Cultivating Tomorrow: Harnessing Gene Editing and Silencing for Precision Horticulture

Abstract

Precision Horticulture, propelled by gene editing and silencing technologies, emerges as a transformative approach to address the demands of sustainable and efficient crop production. Utilizing CRISPR-Cas9 and RNA interference, horticulturists can precisely modify and regulate plant genomes, ushering in a new era of tailored crops. Gene editing enables the development of crops with heightened resistance to pests, diseases, and improved adaptability. This targeted enhancement, coupled with accelerated breeding processes, results in resilient varieties that can meet the challenges of evolving agricultural landscapes. Concurrently, gene silencing technologies allow for the suppression of undesirable traits, extending the shelf life of produce and minimizing post-harvest losses. Integration of these technologies into Precision Horticulture not only optimizes crop traits but also promotes sustainability by reducing reliance on chemical inputs. The approach aligns with environmental conservation, ensuring a more ecologically balanced and resource-efficient cultivation. However, responsible deployment and ethical considerations are paramount for widespread acceptance, highlighting the need for a harmonious balance between technological innovation and ethical utilization in shaping the future of horticulture.

Keywords: gene editing, development, resistance, targeted

Introduction

Understanding the internal genomic mechanism of plants is essential for comprehending the gene silencing process in transgenic crops used in horticulture. Horticulture encompasses a diverse range of plants and crops, such as fruits, vegetables, spices, and ornamental plants **[1]**. Gene silencing is the cellular mechanism that inhibits the expression of a certain gene. The genes that produce messenger RNA (mRNA) molecules are rendered inactive through either cleavage or translational repression. Horticultural plants have developed many mechanisms of gene silencing, involving the use of short RNAs (20–30 nucleotides) to inhibit gene expression either during transcription or after transcription has occurred **[2]**. Horticultural crops have been subjected to gene silencing techniques such as RNA interference technology, transcriptional gene silencing, and virus-induced gene silencing. Double-strand RNAs within the cell trigger the synthesis of tiny interfering RNAs, short hairpin RNAs, and micro-RNAs. The synthesis of these ribonucleic acids results in the suppression of messenger RNA (the RNA that encodes proteins). This methodology is a highly efficient experimental method for suppressing targeted genes to boost stress tolerance, bolster resistance against insects/pests/pathogens, and enhance nutritional status. Artificial means can be used to control and apply this methodology and mechanism, leading to the enhancement of cultivars in various horticulture crops**[3]**.

By employing the RNA interference (RNAi) technology, we specifically targeted two enzymes, α-mannosidase (α-Man) and β-D-N acetyl hexosaminidase (β-Hex), which are involved in altering N-glycoproteins during the ripening process produced by ethylene. As a result, the shelf life of tomatoes was significantly extended, with the fruits becoming 22.5 times firmer **[4]**. The current study demonstrates the involvement of microRNAs in the process of tomato fruit development and ripening. According to the latest study, it has been found that the expression of

CNR is likewise suppressed by APETALA2a, which is a target of miR172. One method by which it exerts a positive influence on the process of fruit ripening **[5]**. Alternatively, it exerts a negative regulatory effect on the synthesis of ethylene. The expression of the chalcone synthase (CHS) gene, which is involved in the ripening process of strawberry fruits (Fragaria x ananassa cv. Elsanta), is suppressed by an ihp-RNA construct. This construct has partial sense and antisense sequences of the CHS gene, which are split by introns obtained from an F. ananassa quinone oxidoreductase gene **[6]**. The decreased expression of CHS mRNA and enzymatic CHS activity resulted in a decrease in anthocyanin levels. Additionally, the precursors of the flavonoid pathway were redirected to the phenylpropanoid pathway, resulting in significant increases in the amounts of (hydroxy) cinnamoyl glucose esters. The use of this method, along with the examination of metabolite profiling, will prove beneficial in the advancement and maturation of strawberry fruit**[7]**.

In the past twenty years, there have been notable breakthroughs in the field of genetic engineering, which involve altering genes from different foreign sources and inserting them into plants to create desirable characteristics **[8]**. The phenomenon of RNA interference (RNAi) was previously identified as an innate mechanism for regulating gene expression in various organisms. The objective is to improve the precision and accuracy in developing resistance to pests and pathogens, enhancing the quality of plants, and changing their architecture **[9]**. Nevertheless, it has recently gained popularity as a commonly utilized approach. RNA interference (RNAi) technologies have the potential to selectively decrease the expression of specific genes without affecting the expression of other genes. RNA interference is now the primary technique for exploring gene activities in different animals by suppressing the expression of genes **[10]**. It is crucial to develop new methods and uses to improve desired traits in crops through gene suppression and a better understanding of the natural RNA interference processes in plants. In recent years, RNAi technology has emerged as a crucial and preferred approach for managing insects, pests, pathogens, and environmental challenges such as drought, salinity, and temperature. Despite several limitations in the effectiveness of this technique**[11]**, such as the selection of gene candidates, stability of the trigger molecule, and the choice of target species and crops. However, during the past decade, researchers have identified several target genes in various crops that might be enhanced to improve their resistance to both biotic and abiotic challenges. This review focuses on highlighting the research conducted on crops subjected to both biotic and abiotic stress utilizing RNAi technology. The review also emphasizes the gene regulatory pathways and gene silencing, RNA interference, RNAi knockdown, RNAi induced biotic and abiotic resistance, and advancements in the comprehension of RNAi technology and the functionality of different components of the RNAi machinery in crops for their enhancement**[12]**.

Nutrition is essential for the survival of human beings on the planet. Meeting the escalating global food demand using conventional crop development technologies is exceedingly challenging due to the rapid population growth. Individuals are persistently striving to enhance crop productivity, nutrient composition, and develop crops that are resistant to diseases through the utilization of traditional methods of crop enhancement. Regrettably, the existing plant breeding techniques are not feasible for meeting the demands of a rapidly expanding population due to their arduous and time-consuming nature**[13]**.

According to assessments, there is a pressing requirement to augment food output by 70% by the year 2060 in order to adequately nourish the growing global population. Currently, several

methods like as crossbreeding, transgenic breeding, and mutation breeding are being used to develop genotypes that are resistant to diseases and can withstand climate change and other forms of stress. Nevertheless, crossbreeding and mutation breeding are non-specific breeding techniques that involve laborious procedures**[14]**. Additionally, the production and commercialization of the resulting genotypes are hindered by various restrictions. On the other hand, transgenic breeding, despite its protracted and expensive commercialization process, also confronts the obstacle of public acceptance due to concerns surrounding genetically modified crops.

In recent times, there have been significant advancements in the field of RNA-based gene regulation, specifically in RNA interference (RNAi). RNAi is a gene regulatory technique that has been extensively developed for enhancing crop quality by modifying gene expression**[15]**. This approach offers the advantage of improved trait quality and reduced biosafety concerns, as it does not involve the use of transgenic lines. RNAi is a process that can silence genes and is used to study gene function, manipulate plant metabolism, and create crops that can withstand stress and resist diseases**[16]**.

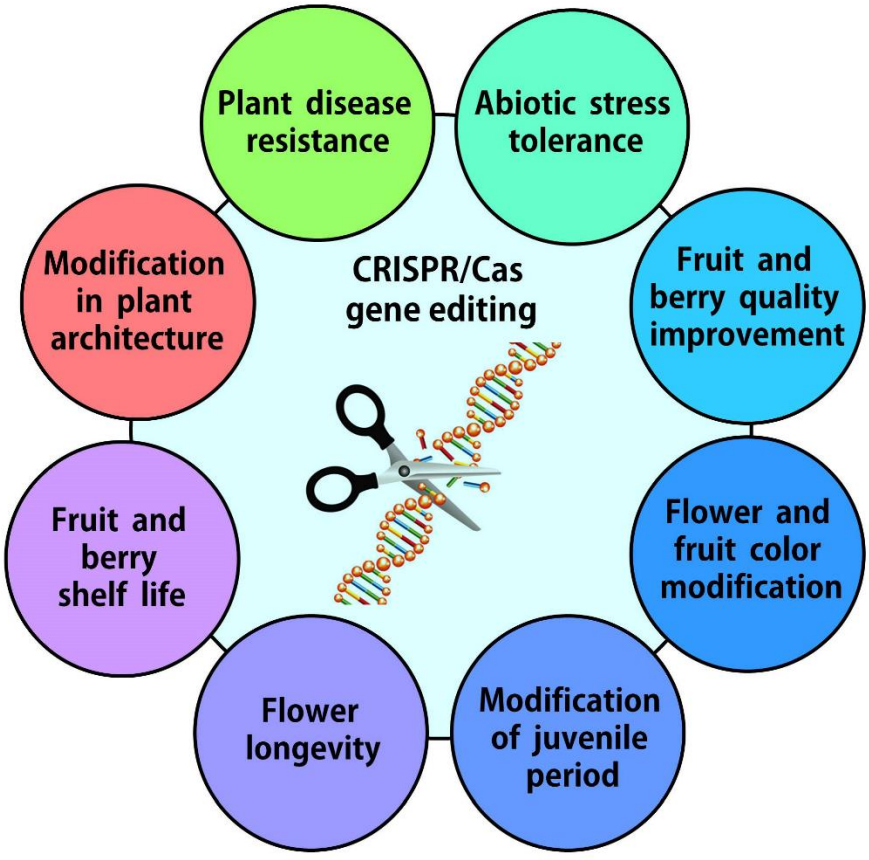


Fig 1 : Gene editing model

Why we need gene silencing

In the last five years, the RNA-guided nucleases-based gene editing technique known as clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein (CRISPR/Cas) has gained recognition as an effective method for precisely modifying genes in crops. CRISPR enables precise manipulation of gene expression by selectively targeting and modifying specific sequences within the genome and epigenome**[17]**. It can be used for gene knockin, knockout, replacement, as well as for watching and controlling gene expression. The genome editing capability of CRISPR relies on three essential components, namely CRISPR RNA (crRNA), CRISPR-associated enzymes (Cas), and trans-activating crRNA (tracRNA). The combination of these three elements can be used to create a unified chimeric synthetic RNA molecule called single-guide RNA (sgRNA) for the purpose of genome editing. CRISPR enables the simultaneous targeting of several genes, while also allowing for easy editing of multiple genes **[18]**. Consequently, it has been extensively employed to modify, control, and oversee genes not only in plants but also in microorganisms and animals. In order to modify the genome, double-stranded DNA breaks are deliberately created at precise locations using site-specific nucleases. This process then triggers DNA repair mechanisms, including non-homologous end joining (NHEJ) and homology-directed repair (HDR), which are responsible for introducing specific alterations to the genome**[19]**. The non-homologous end joining (NHEJ) pathway repairs double-strand breaks (DSBs) by directly joining the damaged ends without relying on homologous DNA. This process can introduce insertions or deletions (InDels) or single-nucleotide polymorphisms (SNPs) at the location of the break, which can cause frameshift or nonsense mutations. During HDR, gene replacement occurs at the breakpoint using a homologous template **[20]**. Thus, both Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR) have significant contributions in nuclease-mediated gene editing. This method produces cultivars in crop breeding that are free of transgenes. This review explores the diverse functions and potential uses of RNAi and the RNA-guided CRISPR/Cas9 system as highly effective technologies for enhancing the productivity and resilience of agriculturally significant crops **[21]**. These technologies have the capacity to significantly increase crop yields and improve tolerance to a wide range of environmental stressors, including both biological and non-biological factors. Furthermore, the discussion has encompassed the constraints, difficulties, and possible future advancements.

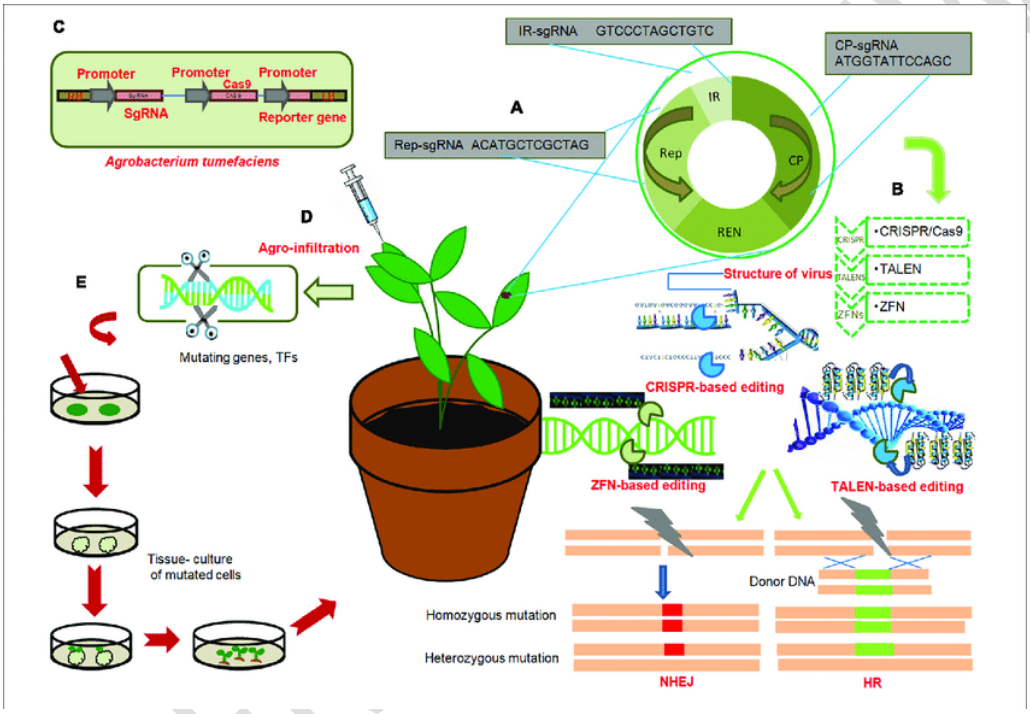
Post harvest loss and wastage in horticulture

Postharvest waste and postharvest loss are occasionally used synonymously, however, this is inaccurate. Postharvest loss is inadvertent. It delineates the consequential losses that arise from occurrences across the entire process of food production, from the farm to the table. These losses encompass physical harm, internal bruising, early decay, and insect infestation, among other factors. Produce loss is considered quantifiable since it can be measured. This does not suggest that data is readily accessible, but rather that it may be obtained**[22]**.

Postharvest waste, on the other hand, is deliberate. It refers to the act of discarding edible produce due to not meeting buyer expectations. Produce can be rejected by producers, distributors, processing businesses, retailers, and consumers if it does not suit their intended or established preferences. Waste production is considered qualitative due to its challenging nature in terms of measurement and assessment **[23]**. However, in the United States, approximately 7% of fruit and vegetable losses after harvest happen on the farm, whereas consumer-facing enterprises and households squander more than double that amount, specifically 17% and 18% respectively **[24]**.

The occurrence of postharvest loss and waste (PLW) poses a significant threat to environmental sustainability, particularly when considering the simultaneous problems of global climate change and population expansion. PLW refers to the improper allocation of financial investments in horticulture, as well as the unsustainable utilization of non-renewable natural resources **[25]**. Implementing technological strategies to control post-harvest losses, such as the establishment of a cold-chain system and the utilization of plastic packaging, also entail energy consumption and carbon emissions. Enhancing the longevity and quality characteristics of harvested crops by genetic modification or intelligent breeding could serve as potential remedies to mitigate the severity of these issues **[26]**.

Fig 2 : Technological strategies to control post-harvest losses



RNA technology

• RNA interference is a naturally occurring gene regulatory phenomenon in eukaryotic cells.

• It protects cells against foreign DNA, maintains genomic stability, and controls cellular processes.

• The phenomenon was discovered in Petunia flowers, where it led to variegated flowers instead of expected deep purple **[27]**.

• In Caenorhabditis elegans, RNA interference (RNAi) was discovered, resulting in efficient silencing of the target endogenous gene homologous to RNA.

• RNAi is a promising tool for gene regulation with greater potential than other post-transcriptional gene regulation technologies like antisense technology **[28]**.

• Small non-coding RNAs (ncRNA) participate in gene regulation, which are the cleavage product of dsRNAs.

• RNAi is associated with the RNA-induced silencing complex (RISC), argonaute (AGO), and other effector proteins, causing complex degradation of the target messenger RNA **[29]**.

• RNAi can be defined as the capability of endogenous or exogenous dsRNA to inhibit the expression of the gene whose sequence is complementary to dsRNA **[30]**.

Components and machinery of RNAi

Over the past two decades, the role of small non-coding RNAs in gene regulatory processes has been explored. Various classes of RNAs have been discovered, including miRNA, siRNA, piRNA, qiRNA, and svRNA. These RNAs have different biogenesis pathways and regulatory mechanisms. miRNA and siRNA have different origins, with miRNA originating from genomic DNA and siRNA derived from viruses, transposons, or transgenes. However, they share similarities in size and sequence-specific inhibitory functions, suggesting a connection between their biogenesis pathways **[31]**.

MiRNAs are 21-24 nucleotide long small RNAs derived from MIR genes. They are biogenesisd in the nucleus by RNA polymerase II-aided transcription of MIR genes, forming a primary miRNA transcript. This is processed into a short stem-loop precursor called pre-miRNA, which is then cropped by DCL1 in the nucleus and generates the RNA duplex (miRNA:miRNA) **[32]**. The 3′-terminals of the RNA duplex are methylated by HUA ENHANCER (HEN1) to prevent degradation. The RNA duplex is exported to the cytoplasm, where mature miRNA is loaded onto the RISC complex with AGO and other effector proteins. If complete base pairing does not occur, miRISC inhibits the translation process **[33]**.

MiRNA-mediated downregulation of gene expression occurs through miRISC-mediated inhibition of translational initiation or ribosome subunit joining, premature degradation of the budding polypeptide chain, or inducing deadenylation and destabilization of the target mRNA. Changes in expression and biogenesis of these RNAs could lead to crop formation with agronomically valuable characteristics **[34]**.

Micro RNA (miRNA)

MiRNAs are small RNAs derived from MIR genes, forming a primary miRNA transcript of about 1000 nt. This is processed into a short stem-loop precursor called pre-miRNA with the help of DCL1 and the dsRNA binding protein DRB1 or HYL1. DCL1 crops this pre-miRNA in the nucleus, generating the RNA duplex (miRNA:miRNA). The 3′-terminals of the RNA duplex are methylated by HUA ENHANCER (HEN1) to prevent degradation **[35]**. The RNA duplex is exported to the cytoplasm, where mature miRNA is loaded onto the RISC complex with AGO and other effector proteins. This miRNA-induced silencing complex (miRISC) base pairs with the complementary target mRNA completely, and the AGO protein degrades the target mRNA **[36]**. If complete base pairing does not occur, miRISC inhibits the translation process. Expression of miRNA occurs during plant growth, development, secondary metabolite synthesis, and abiotic and biotic stress, potentially leading to crop formation with agronomically valuable**[37].** characteristics.

Small Interfering RNA (siRNA)

Gene silencing through RNAi can be triggered by long dsRNA or short hairpin precursors. The RNAi pathway is activated by recruiting Dicer or Dicer-like enzymes, which convert dsRNAs into short 21-24 nt long SiRNA duplexes. The SiRNA-induced silencing complex (SiRISC) is

recruited, degrading the sense strand and loading the antisense strand onto the target mRNA **[38]**. This leads to post-transcriptional gene silencing (PTGS) by cleaving the target mRNA or inhibiting translation. SiRNAs can also participate in co-transcriptional gene silencing. Dicer-independent siRNA genesis has been reported in various organisms, mainly arising from transposable elements, intergenic elements, and transgenes **[39]**.

The significance of RNA interference (RNAi) in enhancing crop quality and productivity

RNAi-Mediated Virus Resistance

• RNAi technology offers a broad-spectrum resistance against viral infections by targeting multiple regions of a viral gene.

• The first RNAi-mediated virus-resistant potato transgenic lines were reported in 1998 **[40]**.

• RNAi technology has been used to develop virus-resistant cultivars, including Beet necrotic yellow vein virus (BNYVV), Plum pox virus (PPV), and Bean golden mosaic virus (BGMV)-resistant Phaseolus vulgaris.

• Si-RNA-mediated silencing of the African cassava mosaic virus (ACMV) resulted in a 66% decrease in ACMV genomic DNA **[41]**.

• Cassava brown streak disease (CBSD) was first developed and provided protection against two causative organisms belonging to two different species **[42]**.

• Tobacco streak virus (TSV)-resistant transgenic lines of both tobacco and sunflower were produced by RNAi technology using a 421-bp-long coat protein gene.

• Rice strip disease caused by the Rice strip virus (RSV) was successfully suppressed in two RSV-susceptible varieties of Japonica **[43]**.

• Soybean mosaic virus (SMV)-resistant transgenic lines of soybean were produced by introducing a hairpin RNAi construct containing the Hc-Pro gene.

• A study conducted on N. benthamiana and Vigna unguiculata plants to develop resistance against the Bean common mosaic virus (BCMV) by exogenous application of RNAi construct containing viral coat proteins to protect plants from aphid mediated transmission of BCMV **[44]**.

RNAi-Mediated Bacterial Resistance

• Bacteria serve as the biggest hurdle in crop production as they are ubiquitous in nature and replicating with great speed and causing infection **[45]**.

• Escobar et al. conducted a study on A. thaliana and S. lycopersicum (tomato) to suppress crown gall disease caused by Agarobacterium tuminifaciens through RNAi technology.

• P. syringae infection in A. thaliana induced biogenesis of endogenous si-RNA i.e., nat-SiRNAATGB2 **[46]**.

RNAi-Mediated Fungal Resistance

• Research findings suggest that RNAi technology can enhance resistance against fungi in genetically engineered crops.

• Gene silencing has been studied using homologous transgenes (co-suppression), antisense or dsRNAs in many plant-pathogenic fungi **[47]**.

• Agarobacterium-mediated transformation (AMT) of RNAi constructs act as a potent approach for investigating the role of the gene involved in pathogenesis **[48]**.

RNAi-Mediated Insects and Nematode Resistance in Crops

Insects and Nematodes' Impact on Crops

• Nematodes like Meloidogyne spp., Heterodera and Globodera spp., Pratylenchus spp., Helicotylenchus spp., Radopholus similis, Ditylenchus dipsaci, Rotylenchulus reniformis, Xiphinema spp., and Aphelenchoides spp. can cause severe damage to crops **[49]**.

• RNAi-mediated expression of dsRNA targeting the housekeeping gene and parasitism gene of root-knot nematodes (RKN) can provide broad-spectrum resistance against nematode infection.

RNAi-Mediated Resistance against Cyst Nematodes

• RNAi-mediated silencing of all four parasitism genes of the sugar beet cyst nematode (Heterodera schachtii) reduced the number of female nematodes **[50]**.

• RNAi-mediated resistance against the soybean cyst nematode H. glycines was reported.

RNAi-Mediated Insect Resistance in Crops

• The success of the cry toxin from Bacillus thuringiensis as an insecticide has led to the foundation of RNAi-mediated insect resistance in crops **[51]**.

• Transgenic lines of Arabidopsis and tobacco plants expressing CYP6AE14-specific dsRNA have shown resistance against gossypol, a polyphenol compound.

• RNAi-mediated resistance against the whitefly population in tobacco plants and lettuce (Lactuca sativa) increased the mortality rate of insects feeding on transgenic plants **[52]**.

RNAi-Mediated Insect-Resistant Cultivars

• 3-hydroxy-3-methyl glutaryl coenzymeA reductase (HMGR) and the chitinase (HaCHI) gene can be utilized as potential targets to produce insect-resistant cultivars using RNAi.

Abiotic Stress Tolerance in Plants

• RNAi interference technology can be exploited to develop transgenic cultivars that can cope with different abiotic stresses **[53]**.

• RNAi-mediated downregulation of RACK-1 gene in transgenic O. sativa plants has shown more tolerance to drought stress.

• MiRNAs are involved in the early stage during seed germination and negatively affect the expression of the post-transcriptional gene **[54]**.

• Overexpression of OsTZF1 gene in rice (O. sativa) can induce the expression of OsTZF1 gene, enhancing tolerance to high salt and drought stresses.

• Overexpression of the dehydrin gene WZY2 provides more tolerance for plants against osmotic damage **[55]**.

Seedless Fruit Production and Shelf-Life Enhancement

Seedless Fruits and Parthenocarpy

• Seedless fruits are appreciated for their quality and shelf life enhancement.

• Parthenocarpy, which involves fruit development directly from the ovary without fertilization, can induce seedless fruits **[56]**.

• Seedless fruits can be produced artificially by disrupting genes involved in seed and seed set formation.

• Seedless fruits show pleiotropic effects such as change in taste and reduced fruit size **[57]**.

Seedless Fruit Development and Auxin Response Factors

• Seed development in fruits limits yield in cucumber and tomato.

• The replacement of seed and seed cavities with edible fruit tissue is highly desirable and appreciated.

• Auxin response factors (ARFs) encode transcription factors that control auxin-dependent plant developmental processes **[58]**.

• RNAi-mediated development of transgenic tomato lines with a downregulated slARF7 gene resulted in the generation of parthenocarpic fruits **[59]**.

Self-Life Enhancement

• Fruits and vegetables are more vulnerable to spoilage than cereals.

• Enhancing the shelf-life of fruits and vegetables is essential to minimize horticultural losses.

• Regulation of ethylene biosynthesis, ethylene-mediated signaling, and ethylene response elements can be achieved by delaying the ripening of the fruit **[60]**.

• RNAi-facilitated suppression of ACC oxidase enzyme, expression of three homologs of 1-Aminocyclopropane-1-carboxylate synthase (ACS), and suppression of SISGR1 gene resulted in fruit softening and extended shelf life.

• Downregulation of the SPP gene through RNAi leads to inhibition of cold-induced hexogenesis in transgenic tubers **[61]**.

Male Sterile Plants Development

• The development of hybrid cultivars has augmented productivity due to hybrid vigor and improved uniformity.

• RNAi has been one of the most efficient tools in the development of male sterile lines by targeting male-specific genes that participate in tapetum and pollen development **[62]**.

• Suppression of SAMDC gene in the tapetal tissue of tomato plants leads to the development of male sterility.

• Cytoplasmic male sterility is the maternally inherited phenomenon present in plants **[63]**.

Flower Color Modification and Nutritional Improvement

• RNA interference technology can be used to modify the color and patterns of flowers, enhancing their appeal and functionality.

• Studies have shown that silencing pigment encoding genes can lead to color changes in various plants, including N. tobacum, Tricyrtis sp., and gentian plants **[64]**.

• The accumulation of a polyacrylate delphinidin, gentiodelphin, in the petals of gentian plants contributes to the color.

• RNAi-mediated silencing of genes like chalcone synthase (CHS), anthocyanidin synthase (ANS), and flavonoid 3′,5′-hydroxylase (F3′5′H), can lead to changes in flower color **[65]**.

Nutritional Improvement

• RNAi can be used to achieve required levels of nutrients in crops by modifying various biochemical and physiological pathways.

• RNAi can be used to decrease the level of α-linolenic acid in soybeans and Camelina sativa, improve oilseed quality, and increase carotenoid content in Brassica napus **[66]**.

• RNAi can also be used to accumulate minerals in crops, as demonstrated by the production of Triticum grains with high Zn and Fe content **[67]**.

Secondary Metabolite Production

• RNAi can be used to suppress the expression of undesirable compounds and manipulate secondary metabolites.

• Examples include the replacement of morphine with non-narcotic alkaloid (S)-reticuline in the opium poppy and the production of decaffeinated coffee beans **[68]**.

• Caffeine, a natural stimulant, can be enhanced by downregulating the initial enzyme in flavonoid biosynthesis in coffee plants.

• In aromatic plants like spearmint, the DE-ETIOLATED-1 (DET1) gene, a negative regulator of photomorphogenesis, was suppressed through RNAi in embryonic callus **[69]**.

Application in transgenic crops

silencing technologies can be used to simultaneously silence multiple genes using transgenes that contain a conserved sequence or a composite sequence of multiple genes, while this would be difficult to achieve using CRISPR / Cas9-like mutagenesis methods. With continued efforts to better understand RNA silencing mechanisms in plants, it can be expected that RNA silencing technologies will be further improved to overcome the potential limitations that allow for wider applications in agriculture **[70]**. RNAi has become a highly effective experimental tool in functional genomics for silencing genes for both basic and applied biological studies in various organisms including plants. RNAi deploys small RNAs, mainly siRNAs, to mediate the

degradation of mRNA for regulating gene expression in plants. However, RNAi stability in plants is critical, but the RNAi-mediated gene suppression approach opens new avenues for the development of eco-friendly biotech approaches for crop improvement **[71]**. By way of knocking out of the specific genes for better stress tolerance and integrating novel traits in different plant species for insect/pest/pathogen resistance and enhanced nutritional status become more convenient rather than convectional practices. This technology having revolutionary capabilities could be further exploited for functional analysis of target genes and regulation of gene expression for crop improvement **[72]**.

Bio-Fortification and Herbicide Resistance in Tomatoes and Potatoes

• Tomato β-Carotene & Lycopene NCED1

• Potato β-Carotene & lutein BCH

• Tomato Vitamin C APX (Zhang et al., 2011)

• Bio-elimination Coffea canephora CaMXMT **[73]**

• Papaver somniferum Morphine Codeine Reductase (COR)

• Altered phenotype Torenia hybrida cv. Summerwave Blue Flower colour: blue to white CHS

• Petunia Scent profile modification PhBSMT **[74]**

• Tomato Parthenocarpy AUCSIA

Insect Resistance in Tomatoes

• Huanong No. 1 (Papaya Rainbow, SunUp)

• Apple Golden Delicious **[75]**

• Granny Smith

• Plum C-5 (NA)

• Tomato Da Dong No 9 (NA), Huafan No 1 (NA), FLAVR SAVR **[76]**

• Brinjal Bt Brinjal Event EE1

• Potato Lugovskoi plus, Atlantic NewLeaf2 potato

• Starch Potato, Innate2 Russet Burbank Potato **[77]**

• New Leaf2 Y Russet Burbank potato

• Sweet pepper PK-SP01 (NA)

• Rose WKS82/130-4-1 (NA) **[78]**

• Carnation Moondust

• Moonshadow Herbicide tolerance 1

• Moonshade

• Moonlite

• Moonaqua

• Moonvista

• Moonique

• Moonpearl

• Moonberry

• Moonvelvet

• Petunia

• Creeping Bentgrass Roundup Ready

Conclusion

The agriculture industry faces the challenge of providing food security to the rapidly expanding global population and addressing malnutrition in developing countries. To ensure balanced food supply, biofortified staple food, vegetables, and fruits must be developed, enriched in essential compounds and mineral elements **[79]**. The development of cultivars resistant to biotic and abiotic stresses is crucial for addressing food security, malnutrition, and famine problems. RNAi and CRISPR/Cas9 technology have gained interest in developing crops with high-value agronomic traits by targeting their broad range of targets, accelerating crop improvement schemes, and increasing their effectiveness.

CRISPR technology has advanced functional genomics research and innovative crop development, with recent advancements in promoter, base editing, and prime editing. The CRISPR-Cas system has three expression strategies: mixed dual promoter system, dual Pol II promoter system, and single transcriptional unit system (STU) **[80]**. However, the STU system has limitations, such as refinement, nonoptimal expression system, and difficult post-transcriptional processes. Base-editing, the newest advancement of CRISPR-Cas-based technology, can directly install point mutations in cellular DNA without inducing a double-strand DNA break. However, base-editing technologies cannot generate precise base-edits beyond four transition mutations. Prime editing, a recent technological evolution of the CRISPER/Cas system, can overcome these limitations. RNAi and CRISPR/Cas will bring a gene revolution in breeding crops with desired traits, supporting food security in both developed and developing countries. Studies on gene silencing and gene deletion or disruption are essential for designing better gene-editing strategies.

References

1. Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food Security: The Challenge of Feeding 9 Billion People. *Science* **2010**, *327*, 812–818
2. Chen, K.; Wang, Y.; Zhang, R.; Zhang, H.; Gao, C. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. *Annu. Rev. Plant Biol.* **2019**, *70*, 667–697.
3. Pathak, K.; Gogoi, B. RNA interference (RNAi): Application in crop improvement: A review. *Agric. Rev.* **2016**, *37*, 245–249.

4. Jinek, M.; Chylinski, K.; Fonfara, I.; Hauer, M.; Doudna, J.A.; Charpentier, E. A Programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **2012**, *337*, 816–821.
5. Pardo, B.; Gómez-González, B.; Aguilera, A. DNA Repair in Mammalian Cells. *Cell. Mol. Life Sci.* **2009**, *66*, 1039–1056.
6. Gill, R.A.; Scossa, F.; King, G.J.; Golicz, A.; Tong, C.; Snowdon, R.J.; Fernie, A.R.; Liu, S. On the Role of Transposable Elements in the Regulation of Gene Expression and Subgenomic Interactions in Crop Genomes. *Crit. Rev. Plant Sci.* **2021**, *40*, 157–189. [] []
7. Mamta, B.; Rajam, M.V. RNA Interference: A Promising Approach for Crop Improvement. In *Biotechnologies of Crop Improvement*; Gosal, S., Wani, S., Eds.; Springer: Cham, Germany, 2018; Volume 2, pp. 41–65.
8. Napoli, C.; Lemieux, C.; Jorgensen, R. Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes in trans. *Plant Cell* **1990**, *2*, 279–289.
9. Fire, A.; Xu, S.; Montgomery, M.K.; Kostas, S.A.; Driver, S.E.; Mello, C.C. Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*. *Nature* **1998**, *391*, 806–811.
10. Johnson, N.; Axtell, M.J. Small RNA warfare: Exploring origins and function of trans-species microRNAs from the parasitic plant Cuscuta. *Curr. Opin. Plant Biol.* **2019**, *50*, 76–81. [] [] [**PubMed**]
11. Dutta, T.K.; Banakar, P.; Rao, U. The status of RNAi-based transgenic research in plant nematology. *Front. Microbiol.* **2015**, *5*, 760.
12. Lunardon, A.; Johnson, N.; Hagerott, E.; Phifer, T.; Polydore, S.; Coruh, C.; Axtell, M.J. Integrated annotations and analyses of small RNA–producing loci from 47 diverse plants. *Genome Res.* **2020**, *30*, 497–513.
13. Lunardon, A.; Kariuki, S.M.; Axtell, M.J. Expression and processing of polycistronic artificial microRNAs and trans -acting siRNAs from transiently introduced transgenes in Solanum lycopersicum and Nicotiana benthamiana. *Plant J.* **2021**, *106*, 1087–1104.
14. Yang, X.; Sanchez, R.; Kundariya, H.; Maher, T.; Dopp, I.; Schwegel, R.; Virdi, K.; Axtell, M.J.; MacKenzie, S.A. Segregation of an MSH1 RNAi transgene produces heritable non-genetic memory in association with methylome reprogramming. *Nat. Commun.* **2020**, *11*, 1–17.
15. Romano, N.; Macino, G. Quelling: Transient inactivation of gene expression in Neurospora crassa by transformation with homologous sequences. *Mol. Microbiol.* **1992**, *6*, 3343–3353.
16. Wilson, R.C.; Doudna, J.A. Molecular Mechanisms of RNA Interference. *Annu. Rev. Biophys.* **2013**, *42*, 217–239.
17. Cerutti, H.; Ma, X.; Msanne, J.; Repas, T. RNA-mediated silencing in algae: Biological roles and tools for analysis of gene function. *Eukaryot. Cell* **2011**, *10*, 1164–1172.
18. Hammond, S.M.; Bernstein, E.; Beach, D.; Hannon, G.J. An RNA-directed nuclease mediates post-transcriptional gene silencing in Drosophila cells. *Nat. Cell Biol.* **2000**, *404*, 293–296.
19. Elbashir, S.M.; Harborth, J.; Lendeckel, W.; Yalcin, A.; Weber, K.; Tuschl, T. Duplexes of 21-nucleotide RNAs mediate RNA interference in cultured mammalian cells. *Nat. Cell Biol.* **2001**, *411*, 494–498.

20. Ali, N.; Datta, S.K.; Datta, K. RNA interference in designing transgenic crops. *GM Crop* **2010**, *1*, 207–213.
21. Hammond, S.M. Dicing and slicing: The core machinery of the RNA interference pathway. *FEBS Lett.* **2005**, *579*, 5822–5829.
22. Hutvagner, G.; Simard, M. Argonaute proteins: Key players in RNA silencing. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 22–32.
23. Redfern, A.D.; Colley, S.M.; Beveridge, D.J.; Ikeda, N.; Epis, M.R.; Li, X.; Foulds, C.E.; Stuart, L.M.; Barker, A.; Russell, V.J.; et al. RNA-induced silencing complex (RISC) Proteins PACT, TRBP, and Dicer are SRA binding nuclear receptor coregulators. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6536–6541.
24. Dash, S.K.; Sushil, K.M.; N, M.H. RNA Interference a fine tuner of gene regulation: A review. *Int. J. Biotechnol. Mol. Biol. Res.* **2015**, *6*, 35–39.
25. Zhang, H.; A Kolb, F.; Jaskiewicz, L.; Westhof, E.; Filipowicz, W. Single Processing Center Models for Human Dicer and Bacterial RNase III. *Cell* **2004**, *118*, 57–68.
26. Aalto, A.P.; E Pasquinelli, A. Small non-coding RNAs mount a silent revolution in gene expression. *Curr. Opin. Cell Biol.* **2012**, *24*, 333–340.
27. Kurihara, Y.; Takashi, Y.; Watanabe, Y. The interaction between DCL1 and HYL1 is important for efficient and precise processing of pri-miRNA in plant microRNA biogenesis. *RNA* **2006**, *12*, 206–212.
28. Kurihara, Y.; Watanabe, Y. From The Cover: Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 12753–12758.
29. Huang, Y.; Ji, L.; Huang, Q.; Vassylyev, D.G.; Chen, X.; Ma, J.-B. Structural insights into mechanisms of the small RNA methyltransferase HEN1. *Nat. Cell Biol.* **2009**, *461*, 823–827.
30. Li, J.; Yang, Z.; Yu, B.; Liu, J.; Chen, X. Methylation protects miRNAs and siRNAs from a 3′-end uridylation activity in Arabidopsis. *Curr. Biol.* **2005**, *15*, 1501–1507.
31. Guo, Q.; Liu, Q.; ASmith, N.; Liang, G.; Wang, M.B. RNA silencing in plants: Mechanisms, technologies and applications in horticultural crops. *Curr. Genom.* **2016**, *17*, 476–489.
32. Huntzinger, E.; Izaurralde, E. Gene silencing by microRNAs: Contributions of translational repression and mRNA decay. *Nat. Rev. Genet.* **2011**, *12*, 99–110.
33. Pareek, M.; Yogindran, S.; Mukherjee, S.K.; Rajam, M.V. Plant MicroRNAs: Biogenesis, Functions, and Applications. In *Plant Biology and Biotechnology*; Springer: New Delhi, India, 2015; pp. 639–661.
34. Mello, C.C.; Conte, D. Revealing the world of RNA interference. *Nat. Cell Biol.* **2004**, *431*, 338–342.
35. Bernstein, E.; Caudy, A.; Hammond, S.M.; Hannon, G.J. Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nat. Cell Biol.* **2001**, *409*, 363–366.
36. Hamilton, A.J.; Baulcombe, D.C. A species of small antisense RNA in posttranscriptional gene silencing in plants. *Science* **1999**, *286*, 950–952.
37. Saurabh, S.; Vidyarthi, A.S.; Prasad, D. RNA interference: Concept to reality in crop improvement. *Planta* **2014**, *239*, 543–564.
38. Aoki, K.; Moriguchi, H.; Yoshioka, T.; Okawa, K.; Tabara, H. In vitro analyses of the production and activity of secondary small interfering RNAs in *C. elegans*. *EMBO J.* **2007**, *26*, 5007–5019.

39. Halic, M.; Moazed, D. Dicer-Independent Primal RNAs Trigger RNAi and Heterochromatin Formation. *Cell* **2010**, *140*, 504–516.
40. Lee, H.-C.; Li, L.; Gu, W.; Xue, Z.; Crosthwaite, S.K.; Pertsemlidis, A.; Lewis, Z.; Freitag, M.; Selker, E.U.; Mello, C.C.; et al. Diverse Pathways Generate MicroRNA-like RNAs and Dicer-Independent Small Interfering RNAs in Fungi. *Mol. Cell* **2010**, *38*, 803–814.
41. Ye, R.; Chen, Z.; Lian, B.; Rowley, J.; Xia, N.; Chai, J.; Li, Y.; He, X.-J.; Wierzbicki, A.T.; Qi, Y. A Dicer-Independent Route for Biogenesis of siRNAs that Direct DNA Methylation in Arabidopsis. *Mol. Cell* **2016**, *61*, 222–235.
42. Tester, M.; Langridge, P. Breeding Technologies to Increase Crop Production in a Changing World. *Science* **2010**, *327*, 818–822.
43. Sharma, V.K.; Basu, S.; Chakraborty, S. RNAi mediated broad-spectrum transgenic resistance in *Nicotiana benthamiana* to chilli-infecting begomoviruses. *Plant Cell Rep.* **2015**, *34*, 1389–1399.
44. Rupp, J.; Cruz, L.F.; Trick, H.; Fellers, J.P. RNAi-Mediated, Stable Resistance to Triticum mosaic virus in Wheat. *Crop Sci.* **2016**, *56*, 1602–1610.
45. Ahmed, M.M.S.; Bian, S.; Wang, M.; Zhao, J.; Zhang, B.; Liu, Q.; Zhang, C.; Tang, S.; Gu, M.; Yu, H. RNAi-mediated resistance to rice black-streaked dwarf virus in transgenic rice. *Transgenic Res.* **2017**, *26*, 197–207.
46. Hameed, A.; Tahir, M.N.; Asad, S.; Bilal, R.; Van Eck, J.; Jander, G.; Mansoor, S. RNAi-Mediated Simultaneous Resistance Against Three RNA Viruses in Potato. *Mol. Biotechnol.* **2017**, *59*, 73–83.
47. Yang, X.; Niu, L.; Zhang, W.; Yang, J.; Xing, G.; He, H.; Guo, D.; Du, Q.; Qian, X.; Yao, Y.; et al. RNAi-mediated SMV P3 cistron silencing confers significantly enhanced resistance to multiple Potyvirus strains and isolates in transgenic soybean. *Plant Cell Rep.* **2018**, *37*, 103–114.
48. Kumari, A.; Hada, A.; Subramanyam, K.; Theboral, J.; Misra, S.; Ganapathi, A.; Malathi, V.G. RNAi-mediated resistance to yellow mosaic viruses in soybean targeting coat protein gene. *Acta Physiol. Plant* **2018**, *40*, 1–12.
49. Senthilraja, C.; Reddy, M.G.; Rajeswaran, J.; Kokiladevi, E.; Velazhahan, R. RNA interference-mediated resistance to Tobacco streak virus in transgenic peanut. *Australas. Plant Pathol.* **2018**, *47*, 227–230.
50. Malathi, P.; Muzammil, S.A.; Krishnaveni, D.; Balachandran, S.; Mangrauthia, S.K. Coat protein 3 of Rice tungro spherical virus is the key target gene for development of RNAi mediated tungro disease resistance in rice. *Agri Gene* **2019**, *12*, 100084.
51. Gao, L.; Luo, J.; Ding, X.; Wang, T.; Hu, T.; Song, P.; Zhai, R.; Zhang, H.; Zhang, K.; Li, K.; et al. Soybean RNA interference lines silenced for eIF4E show broad potyvirus resistance. *Mol. Plant Pathol.* **2019**, *21*, 303–317.
52. Tzean, Y.; Chang, H.-H.; Tu, T.-C.; Hou, B.H.; Chen, H.-M.; Chiu, Y.-S.; Chou, W.-Y.; Chang, L.; Yeh, H.-H. Engineering Plant Resistance to Tomato Yellow Leaf Curl Thailand Virus Using a Phloem-Specific Promoter Expressing Hairpin RNA. *Mol. Plant-Microbe Interact.* **2020**, *33*, 87–97.
53. Escobar, M.A.; Civerolo, E.L.; Summerfelt, K.R.; Dandekar, A.M. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 13437–13442.

54. Katiyar-Agarwal, S.; Morgan, R.; Dahlbeck, D.; Borsani, O.; Villegas, A.; Zhu, J.-K.; Staskawicz, B.J.; Jin, H. A pathogen-inducible endogenous siRNA in plant immunity. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 18002–18007.
55. Enrique, R.; Siciliano, F.; Favaro, M.A.; Gerhardt, N.; Roeschlin, R.; Rigano, L.; Castagnaro, A.; Vojnov, A.; Sendin, L.; Marano, M.R. Novel demonstration of RNAi in citrus reveals importance of citrus callose synthase in defence against *Xanthomonas citri* subsp. citri. *Plant Biotechnol. J.* **2011**, *9*, 394–407.
56. Sanju, S.; Siddappa, S.; Thakur, A.; Shukla, P.K.; Srivastava, N.; Pattanayak, D.; Sharma, S.K.; Singh, B.P. Host-mediated gene silencing of a single effector gene from the potato pathogen *Phytophthora infestans* imparts partial resistance to late blight disease. *Funct. Integr. Genom.* **2015**, *15*, 697–706.
57. Cheng, W.; Song, X.-S.; Xiao-Li, Q.; Cao, L.-H.; Sun, K.; Qiu, X.-L.; Xu, Y.-B.; Yang, P.; Huang, T.; Zhang, J.-B.; et al. Host-induced gene silencing of an essential chitin synthase gene confers durable resistance to Fusarium head blight and seedling blight in wheat. *Plant Biotechnol. J.* **2015**, *13*, 1335–1345.
58. Ghag, S.B.; Shekhawat, U.K.S.; Ganapathi, T.R. Host-induced post-transcriptional hairpin RNA-mediated gene silencing of vital fungal genes confers efficient resistance against Fusarium wilt in banana. *Plant Biotechnol. J.* **2014**, *12*, 541–553.
59. Andrade, C.M.; Tinoco, M.L.P.; Rieth, A.F.; Maia, F.C.O.; Aragão, F.J.L. Host-induced gene silencing in the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*. *Plant Pathol.* **2016**, *65*, 626–632.
60. Bharti, P.; Jyoti, P.; Kapoor, P.; Sharma, V.; Shanmugam, V.; Yadav, S.K. Host-induced silencing of pathogenicity genes enhances resistance to *Fusarium oxysporum* wilt in tomato. *Mol. Biotechnol.* **2017**, *59*, 343–352.
61. Zhu, L.; Zhu, J.; Liu, Z.; Wang, Z.; Zhou, C.; Wang, H. Host-induced gene silencing of rice blast fungus *Magnaporthe oryzae* pathogenicity genes mediated by the brome mosaic virus. *Genes* **2017**, *8*, 241.
62. Tiwari, I.M.; Jesuraj, A.; Kamboj, R.; Devanna, B.N.; Botella, J.; Sharma, T.R. Host Delivered RNAi, an efficient approach to increase rice resistance to sheath blight pathogen (*Rhizoctonia solani*). *Sci. Rep.* **2017**, *7*, 1–14.
63. Majumdar, R.; Rajasekaran, K.; Sickler, C.; Lebar, M.; Musungu, B.M.; Fakhoury, A.M.; Payne, G.A.; Geisler, M.; Carter-Wientjes, C.; Wei, Q.; et al. The pathogenesis-related maize seed (PRms) gene plays a role in resistance to *Aspergillus flavus* infection and aflatoxin contamination. *Front. Plant Sci.* **2017**, *8*, 1–11.
64. Pareek, M.; Rajam, M.V. RNAi-mediated silencing of MAP kinase signalling genes (Fmk1, Hog1, and Pbs2) in *Fusarium oxysporum* reduces pathogenesis on tomato plants. *Fungal Biol.* **2017**, *121*, 775–784.
65. Gilbert, M.K.; Majumdar, R.; Rajasekaran, K.; Chen, Z.-Y.; Wei, Q.; Sickler, C.M.; Lebar, M.D.; Cary, J.W.; Frame, B.R.; Wang, K. RNA interference based silencing of the alpha-amylase (amy1) gene in *Aspergillus flavus* decreases fungal growth and aflatoxin production in maize kernels. *Planta* **2018**, *247*, 1465–1473.
66. Yoshioka, M.; Adachi, A.; Sato, Y.; Doke, N.; Kondo, T.; Yoshioka, H. RNAi of the sesquiterpene cyclase gene for phytoalexin production impairs pre- and post-invasive resistance to potato blight pathogens. *Mol. Plant Pathol.* **2019**, *20*, 907–922.

67. Wang, L.; Wang, H.; He, S.; Meng, F.; Zhang, C.; Fan, S.; Wu, J.; Zhang, S.; Xu, P. GmSnRK1. 1, a sucrose nonfermenting-1 (SNF1)-Related Protein Kinase, Promotes Soybean Resistance to *Phytophthora sojae*. *Front. Plant Sci.* **2019**, *10*, 996.
68. Singh, N.; Mukherjee, S.K.; Rajam, M.V. Silencing of the ornithine decarboxylase gene of *Fusarium oxysporum* f. sp. *lycopersici* by host-induced RNAi confers resistance to *Fusarium* wilt in tomato. *Plant Mol. Biol. Rep.* **2020**, *38*, 419–429.
69. Mamta; Reddy, K.R.K.; Rajam, M.V. Targeting chitinase gene of *Helicoverpa armigera* by host-induced RNA interference confers insect resistance in tobacco and tomato. *Plant Mol. Biol.* **2016**, *90*, 281–292.
70. Malik, H.J.; Raza, A.; Amin, I.; Scheffler, J.A.; Scheffler, B.E.; Brown, J.K.; Mansoor, S. RNAi-mediated mortality of the whitefly through transgenic expression of double-stranded RNA homologous to acetylcholinesterase and ecdysone receptor in tobacco plants. *Sci. Rep.* **2016**, *6*, 1–11.
71. Ibrahim, A.B.; Monteiro, T.R.; Cabral, G.; Aragão, F.J.L. RNAi-mediated resistance to whitefly (*Bemisia tabaci*) in genetically engineered lettuce (*Lactuca sativa*). *Transgenic Res.* **2017**, *26*, 613–624.
72. Bhatia, V.; Bhattacharya, R. Host-mediated RNA interference targeting a cuticular protein gene impaired fecundity in the green peach aphid *Myzus persicae*. *Pest Manag. Sci.* **2018**, *74*, 2059–2068.
73. Shin, Y.H.; Lee, S.H.; Park, Y.-D. Development of mite (*Tetranychus urticae*)-resistant transgenic Chinese cabbage using plant-mediated RNA interference. *Hortic. Environ. Biotechnol.* **2020**, *61*, 305–315.
74. Dutta, T.K.; Papolu, P.K.; Banakar, P.; Choudhary, D.; Sirohi, A.; Rao, U. Tomato transgenic plants expressing hairpin construct of a nematode protease gene conferred enhanced resistance to root-knot nematodes. *Front. Microbiol.* **2015**, *6*, 260.
75. Li, Y.; Wang, K.; Lu, Q.; Du, J.; Wang, Z.; Wang, D.; Sun, B.; Li, H. Transgenic *Nicotiana benthamiana* plants expressing a hairpin RNAi construct of a nematode Rs-cps gene exhibit enhanced resistance to *Radopholus similis*. *Sci. Rep.* **2017**, *7*, 1–11.
76. Chukwurah, P.N.; Poku, S.A.; Yokoyama, A.; Fukuda, H.; Shishido, M.; Nakamura, I. Expression of Meloidogyne incognita PolA1 hairpin RNA reduced nematode multiplication in transgenic tomato. *Plant Biotechnol. Rep.* **2019**, *13*, 591–601.
77. Hu, Y.; You, J.; Li, C.; Pan, F.; Wang, C. The Heterodera glycines effector Hg16B09 is required for nematode parasitism and suppresses plant defense response. *Plant Sci.* **2019**, *289*, 110271.
78. Tian, B.; Li, J.; Vodkin, L.O.; Todd, T.C.; Finer, J.J.; Trick, H.N. Host-derived gene silencing of parasite fitness genes improves resistance to soybean cyst nematodes in stable transgenic soybean. *Theor. Appl. Genet.* **2019**, *132*, 2651–2662.
79. Joshi, I.; Kumar, A.; Kohli, D.; Singh, A.K.; Sirohi, A.; Subramaniam, K.; Chaudhury, A.; Jain, P.K. Conferring root-knot nematode resistance via host-delivered RNAi-mediated silencing of four Mi-msp genes in Arabidopsis. *Plant Sci.* **2020**, *298*, 110592.
80. Shi, Y.; Liu, P.; Xia, Y.; Wei, P.; Li, W.; Zhang, W.; Chen, X.; Cao, P.; Xu, Y.; Jin, L.; et al. Downregulation of the lycopene e-cyclase gene confers tolerance to salt and drought stress in *Nicotiana tabacum*. *Acta Physiol. Plant* **2015**, *37*, 1–15.