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ORIGINAL ARTICLE

Gene silencing RNAi technology: Uses in plants

Çağlar Kaya[1] **Tolga Sarıyer**[2]

1 Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture, Türkiye, e-mail: ckaya@stu.comu.edu.tr

2 Çanakkale Onsekiz Mart University, Faculty of Agriculture, Department of Horticulture, Türkiye, e-mail: tolgasariyer@comu.edu.tr

ABSTRACT

Ensuring sustainable food production in national and global area depends on the determination of plant species and varieties that can survive under the influence of various stress factors that may occur due to global climate changes and other factors that adversely limit growth and development, and depends on the protection and development of existing ones. It is important to develop new plant varieties that are resistant to abiotic stress factors that have occurred as a result of global climate changes. At this point, modern biotechnological methods have been widely needed in plant breeding in recent years. One of these techniques is RNAi technology. The mechanism of RNA interference (RNAi) is defined as post-transcriptional gene silencing or regulation of gene expression, resulting in the degradation of mRNA chain, which is the complement of double-stranded RNA (dsRNA) entering the cell. RNA interference begins when double-stranded RNA is cut into small inhibitory RNAs (siRNA) by an RNase III enzyme called as Dicer. These siRNAs then bind to the RNA-inducing silencing complex (RISC) which is a multiprotein-RNA nuclease complex. RISC uses siRNAs to find complementary mRNA and cuts the target mRNA endonucleolytically. The resulting decrease in specific mRNA leads to a decrease in available protein(s). Post-transcriptional gene silencing, RNA interference and other forms of RNA silencing have been observed particularly in plants. In recent years, RNAi studies, which are among the leading topics in the global area, have shown that non-coding RNAs in plants play a role in the control of tissue differentiation and development, signal transmission, interaction with phytohormones, abiotic (drought, salinity, etc.) and environmental factors such as biotic stress. In this review paper, the basics of RNAi mechanism and the usage of RNAi in plants are explained.

Keywords: Gene silencing, RNAi, siRNA, climate, plants

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Corresponding Author:
Çağlar Kaya
Email: ckaya@stu.comu.edu.tr

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1. INTRODUCTION

RNAi technology, which emerged as a result of genetic transformations, is a mechanism used in the regulation of gene expression after transcription with double-stranded RNA. RNAi is also defined as the post-transcriptional gene silencing mechanism through the degradation of mRNA chain, which is the homolog of dsRNA entering into the cell. This technology is a heavily and newly researched field in gene function (functional genomics) research. RNAi was named as "molecule of the year" and "the most important scientific breakthrough of 2002" by Science in 2001 by Fire et al., (1998).

RNA interference technology has revolutionized the field of functional genomics and is an important tool that allows the function of many different plant genes to be elucidated. Thanks to RNA interference-mediated gene expression suppression studies, the amount of protein synthesized from the gene of interest is reduced and information about the function of the protein can be obtained (Bora, 2020). RNAi is an important breakthrough during genetic transformation studies. The gene silencing mechanism is a very important discovery made by the botanist Napoli et al. (1990) during their research in which they tried to over-express the chalcone synthase gene in petunia plant. These researchers wanted to increase the effects of enzymes that catalyze pigmentation in petunia by adding a gene (*CHS*). However, they declared that whiter petunias were formed instead of more purple petunias, sometimes with unexpected reverse results Napoli et al., (1990). Although, this mechanism was not elucidated at the time, but, it was later discovered by Jorgensen et al., (1990) they report that this is a result of degeneration of the dsRNA region within the chalcone synthase gene (*CHS*), and may be associated with a PTGS (post-transcriptional gene silencing). In recent years, effective gene silencing mechanism has yielded successful results in Petunia, Nicotiana and Arabidopsis plant species Van der Krol et al., (1988).

With the increase in studies in the field of plant genetics & breeding and the development of the methods used, a lot of research has been done on plants as in many other fields. To date, studies on crops of agricultural importance such as wheat, tomato, corn, and beans are carried out by using epigenetic mechanisms such as DNA methylation, histone modification, mRNAs, ncRNA, RNAi. The methods to be applied to take precautions against pests are also important. It is critical that new methods and practices to be used against insects for plant production are environmentally friendly and healthy for humans and animals. RNAi is one such method. Parallel to the developments in the field of biotechnology, RNAi technology, which is one of the latest developments in the field of plant genetics, has a lot of contributions to agricultural applications Ayaz et al., (2018).

2. RNAI MECHANISM AND TYPES OF RNA INVOLVED

During the RNAi mechanism, RNA complementary to the target mRNA binds to the significant sequence of the mRNA on the RISC (RNA Inducing Silencing Complex) factor, an RNA-multiprotein complex with nuclease activity. Gene silencing is controlled through this RISC factor. The mRNA interacting with the protein named as 'Argounate' in the RISC factor is recognized and cut by the 'Dicer' enzyme (Figure 1), which is a ribonuclease in the RNase III family and thus silencing occurs. The RNAi mechanism or gene silencing mechanism is carried out by two different types of molecules in eukaryotic organisms (Zamore et al., 2000). These molecules are consisted of 22 nucleotides with long miRNA (micro RNA) and 21-23 nucleotide long double-stranded small interfering RNA (Figure 2).

Figure 1. Schematic representation of the predicted consensual domain structure for the Dicer enzyme. Helicase: N-terminal and C-terminal helicase domains. PAZ: Pinwheel-Argonaute-Zwille domain. RNase III: bidentate ribonuclease III domains. dsRBD: Double stranded RNA-binding domain (Bernstein et al., 2001).

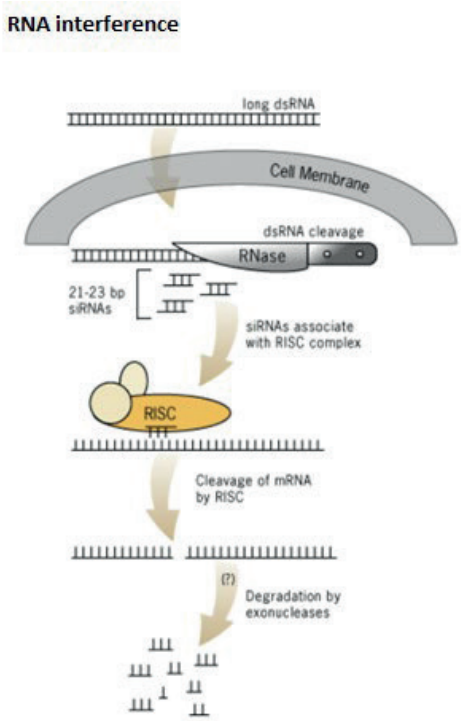

Figure 2. RNAi mechanism; dsRNA recognition and screening process, recognition and cleavage of double-stranded dsRNA in the presence of Dicer-RDE-1 (RNAi deficient-1), an RNase III family enzyme, production of siRNAs: Formation of RNAs with a length of 21-23 nucleotides, fusion of the siRNA-RISC complex pair with the specific complementary mRNA region, destruction of target mRNA by exonucleases in RISC and RISC complex returning for a new process (Ambion, 2007).

miRNAs and siRNAs are very similar to each other, but the differences between them are as follows:

a) The miRNA precursor is single stranded RNA (single strand: ssRNAs) in the hairpin structure, while the siRNA precursor is long dsRNAs.

b) Argounate proteins required for miRNA are AGO1, AGO10, and Argounate proteins required for siRNA are AGO1, AGO4, AGO6, AGO7.

c) miRNA is responsible for mRNA degradation, suppression of translation, while siRNA is responsible for DNA methylation, modification of histones, and mRNA degradation.

d) In plants, miRNA provides partial or full complementarity to the target sequence, while siRNA provides full complementarity.

e) miRNA is involved in cell development and differentiation, developmental processes, and response to biotic and abiotic stresses, while siRNA is involved in protection against transposons and viruses, and in stress adaptation (Zilberman et al., 2003; Bartel, 2004; Kim, 2005; Sunkar & Zhu, 2007; Watanabe et al., 2007; Carthew & Sontheimer, 2009; Pratt & MacRae, 2009).

The miRNA pathway begins with the conversion of pri-miRNAs in the nucleus into pre-miRNAs, which are approximately 70 nucleotides in length and hairpin structure, via the "Drosha enzyme". The pre-miRNAs are transferred to the cytoplasm and translated into miRNA duplexes by another RNase, Dicer. After the Dicer enzyme functions, one of the short dsRNA duplexes interacts with the RISC factor to bind to the target mRNA via base pairing. These miRNAs have the capacity to mediate translation suppression or degradation of mRNAs. With these features, they have taken their place among the interesting subjects of modern molecular biology. The capacity of translation suppression or direct degradation of mRNAs in plants varies according to the region where miRNAs bind on target mRNA. If it binds to the untranslated region (UTR) of mRNA, there is incomplete complementarity and translation is suppressed. Binding to the translated ORF (open reading frame) region shows complete complementarity and mRNA is degraded by Aragunate2 (AGO2). siRNAs are the precursor of dsRNAs, and dsRNAs are cut by the Dicer enzyme in such a way that the 3' end remains protruding to form 20-25 bp siRNAs. Double-stranded siRNAs combine with RISC factors to become single-stranded and function as gene silencing by mRNA degradation Khvorova et al., (2003).

3. USAGE OF RNA INTERFERENCE TECHNOLOGY IN PLANTS

The basis of RNA interference studies in plants was laid by Napoli et al., (1990) when they investigated the overexpression of the *CHS* gene in Petunia plant by the vector *Agrobacterium tumefaciens*. In the research, it was tried to obtain darker petunias by increasing the pigmentation, but colorless, light-colored or variegated plants were obtained.

Further research by the same team showed that the expression of the gene responsible for pigment production and homologous genes were suppressed by the 35S promoter of the virus. It has been determined that gene silencing occurs with the degeneration of the dsRNA region in *CHS*. The miRNA mechanism was first described by Park et al., (2002) discovered by their research on *Arabidopsis* plant. It was thought that the micRNA metabolisms of the *CAF-1* (Carpel Factory) gene and *HEN1-1* genes, which have similar tasks in the study, would also be similar. In order to determine the functions of these genes, miRNA has been isolated from *HEN1-1* and *CAF-1* mutant *Arabidopsis* plants and from *Arabidopsis* plant that did not contain any mutations. In addition, isolation was done from tobacco, rice and maize crop plants thought to carry potential homologous genes. As a result of the research, it was determined that miRNA formation is controlled by growth and its density decreased in *HEN1-1* and *CAF-1* mutant plants. Many details regarding the biogenesis of miRNAs were also determined by this model plant. miRNA has been identified to date in a wide variety of plants such as *Chrysanthemum*, *Gossypium*, *Arabidopsis*, *Sorghum*, *Brassica* and *Vitis* (Adai et al., 2005; Bedell et al., 2005; Billoud et al., 2005; Dezulian et al., 2005; Li & Zhang, 2005; Lu et al., 2005; Sunkar et al., 2005; Qiu et al., 2007; Velasco et al., 2007).

Fruits are important as they are a source of additional nutrients. The nutritional value of the fruit, such as flavor and shelf life, are the factors that determine the quality. For this reason, reducing product loss by increasing fruit shelf life by illuminating the metabolic process in fruit ripening is of great interest for commercial reasons. The 1-Aminocyclopropane-1-carboxylate (*ACC*) oxidase gene, which catalyzes the oxidation of ethylene, a plant growth regulator (PGR), was used in an RNAi study to delay ripening in tomato, one of the most consumed fruits in the world. Successful conversion of double-stranded RNA (dsRNA) of tomato ACC oxidase into tomato variety Hezuo 906 by *A. tumefaciens* using the cauliflower mosaic virus (CMV) 35S promoter produced a transgenic tomato with a shelf life of more than 120 days (Xiong et al., 2005).

Certain studies have been conducted in which plant color changes in transgenic plants as a result of inhibiting the accumulation of anthocyanins by suppressing some structural genes involved in anthocyanin biosynthesis with RNAi. In another study, the 3'UTR region of chalcone synthase (*CHS*) mRNA, is an important enzyme in anthocyanin and flavonoid biosynthesis, was targeted and suppressed with RNAi to obtain plants with a lighter color than normal color in *Torenia hybrida* plant (Fukusaki et al., 2004).

In a study conducted on rice seed, the phytochelatin synthase (*OsPCS1*) gene, which has an important role in heavy metal accumulation and resistance, was suppressed by RNAi to reduce cadmium (Cd) accumulation. For this, the hairpin structure of the PCS gene fragment was designed in pRNAi-OsPCS1 under the control of *ZMM1*, a maize seed-specific promoter, and transferred to rice by *A. tumefaciens* (Li et al., 2007).

As stated by researchers working on the RNAi technology in potatoes, β-carotene content was increased by silencing the β-carotene hydroxylase gene (*BCH*) transcript, which converts β-carotene to zeaxanthin using RNAi technology (Van Eck et al., 2007).

According to the results of a study conducted on the subject in which the two E-class *MADS* box genes, *MaMADS1* and *MaMADS2*, which are homologous to the *RIN MADS* ripening genes in tomato, were functionally characterized, ripening was delayed in transgenic bananas in which these two genes were silenced with RNAi, and the banana ripening process was characterized by at least two *SEPALLATA-MADS* box genes in bananas (Elitzur et al., 2016).

Terpenoid gossypol toxin found in cotton seed and oil, which is an important industrial plant, and the RNAi and cadinene synthase gene in the gossypol biosynthesis pathway were suppressed only in the seed using a seed-specific promoter, and the leaves continued to produce terpenoids at normal levels in order not to affect the defence against insects Sunilkumar et al., (2006).

Plants may encounter a wide variety of stress factors such as biotic factors, infection by microorganisms and stress factors caused by the attacks of harmful animals, and abiotic factors (temperature, water, radiation, chemicals, magnetic and electrical fields) during their life (Levitt, 1980). Plants can respond through a wide variety of stress-related proteins, transcription factors, metabolites, or through epigenetic regulation. Epigenetic changes; encompasses RNA-directed DNA methylation, histone or DNA modifications, which play an important role in gene expression changes, particularly when the plant is exposed to an abiotic stress. It has been predicted that non-coding RNAs in plants may be formed by different mechanisms, such as insertion of a protein-coding gene after duplication or originating from transposons that make up more than 80% of the plant genome, but it has been stated that it is very difficult to determine a precise origin due to mutations accumulated in plants (Cushman and Bohnert 2000). Although there is no definite opinion about its origin. RNAi in plants is a technology that has the potential to be used in many areas such as genomics, determining the functions of genes, controlling tissue differentiation and development, signal transmission, interaction with phytohormones, and determining the response to environmental factors. It is possible to give a wide variety of examples for the application of RNA interference technology in plants (Priyapongsa and Jordan 2008).

According to the obtained results of a study, tobacco plants were manipulated with the tobacco mosaic virus (TMV) coat protein gene (*CP*). While all the features of the infection were observed in the initially transformed plants. It was noted that the plants recovered in the following weeks. As a result of the molecular analyzes performed in the healed tissues, it was understood that the transcription of the TMV sequences continued, but the functions of the mRNAs did not continue. At the end of the study, researchers were agreed with the opinion that gene silencing or co-repression is localized in cytoplasm and occurs at the post-transcriptional stage (Lindbo et al., 1993).

In a recent study on drought stress resistant *Physcomitrella patensis*, 16 miRNAs (DsAmR) associated with drought stress were identified and miR902a-5p and miR414 were reported to be important in inducing drought stress resistance (Wan et al., 2011).

The useage of RNAi technology in increasing resistance to sugarcane mosaic virus (SCMV) in maize (*Zea mays*) was investigated by researchers. As a result of the study, it was seen that the hairpin RNAs formed by the transcription of the sheath protein (*SHP*) gene prevent SCMV infection and it has been stated that the RNA interference-post transcriptional gene silencing approach may be important. It has been emphasized in other studies that the virus resistant plants can be obtained by inhibiting gene expression (Gan et al., 2014).

By silencing the GTPase *MtROP9* gene in *Medicago truncatula*, it was observed that ROS-related enzymes were suppressed and ROS products were reduced in transgenic roots (MtROP9i) infected with pathogenic (*Aphanomyces euteiches*) and symbiotic microorganisms i.e., *Sinorhizobium meliloti* and *Glomus intraradices*. In this study, time-dependent proteome responses were investigated in plants under the same conditions (22°C temperature, 65% humidity and 16hs photoperiod at 220 μE m−2 s−1 in plant growth chamber). When the induced proteins were analyzed as a result of the study, it was determined that the ROS production and clearance mechanism was controlled by the roots, while *MtROP9i* had alternative protection mechanisms Kiirika et al., (2012).

According to the obtained results of a study, it has been found that the MYB transcription factors play a role in plant development, metabolism and stress response. In the study, the function of *OsMYB103L* gene encoding the R2R3-MYB transcription factor in rice crop was investigated. The *OsMYB103L* gene is located in the nucleus and has processing capacity. As a result of the study, it was observed that ovarian expression of this gene caused the curly leaf phenotype in rice. The study further determined that the expression of cellulose synthesis genes (*CESA*) increased by silencing of *OsMYB103L* gene with RNAi technology.

It was observed that the expression of *CESAs* gene decreased the cellulose content in plant and the curl in the leaf decreased (Yang et al., 2014).

RNA interference technology has been successfully used in plant breeding and genetics. Homozygous parental lines can be obtained from heterozygous plants by stopping the recombination of meiosis. Recombination in meiosis can be achieved by RNAi (RNA interference) assisted gene editing. With this method, double haploid lines can be obtained and by crossing these lines, heterozygous lines can be obtained again. Recombination in meiosis can be prevented using different methods, including RNAi technology. For example, *DMC1* and *SPOL1* genes are involved in the recombination processes of meiosis. By silencing these genes with RNAi technology, homozygous parental lines are obtained from a heterozygous line (Schaart et al., 2016; Savadi et al., 2018).

4. CONCLUSION

Molecular technologies such as RNAi technology offer important opportunities to increase agricultural production in order to ensure adequate and balanced nutrition for the rapidly increasing world population. In addition to the application of sustainable agricultural techniques, the development of high yielding and high-quality plant varieties that are resistant to biotic and abiotic stress conditions is an important priority. In the development of these plants, it will be more accurate to focus mainly on molecular plant breeding techniques, not only transgenic plants obtained by transformation, in the short and medium term. It will help developing countries such as Türkiye, which have rich genetic resources, to determine their priority areas and to establish sufficient infrastructure for plant genetics and breeding studies.

It depends on the determination of plant species and varieties that can survive under the influence of various stress factors that may occur due to global climate changes and other factors that adversely limit growth and development, and it depends on the protection and development of existing ones. At this point, new and modern biotechnological approaches are important. In this sense, RNAi technology, which is an innovative approach in plant breeding, has become the preferred biotechnological approach in the international area. RNAi technology is the biggest advance in studying the function of genes in recent years. This mechanism is one that exists in nature, and it also has a wide range of applications in molecular biology, gene-protein function analysis, and functional genomics research. This technology can be used to obtain transgenic plants resistant to stress factors, to develop defence mechanisms against diseases caused by pathogens (fungal, viral and bacterial), etc., made significant contributions in many fields. In terms of the future prospective along with the development of technological tools, a more detailed understanding of post-transcriptional gene regulation mechanisms in different plants under different conditions will be possible.

Compliance with Ethical Standards

Conflict of interest:

The authors declare that they have no conflict of interest.

Ethical approval:

For this type of study, formal consent is not required.

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