 and 2.

Targeted Genetic Manipulation for Cucurbit Cultivation

Modern genome editing technologies rely on sequence-specific endonucleases (EENs), which are engineered to recognize and cleave DNA at precise locations. These endonucleases bind to specific DNA sequences and create targeted cuts, enabling accurate modifications to the plant genome. When DNA double-strand breaks (DSBs) are induced, cellular repair pathways, including homology-directed repair (HDR) and non-homologous end joining (NHEJ), are activated, resulting in targeted gene modifications at specific loci.

A few genome editing tools have been produced, such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas) system, specifically CRISPR-associated protein 9 (CRISPR/Cas9). ZFNs and TALENs function through the fusion of endonuclease domains to modular DNA-binding proteins, which allows the formation of targeted double-strand breaks (DSBs) at designated genomic loci. Conversely, the CRISPR/Cas9 mechanism utilizes a guide RNA that complements the target DNA through Watson-Crick base pairing, which guides the Cas9 enzyme to the intended location to be cleaved.

The CRISPR/Cas9 system, developed from a natural genome editing pathway found in bacteria and Archaea (Wiedenheft *et al.* 2012), is generally preferred because it is simple, cheap, versatile, and highly efficient. It is simpler to establish, less expensive, and less expensive for scaling compared to ZFNs and TALENs and, therefore, has proven to be a strong tool for targeted genome editing. ZFNs and TALENs, on the other hand, are encumbered by their technical technicalities and reduced efficiency.

In plants, DSBs induced by CRISPR/Cas9 are predominantly repaired via the non-homologous end joining (NHEJ) pathway (Li *et al.*, 2013) [20]. This repair process can introduce small insertions, deletions, or premature stop codons, disrupting the open reading frame of a gene and resulting in functional modifications (Belhaj *et al.*, 2013) [4]. Additionally, the rapid degradation of the Cas9-guide RNA complex in regenerating cell cultures enhances the efficiency and precision of CRISPR/Cas9, contributing to its widespread adoption over conventional transgenic approaches.

A significant advantage of CRISPR/Cas9 is that gene-edited crops often do not contain foreign transgenes, allowing them to bypass stringent GM regulations and gain broader consumer acceptance (Jones 2015) [16]. This feature, combined with its precision and efficiency, has made CRISPR/Cas9 a transformative tool in crop improvement and genome editing.

Transgenic cucumber T3 lines designed to target both eIF4E sites were resistant to a number of viruses, such as Ipomovirus, Zucchini yellow mosaic virus, potyviruses, Papaya ringspot mosaic virus-W and Cucumber vein yellowing virus. In a similar study, Malnoy *et al.* (2016) [24] utilized CRISPR/Cas9 ribonucleoproteins (RNPs) applied directly to create mutations in the MLO-7 grape gene to boost resistance against powdery mildew disease and in the DIPM-1, DIPM-2, and DIPM-4 genes in apple varieties to increase resistance to fire blight disease. Tian *et al.* (2017) [36] used CRISPR/Cas9 to induce site-specific mutations in the ClPDS (phytoene desaturase) gene of watermelon to produce an albino phenotype. The transgenic watermelon plants exhibited obvious or mosaic albino phenotypes, showing 100% efficiency in genome editing with the CRISPR/Cas9 system.

Parthenocarpy, a desirable trait in horticultural crops for industrial and consumer purposes, was achieved by Ueta *et al.* (2017) [38] in tomato plants. CRISPR/Cas9 has been utilized to introduce 100% somatic mutations of the SlIAA9 gene that produces seedless fruit with modified leaf morphology. Kishi-Kaboshi *et al.* (2017) [17] also utilized CRISPR/Cas9 to produce transgenic chrysanthemum plants that express the yellowish-green fluorescent protein (CpYGFP) gene of Chiridius poppei, which shows the adaptability CRISPR/Cas9 in plant biotechnology. These researches highlight the promise of CRISPR of /Cas9 for exact genome editing to design crops with increased resistance, new traits, and better quality.

What is Gene Silencing

Gene silencing is a molecular technique used to regulate gene activity by reducing or completely inhibiting gene expression at the transcriptional or translational level. As the term implies, this process "silences" specific genes, preventing the production of their corresponding proteins. The mechanism primarily involves neutralizing targeted mRNA molecules, thereby halting protein synthesis. Additionally, certain DNA elements, such as transposons, can disrupt gene function and effectively inactivate genes. Gene silencing is often referred to as RNA interference (RNAi), as it blocks translation and triggers the degradation of homologous RNA transcripts, significantly reducing gene expression.

Due to its precision and effectiveness, gene silencing is widely employed in functional genomics (Watson *et al.*, 2005) [42]. The technique typically relies on specific proteins or RNA molecules introduced via genetic modification. In transgenic plants, Agrobacterium-mediated transformation is commonly used to deliver the desired gene into the plant genome through T-DNA (transfer DNA). The T-DNA can integrate into the host genome in various orientations head-to-head, tail-to-tail, or head-to-tail. This process not only enables targeted gene silencing but also functions like a built-in defense system, providing resistance against viruses. Moreover, gene silencing can contribute to genomic regulation, as observed in transgenic RNA-guided DNA methylation.

Gene expression can be suppressed via various mechanisms, such as

a. RNA interference (RNAi)-Blocking mRNA translation.
b. DNA methylation-Preventing gene transcription.
c. Histone modification-Changing gene accessibility.
    1. Post translational gene silencing (PTGS)
    2. Virus induce gene silencing (VIGS)
    3. Transcriptional gene silencing (TGS)
    4. MicroRNA gene silencing (miRNA).

Uses of Gene Silencing Approaches

Biotic stressors, including diseases, insects, and nematodes, can substantially reduce crop yields. To mitigate these effects, Ribonucleic Acid (RNA) silencing technologies, in particularly hairpin RNA (hpRNA) transgenes and artificial microRNA (amiRNA) approaches, are widely employed to govern the expression of genes involved in metabolic pathways across various model and crop plant species.

These technologies have been instrumental in uncovering biochemical pathways, understanding gene functions, enhancing resistance to pathogens and pests, and improving other agronomic traits. Beyond agriculture, gene silencing has also brought significant advancements in the industrial and medical fields.

Grafting Approaches for Enhancing Biotic Stress Resistance in Cucurbits

Grafting is a horticultural technique in which two living plant segments are joined to form a single, functional plant. The upper portion, responsible for fruit production, is known as the scion, while the lower portion, which provides the root system, is called the rootstock.

The rootstock provides vigor and resistance to diseases, whereas the scion is selected for its fruit quality and characteristics. This method, a centuries-old practice for propagating fruit and woody plants, has gained popularity in the commercial vegetable industry over the past few decades.

"Grafting Approaches for Enhancing Biotic Stress Resistance in Cucurbits" refers to the different grafting techniques and strategies used in cucurbit crops (like cucumber, watermelon, melon, bottle gourd, pumpkin, squash, bitter gourd, etc.) with the aim of making them more resistant or tolerant to biotic stresses such as:

The practice of grafting vegetable plants began in the late 1920s in Korea and Japan, where watermelon seedlings were grafted onto squash rootstock.

Vegetable production often faces challenges due to various biotic and abiotic stressors, leading to reduced yields and poor quality. To combat biotic stresses, farmers frequently resort to excessive pesticide use, raising concerns among health-conscious consumers. Soil-borne diseases, in particular, significantly impact the vegetable industry (Lee *et al.* 2010) [19]. Grafting provides numerous advantages, including increased yield, enhanced shoot growth, improved resistance to diseases and nematodes, and tolerance to extreme temperatures. It also promotes better nutrient and water uptake, confers tolerance to salinity, waterlogging, heavy metals, and organic pollutants, improves fruit quality, extends harvest periods, and supports multiple or successive cropping cycles. It also facilitates organic waste management and has ornamental and educational value.

Future Prospects and Challenges

Integrating advanced molecular tools with traditional breeding offers significant promise for developing durable, high-yielding, and biotic stress-resistant cucurbit cultivars. CRISPR-based gene editing emerges as a powerful technique, allowing precise genetic modifications to confer broad-spectrum resistance. Nevertheless, challenges such as public acceptance, regulatory hurdles, and the ecological impact of transgenic and gene-edited crops necessitate careful evaluation. Further, comprehensive phenotyping, combined with genomic selection, can enhance the predictability of breeding outcomes, accelerating cultivar development.

Conclusion

Advancements in molecular breeding technologies have substantially improved cucurbit resistance breeding. By harnessing these tools, breeding programs can address current limitations and sustainably enhance crop resilience to biotic stresses. Future research should focus on integrated approaches, combining genomics, phenomics, and innovative breeding methodologies to achieve durable resistance and global food security.

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