Review Article

The function of RNA interference (RNAi) in crop enhancement-Mechanism and Applications: A Review

ABSTRACT

In agricultural biotechnology, RNA interference (RNAi) has become a potent technique that allows for the modulation of gene expression to impart many desirable features in crop plants. ~~The many~~Various uses of RNA interference (RNAi) are examined in this paper, encompassing protection against biotic and abiotic stressors, morphological changes, male sterility modification, nutritional fortifying, and allergy reduction.Through the destruction of certain messenger RNAs (mRNAs) by tiny RNA molecules like microRNA (miRNA) and small interfering RNA (siRNA), RNAi suppresses the expression of genes. The identification of RNA interference (RNAi) has transformed crop growth by providing a non-transgenic method of gene editing.The critical function of RNAi in raising production and resilience in agriculture. Researchers have learned more about plant ~~defense~~defence systems, stress responses, and virus resistance by clarifying the processes of RNA interference. The discovery of regulatory noncoding RNA (ncRNA) has created new opportunities for enhancing crop quality and controlling cellular ~~defenses~~defences. RNA interference (RNAi) and CRISPR technologies offer viable ways to tackle problems in agriculture, such as stress tolerance and disease control.Overcoming obstacles such as nucleases degradation, and developments in RNA-based gene control have resulted in ~~the~~ creation of synthetic oligonucleotides and high-throughput analytic methods. Researchers have created superior transgenic plants with improved nutritional content, disease resistance, and horticultural traits by precisely predicting and synthesizing artificial RNA strands.Moreover, RNA interference (RNAi)-based gene silencing methods provide extensive implications in agriculture due to its conservation across many plant and animal species. Researchers may design crops with enhanced nutritional value, stress tolerance, and commercial appeal by utilizing RNA interference (RNAi) technology. In tackling global agricultural difficulties and opening the road for sustainable food production, this research highlights the revolutionary influence of RNAi on agricultural biotechnology.

keywordsAgricultural biotechnology, Stress tolerance, Gene editing, Crop enhancement, and RNAinterference (RNAi).

1. INTRODUCTION

Numerous desirable traits, such as nutritional fortifications, allergen reduction or allergen substitution, morphological alterations, male sterility alternation, secondary metabolite enhancement, and defense against a variety of biotic and abiotic stresses

in different crop plants, have all been modified successfully through the use of RNA interference.[1] RNA interference (RNAi) is a natural defense mechanism against double-stranded RNA (dsRNA), targeting viral and cellular mRNAs. The cleavage products are microRNA (miRNA) and small interfering RNA (siRNA), produced by DICER enzyme. In recent years, researchers have shifted towards biotechnology methods for crop development, allowing for easy manipulation of gene expression in crops for desirable features.[2]

RNA interference (RNAi), which was aptly named Science's "Technology of the Year" in 2002 and Fortune Magazine's "Billion Dollar Breakthrough"in 2003, works by suppressing gene expression by breaking down particular messenger RNAs (mRNA).[3]

Plants provide food, shelter, and other necessities for life, as well as useful products like wood and medicine. However, constraints like ~~as~~ limited land and changing climate pose a danger to this crucial partnership. Although artificial gene transfer raises difficulties, genetic engineering provides remedies by increasing agricultural yields and nutrition. Let me introduce you to RNAi, a revolutionary technology that silences genes without the need for transgenics, perhaps lowering dangers and winning over people. This breakthrough improves agricultural resilience and illuminates the control of genes, among other areas, and provides access to new technologies. By impacting plant development and stress tolerance, RNA interference (RNAi) is paving the way for a future in which artificial manipulation strengthens rather than upsets the delicate equilibrium between people and plants.[4]

By providing protective armor, HUA ENHANCER1 (HEN1)keeps single-stranded RNA fighters from being destroyed. Thanks to ARGONAUTE proteins, these fighters are able to silence genes they identify. A whispered "quiet!" will silence the target's message, or they can alter its DNA to permanently silence it. Especially for genes introduced by outsiders, this army of silence is powered by a unique enzyme called RdRP. Understanding genes in plants and other organisms is made possible by this superpower of RNA interference (RNAi), which is woven through development, stress responses, and even fights viruses.[5]

Petunia flowers revealed a curious silencing of color genes, sparking the discovery of RNAi in worms. This potent process uses injected double-stranded RNA to dismantle specific genes, even in offspring. The mystery of its function and RNA transport ~~fueled~~fuelled research across various organisms, boosted by the power of genomic data. Today, RNAi remains a crucial tool for understanding and manipulating gene activity in plants and animals.[6]

Advances in RNA-based gene regulation, specifically RNA interference (RNAi), have led to improved crop quality and reduced biosafety issues. RNAi is a gene silencing phenomenon used for gene function assessment, plant metabolic engineering, and developing stress-tolerant and disease-resistant crops.[7]

The discovery of regulatory noncoding RNA (ncRNA) led to the genetic revolution and two new areas of study: understanding ncRNA functions and practical applications like RNA interference (RNAi) to manipulate cellular defenses. RNAi and CRISPR, a bacterial virus

defense system, have been suggested as potential magic bullets for medicine and agriculture. RNAi is still used experimentally for disease management in agriculture, reverse genetics, and functional genomics, despite the use of CRISPR offering new promises and challenges. Recent reviews have explored various aspects of RNAi for plant disease management strategies, including a special issue of Current Opinion in Virology.[8]

Double-stranded RNA (dsRNA) is a widely used gene function analysis tool due to its specificity and effectiveness. It is found in plants and animals and has been used in genome-wide analysis in worms and flies. RNAi techniques have been developed for high-throughput analysis in cultured cells.Recent research has demonstrated the natural functions of RNA interference, including suppression of transposon activity, resistance to viral infection, post-transcriptional and post-translational regulation of gene expression, and epigenetic control of chromatin structure. RNAi is expected to be useful in genetic engineering crop plants, particularly as a knockdown technique.[9]

Antisense oligonucleotide effectiveness is drastically decreased by cellular nucleases, which break down these molecules quickly after they are administered. This barrier might be addressed by using synthetic oligonucleotides that change the phosphodiester bond's composition by substituting sulfur for oxygen. We refer to these altered oligonucleotides as ~~phosphorothionates~~phosphonothioates.[10]

Agriculture is being transformed by RNAi technology. Think about fruits without seeds, fruits with a longer shelf life, and fruits with more nutrition–all perfectly accessible! Plant genes can be altered by this novel approach, which can improve tolerance to adverse conditions, regulate biomass, and change the color of flowers. As allergies are reduced, it even permits the addition of beneficial nutrients. The primary factor controlling important plant characteristics is siRNA. A large portion of our protein and 65% of our calories come from cereals like wheat and maize. In influencing the future of cereal crops, this paper examines the fascinating real-world uses of RNA interference.[11]

Small interfering RNA was found through more research, which prompted computer scientists to become involved in the prediction and synthesis of precise and artificial RNA strands with greater specificity. This process improves the production of superior transgenic plants with higher market values. A greater and superior quality of crops with high nutritional content, resistance to disease and abiotic and biotic stress, and improved horticultural features, such as the color of the flowers, can be obtained thanks to RNAi technology and the accessible genetic pool.[12]

Since RNAi-based gene silencing mechanisms are conserved in both the plant and animal worlds, they have potential uses in agriculture, such as stress tolerance engineering, as well as functional genomics. Furthermore, advancements in the RNAi technique have unlocked its immense potential to address a number of agricultural difficulties.[13]

2. HISTORY OF RNAi

Gene function was previously studied using a variety of techniques, including antisense RNA suppression, mutagen or radiation therapy, T-DNA element and transposon insertion, and more. These techniques, however, were laborious and unreliable. As a result, the RNA interference (RNAi) approach was discovered. Plant scientists first employed antisense RNA suppression as a type of RNA silencing (Williams et al., 2004). However, this strategy did not always completely quiet the target gene, which led to more study into alternate gene silencing techniques. By concurrently introducing sense and antisense strands, Fire et al. (1998) initially reported the RNAi approach in the roundworm Caenorhabditis elegans, generating the double-stranded RNA (dsRNA) necessary for initiating the RNAi pathway.When compared to employing simply sense or antisense strands alone, this unexpectedly led to a tenfold increase in targeted gene silencing. This groundbreaking discovery encouraged researchers to explore the complex RNAi phenomenon in greater depth, gaining insight into the molecular workings of RNAi technology and how it affects the activity of particular genes. Plant breeders are now able to use RNAi as a tool for crop enhancement and plant protection thanks to this significant breakthrough. [10]

Molecular biologists have been working to prevent gene expression at the mRNA level for the last 20 years. Although antisense approaches utilizing DNA or RNA were relatively straightforward ways to investigate gene activity, their efficacy and specificity were limited. Furthermore, it was sometimes difficult to predict the intended effects, and only modest suppression could be achieved. After the discovery of post-transcriptional gene silencing (PTGS) in plants in 1998, RNAi function in flies, mammals, plants, and fungi was also seen. The lengthy dsRNA molecules start the process. dsRNA is produced by viruses and transposon activity during natural RNAi processes; it may also be injected into cells for research reasons. The complementary opposite strand is referred to as the antisense strand, while the dsRNA strand that matches a particular area of the target mRNA molecule is referred to as the sense strand. After identifying dsRNA, a Dicer complex that resembles RNAse III in *D. melanogaster* cleaves it into segments that are approximately 22 nucleotides long. These fragments, referred to as "small interfering RNAs" (siRNAs), continue to exist as short 3' overhanging double-stranded duplexes. The homologous mRNA is degraded and its synthesis is particularly suppressed by the RNA-induced silencing complex (RISC), which uses these duplexes as templates. This kind of RNAi is called PTGS; in some species, other kinds may also have transcriptional or genomic effects. Nonetheless, Fire and Mello made a substantial contribution to this field's growth. Motivated by the previously reported occurrences, they pondered the potential outcomes of introducing both sense and antisense strands into the worms. Surprisingly, this combination of strands was ten times more efficient than either strand alone at reducing the expression of a particular gene. After identifying the uniqueness of the dsRNA-induced effect, Fire and Mello gave the mechanism the moniker RNA interference (RNAi). [14]

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3. MECHANISM OF RNAi

Components of RNAi Machinery:Dicer and the RNA-induced silencing complex (RISC) are the two ribonucleases that take part in the RNAi pathway. Dicer converts dsRNA into active small non-coding RNAs and starts the RNAi pathway, while RISC and the RNase H core enzyme Argonaute (AGO) carry out the gene silencing. The N-terminal helicase

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domain, a PAZ (Piwi/Argonaute/Zwille) domain, dual RNase III domains, and a dsRNA binding domain make up the Dicer family, which is a class 3 RNase III enzyme. These enzymes' main job is to identify the dsRNA precursor from the RNAi pathway and produce short non-coding RNA with a precise length (between 21 and 24 nt).According to the Dicer catalysis concept, the active core of the multidomain dicer enzyme is formed when two RNase III domains dimerize to produce an intramolecular pseudo-dimer. Additionally, it has been proposed that every domain forms a new termination by cutting a single strand of ~~dsRNA .~~dsRNA.The RNA interference process culminates in gene silence by target mRNA degradation, which is carried out by RISC in collaboration with the argonaute (AGO) protein and other effector proteins. Proteins called argonautes are mostly found in eukaryotes, bacteria, and archaea. The Argonaute protein has three important roles: it may identify guide strand termini, use its nuclease ability to cut the target mRNA, or recruit other proteins that are involved in silence. Additionally, RISC with gene silencing takes role in the method of cellular surveillance.[7]

3.1 Silencing Genes with RNAi: A Three-Act Play

A potent biological process known as RNA interference (RNAi) targets messenger RNA (mRNA) for destruction in order to control gene expression. The ideal match between the target mRNA and the inserted "guide" sequence is crucial to this process. Here are the three main steps that it takes:

3.1.1 Act I: Dicer Takes the Stage

Double-stranded RNA (dsRNA) is either inserted into the cell or arises spontaneously at the start of the play. Dicer, an RNase III family enzyme, intervenes in this situation. Like a proficient cutter, the dicer divides the dsRNA into exact 21-nucleotide segments known as small interfering RNAs (siRNAs). The two-nucleotide overhang on both ends of these siRNAs is a distinctive characteristic.

3.1.2 Act II: RISC Assembles and Unwinds

Subsequently, the siRNA joins an organization known as the RNA-induced silencing complex (RISC). Argonaute (AGO) protein is the star of the show among the several proteins that make up RISC. The guide strand and the passenger strand are the two strands that remain after the siRNA unwinds inside RISC. The guide strand, nurtured by AGO's PAZ domain, emerges as the play's principal investigator, while the passenger strand frequently deteriorates.

3.1.3 Act III: Guide Strand Finds and Destroys

Equipped with RISC, the guide strand sets out to locate its complementary target within the cellular mRNA pool. The AGO protein in RISC flexes its muscle when a perfect match is detected. Its PIWI domain cleaves the target mRNA at a precise place, usually ten nucleotides distant from the match, similar to a pair of molecular shears. This successfully silences the specific gene by making the mRNA unintelligible.

Sometimes an enzyme known as RNA-dependent RNA polymerase (RDRP) can be employed to produce even more siRNAs from the passenger strand rather than discarding it. This increases the effect of silence, but it's not a constant feature of the performance.

Through the strategic placement of complementary guide strands, RNA interference (RNAi) enables cells to precisely control gene expression, resulting in a well-tuned cellular symphony.[12]

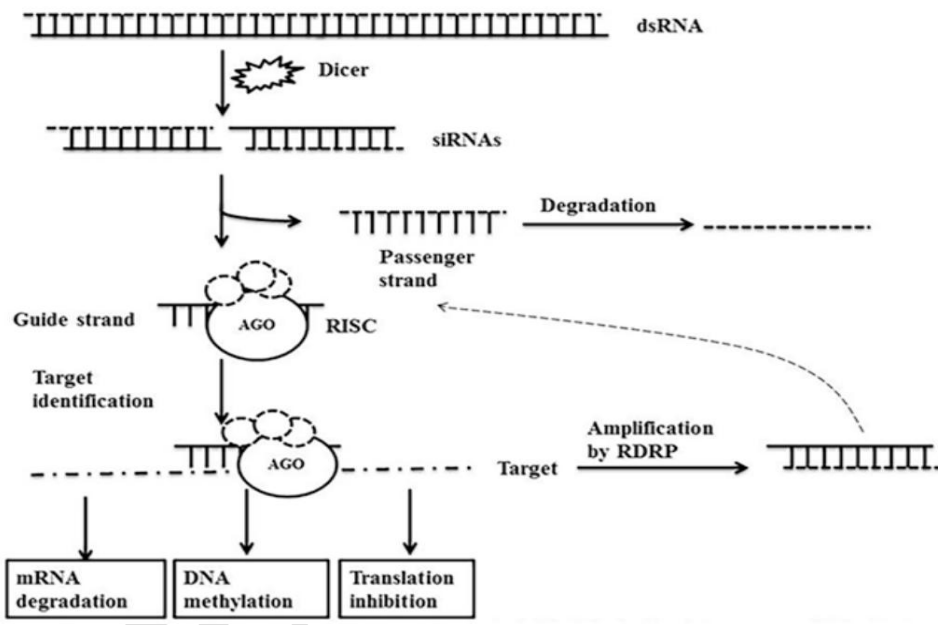

Fig 1. Plant RNAi pathway mechanism.
Dicer cleaves dsRNA to create siRNAs, which are then processed and added to RISC in conjunction with the AGO protein. The target sequence is subsequently identified by the RISC complex, which cleaves it to prevent the desired gene from being expressed.[12]

4. GENE CONSTRUCT FOR RNAi

Gene expression is inactivated by RNA interference in a sequence-specific way. One efficient RNAi trigger is double-stranded RNA (dsRNA). Direct introduction of dsRNAs can produce RNAi in worms, flies, and mammalian cells. On the other hand, hairpin RNA-producing construct transformation is typically used to establish RNAi in plants. Chuang and Meyerowitz (2000) were the first to show that an RNAi construct could effectively transcribe RNA in *Arabidopsis thaliana*. They demonstrated how the insertion of an RNA interference vector led to the silence of a homeobox gene that was identical to the

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corresponding null mutant. An inverted repeat containing target sequences under the direction of a potent promoter makes up the plant RNA interference vector. A spacer fragment lies between the inverted repeat sequences. The substrate of Dicer is a dsRNA structure formed by the inverted repeat region against the target gene. Following transcription, a spacer creates a hairpin RNA's loop and inverted repeat sequences produce a dsRNA structure known as the stem (Fig.2) Since *Escherichia coli* cannot replicate inverted repeat sequences without a spacer, the spacer sequence makes it easier to produce an RNAi vector.[6]

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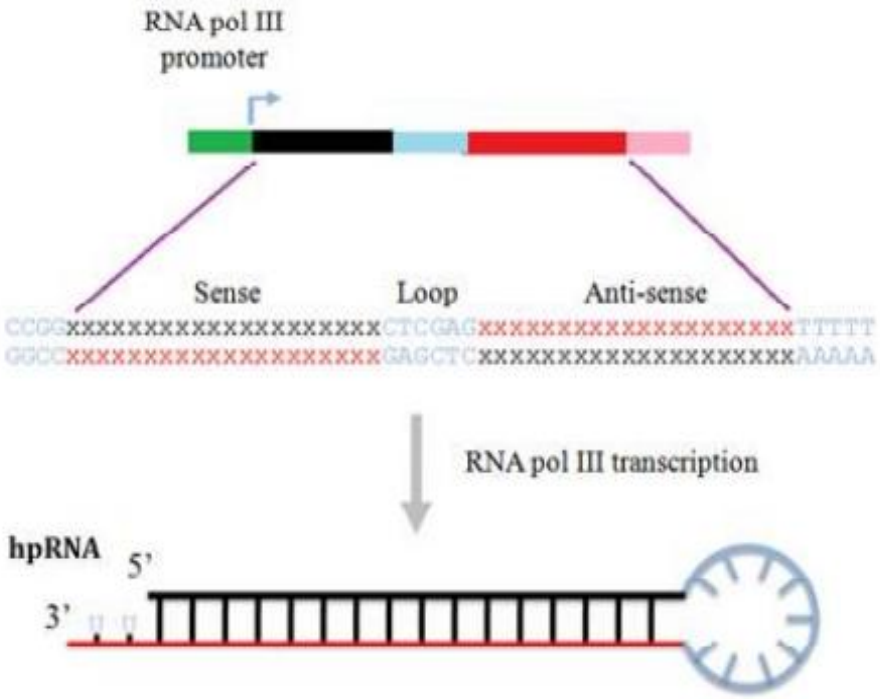

Fig. 2. Gene construct for RNAi

5. APPLICATIONS OF RNA INTERFERENCE TO ENHANCE AGRICULTURAL YIELD

5.1 Seedless Fruit Development: Fruits without seeds are often preferred by customers because they improve fruit quality and prolong their shelf life [21,22]. Parthenocarpy, which is the process by which fruit develops directly from the ovary without fertilization, can produce fruits without seeds. In eggplant, the absence of seeds inhibits browning and pulp texture deterioration [23]. It is possible to intentionally induce the generation of seedless

fruits by interfering with the genes responsible for seed formation and seed set. The phytohormones control the process of seed development both geographically and temporally. Fruit that is seedless and produced by phytohormone mutations typically exhibits pleiotropic consequences, such as altered flavor and smaller fruit size. Hence, new, more effective techniques should be used for the development of parthenocarpic fruits [23]. Cucumber *(Cucumis sativus)* [24,25] and tomato*(Solanumlycopersicum)* [26] yields are limited by the development of their seeds. Therefore, edible fruit tissue is a widely desired and appreciated alternative to seed and seed cavities for customers, breeding firms, and production enterprises. Auxin-dependent plant developmental processes are regulated by transcription factors encoded by auxin response factors (ARFs). It was discovered that mature, unpollinated ovaries had high expression levels of the tomato (*S. lycopersicum*) ARF7 factor, also known as SlARF7. Additional investigation showed that 48 hours after pollination, the expression of *slARF7* falls. This expression is elevated from the beginning stages of flower development to the production of mature blooms. Fruits that are parthenocarpic were produced via RNA interference-mediated development of transgenic tomato*(S. lycopersicum)* lines that had a downregulated slARF7 gene [27]. By suppressing the chalcone synthase (CHS) gene, the initial gene involved in the flavonoid production pathway, using RNA interference (RNAi), Schijlen et al. [28] created seedless tomatoes. Similarly, tobacco (*Nicotiana. tobacum cv. xanthi*) produced fruits with fewer or no seeds when the *flavonol synthase* (FLS) gene was silenced by post-transcription, a crucial enzyme for the synthesis of flavonols [29].

In tomato parthenocarpic flower buds, auxin production is specifically expressed by auxia genes. When these genes were silenced using RNA interference, tomatoes developed parthenocarpic fruits along with a few other auxin-related abnormalities [30]. Small parthenocarpic fruit and flower (spff) mutants in a tomato cultivar were identified and described by Takei et al. [31]. Male sterility with parthenocarpic fruit set development was seen upon linkage analysis and RNAi-based silencing of the *Solyco4g077010* gene, which encodes the receptor-like protein kinase.

5.2 Increasing Nutritional Value: The primary source of necessary nutrients for human diets is plants. Nonetheless, deficiencies in one or more important mineral elements affect about two thirds of the global population [32]. By altering a number of biochemical and physiological processes, RNA interference (RNAi) can be used to raise the quantities of nutrients that crops need. Under the polyunsaturated fatty acid synthesis route, the enzyme omega-3 fatty acid desaturase (FAD3) catalyzes the production of α-linolenic acid (18:3). Soybean (*Glycine max*) and other seed oils become unstable due to α-linolenic acid. By using siRNA to silence FAD3 in soybeans, Flores *et al*. [33] were able to drastically lower the quantity of α-linolenic acid by 1–3% when compared to other non-transgenic lines. Artificial microRNA (amiFATB) has been used to downregulate the fatty acyl-ACPthioesterase (FATB) gene in *Camelina sativa*, improving the oilseed quality. In comparison to wild-type seeds, the results demonstrated a significant drop in the

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concentration of total saturated fatty acids, with a 45% reduction in palmitic acid (16:0) and a 38% reduction in stearic acid (18:0) [34].

By using hairpin RNA to silence the gluB gene, Kusaba*et al*. [35] created an *Oryza- sativa* cultivar with minimal glutenin levels, known as LGC-1. An elevated concentration of amylose Two starch branching enzyme (SBE) II (SBEIIa and SBEIIb) have been suppressed in *Triticum* endosperm using RNAi to create the *Triticum* cultivar [36]. Starch phosphorylation and dephosphorylation are essential steps in the breakdown of starch in plants. The buildup of starch in the leaves of *Arabidopsis thaliana* and *Zea- mays*was caused by the downregulation of phosphoglucan phosphatase (SEX4) and glucan water dikinase (GWD) by RNA interference [37].*Brassica napus* increased its carotenoid content by RNA-mediated silencing of ε-Cyclases (ε-CYC). It was discovered that seeds produced from RNAi transgenic *Brassica* lines have high levels of lutein, violaxanthin, zeaxanthin, and β-carotene [38].

RNAi can potentially be used to help crops accumulate minerals. Aggarwal *et al*. [39] produced *Triticum* grains with high Zn and Fe content and low levels of the antinutrient phytic acid (PA) by RNAi-mediated downregulationof inositol pentakisphosphate kinase (IPK1).

5.3 Abiotic Stress Resistance:Abiotic stress conditions, such as salinity, drought, floods, and temperature changes, are the main causes of the global decline in the production of many important crops. Scientists are under tremendous pressure to produce crop kinds that are resistant to stress due to the ever-changing environment and the increasing need for food from a growing population. The capacity of non-coding RNAs (ncRNAs), specifically microRNAs (miRNAs), to create crops resistant to abiotic stress. ncRNAs regulate critical functions like as transcription, detoxification, and development by adjusting gene expression at various levels. This affects how crops react to oxidative stress, salt, cold, and drought. This process is shown by the overexpression of miR393 in *Arabidopsis* seedlings subjected to different stimuli.

Researchers can create cultivars that are more resilient to stress by using RNA interference (RNAi) technology to target particular non-coding RNAs (ncRNAs) and the genes that are linked to them. Under difficult environmental circumstances, this strategy has great potential to increase the production and sustainability of important crops like rice, wheat, lentils, and sugarcane.plant responses to environmental stressors, particularly salt and drought, and the function of microRNAs (miRNAs).[1]
It indicates that particular miRNAs are expressed by a variety of plant species, including important crops like rice, wheat, and maize, and that these miRNAs affect the expression of these plant species in response to salinity. Important participants in this adaptation are identified as members of notable miRNA families, such as miR156 and miR398, respectively. The paragraph also highlights studies that exploit genome-wide methods to investigate miRNA-mediated gene regulation in drought-stressed plants, including rice and

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sorghum as examples. This work highlights the possibility of comprehending miRNA activities to enhance plant resilience and stress tolerance overall.[1]

Two effective methods for enhancing rice's tolerance to drought through natural processes are:. In the first method, superoxide dismutase (SOD) activity is elevated and drought tolerance is eventually raised as a result of RNA interference (RNAi) silencing the receptor for activated C-kinase 1 (RACK1). The second technique uses maize short interfering RNA (siRNA) to inhibit rice's farnesyltransferase/squalene synthase (SQS), which raises sterol levels, decreases stomata-mediated water loss, and improves rice's resistance to drought. These techniques greatly increase rice's resistance to drought in both the vegetative and reproductive phases, providing important resources for creating crops that can tolerate changing climate conditions. The OsTZF1 gene's critical function in rice stress tolerance. Surprisingly, silencing this gene increased tolerance to low water and high salt environments. It's interesting to note that low OsTZF1 expression regulates hormone synthesis at the molecular and cellular levels in response to salt stress, aiding plants in preserving homeostasis. The study emphasizes the importance of microRNAs (miRNAs) in plant stress responses beyond OsTZF1. Various plant species use distinct miRNAs that are sensitive to heat and cold to control target genes, which increases resistance to a range of abiotic stresses. The ability to develop stress-resistant crops using OsTZF1 and miRNA manipulation.[1]

5.4 Biotic Stress Tolerance: Two core lncRNAs, GhlncNAT-ANX2- and GhlncNAT-RLP7-silenced seedlings, demonstrated greater resistance against *Verticillium dahliae* and *Botrytis cinerea*, according to functional analysis. This enhanced resistance may have been caused by increased expression of the LOX1 and LOX2 genes. Cotton leaf curl virus, which contains a sense transcript BC1 that may cause pathogenicity symptoms, affects cotton, one of the major cash crops. A BC1 antisense construct was inserted into the *Nicotiana tabacum* plant, and upon expression, the BC1 anti-transgenic tobacco exhibited resistance against the Begomovirus-causing viruliferous whitefly (*Bemisiatabaci*)[15].

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5.4.1Bacterial Disease Resistance: Bacteria are basic tiny creatures that infiltrate plants by rain, wind, soil, seed dissemination, or other mechanisms. Unlike viruses, bacteria do not require insects as their vectors. Numerous plant diseases caused by bacterial pathogens have a significant negative impact on crop productivity and result in significant yearly losses for the world's farmers. One of the main problems in the field of crops like tomatoes, soybeans, and bananas is bacterial illness[1].

5.4.2Viral Disease Resistance:Numerous plant viruses are highly transmissible and frequently have severe impacts on plants. Due to their quick growth and dissemination, several of them significantly reduce crop output and quality. Plant resistance to viruses is largely dependent on RNA silencing, with a variety of silencing components contributing to antiviral defense. The small RNA-directed RNA degradation mechanism targets both DNA and RNA viruses. RNA silencing, a key antiviral mechanism in plants, is dependent on

DICER-like enzymes cleaving viral dsRNA into virus-derived small interfering RNAs (vsiRNAs). Even now, lines of virus-resistant squash (*Cucurbita*) and papaya (*Carica papaya*) that were deregulated in the 1990s are still grown. Even though it was previously believed that viruses infect plants through the development of a virus-derived protein (coat protein-mediated resistance, or CPMR) before the viruses really settle and begin to disrupt the plant's RNA silencing mechanism. The first use of gene silencing was to create virus-resistant plant types.Plants have developed antiviral tactics that resemble the RNA silencing processes found in nature. This was initially shown when researchers created the potato virus Yresistance, which caused plants to produce the viral proteinase gene's RN transcripts.[1,5]

Plants can also be engineered to produce dsRNAs that silence vital genes in insect pests and parasitic nematodes. Immunity 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, has also been demonstrated.[5]

5.4.3Fungal Diseases:Fungal diseases pose a serious hazard to crops because they can produce toxic mycotoxins that are dangerous to human and animal health in addition to causing enormous yield losses. Utilizing RNA interference (RNAi) technology to build resistance to fungal infections has ~~being~~been studied by researchers. According to one study, tobacco plants that had their glutathione S-transferase (GST) gene silenced by RNA interference (RNAi) were more resilient to the fungal pathogen *Phytophthora parasitica* var. *nicotianae*, which causes black shank disease. In ~~a different research~~different research, the fungus *Blumeriagraminis*, which causes barley and wheat powdery mildew, was targeted via RNA interference (RNAi). The outcomes demonstrated that using RNA interference to control fungal diseases can be successful.[1]

Table 1.RNA interference to control fungal diseases

Fungal pathogen	Disease	Target gene	Outcome
Phytophthora parasitica var. nicotianae	Black shank disease	Glutathione S-transferase (GST)	Increased resistance in tobacco plants
Blumeriagraminis	Powdery mildew of barley and wheat	Not specified	RNAi can be an effective way to control the pathogen

5.4.4Insect and Nematode Resistance: Insect functional genomics research and insect pest management both benefit from the use of RNA interference (RNAi). Insects should be able to absorb dsRNA ~~for this reason~~ by grazing on plants. According to recent findings, dsRNA that was given to insects as part of their food efficiently down-regulated the genes that the

insects were targeting. More importantly, it has been demonstrated that the hdRNAi-1 approach siles insects that feed on plants, paving the path for the creation of a new breed of insect-resistant crops. Western corn rootworm (WCR) resistance was demonstrated using transgenic corn plants designed to produce dsRNAs for the tubulin or vacuolar ATPase genes. In this study, resistance manifested itself as decreased mortality, stunting, and damage to plants. Larval development of cotton bollworm (*Helicoverpaarmigera*) has been seen to be slowed when they feed on plant material producing dsRNA specific to the cytochrome P450 gene (CYP6AE14). Therefore, species-specific insecticide, or hdRNAi-1 technology, is another name for it and a possible substitute for conventional pesticides. In addition to stable RNAi transgenic plants, insect damage can be reduced by applying dsRNA to plant leaves and dsRNA-enriched soil. The preceding evaluation covered studies evaluating the effectiveness of dsRNA spray against insect pests. However, further knowledge of the RNAi process in insects and their genetic data is required to fully grasp the promise of this technique.[13]

Some crucial questions that need to be answered are: can an insect's dsRNA be transferred back to plants through insect saliva? Can an insect's RNAi mechanism be passed from one feeding stage (larvae or adults) to the next growth stage or generation (pupa or egg)? How long does it take an insect to effectively silence its genes and die from its first meal on an RNAi transgenic plant? Furthermore, dsRNA in vertebrates has the ability to trigger immunological responses on its own, perhaps providing resistance to insect progeny. Further investigation is necessary to gain a deeper comprehension and make appropriate adjustments to the hd-RNAi technique.[13]

Engineering plants to be resistant to nematodes is a crucial component of agricultural progress. Two methods may be used to achieve this: (i) utilizing the hdRNAi-1 approach to increase plant resistance; (ii) using HGS-hpRNAi to silence specific plant genes. When it comes to these two methods, hdRNAi-1 works best. Through the consumption of transgenic plant tissue, dsRNA or its siRNAs are transferred from plant to nematode in this manner. Compared to cyst nematode (CN), transgenic plants expressing hdRNAi-1 have demonstrated greater efficacy against root-knot nematode (RKN). It has been demonstrated that regulating this nematode is possible with transgenic tobacco plants that produce dsRNA targeting a putative zinc finger transcription factor gene (MjTis11) in RKN (*Meloidogyne javanica*). hdRNAi-1 was utilized to target up to four important CN *(Heteroderaschachtii*) genes by expressing several hpRNA constructs in *Arabidopsis*, therefore increasing the efficiency of CN nematode control. As a result, there were less adult CN nematode females eating on this plant. Higher gene silencing effectiveness in CN will be possible with a deeper comprehension of the RNAi system in plant parasitic nematodes.[13]

5.4.5Improving Resistance against Parasitic Weeds: It has been challenging to manage plant parasitice weeds using genetic engineering since no genetic loci granting resistance in agricultural plants have yet to be discovered. Therefore, current study has looked at the possible applications of RNA interference in this field. The transmission of signals from the

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host plant to the parasite weeds is required for hdRNAi-1 to function. Thus, the distribution of the RNAi signal was studied. Transgenic lettuce roots producing hpRNA carrying a portion of the GUS gene were made available for ~~parasitization~~parasitisation by the *Triphysariaversicolor* parasite, which expresses the GUS reporter gene. *Triphysaria* roots connected to non-transgenic lettuce displayed complete GUS activity when stained for GUS activity, whereas those parasitizing RNAi transgenic lettuce lacked GUS activity. This suggests that a silencing signal can quiet a specific gene by traveling from host to parasite.[13]

Mannose 6-phosphate reductase gene (M6PR) hpRNA constructed from *Orobancheaegyptiaca* is present in transgenic tomato plants, and this construct has been shown to be efficient against the parasite by killing its tubercles that are connected to tomato plants. Additional research also demonstrated that parasites may use their haustorium to absorb particular RNAi from their host. These investigations unequivocally showed the potential of hdRNAi-1 technology for controlling plant parasites in the future.[13]

Table 2: Crops with improved stress tolerance through RNAi

Trait(s)	Crop Improved	Resistance Against	Targeted Gene(s)	References
Bacterial resistance	*A. thaliana*	*Agrobacterium tumefaciens*	iaaM ipt	[40]
		Pseudomonas syringae	PPRL	[41]
	Citrus limon	*Xanthomonas citri*	CalS1	[42]
Abiotic stress tolerance	*A. thaliana*	Osmotic tolerance	WZY2	[43]
	T. aestivum	Salt tolerance	TaPUB-1	[44]
	N. tabacum	Salt tolerance	Nt ε-LCY	[45]
	O. sativa	Salt tolerance	OsPEX11	[46]
	B. rapa	Salt tolerance	GIGANTEA (GI)	[47]
	A. thaliana	Drought tolerance	PAD4 LSD1 EDS1	[48]
	O. sativa	Drought tolerance	OsGRXS17	[49]
	O. sativa	Drought tolerance	OsDSR-1	[50]

	O. sativa	Drought tolerance	OsERF101	[51]
	S. lycopersicum	Drought and salt tolerance	SlbZIP1	[52]
	N. tabacum	Drought tolerance	BrDST71	[53]
Virus resistance	*N. bethamiana*	Tomato yellow leaf curl Thailand virus (TYLCTV)	GSA	[54]
	Glycine max	Soybean mosaic virus (SMV)	eIF4E1	[55]
	O. sativa	Rice tungroo bacilliform virus (RTBV) Rice tungroo spherical virus (RTSV)	Coat protein 3 CP3	[56]
	Nicotiana bethamiana	Chilli-infecting begomoviruses	AC1 AC2 βC1	[57]
	Triticum spp.	Triticum mosaic virus (TMV)	Coat protein (CP)	[58]
	Oryza sativa	Rice black streak dwarf virus (RBSDV)	S7-2 S8	[59]
	Glycine max	Soybean mosaic virus (SMV)	SMV P3 cistron	[60]
		Mungbean yellow mosaic virus (MYMIV)	CP	[61]
	Arachis hypogaea	Tobacco streak virus (TSV)	CP	[62]
	Solanum tuberosum	Potato virus X (PVX), Potato virus Y (PVY) Potato virus S (PVS)	CP	[63]
Virus resistance	*Brassica rapa*	*Tetranychusurticae*	COPB2	[64]

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Insect resistance				
	A. thaliana	*Myzuspersicae*	MyCP	[65]
	Lettuce	*B. tabaci*	V-ATPase	[66]
	N. tabacum	*Bemisiatabaci*	AChE EcR	[67]
	Nicotiana bethamiana	*Chilli-infecting begomoviruses*	AC1 AC2 βC1	[68]
	S. lycopersicum	*Helicoverpaarmigera*	HaCHI	[69]
Fungal resistance	*Glycine max*	*Phytophthora sojae*	GmSnRK1.1	[70]
	S. lycopersicum	*F. oxysporum*	ODC	[71]
	S. tuberosum	*Phytophthora infestans*	Avr3a	[72]
	T. aestivum	*Fusarium graminearum*	Chs 3b	[73]
	Musa spp.	*F. oxysporum f. sp. cubense (Foc)*	Foc velvet protein	[74]
	N. tabacum	*Sclerotinia sclerotiorum*	Chs	[75]
	S. lycopersicum	*F. oxysporum*	Fow2 chs V	[76]
	Z. mays	*A. flavus*	Amy1	[77]
	Zea mays	*Aspergillus flavus*	ZmPRms	[78]
	S. tuberosum	*Phytophthora infestans Alternaria solani*	PVS1 PVS2 PVS3 PVS4	[79]
	S. lycopersicum	*F. oxysporum*	Fmk1 Hog1 Pbs2	[80]

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	O. sativa	*Magnaporthe oryzae*	MoABC1 MoMAC1 MoPMK1	[81]
		Rhizoctonia solani	RPMK1-1 RPMK1-2	[82]
Nematodes Resistance	*A. thaliana*	*M. incognita*	Mi-msp3 Mi-msp 5 Mi-msp18 Mi-msp24	[83]
	S. lycopersicum	*Meloidogyne incognita*	Mi-cpl1	[84]
	N. benthamiana	*Radopholussimilis*	Rs-cps	[85]
	S. lycopersicum	*M. incognita*	PolA1	[86]
	Glycine max	*Heteroderaglycines*	Hg16B09	[87]
			HgY25 HgPrp17	[88]

5.5 Development of Male Sterile Lines: Male sterility has also been produced by RNA interference, which is useful for the hybrid seed market. With RNA interference (RNAi), genes that are expressed only in tissues involved in pollen generation may be targeted. For example, TA29 gene expression, which is required for pollen formation, can be inhibited in order to create male sterile tobacco strains.Additionally, RNA interference (RNAi) was utilized to stop Msh1 from expressing in tobacco and tomatoes, which led to changes in the mitochondrial DNA linked to cytoplasmic male sterility that occurs spontaneously.[5]

5.6 Modification of Flower Color and Scent by RNAi-Mediated Gene Silencing: A branch of horticulture called "flower farming" is dedicated to the production of flowers and other decorative plants. The need for flowers in a variety of hues and designs for smells and decorating has grown in the modern day. This may be done by employing RNA interference technology to silence the genes that code for pigment. By utilizing RNA interference (RNAi) to repress a cDNA encoding the chalcone isomerase (CHI) gene that was obtained from *N. tobacum* petals, flavonoid components in flower petals were altered and pigmentation was reduced [89]. Similarly, the RNAi construct TrCHS1 targeting chalcone synthase (CHS) has been shown to cause flower color modification in liliaceous ornamental *Tricyrtis* sp. [90]. Ornamental gentian plants' flower colors changed as a result of RNA interference-mediated silencing of three anthocyanin biosynthesis genes: chalcone synthase (CHS), anthocyanidin synthase (ANS), and flavonoid 30,5 0-hydroxylase (F305 0H) [91]. The bloom of gentian

plants is naturally bright blue in hue. The pigment known as "gentiodelphin," a polyacrylate delphinidin, accumulates in the petals of gentian plants and imparts color to the flowers. Important enzymes for gentiodelphin production are flavonoid 30,50-hydroxylase (F305 0H) and anthocyanin 5,30-aromatic acyltransferase (5/30AT), whose downregulation by RNA interference modifies floral color [92]. In order to reconstruct the delphinidin pathway, He *et al*. [93] initially identified two chrysanthemum cultivars and separated seven genes involved in anthocyanin biosynthesis: CmCHS, CmF3H, CmF30H, CmDFR, CmANS, CmCHI, and Cm3GT. Furthermore, cyanidin concentration was elevated in chrysanthemum flowers with brighter red petals due to the overexpression of the *Senecio cruentus* F3050H (PCFH) gene and the inhibition of the CmF30H gene; however, no reports of delphinidin accumulation have been made.

5.7 Enhanced Fruit Shelf Life: Fruits and vegetables are more prone to spoiling owing to their nature and composition than grains. India loses between thirty and forty percent of the overall yield of fruits and vegetables to spoiling, according to the Agricultural Research Data Book 2004. Consequently, as another essential agronomic attribute that may reduce fruit and vegetable spoiling and, in turn, lessen agricultural loss, we must use strategies that might extend the shelf life of fruits and vegetables. By focusing on the genes that code for the ethylene production pathway, RNA interference technology (RNAi) extended the tomato's shelf life. When a tomato was given a dsRNA unit, the ACC oxidase gene's expression was inhibited. It was discovered that the transgenic plants' matured fruits significantly decreased the rate at which ethylene was produced, extending the tomato's shelf life. Using RNA interference technology, two ripening-specific N-glycoprotein modifying enzymes were identified in tomatoes: α-mannosidase (α-Man) and β-d-N-acetylhexosaminidase (β-Hex). This demonstrated how suppressing these genes lengthens the tomato's shelf life by slowing down the process of ripening.[1]

5.8 Secondary Metabolite Production:Secondary metabolites from plants are utilized in medications, food additives, perfumes, colors, and insecticides. Many different genes control the biosynthesis of secondary metabolites, however occasionally certain unwanted substances can impede this process. In addition to being useful for manipulating secondary metabolites, RNA interference (RNAi) may be employed to effectively decrease the production of those molecules [94]. In the opium poppy (*Papaver somniferum*), Allen *et al*. [95] revealed that RNA interference (RNAi)-mediated silencing of numerous genes involved at different points in a complicated biochemical process replaced morphine with the non-narcotic alkaloid (S)-reticuline. To inhibit the expression of every member of the codeine reductase (COR) gene family, they created hpRNA. As a result, morphine, codeine, and opium were substituted with (S)-reticuline, a non-narcotic alkaloid precursor, to create transgenic lines. The central nervous system, respiratory system, and circulatory system are all naturally stimulated by caffeine. Moreover, it lowers the risk of throat, mouth, and liver cancer. In addition, consuming too much of it can lead to health problems including tremors in the muscles, anxiety, upset stomach, sleeplessness, and restlessness. Three enzymes, namely CaXMT1, CaMXMT1 (theobromine synthase), and CaDXMT1 (caffeine synthase),

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are involved in the manufacture of caffeine in coffee plants. The CaMXMT1 gene was silenced by RNA interference (RNAi) and the resulting 70% reduction in caffeine concentration suggests that RNAi technology can be used to produce decaffeinated coffee beans [96]. Similarly, RNA interference (RNAi) was used to down-regulate the caffeine synthase (CS) gene in order to create transgenic tea (*Camellia sinensis*) lines with low caffeine content [97].

A well-known Chinese herb that is also utilized in other Asian nations is salvia miltiorrhiza. By down-regulating the Chalcone synthase (CHS) gene, the first enzyme in flavonoid biosynthesis, and treating it with salicylic acid as an elicitor, phenolic acid production was increased. The synthesis of flavonoids was significantly reduced while the level of phenolic acid increased, according to the results [98]. Peltate glandular trichomes (PGTs) are small, specialized structures that are present in various aromatic plants, including spearmint (*Mentha spicata*), and are involved in the generation of secondary metabolites. In their investigation into the activity of transcription factors in the pathway leading to the manufacture of secondary metabolites, Wang *et al.* [99] identified and described the MsYABBY5 gene that is expressed in PGT. Following the inhibition of the MsYABBY5 gene, terpene synthesis increased, indicating that transcription factors with encodes function as a negative regulator of secondary metabolite production. The DE-ETIOLATED-1 (DET1) gene, a negative regulator of photomorphogenesis, was suppressed through RNA interference (RNAi) in the embryonic callus of papaya (*Carica papaya* L.) plants in order to examine its effects on the expression of a gene involved in the secondary metabolite biosynthesis pathway. The findings suggested a connection between the photo-regulated pathway and secondary metabolite synthesis [100].

5.9 Deletion of Allergens from Food Crops:Additionally, plants have a large number of toxic and undesired substances, the removal of which is an expensive and time-consuming procedure. An effective technique for ridding the plants of toxins is the RNAi approach. By down-regulating three different methylation stages of the caffeine biosynthesis pathway and suppressing the expression of the 7-N-methylxanthine-methyl-transferase gene (CaMXMT1), the amount of caffeine in transgenic coffee beans ~~is~~ was lowered by up to 70%.~~Although cassava (*Manihot esculenta*), is a popular staple meal in the tropics, its tuber~~ Tuber of Cassava (*Manihot esculenta*) contain~~s~~ harmful substances called cyanogenic glucosides. The amount of cyanogenic glucosides can be decreased by about 90% by down-regulating the enzymes CYP79D1 and CYP79D2, which are involved in the production of linamarin and lotaustraline. By simultaneously suppressing the ASPARAGINE SYNTHETASE genes (StAs1 and StAs2), commercial potatoes with much decreased asparagine—a primary precursor of the brain toxin acrylamide—can be created using a similar strategy. A hypersensitive reaction to ordinarily safe protein components in food, primarily via the immunoglobulin (IgE) mechanism, is known as a food allergy. Technology based on RNA interference is also helpful in reducing these allergies. It has been utilized to suppress Mal-d-1, an apple allergen that exhibits IgE antibodies. Intron spliced hpRNA constructs containing the Mal-d-1 inverted repeat sequence were used to alter apple plants.

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This resulted in a 10-fold reduction in Mal-d-1 leaf expression without affecting normal plant development. Tearless onions may also be created by down-regulating the conversion of 1-propenylsulfenic acid to propanthial S-oxide, a factor that causes tears and is lachrymatory, using RNA silencing technology.[10]

5.10 Change/Alteration in Plant Architecture:A plant's success is largely dependent on its architecture, or the blueprint for its form and structure. Every plant, no matter how big or ~~little~~small, has a distinct design that determines important agronomic characteristics including height, branching, yield, and even stress tolerance. Scientists can enhance crops by fine-tuning plant architecture by comprehending this relationship. A potent technology that provides fine control over genes determining architecture is RNA interference (RNAi). For example, rice that has had the OsDWARF4 gene silenced has stronger, shorter plants with erect leaves, which facilitates harvest. RNAi that ~~target~~targets the ODC gene in tobacco change the structure of the plant, decreasing its ability to withstand stress while providing information about its function in development. Plant architecture is likewise shaped by microscopic regulators called microRNAs (miRNAs). In maize, modifying Cg1 miRNA postpones blooming, which may be advantageous for breeding techniques. It's interesting to note that changing Cg1 in poplar trees improves the generation of biofuel by encouraging desirable features like shorter internodes and less lignin. Additionally, RNAi can meet certain demands.

Plants with low lignin content that is simpler to convert to bioethanol are produced when lignin production genes are silenced.In a similar vein, RNAi can enhance leaf plucking in mulberry and tea plants and make roses thornless. This intriguing look into the field of manipulating plant architecture emphasizes the possibilities of RNA interference. Through adjustments to the hidden architect, crops with enhanced stress tolerance, yields, and customized traits may be produced, guaranteeing food security and sustainable agriculture in an evolving global landscape.[1]

5.11. Quality Improvement: The RNAi technology not only increases the food's nutritional value but also fortifies plants against biotic and abiotic stress. One of the most crucial indicators of food quality is thought to be the amount of amino acids it contains.A significant advancement in the use of RNA interference (RNAi) in plants has been made possible by research into the metabolic processes involved in the production of the main amino acids. The model tobacco plant containing the *Arabidopsis* S-adenosyl-L-methionine synthase gene was repressed with RNA interference (RNAi), which led to an increase in L-methionine content in transgenic plants. ~~maize~~ Maize plants by inhibiting the lysine-degrading gene in maize plants~~, which~~ led to transgenic plants having a greater lysine content than wild-type plants. Numerous additional investigations have also noted the silencing impact on plastid genes that increase the levels of aspartate, glutamate, and lysine in plants, such as NbAsp5 and NbPAT.[12]

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