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Gene Silencing and its Applications in Plants

Review Article

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Abstract

Gene silencing (GS) is considered a promising tool for studying gene functions, improving various crop traits as well as providing resistance to pathogens. Silencing may be done at the transcript or at the post-transcript level. Gene silencing can be done using either RNAi or CRISPR. RNAi silences genes by generating knockdowns at the mRNA level, while CRISPR generates knockouts at the DNA level. Genetic silencing played an important role in protecting plants from pathogens, silencing the synthesis pathways of many compounds in plants such as nicotine, caffeine and gluten, as well as improving the quality of fruits and prolonging their shelf life, but silencing by traditional methods requires genetic modification of the plant and this takes a long time in addition to these, genetically modified plants has faced great rejection from most societies. Induction of gene silencing by external spraying of dsRNA molecules complementary to the pathogen's gene on plant is one of the modern, fast, inexpensive, and environmentally friendly methods that do not require genetic modification of plant and will enhance plant resistance against many pathogens. This application is recognized as spray-induced gene silencing (SIGS).

Keywords: Gene silencing;RNAi; miRNA; siRNA; HIGS; SIGS

Introduction

Gene silencing is defined as an epigenetic modification of gene expression leading to inactivation of previously active genes. Epigenetic modification does not alter the DNA sequence and, although it is heritable, variable frequencies of reversions to expression are observed. Gene silencing is used in the course of normal development and differentiation to repress genes whose products are not required in specific cell types or tissues. This may apply to individual genes or larger chromosome regions [1]. Mechanisms responsible for repression of genes involve changes in levels of DNA methylation, alterations in covalent modifications of histone proteins, chromatin compaction, or destabilization of mRNA. Particular patterns of modifications of chromatin proteins and DNA template make genes inaccessible to the transcription machinery. mRNA destabilization and repression of mRNA translation are often mediated by small RNA regulators such as microRNAs (miRNAs) and small interfering RNAs (siRNAs) [2,3].

Gene silencing can act at the transcriptional or posttranscriptional level; the two phenomena being referred to as transcriptional gene silencing (TGS) and posttranscriptional gene silencing (PTGS). Genes affected by TGS are not transcribed at all, or transcripts are produced at very low levels. TGS has been observed in fungi, plants, and animals. In PTGS, also referred to as cosuppression in plants, quelling, or RNA interference (RNAi), the affected gene is transcriptionally active but its transcripts undergo rapid degradation, resulting in the absence of translatable mRNA [1-4].The discovery of mechanisms that suppress gene activity in plants has extended the horizon for research on control of gene expression (Mansoor *et al.*, 2006) [5]. Gene silencing has also been used in food quality modification such as the reduction of caffeine levels in coffee beans [6], and to increase the nutritional value of corn protein and tomatoes [7,8]. Research on forest tree yield and quality has included the study of gene silencing related to lignin synthesis. On the other hand, research on fruit crops has targeted applications of gene silencing on viral and bacterial resistance, and physiological aspects such as self-fertility. The study of plant gene function by affecting gene expression through silencing techniques (PTGS / RNAi and VIGS) has also been present in recent lines of investigation. This review reports and discusses the main molecular mechanisms involved in plant gene silencing, compares the mechanisms and experimental workflow and the applications of this technology in plant improvement.

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Literature Review

The world population is estimated to reach 11.2 billion by 2100 and the global food supply must be continuously improved to meet such population growth [9]. Minimizing the crop losses due to pests and diseases and alter the gene expression for better quality traits are crucial for future sustainability of global crop production. The global agricultural direct yield loss is estimated between 20 and 40%, which are mainly contributed by pathogens, animals, and weeds [10-13].

The initial idea of gene silencing was discovered when an effort to overexpress chalcone synthase (CHS) in pigmented petunia petals by introducing a chimaeric petunia CHS gene has blocked the biosynthesis of anthocyanin, resulting in totally white flowers and/or patterned flowers with white or pale non-clonal sectors on a wild-type pigmented background [14]. However, the mechanism causing suppression of the target gene was unknown. The RNA gene silencing mechanism was later discovered by injecting double-stranded RNA (dsRNA) into the worm Caenorhabditis elegans which triggered the silencing of genes with identical sequences to that of the dsRNA [15].

Since the discovery of RNA-mediated gene silencing mechanism, this approach has been employed for elucidation of gene function in plant [16]or to alter the gene expression for better quality traits, such as development of seedless fruits [17], enhancement of shelf life [18], development of male sterility and fertility[19] , nutritional improvement, allergen and toxin elimination [20,21], and plant protection.

Methods of Gene Silencing

RNA Interference (RNAi): According to the present model, the RNA interference pathway starts with the presence of dsRNA in the cytoplasm that vary in length and origin)[22-24] (Figure1). This particular molecule is recognized by the Dicer enzyme, a member of the RNase III family of nucleases that specifically cleave double-stranded RNAs This enzyme cleaves the dsRNA into shorter RNA duplexes of 21 to 28 nucleotides, which have 5' phosphate and 2-nucleotide 3' overhangs [23-25]. These short RNA duplexes are known as short interfering RNA (siRNA) [26].Several organisms contain more than one Dicer gene, with each Dicer preferentially processing dsRNAs that come from specific source [27].

After Dicer processes the dsRNA, the siRNAs are subsequently rearranged into the RNA-induced silencing complex (RISC) [28,29]. The characterization of RISC includes the presence of an Argonaute protein family member and a guide strand (antisense to the target RNA) of a small RNA. The RISC complex is responsible for the targeting and cleavage of sequence specific mRNA within the cell. RISC acts by cleaving the target mRNA in the middle of the complementary region, ten nucleotides upstream of the nucleotide paired with the 5' end of the guide siRNA [30]. At least one protein from the Argonaute family, present in the RISC complex, probably acts as endonuclease, cleaving the target mRNAs (often referred to as the Slicer function) [31,32]. This cleavage leads to silencing of the target mRNA by preventing read-through of the message by the translational machinery, resulting in mRNA destruction.

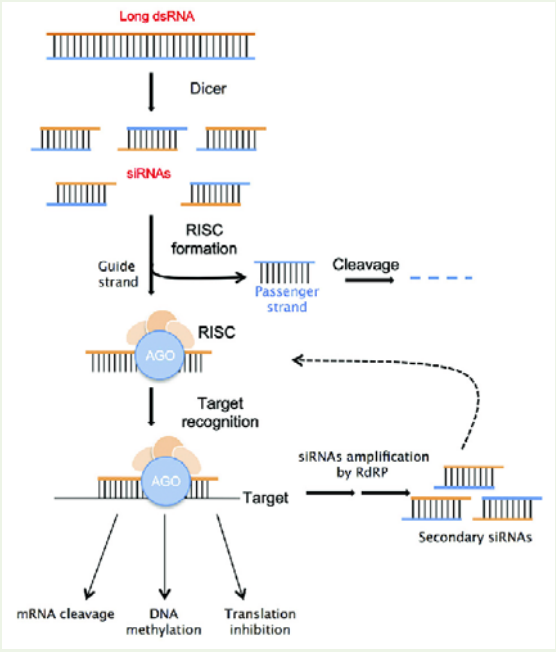

Figure 1: Schematic illustration of RNAi mechanism. Double stranded RNA (dsRNA) molecule binds to a Dicer protein, which cleaves it into small interfering RNAs (siRNAs); these siRNAs bind to an Argonaute (AGO) protein, part of the RNA-Induced Silencing Complex (RISC). The RISC separates the siRNAs into two strands: the passenger strand (blue) is degraded while the guide strand (orange) serves as a search probe, which links RISC to complementary RNA targets. After this recognition target's expression can be regulated through several different mechanisms. In plants, the silencing signal can be perpetuated by the action of the RNA-dependent RNA polymerase (RdRP) . Adapted with permission [87].

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9): The CRISPR-Cas9 system is an adaptive immune mechanism present in 40% of the sequenced bacterial genomes and 90% of the archaea [33], and has been identified to protect these microbes from the future invasions by bacteriophages [34]. After many years, the essential components of this system, including the guide RNA (gRNA) which is essential to direct the Cas9 protein (an important enzyme for induction of DNA double-strand break) to the targeted region on the genome, have been identified. The CRISPR-Cas 9 system is now broadly applied to eukaryotes for editing genes, including creating knock-in, knock-out, and also to correct the mutated genes in the genome [35]. After the CRISPR-Cas9 system induces a double strand break (DSB) at the targeted site(Figure 2), the endogenous cellular repair mechanisms will be activated, and can naturally attempt to repair and rejoin the broken DNA strands through either of two mechanisms: (i) non-homologous end joining (NHEJ) or (ii) homology-directed repair (HDR)[36,37]. Through NHEJ, insertions and deletions (called indel mutations) of a small number of the nucleotides is possible, and this might trigger a frame-shift mutation which can lead to the "loss-of-function" of protein-coding genes via the disruption of open reading frame (ORF) [38]. NHEJ-induced mutations can lead to silencing of a gene [39]-whereas, using HDR, a large portion of the gene (2–10 kb) can be deleted and, simultaneously, an incorporation of the exogenous DNA at the target region of the genome is possible.

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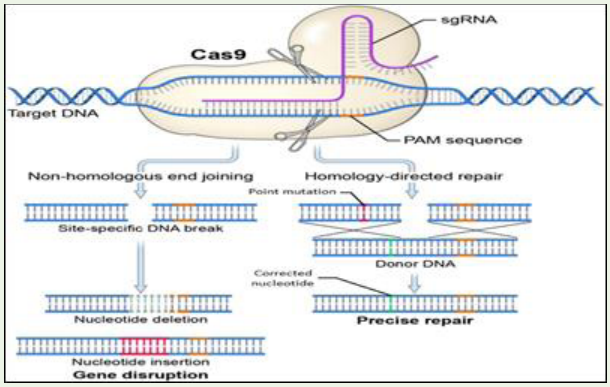

Figure 2: Schematic illustration of the CRISPR/Cas9 system structure. sgRNA complexes with Cas9 nuclease to hone in on the targeted genomic site containing an adjacent PAM sequence. Nucleotide hybridization of sgRNA-Cas9 complex to targeted loci creates a conformational change that activates Cas9 nuclease activity, resulting in DNA double-strand breaks. Induced DSBs of the target DNA are repaired by either NHEJ or HDR, producing gene mutations that include nucleotide insertion, deletion or substitution around the cleavage sites. Adapted with permission from) [88].

Experimental Workflow in RNAi and CRISPR

Design of siRNAs easiar than design of sgRNAs for CRISPR. In order to design siRNAs, one only needs the sequence of the corresponding mRNA transcript. While design for CRISPR requires knowledge of the genomic DNA sequence, and CRISPR also depends on the presence of a PAM sequence in the gene of interest. Depending on the type of Cas9 used, the PAM sequence may be very common within the genome (i.e. spCas9's 5'-NGG-3`), or not as common (i.e. saCas9's 5`-NNGRRT-3`). In almost all cases a 5`-NGG-3` PAM sequence will be present within the gene of interest.

RNAi has the simplest experimental set up and siRNA treatment can cause significant gene repression in only 24 hours [40]. Only one transgene needs to be delivered into the cell. It can be prepared as a ~20 bp double-stranded siRNA, or a ~80 bp shRNA cloned into a vector. In comparison, CRISPR rely on exogenous nucleases that must be delivered into the cell. This limits their effectiveness for use with viral expression systems such as AAV (Adeno-Associated Virus), which have limited packaging capacity.

No matter the method used, gene silencing must be verified before conclusions can be made. The process for verifying gene silencing varies depending on the technique used. When using RNAi, it's best to use two validation methods: one measuring mRNA levels (such as qRT-PCR), and another measuring protein levels (such as Western blot). A decrease in mRNA levels seen without a corresponding decrease in protein levels indicates that protein turnover may be slow. A decrease in protein levels without a corresponding decrease in mRNA levels indicates that the siRNA may be exerting its effects via translational inhibition instead of mRNA degradation. CRISPR gene silencing can be verified with methods that target the DNA. Initial screening is usually performed using the Mismatch Cleavage Detection Assay (a.k.a. Surveyor or T7E1), or by using Sanger sequencing(Figure 3).

Applications of Gene Silencing in Plants

Gene silencing was first used to develop plant varieties resistant to viruses. Engineered antiviral strategies in plants mimic natural RNA silencing mechanisms. This was first demonstrated when scientists developed Potato virus Y- resistant plants expressing RNA transcripts of a viral proteinase [41]. Immunity has since been shown to other viruses such as the Cucumber and Tobacco Mosaic Virus, Tomato Spotted Wilt Virus, Bean Golden Mosaic Virus, Banana Bract Mosaic Virus, and Rice Tungro Bacilliform Virus among many others.

In addition, plants can also be modified to produce dsRNAs that silence essential genes in insect pests and parasitic nematodes. This approach was used to develop root-knot nematode, corn rootworm and cotton bollworm resistant varieties.

A spectacular example using gene silencing is the rescue of the Hawaiian papaya industry by conferring resistance to papaya ringspot virus (PRSV)[42]. Another notable achievement is the bioengineered resistance of "NewLeaf Plus" potatoes to Potato leafroll virus, released by Monsanto [43]. In Australia, Peter Waterhouse and his CSIRO group pioneered the use of RNAi technology to develop varieties of barley that are resistant to barley yellow dwarf virus(BYDV) [44].

Kusaba and colleagues applied RNAi to reduce the level of glutenin in rice and produced a LGC-1 (low glutenin content 1) rice variety. This low-protein rice is useful for patients with kidney disease whose protein intake is restricted. The trait was stable and was transmitted for a number of generations[45].

During chemical pulping of wood, one of the most expensive and environmentally hazardous processes is to separate lignin from cellulose and hemicellulose[46]. The production of plant material with lower contents of lignin would mean a significant reduction of cost and pollution to the paper industry. One of the approaches to obtain reduced lignin forest trees has been the down regulation of lignin biosynthesis pathways [47]. The main genes involved with genetic transformation targeting lignin reduction are 4-coumarate: coenzyme A ligase (Pt4CL1) cynnamyl alcohol deshydrogenase (CAD - the final enzyme in the biosynthesis of lignin monomers) [48]and caffeate/5-hydroxyferulate O-methyltransferase (COMT - enzyme involved in syringyl lignin synthesis) [49].The downregulation of the Pt4CL1 gene in PopulustremuloidesMichx., produced trees with a

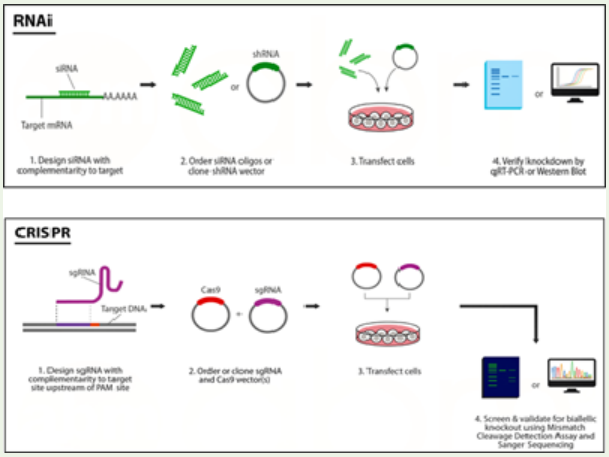

Figure 3: Workflows for using RNAi and CRISPR to achieve gene silencing.

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45% reduction of the lignin content compensated by a 15% increase in the cellulose content. In the transgenic lines obtained plant growth was substantially enhanced, and structural integrity maintained both at the cellular and whole-plant levelIn some woody plants, self-incompatibility stands as a major problem in fruit set and breeding programs[50], reported the production of transgenic apple trees able to self-pollinate and develop fruit. This break through was achieved by silencing of the S-gene responsible for self-incompatibility. The self-compatible transgenic plants lacked the pistil S-RNase protein, which is the product of the S-gene.

Fruit quality has also been addressed by silencing experiments. Several characteristics are involved in fruit quality. Transgenic apple fruits silencing key enzymes involved in autocatalytic ethylene production were significantly firmer and displayed an increased shelf-life [51].

Asparagine plays an apparently important role in the assimilation and storage of nitrogen [52], and is particularly abundant in the products of wheat (Triticum aestivum) [53], coffee and potato [54,55]. On heat processing, the amide amino acid reacts with reducing sugars to produce acrylamide [56].In humans, oral intake levels believed to be without an appreciable risk of deleterious effects are currently estimated to be 3.0 μg acrylamide/day (http://www.epa.gov/iris). This level of dietary intake is exceeded in small subsets of the population, particularly in young children and adolescents[57]. The Joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives and Contaminants has therefore recommended reducing the acrylamide content of processed starchy foods.

The faster route to decrease the acrylamide potential of food crops was established through gene silencing. Simultaneous silencing of two tuber-expressed genes in starch degradation, which encode water dikinase R1 and amyloplast-targeted phosphorylase-L, led to a decrease in the accumulation of glucose and fructose by approximately twofold [58]. These modified tubers correlated with an approximately two- to threefold decrease in acrylamide levels.

Among 12,000 alkaloids which are produced in plants, caffeine (1,3,7-trimethylxanthine) is one of the best known. The demand for decaffeinated coffee is growing globally, because of the possible adverse health effects of caffeinated coffee. Caffeine can trigger palpitations and increase blood pressure in sensitive individuals.

In coffee plants, caffeine is synthesized from xanthosine through three successive methylation and ribose removal steps (Figure 4). Ogita and his colleagues isolated all key genes for making caffeine in coffee plants (Figure 5) and they were able to reduce decrease the caffeine content in coffee plants by using an RNAi for MXMT, yielding a 70% suppression of the caffeine level in leaves of transgenic coffee plants (Figure 6).

Worldwide, approximately 1.1 billion people are smokers and more than 7 million people die from the negative effects of smoking every year (WHO report, 2017). One of the main natural ingredients causing dependence on tobacco is nicotine. Tobacco with a lowered nicotine content could help people to overcome their

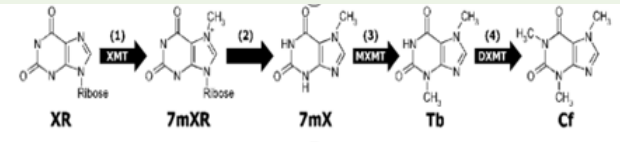

Figure 4: Caffeine biosynthetic pathway in coffee plants. The first (1), third (3), and fourth (4) steps feature methyl group transfer, and the second (2) step involves ribose (Rib) removal. XR, xanthosine; 7mXR, 7-methylxanthosine; 7mX, 7-methylxanthine; Tb, theobromine; Cf, caffeine; XMT, xanthosine methyltransferase; MXMT, 7-methylxanthine methyltransferase; DXMT, 3,7-dimethylxanthine methyltransferase. Adapted with permission from [89].

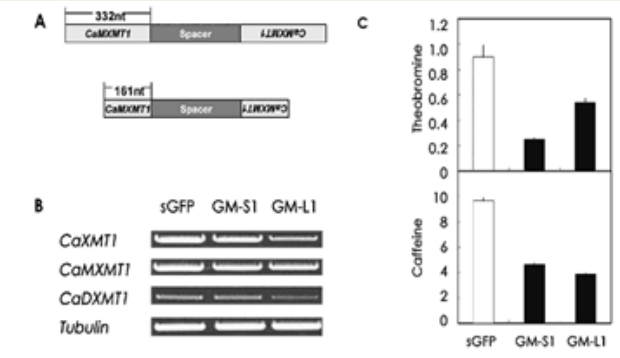

Figure 5: Genetic transformation of coffee plants. (A) Design of RNAi constructs. (B) Transcript levels of methyltransferase genes in young leaves of regenerated plantlets of C. canephora. Total RNA was isolated and analyzed for transcript levels of CaXMT1 and CaMXMT1, and CaDXMT1 by 32 and 35 cycles of RT-PCR, respectively. As an internal control, transcripts for a-tubulin were simultaneously measured by 32-cycle PCR. (C) Purine alkaloid contents in trangenic plantlets. Endogenous theobromine and caffeine levels (mg g1 fresh weight) were estimated in young leaves of transgenic sGFP (control), and indicated transgenic RNAi plantlets of C. canephora. Adapted with permission from [6].

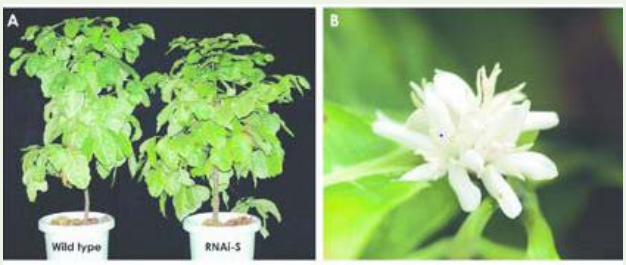

Figure 6: GM decaffeinated coffee plants growing in a greenhouse. (A) 4-year old transgenic coffee trees. Samples are RNAi (right side) and wild type (left side). (B) Flowering of RNAi transgenic coffee plants. Adapted with permission from (Ogita et al., 2005)

nicotine addiction. Nicotine free (or nicotine reduced) cigarettes may contribute to reduce the number of smokers and nicotine consumption, thus reducing the risk of death from tobacco use. The knockdown of the three most highly expressed BBL genes (BBLa–BBLc) by RNAi or the knockout with EMS induced mutations resulted in a reduction of the nicotine content without increasing the content of other alkaloids [59,60].Recently, the BBL gene family in tobacco was expanded by the identification of BBLd.2 and BBLe, leading to six known isoforms [61]. Thus, the simultaneous knockout

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of these BBL genes is a promising approach to generate a nicotine free tobacco plant.

Host-induced gene silencing (HIGS) is one of the methods that have been used to enhance resistance against pathogens, by expressing dsRNAs that target essential pathogen genes in host plant species leading to disease resistance. In recent study, the HIGS approach was successfully applied in maize and soybean to control the polyphagous mirid bug, *Apolyguslucorum*(Liu *et al.*, 2019). The selection of target was based on a previous work of injection-based RNAi of seven candidate genes in A. lucorum. The AlucV-ATPase-E gene was selected as *A. lucorum* fed with dsRNA corresponding to this gene has produced mortality rates of 46.01–82.32% at day 7 after injection. Based on the above finding, the populations of *A. lucorum*were significantly decreased after feeding on the transgenic maize and soybean expressing the dsRNA targeting AlucVATPase-E gene.

In a recent study camerlengo and his colleagues used a multiplex genome editing strategy to silent simultaneously two ATI genes (CM3 and CM16) in durum wheat, both indicated as major allergens in bread and durum wheat [61-63], and likely to be involved in Non-Coeliac Wheat Sensitivity (NCWS). This edited plants have potential to be grown as safer durum wheat lines for individuals predisposed to bakers' asthma, food allergies, and NCWS[64].The study confirmed that the multiplex genome editing system is an effective strategy to suppress simultaneously more than one gene. A similar strategy has been used to target two or more genes in wheat, rice and maize [65-67].

In another paper [68], the same ATI genes, plus the 0.28 gene, were silenced in the bread wheat cultivar Bobwhite by using RNAi. Different parameters related to yield resulted not affected, although one related to dough quality was strongly affected due to the lower expression of high molecular weight glutenin subunits, as an unpredictable effect likely due to RNAi procedure.

Mechanisms of Higs and Sigs

Host-induced gene silencing (HIGS) is an RNAi-based technique, expressing sequence-specifcdsRNAs in the host plant to silence target genes of plant pathogens. A dsRNA or a hairpin-structured dsRNA construct targeting a specific pathogen gene is transformed into the host plant. The transgenic plant produces dsRNAs and siRNAs, which find their entry into the plant pathogens during hostpathogen interactions (Figure 7a). The siRNAs degrade the pathogen mRNAs to protect the host plant against the pathogen[69-71].

Spray-induced gene silencing (SIGS) is a novel non-transformative strategy for plant protection. The dsRNA targeting a pathogen gene is sprayed onto plant surfaces. The fungal pathogen directly takes the dsRNAs up and induces the fungal RNAi machinery, and/or the host plant takes dsRNAs up first, induces the plant RNAi machinery, and then dsRNAs or siRNAs are transferred into fungal cells and induce the fungal RNAi machinery (Figure 7b). Thus, this approach silences pathogen's gene without introducing heritable modifications into the plant genome [72-74].

Applications of Gene Silencing in Plant via Non-Transgenic Approach

Traditional application of RNA-mediated gene silencing to control various pests and diseases or improve crop features is through transgenic approach, whereas the dsRNA/hpRNA construct must be prepared and the transgenic plants need to be generated.However, generation of these resistant transgenic plants may cause substantial delay as it is highly dependent on the transformability and genetic stability of the target crop plant species. Furthermore, limited acceptance of the genetically modified organism (GMO) by consumer will make the application less favourable. Recently, a number of studies showed that the RNA gene silencingcould be induced by just spraying dsRNA corresponding to the pathogen's gene on plant. For instance, topical applications of dsRNAs or siRNAs that target genes involved in the ergosterol biosynthesis in *Fusariumgraminearum* (CYP51A, CYP51B, and CYP51C), suppressed the fungal growth in barley [75]. The study found that the dsRNA was delivered to the distal parts of detached leaves via the plant vascular system and processed by the fungal DICER-LIKE1 (FgDCL-1) into siRNAs after being taken up by the pathogen.Similarly, spraying wheat plants with the dsRNA targeting myosine 5 gene of *F. asiaticum*reduced fungal virulence [76]. In *Brassica napus*, exogenous applications of dsRNAs targeting various genes of B. cinerea also decreased the gray mold disease severity [77]. The dsRNAs or siRNAs targeting the *B. cinereal*siRNA biosynthesis-related genes, such as Dicer-like 1 and 2 (DCL1 and DCL2), significantly reduced the gray mold diseases in various fruits and vegetables[78].This application is recognized as spray-induced gene silencing (SIGS).

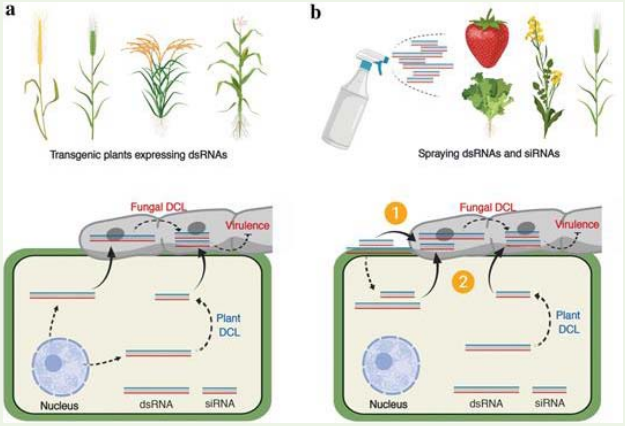

Figure 7: Host-induced gene silencing (HIGS) and spray-induced gene silencing (SIGS) for crop protection against fungal pathogens. a Mechanism of HIGS. Transgenic plants (e.g. wheat, barley, rice, and maize) expressing sequence-specific dsRNAs targeting fungal gene(s) are generated. The dsRNAs produced by transgenic plants are cleaved into siRNAs by the plant Dicer-like (DCL) proteins and both uncleaved dsRNAs and siRNAs are transferred into fungal cells when a fungal pathogen infects. dsRNAs are also cleaved into siRNAs by the fungal DCL proteins. The siRNAs in the fungal cells degrade the fungal pathogen mRNAs to counteract pathogen virulence (or mycotoxin). b Mechanism of SIGS. The dsRNAs and/or siRNAs targeting pathogen gene(s) are sprayed onto surfaces of plants (e.g. strawberry, lettuce, canola, and barley). There are two possible pathways to silence pathogen gene(s) through spraying dsRNAs and/or siRNAs. (1) A fungal pathogen directly takes the dsRNAs and/or siRNAs up and the dsRNAs are cleaved into siRNAs by the fungal DCL proteins. (2) The host plant takes the dsRNAs and/or siRNAs up first and the dsRNAs are cleaved into siRNAs by the plant DCL proteins. Both uncleaved dsRNAs and siRNAs are then transferred into the fungal cells in which the dsRNAs are cleaved into siRNAs by the fungal DCL proteins. In both cases, the siRNAs in the fungal cells degrade the fungal pathogen mRNAs to counteract pathogen virulence. BioRender (www.biorender.com).

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The dsRNA molecules applied exogenously to the leaves showed a fast-systemic spread from the treated (local) to non-treated (systemic) leaves and was present for up to 9 days in local leaves and 6 days in systemic leaves post-application. This approach offers another simple and environmentally safe way for application of dsRNA in control of pathogen virus. The possible workflow of the SIGS application was summarized in (Figure 8) by giving an example to control the insect pest.

However, there are a number of limitations in the application of dsRNA which could disfavour its commercial potential. One of the major limitations of the application of SIGS is the instability of naked dsRNA sprayed on plants. The naked dsRNA is easily degraded with the presence of soil or water. Several approaches, such as loading the dsRNA into a layered double hydroxide (LDH) clay nanosheet, namely the 'Bioclay' technology or guanidine-containing polymers or encapsulation of dsRNA in liposome complexes, were found to prolong the dsRNA shelf life under field condition [79-81]. A recent study demonstrated that the dsRNA loaded in BioClay was not easily washed of, showed sustained release under ambient conditions, and could be detected on sprayed leaves even 30 days after application [82]. In addition, the BioClay will provide RNAi-based systemic protection to newly emerge unsprayed leaves as the SIGS approach is effective on local and distal parts of the target plants[83,84]. The guanidine-containing polymers could protect dsRNA against nucleolytic degradation especially under the high pH environments.

The dsRNA production cost is another concern. However, production of the dsRNA using the in vitro or in vivo systems could also contribute to the production cost saving [85-87]. Production of dsRNA via in vivo expression in bacteria is preferable for the large-scale production [88-90]. An Apse RNA Containers™ (ARCs) was developed recently by using the bacterial system for the biosynthesis of dsRNA and capsid protein [91-95]. The bacteria co-transformed with plasmids encoding the target dsRNA and capsid protein will produce both components concurrently in the bacteria and the protein subunits will be self-assembled around the dsRNA[96-101]. The encapsulated dsRNAs can be purified from the bacteria and ready for field application. This technology will accelerate the mass production of the dsRNA with longer stability, without involving high production cost and complex procedures[102-107].

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