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Research Article

In silico determination of potential miRNAs encoded in wheat genome that target *AvrSr35* in *Puccinia graminis* f. sp. *tritici*

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ABSTRACT

Wheat (*Triticum aestivum* L.) belonging to Poaceae family is a valuable staple food all over the world. *Puccinia graminis* f. sp. *tritici* is one of the major limiting factors in wheat production, causing stem rust disease. RNA interference (RNAi) is an effective technique for plant production against biotic stresses. One of the most investigated RNAi molecules is microRNAs (miRNAs). It is well established that miRNAs play an important role in the regulation of gene expression at the post-transcriptional level. In the present study, wheat miRNAs-stem rust interactions were investigated by using *in silico* methods. For this purpose, reference mature wheat miRNAs were retrieved from miRBase, and the sequences of avirulence gene (*AvrSr35*) in *P. graminis* f. sp. *tritici* were obtained from NCBI. RNAhybrid algorithm was used to predict the relationships between miRNAs and avirulence gene targets. Moreover, a phylogenetic tree was constructed by using miRNAs via MEGA X. It was determined that *AvrSr35* gene was targeted by a total of 12 miRNAs including tae-miR9657a-3p, tae-miR9671-5p, tae-miR1122c-3p, tae-miR1130b-3p, tae-miR9678-3p, tae-miR9781, tae-miR9666b-3p, tae-miR531, tae-miR9773, tae-miR9778, tae-miR9677b and tae-miR10516. As a result of phylogenetic analyses, we observed that all miRNAs were separated into three major groups and they showed close relationships. The first main group consisted of only tae-miR9778. Obtaining findings are expected to contribute roles of miRNAs in the interaction between disease-related miRNAs in plants and pathogens.

1. Introduction

Wheat (*Triticum aestivum* L.) belongs to the *Poaceae* family and is the most widely cultivated cereal crop in the world. Due to cultivation for tens of thousands of years, wheat is one of the field crops with the largest agricultural area and the highest production amount [1, 2]. It is one of the most important nutritional sources and indispensable main products in food industry [3]. More than 40% of the daily calorie intake is provided by this product [4]. The wheat production area is 7 million hectares in Turkey, while its production is 221 million hectares worldwide [5].

Wheat is infected by many pathogens during the life cycle [5]. They are the main constraints for wheat production all over the world [1]. About 50 wheat diseases are widely distributed and cause economic losses. Approximately 20% of wheat is lost because of wheat diseases. Some of them occur as a result of bacteria and virus infections [5]. However, fungal phytopathogens are the primary cause of wheat diseases. These are rust, smut, spot blotch, *Fusarium* head blight, root rot, septoria blotch, powdery mildew, and blast. Fungi-borne rust infections in wheat crops result in significant losses [6, 7]. The stem rust caused by *P. graminis* f. sp. *tritici* is one of the most destructive diseases [8]. The other rust diseases of wheat such as leaf rust and stripe rust cause 60% yield losses, whereas stem rust causes up to 100% losses in case of epidemic [9].

A variety of defence mechanisms for recognizing pathogen invasions are evolved by plants. These defence responses alter host cellular function and can be regulated by miRNAs [9]. miRNAs are a type of short non-coding single-stranded regulatory RNAs with 18-24 nt in length [10]. They regulate the gene expression, binding target mRNA through direct cleavage or its suppression at the post-transcriptional level [11]. Recently, the determination of miRNAs on different plant species has gained considerable momentum to figure out the biotic stress defence [7, 12-14]. In addition, miRNAs have important roles in regulating plant-pathogen interactions [15, 16]. Several miRNAs responding to biological stresses such as fungi, bacteria and viruses have been determined thanks to recent advances in high-throughput technologies [15, 17]. Previous studies have demonstrated that miRNAs are involved in the control mechanism of biotic stresses in wheat plants [9, 18-21]. This study aimed to investigate the interactions between miRNAs in wheat genome and the rust disease avirulence gene *AvrSr35*.

2. Material and Method

2.1. Obtaining Wheat miRNAs and Pathogen *AvrSr35* Gene

Previously identified mature miRNAs in wheat genome were obtained from miRBase (https://www.mirbase.org) [22]. Moreover, *AvrSr35* gene sequences of *P. graminis* f. sp. *tritici* (Accession Number MF474174.1) were retrieved from NCBI.

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2.2. Target Identification and Data Visualization

RNAhybrid web tool was operated to predict wheat miRNAs targeting *AvrSr35* as stated by Krüger and Rehmsmeier [22]. To illustrate the graphical representation, all miRNAs targeting the related gene were analysed with the ggplot2 package (version 3.4.4) of R studio (version 4.3.2) [23].

2.3. Phylogenetic Analyses

The multiple sequence alignments of wheat miRNAs targeting *AvrSr35* gene were conducted by using Clustal Omega program (www.ebi.ac.uk/Tools/msa/clustalo). By using the acquired sequence alignments, evolutionary analysis was performed via MEGA X [24] with adjusted parameters with the neighbor-joining (NJ) method [25], p-distance model [26] and 10,000 repeats of the bootstrap resampling [28].

3. Results and Discussion

In this investigation, a total of 125 tae-miRNAs previously identified in the wheat genome were examined and 12 of them were predicted to target *AvrSr35* gene (Table 1). All wheat miRNAs obtained from target identification by using RNAhybrid algorithm were analysed using R-language through R studio to describe as a graphical representation. Twelve miRNAs targeted *AvrSr35* at nucleotide positions among 132-11.168. Among them, tae-miR1122c-3p and tae-miR1130b-3p targeted the gene at nucleotide position 7692 (Figure 1).

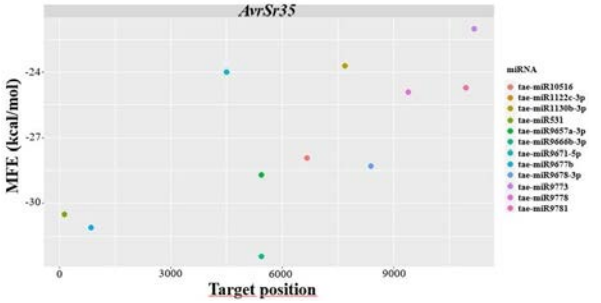

Fig. 1. Estimated target binding positions and MFEs (Minimum Free Energy, kcal/mol) of tae-miRNAs

Furthermore, the multiple sequence alignment analyses showed similarities among tae-miRNAs (Figure 2). A phylogenetic tree was created by using 12 miRNA sequences. In the phylogenetic tree, tae-miRNAs were clustered into three distinct major groups. The first group consisted of tae-miR9778 in one clade. The second group consisted of five miRNA sequences including tae-miR9666b-3p, tae-miR9677b, tae-miR531, tae-miR9678-3p and tae-miR10516. The other tae-miRNAs formed the third group (Figure 3).

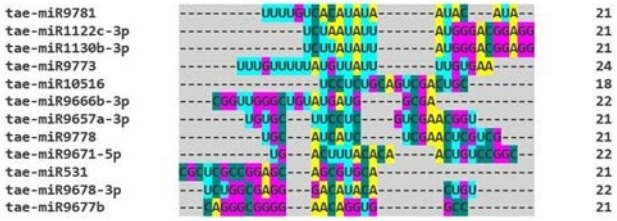

Fig. 2. The multiple sequences alignments of tae-miRNAs

Table 1. RNAhybrid results of tae-miRNAs which target *AvrSr35* gene

Wheat miRNAs	RNAhybrid results
tae-miR9657a-3p	target 5' C CG UCAAGCGAAA U A3' GCCGU CGAUG AGGAAGC CA UGGCA GCUGC UCCUUCG GU miRNA 3' A U 5'
tae-miR9671-5p	target 5' A AAUUAGGCCCCAUUU CCAUAAUUU AUUUU A 3' GCUGGAU GGUUGU GUA GAGUCA CGGCCUG UCAACA CAU UUCAGU miRNA 3' 5'
tae-miR1122c-3p	target 5' G UAAUCAAA CUGUUUGA U 3' CUCUG UCCCGUGAUGUU AGA GAGGC AGGGUAUUAUAA UCU miRNA 3' G 5'
tae-miR1130b-3p	target 5' G UAAUCAAA UCUGUUUG U 3' CUCUG UCCCGUGAUGU AAGA GAGGC AGGGUAUUAUA UUCU miRNA 3' G 5'
tae-miR9678-3p	target 5' U ACU GAAACU A U A 3' GCAG UG UGUCCC CG CCAGA UGUC AC ACAGGG GC GGUCU miRNA 3' AU A 5'
tae-miR9781	target 5' U CACAAAUAAUC C 3' UAUGUGU UAUGUGUGACAAA AUACAUA AUAUACACUGUUU miRNA 3' U 5'
tae-miR9666b-3p	target 5' C CG GUCAAGCGAAAAGGA G 3' CGCCGUCG AU AGCUCAACCG GCGGUAGU UG UCGGGUUGGC miRNA 3' A A 5'
tae-miR531	target 5' A A UCGCUACU CUC A 3' GCAUG UGCUC CGGCGA GC CGUGC ACGAG GCCGCU CG miRNA 3' A G C 5'
tae-miR9773	target 5' G GU U 3' U UGAAGUGGCAUGAGGAUAGA A GUUUUAUUGUAUUUUUGUUU miRNA 3' A GU 5'
tae-miR9778	target 5' G A GA CGAC U 3' GGC GAG UCGGGAUGAU GC CUG CUC AGCUCUACUA CG miRNA 3' G A U 5'
tae-miR9677b	target 5' A UCGACCCUUAUCG GCG G A 3' GGCUACCU GUUUC CCG CCUG CCGGUGGA CAAGG GGC GGAC miRNA 3' G 5'
tae-miR10516	target 5' U U GAAUGUGUGACGA C 3' GC GUCGAUUGCAG GGGA CG CAGCUGACGUC UCCU miRNA 3' U 5'

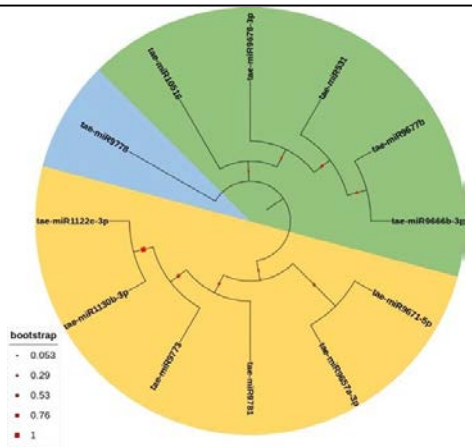

Fig. 3. Phylogenetic tree of tae-miRNAs

Plant pathogens pose serious threats in agricultural areas, causing both crop and yield losses [27]. Because of this, disease management strategies must be regulated in terms of modern plant protection methods. The plant miRNAs are known to be involved in disease modulation by interacting with several regions of related genes during pathogen infections [28-30]. Therefore, we examined the interactions between wheat miRNAs and *AvrSr35* gene of *P. graminis* f. sp. *tritici* in terms of *in silico* analyses. There are many studies related to miRNA-phytopathogen interaction in wheat and other plants [31-36]. One of them was performed by Gupta et al. [9]. They investigated the regulatory functions of 8 miRNAs (miR159, miR164, miR167, miR171, miR444, miR408, miR1129 and miR1138) against stem rust caused by *P. graminis* f. sp. *tritici* in wheat. The result showed that all miRNAs played key roles in suppressing the pathogen through hypersensitive reactions (HR).

We found that tae-miR1130b-3p targeted the stem rust in stress response. Moreover, tae-miR1130b-3p was a sister group to miR1122c-3p. A similar study was conducted by Nair et al. [37]. They investigated disease-related miRNAs and their targets involved in the defence of wheat against leaf rust, powdery mildew and wheat blast disease. Their results showed that tae-miR1130b-3p targeted both blast disease and powdery mildew. In addition, previous studies have also reported the role of miR1122c-3p in abiotic stress response [38].

As a result of our findings, the avirulence gene of *P. graminis* f. sp. *tritici* was also targeted by tae-miR9666b-3p. Concordant with this result, Ramachandran et al. [17] showed that tae-miR9666b-3p is involved in resistance to stripe rust. They investigated the defence responses of a total of 23 tae-miRNAs against stripe rust caused by *P. striiformis* f. sp. *tritici* in the wheat-rust phyto-pathosystem. Another miRNA investigated in this study was miR9657a-3p. In a similar study, Jin et al. 2020 [39] evaluated miRNA-*Fusarium graminearum* interactions in wheat. They examined tae-miR9657a-3p, tae-miR9670, tae-miR9655, and tae-miR9676, and reported that in particular, tae-miR9657a-3p positively regulated the plant response to the pathogen by repressing its target genes. Similar findings for miR9657a-3p were also obtained by Hu et al. [40], investigating the regulatory roles of miRNAs against powdery mildew in wheat. Moreover, Feng et al. [35] identified a novel wheat miRNA, named PN-2013. Research data shows that this miRNA contributes to resistance against stripe rust, regulating the monodehydroascorbate reductase gene through reactive oxygen species metabolism.

miRNAs activated by pathogens can target multiple genes. Each of these can control a cascade of other pathways, regulating the whole cellular process [43, 44]. For example, tae-miR531, tae-miR9778, tae-miR9781 and tae-miR9773 examined in our study were found to be associated with drought stress [41-43]. Samavatian et al. [21] analysed the potential roles of 9 tae-miRNAs including tae-miR156, tae-miR159, tae-miR167, tae-miR171, tae-miR393, tae-miR166b, tae-miR169, tae-miR408, tae-miR444 in response to *Zymoseptoria tritici*. As a result, they reported that tae-miRNAs, especially miR171, miR408 and miR444, played active roles in disease resistance in wheat. In addition, these miRNAs have been reported to respond to stress by regulating the transcripts of several laccase genes involved in cell-to-cell signaling, lignin formation, and abscisic and gibberellic acid pathways. The other study was conducted by Xin et al. [45]. The results indicated that 24 miRNAs in wheat genome respond to powdery mildew infection caused by *Blumeria graminis f. sp. tritici*. Moreover, it was also revealed that some of these miRNAs were involved in defence mechanism against heat stress in wheat. Although miRNAs are conserved molecules among plants, pathogen species differentially influence miRNA regulation in terms of defence response. It may depend on various types of pathogen effectors or how plants identify distinct pathogen effectors [46].

4. Conclusions

Gaining new insights into the regulatory functions of miRNAs in the interactions between plants and fungi holds potential for unveiling the molecular mechanisms behind plant defence responses. Therefore, target gene identification by computational studies is an important step to reveal miRNA-mediated complex regulatory networks. In this study, the roles of wheat miRNAs in the arms race between host and pathogens were investigated *in silico* methods. The findings of our study provide considerable knowledge related to disease resistance in the wheat-stem rust pathosystem. In this sense, further experimental expression studies need to understand the function of miRNAs in phyto-pathosystems. The studies related to miRNA-mediated processes in different pathosystems have important effects to improve new insight for disease control, and result in crop productivity.

Declaration of conflicting interests

The authors declare no competing interests.

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