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Review Article

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RNAi, It's Mechanism and Potential use in Crop Improvement : A review

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ABSTRACT

RNA interference (RNAi) is a promising gene regulatory approach in functional genomics that has significant impact on crop improvement which permits down-regulation in gene expression with greater precise manner without affecting the expression of other genes. RNAi mechanism is expedited by small molecules of interfering RNA (siRNA & miRNA), mostly 21 or 24 nucleotides in size that repress the expression of sequence homologous genes at the transcriptional, post-transcriptional and translational levels effectively. Although the molecular basis of these complicated and interconnected pathways has become clear only in recent years and has been exploited in plants for resistance against pathogens, insect/pest, nematodes, virus and removal of allergenics and poor quality metabolites that cause significant economic losses. This review discusses mechanism of silencing, pathways in plants including post –transcriptional, transcriptional, miRNA, transitive and non-cell-autonomous and roles of RNA silencing in plant improvement.

Key words: *RNAi, siRNA, miRNA, silencing pathway*

INTRODUCTION

RNA interference (RNAi) is a biological process in which RNA molecules such as RNAi & miRNA collectively called small RNAs, inhibit gene expression or translation, by neutralizing targeted mRNA molecules based on homology with small RNAs molecules. This phenomenon was identified by Andrew Fire and Craig C. Mello working with *Caenorhabditis elegans* and for this great contribution they awarded Nobel Prize in Physiology and Medicine in 2006. RNA interference (RNAi) in eukaryotes is example of phenomenon called RNA silencing[32,40,79,98,114,122]. The unifying features of RNA silencing phenomenon are the production of small 21–24 nt short single-stranded non-coding small RNA (siRNA & miRNAs) the hallmark molecules of silencing that act as specificity determinants for down-regulating gene expression[36,57,62,63,65,67,86,93]. The Dicer (in plants Dicer-Like,DCL) enzymes, dsRNA specific endonucleases (RNase III-type ribonucleases) acts upon dsDNA and give rise to small RNAs[10,11,36,49,57] in collaboration with their partner DOUBLE-STRANDED RNA BINDING (DRB) protein[29,33,45,88].

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sRNAs are methylated by HUA ENHANCER1 (HEN1) at their 3' terminal[48,93]. This reaction protects them against poly-uridylation and subsequent degradation. sRNAs then associate with ARGONAUTE (AGO) family proteins[16,31,38,56,76,85,92,100,124,131] the central effectors of RNA-induced silencing complex (RISC) which mediates the cleavage of target RNAs at sequences with extensive complementarity to the siRNA[30,37,38,68,77,89,97,130]. Based on the sequence complementarity, sRNAs guide RISC to silence cognate RNAs through cleavage or translational repression (post-transcriptional gene silencing, PTGS) or induce chromatin/DNA modifications of the specific genomic locus (transcriptional gene silencing, TGS)[46,78]. In addition to Dicer and Argonaute proteins, RNA dependent RNA polymerase (RdRP) genes are required for RNA silencing[18,75,107,121]. In plants, PTGS initiated by transgenes that over express an endogenous mRNA also requires a putative RdRP, *SGS2*, *SDE1*[24,87], although transgenes designed to generate dsRNA bypass this requirement[9]. sRNAs are non-cell autonomous, they can move within the plant to transmit gene silencing from cell to cell or systemically on long distance as mobile silencing signals[28,44,83,84,111]. RNA interference (RNAi) has become a powerful and widely used tool for the analysis of gene function in invertebrates and plants and it is involved in almost all cellular processes like development, stress responses and antiviral defense.

Mechanism of RNAi

1. The entry of long double stranded RNA, such as an introduced transgene, a rogue genetic element or a viral intruder, triggers the RNAi pathway of cells. This results in the recruitment of the enzyme Dicer.
2. Dicer cleaves the dsRNA into short, 2025 basepairs long, fragments, called small interfering RNA (siRNA).
3. An RNA induced silencing complex (RISC) then distinguishes between the two siRNA strands as either sense or antisense. The sense strands (with exactly the same sequence as the target gene) are degraded.
4. The antisense strands on the other hand are incorporated to the RISC. These are used as guide to target messenger RNAs (mRNA) in a sequence specific manner.
5. Messenger RNAs (mRNA), which codes for amino acids, are cleaved by RISC. The activated RISC can repeatedly participate in mRNA degradation, inhibiting protein synthesis.

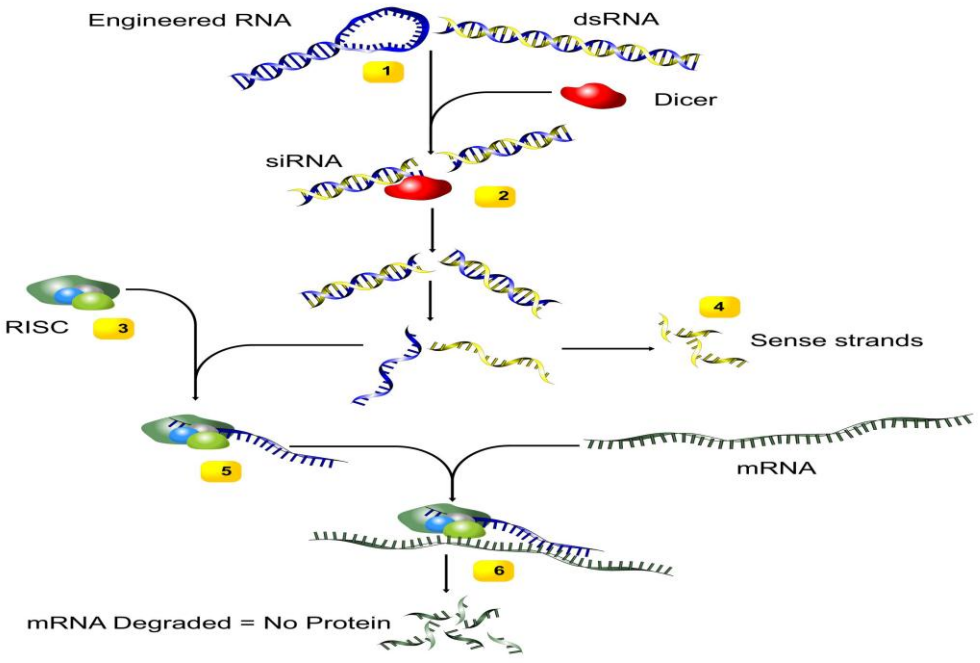

Fig. 1: Mechanism of RNAi

RNA silencing pathways in plant

Although silencing phenomenon have been mostly uncovered by studying plant–virus interactions[15,23,26,41,59,72]. In plants, RNA silencing has evolved as a defence system against invading nucleic acids including viruses, transposons and repetitive genomic sequences. As transgenes often contain viral promoters, they are expressed at very high levels and inserted repetitively into the genome, they can be perceived as 'invading nucleic acids' by host plants. The host plant responds by triggering RNA silencing, resulting in transgene silencing. Therefore, it should not be a surprise that the molecular mechanisms of plant transgene silencing and virus defence have merged to some large extent. However, many other processes Fin plant growth and development have also been found to employ RNA silencing. Examples of reviews describing these increasingly diverse RNA silencing pathways, and their implications in different biological processes[6,9,13,18,74,80,112,113,114,118,120].

1. Post-transcriptional Gene Silencing Pathway (PGTS)

As shown in Figure the PTGS pathway is elicited by aberrant RNAs, RNA molecules lacking a polyA tail or 5' capping. They are thought to be derived from highly abundant transgenic sense or antisense RNA, viral RNA or truncated transcripts from complicated gene insertions or duplications. The mRNA cleavage products at the end of the pathway can also be perceived as aberrant RNAs and further enhance the pathway. These aberrant RNAs are converted into dsRNA by the RNA-dependent RNA polymerase, RDR6[24,87] with the involvement of SGS3, SDE3 and WEX[25,35,87]. Originally identified in Drosophila, Dicers are RNase III enzymes that cleave (or 'dice') dsRNA into siRNAs. In Arabidopsis, a Dicer-like protein, DCL4, digests dsRNA into 21-nt siRNA duplex that are then methylated at the 3' terminal nucleotide by a RNA methyltransferase (HEN1) used in all known small RNA pathways[12,28,48]. One strand of the siRNA duplex is subsequently incorporated into the so called RNA-induced silencing complex (RISC). Although many of the components of the plant RISC have yet to be identified, the Argonaute protein (mainly AGO1) is known to be an integral part of the complex[18,31,85,121] guided by the complementary 21-nt siRNA, Argonaute cleaves the mRNA target. As it takes place mainly in the cytoplasm, this pathway is often known as 'the cytoplasmic RNA silencing pathway'.

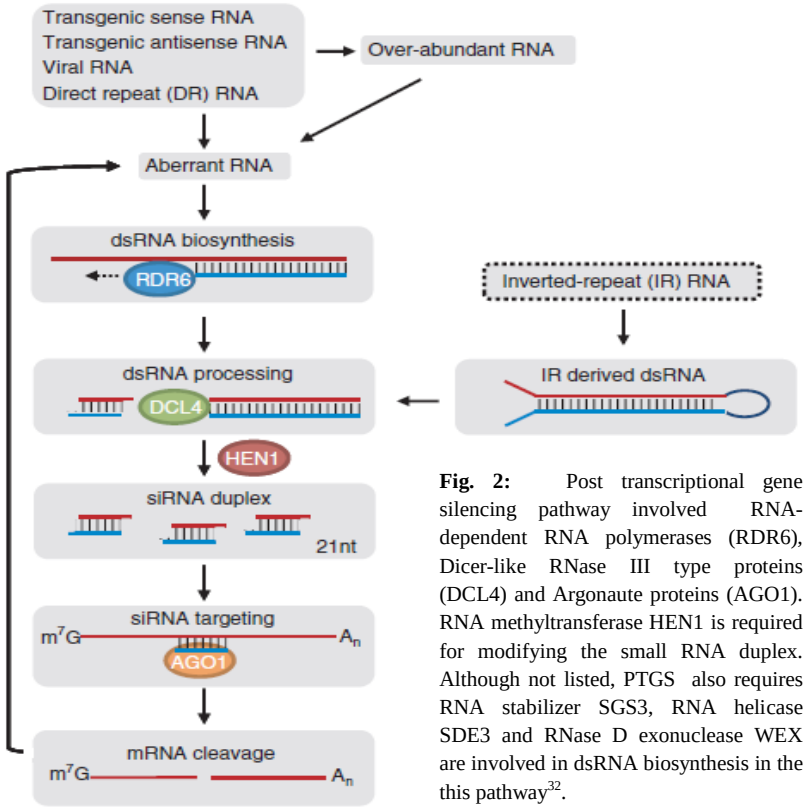

Fig. 2: Post transcriptional gene silencing pathway involved RNA-dependent RNA polymerases (RDR6), Dicer-like RNase III type proteins (DCL4) and Argonaute proteins (AGO1). RNA methyltransferase HEN1 is required for modifying the small RNA duplex. Although not listed, PTGS also requires RNA stabilizer SGS3, RNA helicase SDE3 and RNase D exonuclease WEX are involved in dsRNA biosynthesis in the this pathway[32].

2. Transcriptional Gene Silencing Pathway (TGS)

Transcriptional gene silencing shares similar components and uses a similar siRNA-guided process as PTGS. However, this process targets sequence homologous DNA and results in DNA methylation that suppresses the transcription. Originally discovered in transgene silencing and viral defence, TGS is now believed to be responsible for various epigenetic effects and maintenance of genome integrity. Like PTGS, the pathway is mediated by dsRNA synthesized by another RNA-dependent RNA polymerase, RDR2[126]. This process also requires an isoform of a plant-specific DNA-dependent RNA polymerase (PoI IVa) which contains subunits NRPD1a and NRPD2 (not shown in Figure)[43,54,91,99]. The dsRNA is processed by a different dicer (DCL3) into 24-nt siRNA duplexes that are methylated by HEN1[126]. These 24-nt siRNAs can be loaded into a RISC-like complex, quite similar to the RNA-induced transcriptional silencing complex (RITS) described in fission yeast[116]. This complex contains another Argonaute protein (AGO4) though its biochemical activity remains unclear[134]. Additional components such as Pol IVb (containing subunits NRPD1b and NRPD2)[54,99], DNA methyltransferases (MET1, CMT3 and DRM1⁄ 2)[17], histone deacetylase HDA6[4], histone methyltransferases KYP (SUVH4) and SUVH2[51], and chromatin-remodelling factor DRD1[54], have all been implicated in this pathway, which is now referred as chromatin targeted RNA silencing.

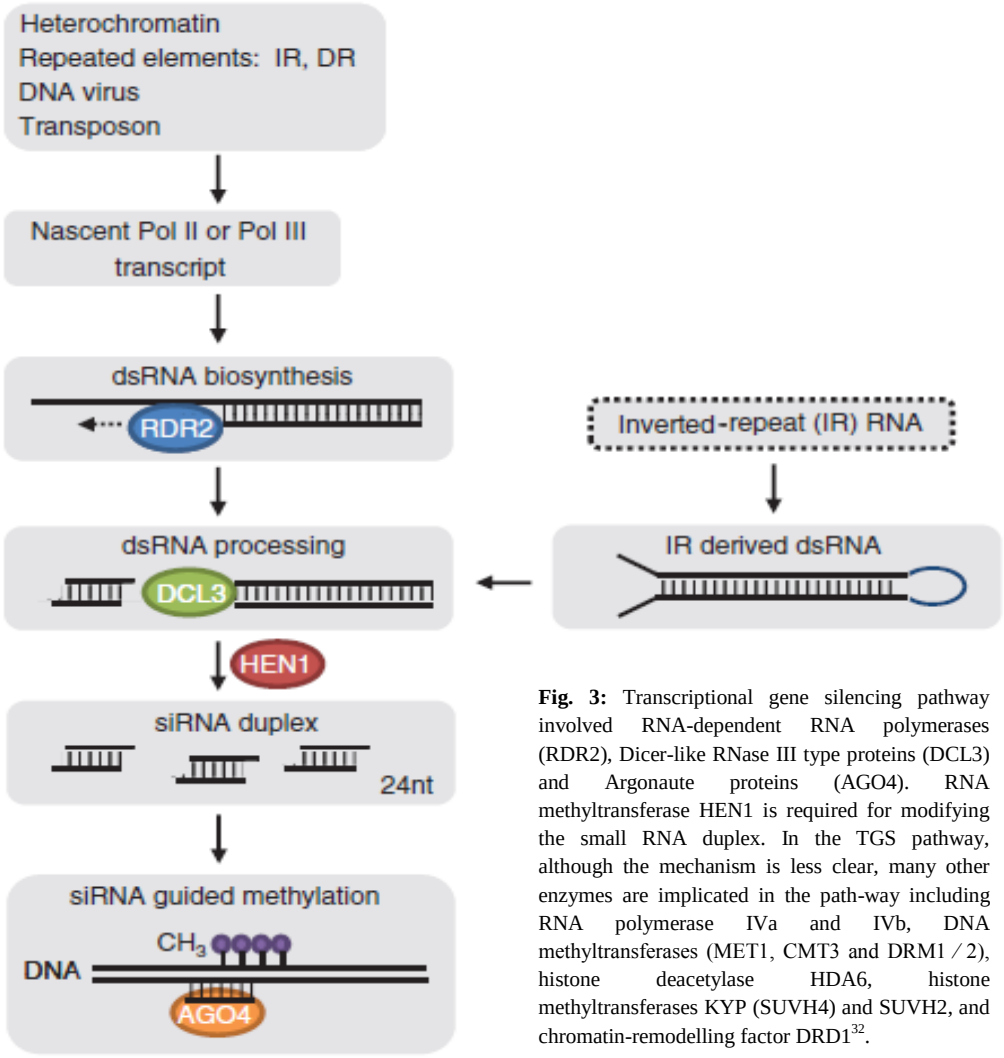

Fig. 3: Transcriptional gene silencing pathway involved RNA-dependent RNA polymerases (RDR2), Dicer-like RNase III type proteins (DCL3) and Argonaute proteins (AGO4). RNA methyltransferase HEN1 is required for modifying the small RNA duplex. In the TGS pathway, although the mechanism is less clear, many other enzymes are implicated in the path-way including RNA polymerase IVa and IVb, DNA methyltransferases (MET1, CMT3 and DRM1 ⁄ 2), histone deacetylase HDA6, histone methyltransferases KYP (SUVH4) and SUVH2, and chromatin-remodelling factor DRD1[32].

3. MicroRNA (miRNA) Pathway

MicroRNAs are another class of small RNAs, best characterized for their roles in developmental regulation and timing. Many miRNAs and targets are conserved in plants. Although part of the PTGS pathway, miRNA biogenesis differs from the described siRNAs pathways, and therefore the use of miRNA for RNA silencing requires different transgene design. MicroRNAs are small RNAs derived from transcripts with a distinctive RNA stem-loop secondary structure. Because the portions of the mature, folded transcript are dsRNA, the miRNA pathway does not require an RDR. The primary transcripts which are ultimately processed to produce miRNAs are termed primary miRNAs (pri-miRNAs). They are synthesized by RNA polymerase II (Pol II) and have typical Pol II 5' caps and polyA tails similar to protein coding transcripts[125]. The base of a pri-miRNA is cut to produce another miRNA precursor (pre-miRNA) which is further processed into a miRNA / miRNA* duplex by DCL1[93,101]. Although this stepwise processing was observed in plants but it is unclear whether DCL1 catalyzes all these steps[60]. Not shown in Figure, is an exportin-5 homologue HASTY[94] and a dsRNA-binding protein HYL1[39,115] which are also involved in miRNA biogenesis. The duplex is methylated by HEN1[12]. The 5' end of the miRNA strand is less stably base-paired than the other end of the duplex (the 5' end of the miRNA* strand) which establishes the loading priority of the miRNA strand into RISC and leaves the miRNA* strand unbound and rapidly degraded[104]. Similar to PTGS, the miRNA pathway mainly uses AGO1 as the RNA slicer in miRNA-loaded RISCs to promote the cleavage of their mRNA targets in plants[8]. In animals, however, miRNAs usually guide translational repression[49]. In plants, in addition to mRNA cleavage and translational repression[20,64], they were found to direct DNA methylation[5] and initiate the phased cleavage of trans-acting siRNAs (tasiRNAs)[1] as well. The discoveries of virus-encoded miRNAs and the virus target of an endogenous miRNA also suggest their role in antiviral defence[66,96]. Given their diverse activities and growing number of species, it is no wonder that miRNAs have wide ranging biological functions in plants and animals.

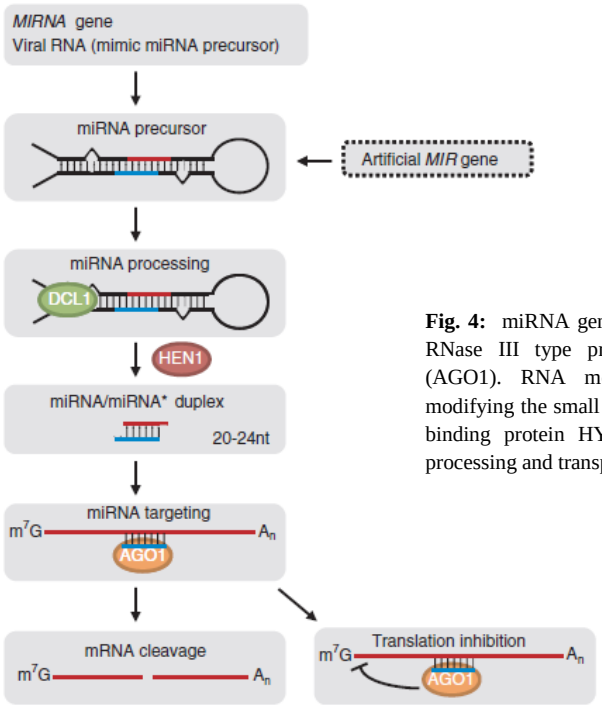

Fig. 4: miRNA gene scilencing pathway involved Dicer-like RNase III type proteins (DCL1) and Argonaute proteins (AGO1). RNA methyltransferase HEN1 is required for modifying the small RNA duplex. Although not listed, dsRNA-binding protein HYL1 and HST are essential for miRNA processing and transport respectively in miRNA pathway[32].

4. Transitive and Non-cell-autonomous RNA Silencing

Although RNA silencing appears to be ubiquitous and conserved among eukaryotes, transitive and non-cell-autonomous RNA silencing is only observed in plants and *Caenorhabditis elegans*[105,106,111]. In plants, these mechanisms amplify and broaden the RNA silencing response from the site of initiation. RDR6, DCL4 and others PTGS components are thought to be involved in these mechanisms[28,44,111]. While greatly enhancing plants systemic defences against viruses, these mechanisms might pose concerns for biotech applications that require tissue or target specificity of RNA silencing. As illustrated in Figure, the sequences of primary siRNAs derived from the initial dsRNA correspond to the region of the mRNA which they were designed to target. As the result of the action of primary siRNAs, secondary siRNAs are generated, which have the sequences spreading to both the 5′ and 3′ sections of the target mRNA. This transitivity is possibly through the primer-dependent and primer-independent activities of RDR6, followed by DCL4-dependent siRNA production[13]. This amplification of RNA silencing by secondary siRNAs is also responsible for extensive cell-to-cell movement of silencing, but the long-distance RNA silencing signal travelling through phloem has yet to be revealed (Figure). It seems that the production of this long-distance silencing signal does not require RDR6 or DCL4[14,102].

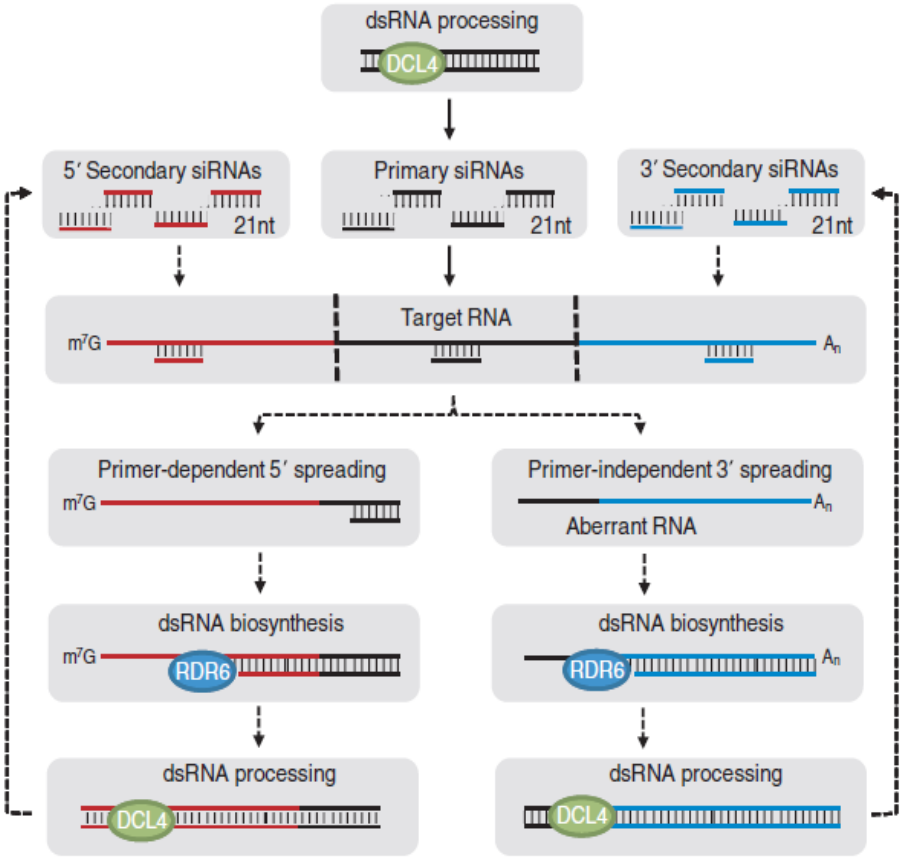

Fig. 5 : Transitive RNA silencing amplifies the effect of RNA silencing. Indicated by solid arrows, primary siRNAs (in black) derived from the initial dsRNA cleave the corresponding region (in black) of the mRNA target. The cleavage products trigger the process of generating secondary siRNAs (dotted arrows). As part of PTGS pathway utilizing RDR6, DCL4 and other PTGS components, primer-dependent spreading generates secondary siRNAs along the 5′ end of the mRNA target (in red); primer-independent spreading generates secondary siRNAs towards the 3′ end of the mRNA target (in blue)[32].

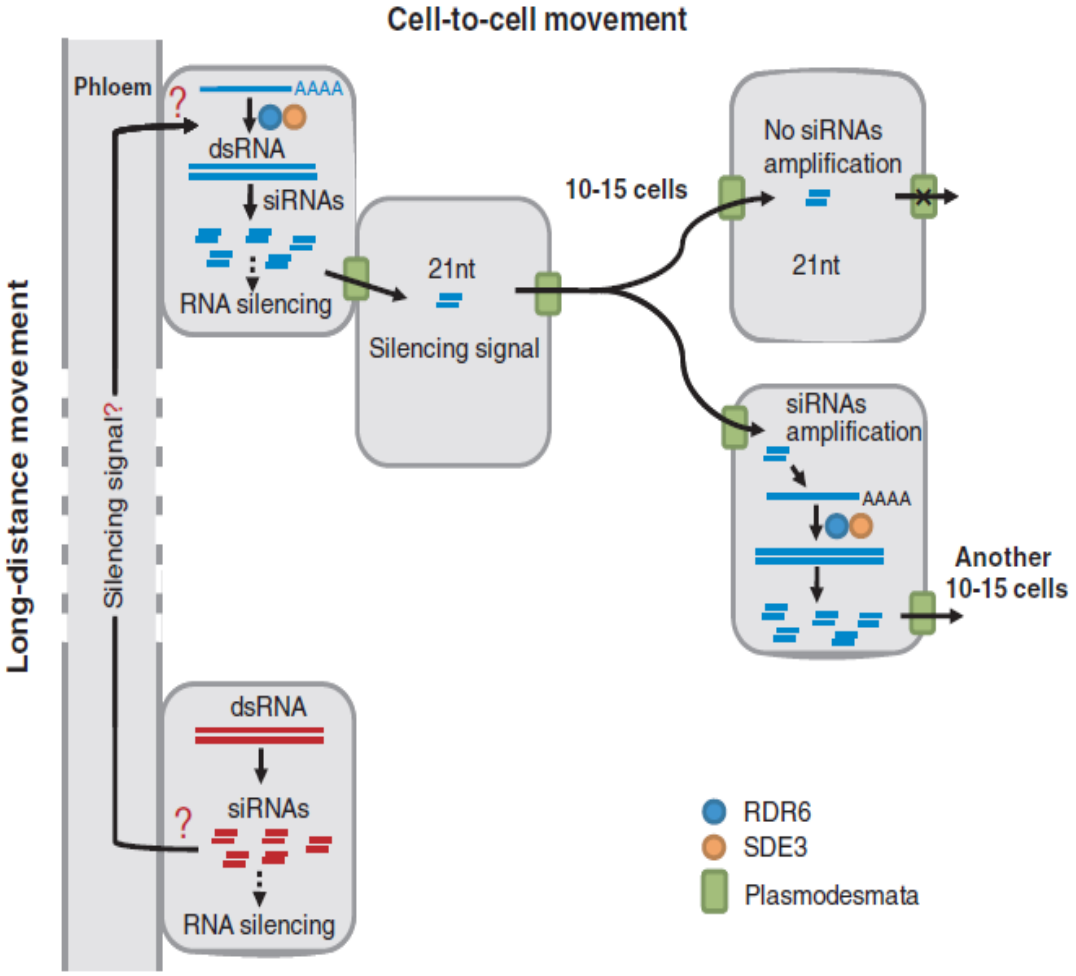

Fig. 6: Non-cell-autonomous RNA silencing results in broadening the effect of RNA silencing. In the long-distance movement, silencing travels through phloem, though the silencing signal has yet to be identified; in the cell-to-cell movement, 21-nt siRNAs are the silencing signals roaming through plasmodesmata to adjacent cells. Silencing spans only about 10–15 cells, unless in the presence of the mRNA target, in which case the signals can be amplified and advanced another 10–15 cells further. RDR6 and SDE3 are required for such amplification[32].

Potential Uses of RNA Silencing for Crop Improvement

Based on RNA silencing technology the metabolic enzymes have been down regulated and used for accumulation of beneficial plant metabolites, the food is improved by removing poor proteins and allergenic metabolites and accumulation of beneficial metabolites in cassava, maize and cotton.

1. Reduction of allergenic metabolites

(i) Reduction of Gossypol from Seeds of cotton

Cotton seed can be protein source for human if the toxic compound, gossypol down regulated in seeds although this is the source of resistance in plants against herbivores, insect and pathogen. So Embryo specific suppression of delta-cadinene synthase by using RNAi technology. This is involved in gossypol biosynthesis significantly reduced the accumulation of gossypol in cottonseed to the level deemed safe for human consumption[108]. In non-targeted tissues, such as foliage and floral portions, the levels of gossypol and related terpenoids were not diminished, and thus their attributes in plant defense remained.

(ii) Reducing linamarin from Cassava

Linamarin is synthesized through two cytochrome P450 enzymes, CYP79D1 and CYP79D2, in leaves and transported to roots. The leaf-specific inhibition of these enzymes lowered the linamarin content of roots in transgenic plants by 99%. Meanwhile, the potential production of novel non-narcotic

alkaloids in *Opium poppy* and low-caffeine coffee beans has been demonstrated in transgenic plants by suppressing the enzymes involving in the respective biosynthesis pathways[2,90]. Similarly non-narcotic alkaloids in *Opium poppy*, low-caffeine coffee beans has been demonstrated in transgenic plants by suppressing the enzymes involving in the respective biosynthesis pathways[2,90]. Removing allergenic or poor protein P34 from soybean[42], 14-16 kDa allergen proteins from Rice[110], Lol p 1 and Lol p 2 from Ryegrass[95], Mal d 1 from Apple[34], Ara h 2 and Ara h 6 from groundnut[21] also have been demonstrated.

(iii) Altering Potato Starch Composition

The potato granule-bound starch synthase (GBSS), responsible for the synthesis of amylase, was suppressed to generate virtually amylase free potato starch[117]. On the other hand, very high amylase potato starch was produced by simultaneously suppressing both starch branching enzymes A and B (Starch branching enzyme A and B)[103]. Because both amylase and amylopectin each type of starch has different fields of application. It requires complicated and expensive processing steps to separate them if both are produced in same potato. Therefore, the possibility of producing either pure amylose or pure amylopectin through a transgenic modification in potato has been investigated.

2. Quality Improvement

(i) Quality Protein Maize

Despite being a major food source for humans and animals, corn protein is largely (60%) made of zein, a storage protein that is devoid of essential amino acids such as lysine and tryptophan[22]. Conventional genetic approaches led to the isolation of a lysine and tryptophan mutant corn line, opaque-2 (o2)[82], and later to the development of quality protein maize (QPM). O2 encodes an endosperm transcriptional factor that is responsible for regulating zein genes along with other endosperm genes. The loss-of-function o2 mutant has a greatly reduced level of zein, but this is accompanied with an increase in non zein protein in kernels. It is believed that this protein replacement results in richer lysine and tryptophan content in o2 kernels. By using a fusion IR transgene that targets zein gene families, similar nutritional improvement was observed in transgenic corn kernels[47]. Specific zein reduction by RNA silencing in principle can reduce the pleiotropic effects associated with the o2 mutation. The lysine increase achieved by this approach is mostly in the protein fraction, distinguishing it from the examples given earlier of free lysine increases by manipulating the lysine metabolic pathway.

(ii) Healthful Vegetable Oil

The fatty acid composition of oilseeds can be modified to create desirable properties for various food applications. For example, lower content of polyunsaturated fatty acids stabilize the oil and render it suitable for uses in margarines and deep frying. Through RNA silencing, two key desaturases, stearoyl-ACP delta(9) desaturase (SAD) and oleoyl-PC delta(12) desaturase (FAD2), have been successfully suppressed in various oilseed species to generate more stable vegetable oils[19,58,70]. SAD converts stearic acid (18 : 0) into oleic acid (18 : 1), and FAD2 converts oleic acid into linoleic acid (18 : 2). In the conventional production of soy-bean oil, hydrogenation is used to increase the content of saturated fatty acids for longer shelf life and better high-temperature stability. This process also produces trans-fatty acids (transfats) that are linked to elevated risk of cardiovascular disease. Inhibition of FAD2-1 in transgenic soybean seeds by RNA silencing increases the level of oleic acid and reduces the level of linoleic acid. The transgenic soybean oil can be directly used without hydrogenation, which is naturally devoid of trans fats and thus healthier for human consumption.

3. RNAi for Biotic and Abiotic Stresses

A. Biotic stress

(i) Virus Resistance

RNA silencing plays a critical role in plant resistance against viruses, with multiple silencing factors participating in antiviral defense. Both RNA and DNA viruses are targeted by the small RNA-directed RNA degradation pathway[119]. A major antiviral

mechanism in plants is mediated by RNA silencing, which relies on the cleavage of viral dsRNA into virus-derived small interfering RNAs (vsiRNAs) by DICER-like enzymes. Virus-resistant papaya and squash lines deregulated in 1990s are still being cultivated today. Although the expression of virus-derived protein (coat protein-mediated resistance; CPMR) was thought to be the viruses upon infection before the viruses establish and exert their inhibition on the plants RNA silencing mechanism. 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 scientist developed Potato virus Yresistan plants expressing RN transcripts of a viral proteinase gene[61,81]. Immunity has 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, Rice Tungro Bacilliform Virus and many others in addition, plants can also be modified to produce dsRNAs that silence essential genes in insect pests and parasitic nematodes.

(ii) Insect Control

Current biotech approaches for controlling insect pests on crops rely mostly on the expression of *Bacillus thuringiensis* (Bt) insecticidal proteins. As RNA silencing is known to occur in insects, it can be used as an alternative mode of insect control mechanism. Transgenic corn plants expressing dsRNAs corresponding to western corn rootworm (WCR) V-ATPase has showed a significant reduction in WCR feeding damage in a growth chamber assay[7]. This example demonstrates the potential of RNA silencing applications in insect control, which would be valuable for managing the emergence of insect resistance against Bt proteins. RNA silencing was also reportedly providing resistance to nematodes in transgenic Arabidopsis and tobacco plants[47,128]. For pest control, the insect should be able to autonomously take up the dsRNA, for example through feeding and digestion in its midgut. At least two pathways for dsRNA uptake in insects are described: the trans membrane channel-mediated uptake mechanism based on *Caenorhabditis elegans'* SID-1 protein and an 'alternative' endocytosis-mediated uptake mechanism[50]. In 2007, Baum *et al*[7]., published a break through paper on insect control through dsRNA feeding experiments. They provided evidence for the potential use of RNAi to control pest insects in crop protection and demonstrated the fact that it is possible to silence genes in insects when they consume plant material expressing hairpin dsRNA constructs against well chosen target genes. They reported the reduction of corn root damage in transgenic maize plants producing vacuolar H+ ATPase dsRNA after infestation of the plant with the western corn rootworm (*D. virgifera virgifera*). In another report, the model plants *Nicotiana tabacum* and *Arabidopsis thaliana* were modified with the cytochrome P450 gene of *H. armigera*. When the cotton bollworm larvae were fed transgenic leaves, the levels of cytochrome P450 mRNA were reduced and larval growth retarded[73].

B. RNAi for Abiotic Stress Tolerance

RNAi is an ultimate appealing and an invigorating phenomenon in which short double strand RNA (dsRNA) averts the specific gene expression by inducing degeneration in the chain sequence of particular target messenger RNA in the cytoplasm. Current findings manifested that RNAi is playing an imperative role in abiotic stresses stimulation in different crops. The function of miRNAs (microRNA) in relation to abiotic stress like oxidative stress, cold, drought, and salinity were reported by Sunkar and Zhu[109] in *Arabidopsis* plants under various abiotic stress and confirmed miR393 was sturdily up-regulated when exposed to higher salinity levels, dehydration, cold, and abscisic acid (ABA). Additionally, miR402, miR319c, miR397b, and miR389a were controlled by abiotic stress under varying levels in *Arabidopsis*[52]. RNAi technology may be a substitute of

complex molecular techniques because of containing several benefits like its specificity and sequence-based gene silencing. This ability of RNAi has been efficaciously utilized for incorporating desired traits for abiotic stress tolerance in various plants species[52].

(i) Drought Stress Tolerance

In relation to drought responses, miR169g and miRNA393 genes have been observed in rice crop which were stimulated under drought conditions[132]. Among genetically engineered plants the rice exhibited gene expression of RACK1 inhibition caused by RNAi, which explained the potential role of RACK1 to drought stress in rice crop. The transgenic rice was observed with a superior level of tolerance in contrast to non-transgenic rice plants[53]. In many plants such as *Arabidopsis*, *Populus trichocarpa*, and *Oryza sativa* the miRNA expression profiling has been performed under drought stress. miR169, miR396, miR165, miR167, miR168, miR159, miR319, miR171, miR394, miR393, miR156, and miR158 were made known to be drought-responsive[69]. Analysis of miRNAs and genome sequencing profiling were executed in drought-studied rice at a various range of growth stages, from tiller formation to inflorescence, utilizing a microarray platform. The results suggested that 16 miRNAs (miR1126, miR1050, miR1035, miR1030, miR896, miR529, miR408, miR156, miR171, miR170, miR168, miR159, miR397, miR396, miR319, miR172 and miRNA1088) were remarkably involved in down regulation in response to drought stress[69]. In contrast, 14miRNAs (miR1125, miR159, miR903, miR169, miR901, miR171, miR896, miR319, miR395, miR854, miR851, miR474, miR845, and miRNA1026) were found in up-regulation under drought stress. Few miRNAs gene families, like miR319, miR896, and miR171 were recognized as both up- and down regulated groups[133]. In *P. vulgaris*, miR2119, miR1514a, and miRS1exhibted a gentle but obvious increase in accretion upon drought treatment, on the other hand, the accumulation was higher for miR2118, miR159.2, and miR393 in reaction to the identical treatment[3]. In recent studies, miRNA expressing patterns of drought tolerance wild emmer wheat in relation to drought-stress explored by utilizing a plant miRNA microarray platform[55]. At the same time, up regulation throughout drought stress in maize crop has been studied by miR474, which interact with proline dehydrogenase (PDH)[123].

(ii) Salt Stress Tolerance

Many regulated miRNAs have been reported in salinity stressed plants. In *Arabidopsis*, miR397, miR156, miR394, miR158, miR393, miR159, miR319, miR165, miR171, miR167, miR169, miR168, and miR398 were up-regulated in reaction to salinity stress, whilst the accumulation of miR398 was reduced[69]. In *P. vulgaris*, it was reported that increment in accumulation of miR159.2 and miRS1 with the addition of $NaCl$[3]. In *P. trichocarpa*, miR171l-n, miR530a, miR1446a-e, miR1445, and miR1447 were down regulated; on the other hand, miR1450 and miR482.2 were up-regulated in salt stress period[71]. Recently, a research investigation was carried out by using microarray to elucidate the miRNA profile salinity- tolerant and a salt-sensitive line of maize; the findings indicated that members of the miR396, miR156, miR167, and miR164 groups were down-regulated, while miR474, miR162, miR395, and miR168 groups were up-regulated in saline-stressed maize roots[27].

(iii) Cold and Heat Stress Tolerance

miRNA in wheat showed variant expression in heat stress response; researchers cloned the miRNAs from the leaves of wheat after treating with heat stress, with the help of Solexa high-throughput sequencing. In wheat, 32

families of miRNA distinguished, among them 9 identified miRNAs were supposed heat responsive. For instant, miR172 was distinctly decreased, while miRNAs including (miR827, miR156, miR169, miR159, miR168, miR160, miR166, and miR393) were noticed with up regulation in response to heat[127].

4. RNAi for Male Sterility

RNAi has also been used to generate male sterility, which is valuable in the hybrid seed industry Genes that are expressed solely in tissues involved in pollen production can be targeted through RNAi. For instance, scientists have developed male sterile tobacco lines by inhibiting the expression of TA29, a gene necessary for pollen development[73]. RNAi was also used to disrupt the expression of Msh1 in tobacco and tomato resulting to rearrangements in the mitochondrial DNA associated with naturally occurring cytoplasmic male sterility[7].

CONCLUSIONS

RNA interference (RNAi) has recently become a highly effective and powerful tool of functional genomics for silencing the gene expression for crop improvement. Nevertheless, RNAi stability in plants is critical, but RNAi-mediated gene suppression approach open new avenues in the development of eco-friendly biotech approaches for crop improvement by knocking-out the specific genes for better stress tolerance and integrating novel traits in various plant species including insect/pest/pathogen resistance and enhanced nutritional status. The RNAi is a sophisticated technology having revolutionary capabilities could be further exploited for functional analysis of target genes and regulation of gene expression for crop protection and improvement.

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