Agriculture Association of Textile Chemical and Critical Reviews Journal (2022) 59-68

AATCC Review

29 August 2022: Received
28 October 2022: Revised
11 December 2022: Accepted
27 December 2022: Available Online

http://aatcc.peerjournals.net/

Review Article Open Access

RNA Interference (RNAi) Induced Gene Silencing To Enhance Crop Resilience

Kasthuri Rajamani[1*], Lydia Chintagunta[2], G. Bhupal Raj[2]

[1]Professor Jayashankar Telangana State Agricultural University, Rajendranagar, Hyderabad, Telangana, India,
[2]School of Agriculture, SR University, Warangal, Telangana, India

Abstract

RNA interference (RNAi) is an important mechanism for gene expression and epigenetic regulation. It involves the double-stranded, small interfering RNAs (siRNA) that bind to mRNA molecules and prevent translation into proteins. This process can be used to repress gene expression and thus has potential applications in improving crop production and productivity. RNAi has been successfully used to increase resistance to abiotic and biotic stresses, such as drought, salinity, temperature, pests and diseases to improve the quality by increasing the nutritional value of food crops. Further, it also be used to increase the efficiency of photosynthesis and reducing the amount of water used for irrigation, enviromental impact on crop production, such as increasing carbon sequestration and reducing the amount of fertilizers used. The application of RNAi in crop improvement has seen considerable success in recent years, where the researchers used this technology to reduce the need for chemical pesticides, as it used to target and reduce the expression of genes associated with pest resistance. Also it has used to reduce the expression of genes that are responsible for undesirable traits, such as production of toxins in potatoes. Furthermore, RNAi can be used to reduce the spread of infectious diseases and viruses in crops, such as the Potato Virus Y, which can reduce crop yields drastically. This article is an attempt to review RNAi mechanism and its achievements which attributed for crop improvement.

Keywords: *RNA interference (RNAi), micro RNAs, small interfering RNAs (siRNAs), double-stranded RNA(dsRNA), viral mRNAs, abiotic and biotic stress resistance, and climate change*

Introduction

RNA interference (RNAi) is a biological process in which RNA molecules inhibit gene expression or translation, by neutralizing targeted mRNA molecules, and leads to post-transcriptional gene silencing (PTGS) which triggered by double-stranded RNA (dsRNA) molecules to prevent the expression of specific genes [3]. RNAi is an important cellular mechanism for controlling the expression of genes, also defined as sequence-specific silencing of target gene. A similar homology dependent gene silencing phenomenon was found in plants known as 'co-suppression' or 'post transcriptional gene silencing', and this process occurs when the homologous gene produces a double-stranded RNA molecule that is similar to the mRNA of the target gene. This double-stranded RNA molecule is then recognized by an enzyme called Dicer, which cleaves it into small interfering RNAs (siRNAs). These siRNAs then bind to a protein complex called the RNA-induced silencing complex (RISC). The RISC then uses the siRNA as a guide to identify and cleave complementary mRNA molecules, thus preventing the mRNA from being translated into a protein.

In plants, co-suppression occurs when an identical or closely related sequence of DNA is expressed in the plant cell, resulting in the silencing of the expression of the homologous gene. In fungi, quelling occurs when the homologous gene is expressed at high

*Corresponding Author: Dr Kasthuri Rajamani
E-mail Address: kasthuri.agrico114@gmail.com
DOI:https://doi.org/10.58321/AATCCReview.2022.10.04.59

level, resulting in the silencing of the expression of the homologous gene. This process is used to create transgenic fungal strains that can produce proteins, antibiotics, and other compounds of interest. The bacteria and archaea exhibited RNA-based immune system known as Clustered Regularly Inter Spaced Short Palindromic Repeats (CRISPR). It is composed of short, repetitive sequences of DNA that are interspersed with short sequences of foreign DNA. The CRISPR system allows bacteria to recognize and destroy invading viruses and other foreign genetic material by using CRISPR array mechanism and CRISPR-associated protein (CAS). The CRISPR system has been adapted for use in gene editing, allowing researchers to make precise and targeted changes to the DNA of living cells of organisms, including plants, animals, and even humans. CRISPR gene editing has the potential to revolutionize the way to treat diseases and could even lead to the development of new treatments for genetic disorders, as well as to develop more efficient and productive crops.

Abiotic stresses *viz.,* cold, heat, drought, and salinity are the main environmental stresses can cause damage to the plant by reducing crop growth rate, altering metabolic pathways, and reducing the photosynthetic activity to restrict the crop productivity [17]. As well the biotic stresses caused by viral, bacterial and fungal pathogens, insects, nematodes, and parasitic weeds leads to decrease the crop production and productivity, further cause severe economic losses [6]. Among the crops, some genotypes are auto-immune to potential bacterial, viral, and fungal strains, but some of them can be extremely problematic to control. In such situation, traditional crop breeding methods have been used to develop resistant cultivars against various biotic and abiotic factors, and these approaches are time-consuming because of limited availability of genetic resources for various crops. Additionally, climate change factors aggravate the global warming and increased production of new virulent strain's to attack resistant cultivars which calls urgent need for development of novel approaches to combat climate change elements to manage pests and diseases [23]. In addition, genetic engineering approaches have been used to develop transgenic plants with enhanced tolerance to biotic and abiotic stresses. These transgenic plants can be used to improve crop yield and quality in areas with unfavorable climatic conditions. In recent years, development of new technologies such as gene editing, molecular breeding, and marker-assisted selection has enabled the development of crop varieties with improved resistance to biotic and abiotic stresses. These technologies allow for the rapid identification and transfer of desirable traits from wild relatives or other crops. In this review, we discuss the various strategies employed by plants to cope with biotic and abiotic stresses and the genetic engineering approaches used to enhance the tolerance of plants to these stresses.

RNAi: RNAi is a defense mechanism occuring naturally against double-stranded RNA (dsRNA) that can target cellular and viral mRNAs. In this process, small RNA interferes with the translation of the target mRNA transcript which usually suppresses the gene expression [22]. The small non-coding RNAs are the division products of dsRNA called micro-RNA (miRNA) and small interfering RNA known as siRNA which carried out by a ribonuclease called DICER or Dicer-like enzyme. The small non-coding RNAs (miRNAs and siRNAs) in coalition with RNA-induced silencing complex (RISC), Argonaute (AGO), and other effector proteins lead to a phenomenon called RNAi [1 & 10].

Micro RNAs (miRNA): The first micro-RNA (miRNA) was discovered in 1993 by the Ambrosand Ruvkun group in *Caenorhabditis elegans* called *lin-4* and was a milestone in the field of molecular biology. Further, these studies assisted to regulate several developmental transitions in plants like Arabidopsis thaliana [32], Zea mays [8], Antirrhinum majus and Petunia hybrid [7]. The miRNA's consist 22 nucleotides in length, and most of miRNAs are transcribed from DNA sequences in to primary miRNAs (pri-miRNAs) and processed into precursor miRNAs (pre-miRNAs) which further mature as miRNAs. Predominantly these miRNAs interact with the 3′UTR of target mRNAs to suppress expression [15].

Small interfering RNAs (siRNAs): Small interfering RNAs also known as short interfering RNA or silencing RNA and are a class of double-stranded RNA molecules that are used to inhibit gene expression by using post-transcriptional gene silencing (PTGS) to break down mRNA. They are typically 20-25 nucleotides in length having 5-phosphorylated dsRNAs overhangs with two nucleotides at the 3'ends generated by dicer from longer dsRNAs and are designed to target a specific mRNA sequence. The siRNA-induced silencing complex (siRISC) is engaged to distinguish between two siRNA strands as

same sense or antisense resulting in the degradation of sense strands. The siRISC is then incorporated into the antisense strand of siRNA with target messenger RNAs (mRNAs) in a sequence-specific manner. The target mRNA is broken down by RISC having Argonaute (AGO) and other proteins which inhibit the translation process. The activated RISC can continuously participate in mRNA degradation to inhibit protein synthesis and result in post-transcriptional gene silencing (PTGS).

The discovery of RNAi has transformed into a powerful tool for the functional genomics of insects and nematodes. Further, this technology has been used to engineer virus resistance in plants by expressing viral sequences as transgenes, and papaya ring spot virus was the best example to inhibit virus growth in papaya. Host-induced gene silencing (HIGS), also defined as host-induced RNAi by expressing dsRNA in plants which shown its potential in conferring resistance to insects, fungi, parasitic plants and nematodes [9]. The function of miRNAs (microRNA) concerning abiotic stress like drought, oxidative stress, cold, and salinity [27] in arabidopsis plants under different abiotic stress conditions and validated miR393 was actively up-regulated when exposed to dehydration, cold, higher salinity levels, and abscisic acid (ABA).

Later on, RNAi phenomenon has been studied in diverse abiotic environments such as heavy metals, nutrition deprivation and radiation impacts in various crops. Other abiotic stresses such as temperature, drought, and salinity can cause significant yield losses in crops. In many cases, these stresses have a greater impact on crop yields than other abiotic stresses, such as nutrient deficiencies or other soil related aspects. Temperature extremes can cause reduced photosynthesis and respiration rates, leading to stunted growth and reduced production. Drought stress can cause water stress, which can reduce the uptake of water and nutrients and reduce the availability of carbohydrates for energy production. Salinity stress can cause nutrient deficiencies, decreased leaf area, and reduced crop yield. All of these stresses can reduce crop yield, and in some cases can even lead to complete crop failure. Under such circumstances, numerous advancements emerged in analytical sector to utilize in biochemical, molecular, genomic, proteomic, and other metabolic segments which further allowing better understanding of complex regulatory networks of stress-mediated responses. Along with these tools, the involvement of RNAi (small RNA species and their respective targets) in abiotic stress responses is well documented [19] in many crops.

Abiotic Stress Resistance: Abiotic stress resistance is the ability of a plant or organism to withstand environmental stressors such as drought, extreme temperatures, and soil salinity. Plants and organisms have evolved various strategies to cope with abiotic stress, including the production of protective compounds, the development of specialized root systems, and alteration of gene expression. Research into abiotic stress resistance has been conducted in order to improve crop yields and increase food security. Furthermore, RNA silencing and its coding is one of the recent technologies that can overcome abiotic stress in crops. In addition, regulating endogenous levels of regulatory RNAs is useful for the expression of target genes that are closely involved in specific stresses.

Cold and Heat Stress Tolerance: Changing environmental conditions like cold and heat stresses influence the crop growth and production and drastically decrease the quality which further aggravate severe economic loss. Plants have evolved a variety of mechanisms to tolerate cold and heat stress impacts. To tolerate cold stress, plants may produce antifreeze proteins which prevent ice crystal formation, use cold acclimation to increase the production of protective proteins and increase levels of protective metabolites, and regulate the expression of genes involved in cold tolerance. The transgenic plants use RNA silencing and coding approaches with subdue of abiotic stresses. RNA silencing involves the use of short interfering RNAs (siRNAs) to suppress the expression of a target gene. This process is used to control the expression of genes involved in abiotic stress responses, such as drought or cold tolerance. Coding approaches involve the introduction of genes that encode proteins to help the plants to survive under abiotic stress conditions. The genes that encode enzymes that help the plant break down stress-induced molecules and genes that encode proteins that protect the cell from stress-induced damage. Further it has been proposed that miR319 changes its expressions in response to cold stress in arabidopsis, rice, and sugar cane [20] to assess the effects of cold stress on the expression of miR319. The results of the study showed that miR319 expression levels were significantly higher in plants exposed to cold stress compared to control plants that were not exposed to cold stress. This suggests

that miR319 plays an important role in regulating gene expression in plants in response to cold stress. Additionally, the authors showed that cold-induced changes in miR319 expression were conserved across the three plant species studied, indicating that miR319 is a conserved regulator of cold stress response in plants. The findings of this study provide important insights into the role of miRNAs in regulating gene expression in response to environmental stressors. The upregulation of the OsamiR319 gene increased cold stress tolerance (4°C) after cold acclimatization (12°C) in many crops. The upregulation of OsPCF5 and OsTCP21 in rice led to production of cold-resistant transgenic plants.

To tolerate heat stress, plants may limit their exposure to direct sunlight, increase the amount of water they absorb through their roots, increase the production of antioxidants and to use heat shock proteins. A thermo-tolerance mechanism [12] to protect reproductive organs, which involves the induction of miR398 to down-regulate to its targeted genes CSD (copper/zinc superoxide dismutase), CSD1and CSD2, and CCS (a gene that codes for copper chaperones for CSD1and CSD2).

Further, noticed that CSD1 and CSD2 showed a higher heat stress tolerance than wild plant types and increased accumulation of heat stress transcription factors, heat shock proteins lead to less damage in crop plants, especially in corn and cassava crops [26] showed resistance to abiotic stresses particularly heat, cold, salt, and drought.

Drought and Salinity Tolerance: Drought and salinity tolerance are important traits for plants to have in order to survive in arid climates and in areas where the soil is salty. The plants have drought and salinity tolerance can survive and thrive in harsh arid environments. There are several strategies plants use to become drought and salinity tolerant such as deep root systems to access water and nutrients deeper in the soil, forming thick cuticles and waxes to reduce water loss, and production of osmolytes to maintain turgor pressure. Other strategies include increased stomata regulation, better utilization of light energy for photosynthesis, and the production of specialized root-like organs for water and nutrient uptake. All of these strategies are important for plants to be able to cope with drought and salinity stress. RNA interference is used in oil seed crops to supply the AtHPR1 promoter which is resistant to seed break offs during drought induced conditions and enhances flowering without affecting yield in drought stress environments. Drought stress tolerant transgenic rice plants have been developed with activated C-kinase1 (RACK1) with knock-down of RING finger E3 ligase gene OsDSG1, and silencing OsDIS1gene for drought-induced SINA protein in rice crop. Wide varieties of miRNAs have been recognized in Brassica, and identified its effects against drought, salinity, and temperatures. Around 126 new miRNAs like miR164, miR160, and miR156 were experimentally approved to target NAC domain-containing proteins *viz.,* ARF17 and SPL2 proteins [5]. RNA interference also regulated the genes to respond similar way to the salinity tolerance in plants. The up-regulation of Gm NFYA3 in arabidopsis led to an increased sensitivity to salinity stress, and exogenous ABA and transgenic *Agrostis stolonifera* plants up-regulated to rice miR319 gene (OsamiR319) for drought and salinity tolerance with increased leaf wax content, water retention, and decreased sodium intake. In tomatoes, upregulation of miR169c gene led to a reduction in stoma openings, transpiration rate, and loss of leaf water content which improved the drought tolerance in transgenic plants compared to wild varieties of crop plants. Similarly, a knock down of the OsTBP2.2 gene was introduced in rice to increase sensitivity to drought stress [35].

Biotic stress resistance: Biotic stress resistance is the ability of a plant to resist damage caused by organisms, such as insects, fungi, bacteria, and viruses which are severely damage the crops and crop products. These pests and diseases affect the crop at different stages *viz.,* seedling, vegetative, budding, flowering, and ripening stages. Plants can develop biotic stress resistance through a variety of mechanisms, including genetic engineering, breeding for resistance, and the use of chemical pesticides. Breeding for resistance involves selecting plants with desirable traits that are resistant to certain pests or diseases, whereas the genetic engineering involves introducing genes from other organisms into the plant to confer resistance to specific pests and diseases. Furthermore, RNAi strategies have been employed to improve the small RNA-mediated defense mechanism in crop plants against various biotic stresses [24].

Insect resistance: According to FAO pests and diseases account 20-40% of yield losses worldwide, costing a global economic loss of 290 billion dollars. Insect resistance is a trait that has been developed in many crops through the use of genetic engineering. This trait is designed to make crops

more resistant to insect pests, which can reduce the need for chemical insecticides to increase crop yields. Insect resistance is achieved by introducing genes from other organisms into the crop's genome, which can produce proteins that are toxic to certain insects and can result in improved crop quality, and greater environmental sustainability. Further, RNA-mediated crop improvement is an innovative technology that utilizes RNA interference (RNAi) to target and disrupt specific genes in plants. RNAi is a natural process that plants use to combat viruses and other foreign invaders. By using this technology, scientists can introduce new traits or suppress undesired traits in plants. Through this process, plants can be made more resistant to pests, diseases, and environmental stressors. Additionally, RNA-mediated crop improvement can be used to produce crops with higher yields and improved nutritional qualities. This method is also known to have minimal environmental side effects since it does not require the use of harsh chemicals or genetic modification.

The RNA-dependent RNA Polymerase (RdRP) mediated amplification of siRNA molecules were identified in plants, nematodes, fungi, and found to be absent in humans and insects. The amplification of RNAi in insects might be due to the presence of some unknown mechanisms analogous to RdRP, leads to the production of large amounts of siRNA molecules, which in turn leads to the further silencing or degradation of the targeted gene to produce devastating effects on insect growth, development, and survival. This mechanism is believed to be responsible for the production of additional dsRNA molecules from the original dsRNA, resulting in a cascade of gene silencing. This cascade of gene silencing can have devastating effects on the organism, leading to death or severe developmental defects. Additionally, the process of RNAi can also be used to target specific gene sequences, allowing researchers to study the effects of specific genes on the organism. In some cases, the amplified RNAi response has been linked to the production of secondary siRNAs. The secondary siRNAs are produced from the mRNA of the targeted gene, resulting in the further silencing of the gene. Finally, some insect species have evolved a form of transgenerational RNAi where the effects of RNAi can be passed from one generation to the next. This form of RNAi amplification can be observed in honeybees, where the effects of RNAi can be passed down through multiple generations. In Host-induced RNAi (HI-RNAi) approach, a crop plant is engineered with hair-pin RNAi vector to produce dsRNA against the target gene of insect pest. Upon feeding on plant parts, dsRNA enters into the insect gut, leading to the induction of RNAi machinery and then, silencing of the target gene in the insect pest. The success of HI-RNAi [2] was first demonstrated on *DiabroticavirgiferavirgiferaLeConte* and *Helicoverpa armigera* respectively. The dsRNAs were expressed in *Nicotiana tobacum* against *H.armigera* cytochrome P450 gene CYP6AE14and CYP6AE14 gene involved in detoxification of gossypol, further expressed in the midgut areas.

The use of RNAi has been confirmed to be an essential approach for developing pests and disease tolerance in various crops. Insect-resistant plants produced sRNA which attacks insects, and gene expresses itself after insects swallow the dsRNA. The transgenic tobacco lines expressing dsRNA of v-ATPase increased the whitefly mortality rate more than 70% in experiments at field level. Further, the downregulation of CsKrh1-mediated RNA interference substantially reduced the transcription of vitellogenesis (Vg) in *C.Suppressalis*, which is very essential for suppression of rice pests [28]. For pest management in field conditions, topical application or spraying of dsRNA shows high potential on soya bean aphids [33], and mortality rate of Aphis glycines was 81.67%, whereas in *Nezara viridula it was 90% [25].*

Additionally, the development of artificial microRNA (amiRNA) sequence-induced transgenic tomato varieties has been shown to confer resistance to aphids, a major agricultural pest. Transgenic tomatoes that express amiRNA sequences targeted against specific genes related to aphid growth and development can help to reduce the damage caused by aphids in tomato plants. The development of transgenic tomato varieties with amiRNA sequences provides an environmentally friendly approach to controll aphid populations on tomato crops [11]. RNAi technologies also used to knock down specific CP genes in BPH by injecting specific double-stranded RNAs (dsRNAs). This technique has been used to knock down up to 135 CP genes in BPH, and it was found that 32 of these genes are important for the development and egg production of the pests.

Virus resistance: Virus resistance of plants is determined by their genetic makeup, which includes traits that can be inherited by progeny. Plants can acquire virus resistance through the process of infection and selection, or through the introduction of foreign genes that code for proteins that can

inhibit viral replication. Additionally, plants can be genetically engineered to possess virus resistance. In this case, viral genes that code for proteins can inhibit the viral replication and are introduced into the plant's genome. Another method of virus resistance is biotechnology. This includes the use of genetic engineering to produce plants with improved resistance to specific viruses. For example, scientists have been able to introduce genes from other plants that code for proteins capable of inhibiting viral replication into plants, thus conferring a degree of virus resistance.

RNA interference is the most advanced approach to keep virus-resistant traits in many crops. The RNAi-based transgenic plants have been used to express genes encoding viral coat proteins that trigger RNA interference (RNAi) to provide resistance to virus infection. The transgenic plant can produce viral sequences that match coat proteins, replication-associated proteins, and ATPases of viral genomes can produce transgenic siRNAs to target viral RNAs for degradation upon infection. RNAi is a sequence-specific gene silencing mechanism that reduces the expression of a specific gene or gene product. The expression of viral coat proteins in transgenic plants creates a "decoy" that attracts and binds incoming virus particles, thus preventing them from entering the cell and replicating. In addition, the expression of these proteins triggers an RNAi pathway that further silences virus replication. This technology has been used to create transgenic plants that are resistant to a wide range of viruses, including those that cause crop diseases such as potato virus Y, tomato spotted wilt virus, and cucumber mosaic virus. Virus resistant transgenic plants have been produced by the method of sense transgene-triggered RNAi against the viral RNAs [30]. There are many other transgenic plants with enhanced defense by RNAi-mediated gene silencing, such as TomatoYellow Leaf Curl Virus in tomatoes [21], Rice Tungro Bacilli form Virus in rice and Citrus Tristeza Virus in Mexican lime.The virus resistance mechanism is based on climate change when carbon dioxide concentrations are increased in atmosphere the viral suppressor named RNAi (VSR) 2b protein of CMV reduces the accumulation of cucumber mosaic virus in *Nicotiana tabacum* [13]

Bacterial resistance: Bacterial diseases are more prevalent in crops such as tomato, soybean, and banana and these diseases spread very fast, and control becomes very difficult when disease progresses. Bacterial resistance is a phenomenon that occurs when bacteria become resistant and this resistance can be caused by mutations in the bacteria that further make bacteria resistant to the drugs, or by the bacteria developing mechanisms to inactivate or efflux the drugs. Employing RNAi for enhancing bacterial resistance in *Agrobacterium thaliana* plants by targeting and silencing genes that are involved in the infection process. By silencing the expression of certain genes, it is possible to reduce the susceptibility of the plants to certain pathogens. For example, silencing the expression of the gene encoding a type III secretion system increases the resistance of *A. thaliana* plants to the pathogen *Ralstonia solanacearum*. Other genes that have been targeted for RNAi-mediated enhancement of bacterial resistance include the genes encoding for chitinase, glucanase, and a LysM receptor-like protein. These genes are involved in the recognition and uptake of bacterial cell wall components, and their silencing increases the resistance of *A. thaliana* plants against a variety of bacterial pathogens. Furthermore, RNAi-mediated enhancement of bacterial resistance has also been successful in other plant species, suggesting that it could become a useful tool for disease control in agricultural crops. Application of RNAi mediated silencing suppressed two major genes of *Agrobacterium tumefaciens* by introducing complementary RNA molecules (siRNA) into the cell, which bind to the target gene and prevent it from being translated into proteins and responsible for the formation of crown gall tumors. The two genes, VirA and VirG, are important components of the bacterial type IV secretion system and are responsible for the recognition and attachment of the bacterium to the plant cell. By silencing these two genes, the researchers were able to significantly reduce the formation of tumors in Arabidopsis plants. This study demonstrates the potential of RNAi-mediated gene silencing as a method for controlling the expression of genes in *Agrobacterium tumefaciens* and minimizing the formation of crown gall tumors. Upregulation of the rice chorismate mutase (OsCM) gene changed the downstream pathway of aromatic amino acids, similarly bacterial leaf rot changing stress-sensitive genes and hormonal accumulation. The RNAi downregulation of OsSSI2 meant for fatty acid desaturation activity which causes increased resistance against the bacterial pathogens as *Xanthomonas oryzae pv. Oryzae* [16]. RNAi genes incorporated plants that showed resistance to bacteria are Arabidopsis [14], soybean [29], citrus [34], and tomatoes [4].

Fungal resistance: Fungal resistance is a type of resistance developed by organisms to protect themselves against infection by fungi. This can occur naturally, or it can be induced by treating the organism with antifungal agents. Genetic engineering based RNA silencing has revolutionized in crop resistance against fungal pathogens by enabling plants to produce specific small interfering RNAs (siRNAs) that can target and suppress the expression of fungal genes. This has enabled researchers to identify and silence specific genes responsible for fungal pathogenicity. By using genetic engineering, researchers have been able to engineer crops with resistance to a wide range of fungal pathogens, such as powdery mildew, fusarium, and rusts. This technique has been used to develop a variety of transgenic crop varieties that are resistant to a wide range of fungal pathogens. RNAi has been proven to be a essential strategy for producing tolerance in various food crops, and first it was reported in wheat. Wheat crop has 24 miRNAs which are responsive to powdery mildew infection caused by the fungus *Blumeriagraminis f. sp. tritici*. At the same, 149 target genes were predicted which are potentially regulated by the novel wheat miRNA. Gene silencing was obtained by a host-induced gene Avra10, which reduced fungal diseases in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) through transient gene expression which resistant to RNAi because of silent point mutations. In rice, the up-regulation of genes like OSAmiR7695, miR160a or miR398b, miR169, and OsamiR167d [36] were identified to get resistance against blast fungus *magnaporthe grisea*; for rapeseed upregulation noticed with BnaNPR1 gene which has an effective resistance against *Sclerotinia sclerotiorum* [31] and the tobacco GST gene was highly effective against *Phyto phthoraparasitica var. nicotine*.

Nematode resistance: Nematode resistance is a trait in plants that allows them to resist the damage caused by nematodes, which are microscopic roundworms that feed on plant roots. This trait can be bred into plants through traditional breeding methods or through genetic engineering. Plant breeders have developed varieties of crops that are resistant to specific species of nematodes, such as root-knot nematodes, cyst nematodes, and lesion nematodes. RNAi has also been exploited in plants to develop resistance against nematodes and this approach has appeared as a novel tool to control plant parasitic nematodes [18]. The delivery of dsRNAs from plant to nematode occurs by the ingestion process of plant cytoplasm, after injection the nematode body accelerate the RNAi that results in inactivation of targeted genes through dsRNA. In vitro ingestion of 16D10 dsRNA gene results the target parasitism gene silencing in root knot nematodes and reduced nematode lethality, and in arabidopsis, in vivo expression of 16D10 dsRNA gene also increase the resistance against four species of root knot nematodes. The nematode-resistant varieties are often more productive and obtain higher yields than non-resistant varieties by reducing the risk of nematode-borne diseases. Furthermore, nematode-resistant varieties can improve the overall quality of the crop, reduce the need for chemical nematicides more tolerant of environmental stresses.

Conclusion

Food is the basic requirement of human beings, but food insecurity and malnutrition is the most serious concern for human health and may cause severe loss of countless lives in developing countries. Therefore, there is a urgent need for innovative technologies to improve crop production strategies *viz.,* precision agriculture based sensors, artificial intelligence and machine learning, hydroponics, aquaponics, and vertical farming along with advanced breeding technologies. There are several techniques which has been adapted to improve the production of crops under biotic and abiotic stress conditions and among them one of the technique is RNAi technology. In this review we have described several advancements of RNAi technology for improving the crop productivity and their traits to withstand under biotic and abiotic stress environments. The biotic and abiotic stresses are the biggest problem in the current agriculture, and the RNA silencing technology is a advanced application that can resolve agricultural issues in a short period. Similarly, RNA interference (RNAi) is becoming one of the powerful tool of functional genomics for silencing gene expression for crop improvement.

But, the RNAi's are not stable in plants, and mediated gene suppression method which opens new paths for the development of eco-friendly biotech approaches to knock out specific genes with high quality and stress tolerant traits in various crops including pathogen/ insect/pest resistance for enhanced nutrition. RNAi efficiency is directly related to the dsRNA delivery in the biotic agents, and it could be achieved by micro injection, feeding, soaking in dsRNA solution, HIGS or SIGS. Apart from these, the growing use of nanoparticles as dsRNA delivery systems has displayed immense potential in achieving protection

against insect pests, and its applications can be exploited to obtain protection against other plant stresses as well. These are relatively newer approaches to RNAi have made the concept of developing RNA-based biopesticides a near reality and are rapidly gaining popularity as broad-spectrum alternatives to chemical-based protection measures. RNAi has been instrumental in the development of new crops with improved traits in plant breeding. By targeting specific genes, breeders can introduce novel traits in various plant species for biotic (pathogen/pest/insect) resistance with improved nutritional quality. This is a much more precise and efficient way of breeding than traditional methods which involves crossing of plants with different traits and selecting for desirable qualities. Further, this revolutionary technology can be used for functional analysis of target genes and regulation of gene expressions for crop improvement in the future. The involvement of RNAi to control of plant physiological functions is an intriguing subject area that requires further exploration to understand its mechanisms. This technology also has few limitations which include the identification of appropriate target genes, off-target effects, and the application of RNAi is more sophisticated and required highly skilled human power. If we use this technology efficiently, we can produce more varieties that are resistant to abiotic and biotic stresses.

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