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Non-Coding RNAs in Plant Stress Responses and Their Implications for Agriculture

Büşra YİRMİBEŞ[1,2*], Nur ÜLGER[2]

[1] Department of Biotechnology, Institute of Science and Technology, Akdeniz University, Antalya, Türkiye.
[2] Multi Tohum Tar. San. Tic. A.ş., Antalya, Türkiye.

ABSTRACT

Agriculture's global significance encompasses food security, economic growth, preservation of biological diversity, and employment generation. However, the stresses such as abiotic and biotic stresses generate substantial challenges in terms of crop yield and quality. Plants respond to these stressors by means of physiological and genetic mechanisims. Notably, small non-coding RNAs (ncRNAs), such as microRNAs (miRNAs) and small interfering RNAs (siRNAs), play essential regulatory roles in adaptation to these stresses. Abiotic stresses include temperature, drought, and salinity and leading to changes in miRNA expression levels. miRNAs like miR167, miR159, and miR171 actively participate in salt, drought, and cold stress responses. Similarly, miR395 responds to sulfate deficiency stress, while miR399 is involved in phosphate homeostasis. Some biotic stresses, like pathogen infections, also affect miRNA modulation and resistance mechanisms siRNAs effectively contribute resistance to biotic stress such as pathogen, bacteria, fungi, virus and abiotic stresses. Virus-derived siRNAs (vsiRNAs) activate plant immunity, and on the other hand, in vitro synthesized siRNAs can be used in controlling pest. Heat stress triggers differential siRNA expression, particularly associated with flowering regulation, while plant species exhibiting drought tolerance display pronounced siRNA regulations in response to water deficiency. In conclusion, understanding plant responses to adverse stress conditions is pivotal for advancing plant resilience, yield, and quality. ncRNAs like miRNAs and siRNAs are key molecular players in these adaptation processes. When combined with gene editing technologies such as CRISPR/Cas9, these approaches offer promising strategies for developing stress-tolerant agricultural products. These strategies hold significant potential in supporting sustainable agriculture and addressing global food security challenges.

Keywords: Agriculture, microRNA, siRNA, plant stress

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[*] Corresponding Author's email: yirmibesbusra@gmail.com

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Bitki Stres Cevaplarında Kodlanmayan RNA’lar ve Tarımdaki Önemi

ÖZ

Tarımın küresel önemi, gıda güvencesi, ekonomik büyüme, biyolojik çeşitliliğin sürdürülmesi ve istihdam sağlanması gibi çok yönlü katkıları içermektedir. Ancak, biyotik ve abiyotik stres unsurları, ürün verimliliği ve kalitesi açısından ciddi zorluklar sunmaktadır. Bitkiler, bu tür streslere fizyolojik ve genetik mekanizmalarla yanıt verirler. Özellikle mikroRNA'lar (miRNA'lar) ve küçük interferan RNA'lar (siRNA'lar) gibi küçük kodlamayan RNA'lar (ncRNA'lar), bu streslere adaptasyonda önemli düzenleyici roller üstlenirler. Abiyotik stres faktörleri arasında sıcaklık, kuraklık ve tuzluluk gibi faktörler bulunmaktadır ve bu faktörler miRNA ekspresyon düzeylerinde değişikliklere yol açarlar. miR167, miR159 ve miR171 gibi miRNA'lar tuz, kuraklık ve soğuk stres yanıtlarında etkin rol oynarlar. Aynı şekilde, miR395 sülfat eksikliği stresine, miR399 ise fosfat homeostazına yanıt verir. Patojen enfeksiyonları gibi bazı biyotik stresler de miRNA modülasyonunu ve direnç mekanizmalarını etkilemektedir. siRNA'lar, patojenler, bakteriler, mantarlar ve virüsler gibi biyotik streslere karşı direnç sağlamada etkin rol oynarlar. Virüs kaynaklı siRNA'lar (vsiRNA'lar), bitki bağışıklığını harekete geçirirken, in-vitro sentezlenen siRNA'lar zararlı organizmaların kontrolünde kullanılabilir. Isı stresi, özellikle çiçeklenme düzenlemesi ile ilişkilendirilen farklı siRNA ekspresyonunu tetiklerken, kuraklık toleransı gösteren bitki türleri su eksikliği yanıtlarında belirgin siRNA düzenlemeleri sergilerler. Sonuç olarak, bitkilerin olumsuz stres koşullarına verdiği tepkileri anlamak, bitki verimliliği, dayanıklılığı ve kalitesini artırmak açısından hayati önem taşır. miRNA'lar ve siRNA'lar gibi kodlanmayan RNA'lar, bu adaptasyon süreçlerinde kilit rol oynayan moleküler oyunculardır. CRISPR/Cas9 gibi gen düzenleme teknolojileri ile birleştirildiğinde, stres toleransına sahip tarım ürünleri geliştirme konusunda umut verici stratejiler sunar. Bu yaklaşımlar, sürdürülebilir tarımı desteklemek ve küresel gıda güvenliği zorluklarına çözüm sağlamak adına önemli bir potansiyel sunmaktadır.

Anahtar Kelimeler: Tarım, miRNA, siRNA, bitki stresi

1 Introduction

Agriculture has a significant importance worldwide due to reasons such as providing food, economic development, preserving biological diversity and ecosystem health, and generating employment opportunities. It is essential for ensuring sufficient and balanced nutrition for human health, as the crops required for this purpose are obtained through agricultural practices. Industries based on agriculture and the export of agricultural crops contribute to economic growth. Furthermore, agricultural areas are important for conserving biological diversity and ensuring the continued health of ecosystems. Proper practices help preserve soil fertility, prevent erosion, and protect habitats [1][2]. Therefore, it is crucial to obtain high-quality and yielding agricultural crops, particularly in terms of health and economic contributions. To achieve this goal, careful work and efforts are required in both growing and crop storage stages. Nevertheless, numerous challenges are encountered in pursuit of this objective.

One of the most significant challenges affecting plant yield and quality, and thus agricultural productivity, is abiotic stresses resulting from temperature, drought, cold stress, as well as soil-related factors including salt accumulation and mineral deficiencies. Another challenge involves biotic stresses caused by harmful organisms such as insects, viruses, and fungi [3][4].

Plants responds to the aforementioned stress with physiological responses containing proteins, some transcription factors and metabolites and genetic responses. Non-coding RNA mechanisms are involved in genetic response [5][6]. These mechanisms do not change DNA sequences but cause the alterations arising from environmental factors in gene expression [7]. Small non-coding RNAs (sRNAs) have significant roles in the plant development and the response to biotic and abiotic stresses. They are also valuable tools in functional genomic studies and biotechnology field [8]. This article focuses on the studies related to small non-coding RNAs (ncRNAs) conducted to enhance the resilience against the mentioned stress factors.

2 Small Non-coding RNAs in Plants

RNA can be categorized as messenger RNA (mRNA) which can be translated into proteins, and non-coding RNA (ncRNA) which does not undergo translation. Around 98% of RNA is not converted into proteins, with introns accounting for 70% of this portion [9][10]. ncRNAs play crucial regulatory roles in cellular functions by facilitating post-transcriptional gene regulation [6]. ncRNAs can be classified into two groups based on their size: long ncRNAs and short ncRNAs. In this context, the focus will be on short non-coding RNAs. microRNAs (miRNAs) and small interfering RNAs (siRNAs) are the two most commonly researched types of regulatory short ncRNAs [11][12].

2.1 micro RNAs (miRNAs) and small interfering RNAs (siRNAs)

miRNAs are approximately 19-25 nucleotides in length and are found in various regions of the genome. In plants, they are mostly derived from intergenic regions. miRNAs bind to specific target gene regions on mRNA, creating either perfect or near-perfect complementarity, which leads to the degradation or, in rare cases, repression of mRNA. This modulation of gene expression, in turn, affects the function of the target gene. miRNAs have specific roles, some of which are conserved across species or tissues, while others are involved in developmental processes [11]. A study conducted in Arabidopsis and maize compared miRNA processes between monocots and dicots and found similarities, indicating a high degree of conservation [13]. It is known that this conservation also exists in target genes. In their study, Floyd and Bowman 2004 [14] stated that the HD-ZIP genes targeted by miR-166 were also conserved.

dsRNAs, which are precursors of siRNAs, are cleaved by the Dicer enzyme, resulting in 20-25 nt siRNAs with double-stranded and 3' overhangs. According to their biogenesis, they are classified as phasiRNAs (phased secondary small interfering RNAs) and hc-siRNAs (heterochromatic small interfering RNAs). PhasiRNAs are derived from double-stranded RNApol II and RDR6 (RNA-dependent RNA polymerase VI) transcripts and are converted into 21-24 nt mature duplexes by DCL4 or DCL5 enzymes. This process is triggered by 22 nt miRNAs. On the other hand, hc-siRNAs are typically 24 nt long and originate from repetitive regions in the genome. These structures, when associated with RISC factors, lead to the degradation of target mRNAs, thereby performing a gene silencing role [15][16]. miRNAs generally play a role in processes such as cell development, differentiation, and response to biotic and abiotic stresses, while siRNAs are also effective in stress adaptation and defense against viruses [5][9].

miRNAs and siRNAs are processed by various processes and proteins in their biogenesis, which play crucial roles in their functional mechanisms. The DCL (Dicer-like) proteins are enzymes responsible for cleaving precursor miRNAs and siRNAs to generate mature small RNAs. These proteins include specialized members such as DCL1, DCL2, DCL3, and DCL4, each of which contributes to the formation of small RNAs of different lengths, facilitating their involvement in gene silencing. DCL1, for instance, cleaves imperfectly complementary double-stranded RNAs to produce approximately 21 nt miRNAs, which can participate in post-transcriptional regulation. DCL2 and DCL4 are two other enzymes involved in antiviral defense and post-transcriptional gene silencing (PTGS). DCL2 generates

22 nt small RNAs, while DCL4 produces 21 nt small RNAs. On the other hand, DCL3 is involved in the biogenesis of 24 nt siRNAs, which play a role in RNA-directed DNA methylation process targeting endogenous double-stranded DNA transcripts. Additionally, DCL4 is also involved in the formation of tasiRNAs (trans-acting small interfering RNAs [17][18]. siRNAs lead to PTGS or RNAi and participate in mRNA degradation are typically 21 nt long small RNAs.

3 Response of miRNAs to Stress Conditions

Plants exhibit similar responses when exposed to abiotic stresses, indicating that they affect common pathways involved in stress response. So far, the response of miRNAs to these stresses has been observed either as upregulation or downregulation of expression levels [5]. The first study on the response of miRNAs in plants to stress was related to nutrient mineral uptake and was conducted in Arabidopsis in 2004. According to the study, an increase in the expression of miR395 targeting the sulfate transporter gene was observed under sulfate deprivation, indicating that miRNAs can be induced by environmental stresses. The ATP sulfurylase genes, such as *APS1*, *APS3*, and *APS4*, targeted by miR395, play a key role in assimilating inorganic sulfate. Under sulfate deprivation, miR395 expression increases while the expression of the *APS1* gene decreases [19]. It is known that insufficient uptake of sulfate leads to impaired plant growth [20], which can negatively affect both yield and quality, causing problems in agricultural production.

After the realization that miRNAs respond to sulfate deprivation, studies have also been conducted on other essential nutrients in plant nutrition and development. Phosphorous is an essential mineral that contributes to the structure of nucleic acids, membranes, and ATP, thereby influencing cellular activities. They also have a central role in protein phosphorylation and signal transduction. Plants incorporate P as Pi (inorganic phosphate) through their roots. miR399 targets two different genes about P transports. One of its targets is a phosphate transporter gene (*PHO2*), and the other is an ubiquitin-conjugating enzyme (*UBC24*). These proteins are important for maintaining Pi homeostasis. When Pi is sufficient, miR399 is suppressed, leading to an increase in the expression of *PHO2* and *UBC24* genes. Hormonal signals regulate root development to prevent excessive Pi loading. However, under Pi starvation conditions, miR399 shows high upregulation, resulting in a decrease in the expression of the two aforementioned proteins [21].

It is known that miRNAs also play a significant role in response to salt stress [22][23]. The excessive accumulation of salts in the soil can disrupt the hydraulic conductivity and osmotic balance of plants, thereby limiting their physiological capabilities associated with growth and nutrient uptake. This phenomenon has the potential to undermine the normal developmental and nutritional functions of plants [24]. Ding et al. (2009) identified a total of 98 miRNAs, with 27 of them showing high expression, in maize through microarray analyses under salt treatments. For example, miR167 and miR164 were found to be downregulated in the NC286 maize variety, suggesting their potential involvement in salt stress signaling. Increased regulation was observed in the regulation of miR168, miR162, and miR395. miR168 was induced in salt-tolerant plants in the NC286 line but suppressed in salt-sensitive plants in the Huangzao4 line [22]. miR156, miR159, miR170, miR171, miR319, and miR396 are among the miRNAs whose expression changes in response to salt stress [25].

In broccoli, a winter vegetable, miRNAs respond to salt stress. Exposure to salt stress led to a decrease in the expression levels of miR393 and miR855, which are conserved in other plants, as well as two putative candidate miRNAs, miR3, and miR34. On the other hand, the expression of conserved miRNAs, miR396a and candidate miR37, was high. The target genes of these differentially expressed miRNAs were found to be involved in cell cycle regulation, hormone signal transduction, and metabolic processes. Therefore, miRNAs have a significant impact on salt stress in broccoli [23].

Drought stress or excessive water loss in plants leads to morphological and physiological changes [5]. Drought stress slows down plant development, resulting in negative effects on yield and quality. Molecular studies conducted on plants exposed to this stress have shown changes in miRNA expression. Zhou et al. (2010) reported the downregulation of 16 miRNAs (miR156, miR159, miR168, miR170, miR171, miR172, miR319, miR396, miR397, miR408, miR529, miR896, miR1030, miR1035, miR1050, miR1088, and miR1126) in response to drought stress in rice, while miR159, miR171, miR319, miR169, miR395, miR474, miR845, miR896, miR851, miR854, miR901, miR903, miR1026, and miR1125 were upregulated. Among these, miR159 and miR171 seem to be both upregulated and downregulated. This is due to the clustering of miRNAs, which is referred to as miRNA families. For example, the miR171 family may have different members such as miR171a and miR171b, which can generate different responses. High expression of different members of the same family can be observed during shoot and reproductive stages. However, only miR854 is highly expressed during shoot and flower formation [26], indicating its significant role in these processes. Since flower formation has a considerable impact on yield, this information is crucial for breeding programs. In drought-tolerant tomato plants, increasing in the expression of miR2118a is 80%. The target of this miRNA is the pectate lyase protein, which is involved in plant elongation [24].

Another stress that leads to significant crop losses is cold stress. Cold stress can impose severe damage upon cell membranes, organelles, and plant organs. It can notably suppress pollen germination and inhibit pollen tube formation. Furthermore, it can instigate flower shedding, leading to substantial reductions in yield [28]. Cold stress can be classified as chilling (approximately 4 °C) and freezing (-18 °C), both of which negatively affect plant growth. Plants under cold stress experience reduced water uptake or cellular dehydration. This leads to the formation of reactive oxygen species, causing osmotic stress [29][30]. Some miRNAs that respond to cold stress have been identified. In a study conducted to investigate the response to cold stress, it was noted that miR167, miR319, and miR171 showed different expressions. The study also emphasized that miRNAs could be located in different regions [10]. It is known that miR397, miR169 and miR172 also response to cold stress and upregulate in Arabidopsis [25][31].

miRNAs also play a role in response to biotic stresses. It has been shown that 99 miRNA families are induced in response to *Verticillium dahliae* infection, which causes significant yield losses in eggplant. Twelve hours after fungal infection, a decrease in the expression of miR156, miR159, miR160, miR162, miR166, miR167, miR169, miR171, miR172, miR319, and miR396 was observed in the plants. Real-time PCR analyses revealed a significant decrease in the expression of miR393 with increasing infection duration. miR393 targets an auxin receptor called as *TIR1*, and they have a negative correlation. An abundance of miR393 leads to a decrease in the expression of the target gene *TIR1*. When *TIR1* expression decreases, the expression of auxin response genes decreases. This negative effect contributes to resistance against necrotrophic fungal diseases [32][33].

4 Responses of siRNAs to Stress Conditions

Viruses are among the most significant stressors for plants. In response to viral infections, plants can activate resistance mechanisms by generating of siRNAs that target viral RNA. These siRNAs facilitate the degradation of viral RNA, thereby inhibiting the spread of the virus. The discovery of virus-derived siRNAs (vsiRNAs) in tobacco plants infected with potato virus led to further investigations in other plants. vsiRNAs derived from Wheat yellow mosaic virus (WYMV) were found to activate plant immunity. In transgenic wheat plants expressing vsiRNA1, high levels of this RNA were produced, conferring resistance against WYMV. It was also reported that a wheat thioredoxin-like gene (TaAAED1) was targeted by vsiRNA, leading to its silencing [34]. For resistance against bean golden mosaic virus, siRNAs targeting the AC1 gene, responsible for viral replication, were generated in bean plants, resulting in significant resistance [35].

Plants can employ siRNAs to combat pathogens. For instance, as part of defense mechanisms against fungal infections, plants can produce siRNAs that suppress the expression of genes in pathogens, curbing their proliferation. For example, it is well-known that siRNAs are induced for protection against various fungal species. *Sclerotinia sclerotiorum* is a fungal disease that causes economic losses in around 500 different plant species. Leaf lesions caused by this disease in canola plants have been suppressed using siRNA [36]. In another study, siRNAs were utilized to suppress three cytochrome P450 genes in *Fusarium graminearum*, inhibiting fungal growth in barley leaves (Koch et al., unpublished). siRNAs originating from natural antisense transcript (NAT) regions, called nat-siRNAs, are considered an important source for siRNAs induced by environmental stress factors. It is known that nat-siRNAs are induced in response to *Pseudomonas syringae* carrying AvrRpt2 receptor [38].

In vitro synthesis of siRNAs targeting disease-causing genes is feasible. For example, a library of genes related to corn rootworm was created to identify potential targets, and siRNAs specific to these genes were produced in vitro. Feeding these siRNAs to corn rootworm larvae resulted in reduced expression of some genes, leading to retardation in larval development and their death [39].

Although siRNAs are predominantly induced under biotic stresses, they can also be activated in response to abiotic stresses such as heat, salt and drought. siRNAs display differential gene expression under heat stress. In cucumber leaves exposed to 38°C at different time intervals, 536 (1 h), 816 (6 h), and 829 (12 h) siRNAs were shown to be expressed differently. The study identified 795 target genes, including known genes such as serine/threonine-protein kinase SRK2I, CTR-1 like resistance protein RML1A-like, and RPP1, which are associated with heat tolerance and involved in the regulation of flowering [40].

In Arabidopsis, under conditions of salt stress, a nat-siRNA derived from the cis-natural antisense gene pair SRO5 and P5CDH (Δ1-pyrroline-5-carboxylate dehydrogenase) regulates oxidative stress and osmolyte accumulation. The expression of SRO5 increases in response to salt stress, leading to the formation of SRO5-P5CDH dsRNA. This dsRNA is processed by DCL2, RDR6, SGS3, and DNA-dependent RNA polymerase IV (NRPD1A) to generate a 24-nt nat-siRNA. This siRNA targets P5CDH. Consequently, proline is accumulated. This also contributes to the reduction of reactive oxygen species generated under stress conditions [41].

In drought-resistant rice plants, 21-24 nt siRNAs are significantly expressed, and their targets are related to oxidation-reduction and proteolysis processes, which contribute to the phenotypic response under water deficit conditions [42].

These examples illustrate how siRNAs are harnessed by plants to respond to various stressors. SiRNAs form a pivotal component of plants' adaptation mechanisms, aiding in their more effective response to stressors. Through regulating gene expression and influencing interactions, siRNAs contribute to plants' ability to navigate and cope with stress conditions.

5 Conclusions

High crop yield and quality in agriculture are desired conditions for both consumers and producers. Therefore, the investigation of how plants respond to adverse stress conditions is of great importance. Biotic stresses (caused by pathogens, pests, and other living organisms) and abiotic stresses (such as drought, salinity, temperature extremes, and toxic substances) can negatively impact plant growth, development, nutrient uptake, and metabolic processes. However, plants have evolved various molecular mechanisms to cope with these stresses. Plant responses to stress involve complex molecular and physiological changes. These adaptive responses are regulated by signaling pathways, gene expression, protein modifications, and the accumulation of specialized metabolites. For instance, small non-coding RNAs, like microRNAs (miRNAs) and small interfering RNAs (siRNAs), play pivotal roles in post-transcriptional gene regulation, enabling plants to adapt to stress conditions. Understanding stress tolerance and adaptation is instrumental in breeding resilient plant varieties with increased yield and quality. Ongoing research in agriculture has shown that by elucidating the mechanisms underlying plant responses to stress and leveraging modern biotechnological tools, such as gene editing techniques like CRISPR/Cas9, it is possible to engineer plants with improved stress tolerance and overall performance.

In conclusion, investigating how plants respond to adverse stress conditions is of paramount importance in agriculture. Such research can lead to the development of stress-tolerant plant varieties, ultimately benefiting both consumers and producers by enhancing crop yield and quality and contributing to the sustainability of agriculture.

6 Declarations

6.1 Study Limitations

None.

6.2 Funding source

None.

6.3 Competing Interests

There is no conflict of interest in this study.

6.4 Authors’ Contributions

Developing ideas for the article, B.Y. and N.Ü.; Taking responsibility for the literature review during the research, B.Y.; Writing-review and editing, B.Y.; Taking responsibility for the creation of the entire manuscript, B.Y.; approving the final editings of the text B.Y and N.Ü.

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