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In silico analyses of miRNAs that Target Odorant Binding and Chemosensory Proteins in *Bemisia tabaci*

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ABSTRACT

The whitefly, *Bemisia tabaci*, damages various crops by releasing honeydew and spreading. Although farmers and pest control experts primarily rely on insecticides to manage whiteflies, the notable issue is their tendency to develop resistance to major insecticide categories, posing a significant challenge. This result has led to the improvement of new drugs or insecticide mixtures. In addition, some plant-based studies have been conducted to control whiteflies, and RNA interference (RNAi) technology has been used in recent years. This study aimed to identify the relationships between tobacco, cotton, tomato, and linen miRNAs and odorant-binding protein (*OBP*) and chemosensory protein (*CSP*) genes in whiteflies by using *in silico* approaches. We determined that 115 miRNAs belonging to these plants targeted 13 *CSP* and 8 *OBP* genes of *B. tabaci*. Obtaining findings are important to reduce dependency on chemicals and pesticides in pest management.

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Introduction

Various pests and pathogens cause a reduction in plant growth and development. These pathogens, including viruses, bacteria, fungi and oomycetes, can be commensal, symbiotic, and pathogenic characteristics (Wang et al., 2022; Karanfil et al., 2023; Randa-Zelyut et al., 2023). Certain pathogens can directly penetrate plant tissues, while others enter through wounds or natural openings. Vector-borne pathogens can be introduced directly into vascular tissues. Each tissue invasion method differs in different cells and tissue types (Faulkner et al., 2012; Huang et al., 2020; Kashyap et al., 2020).

Besides plant pathogen microorganisms, plant pests are also responsible for 18-26% of worldwide crop loss annually (Culiney et al., 2014). These pests include insects, mites, nematodes, birds, and others. They can cause damage to crops by feeding on plant tissues, transmitting diseases, competing for nutrients, or inhibiting plant growth and development. Effective management of plant pests is essential for maintaining agricultural productivity and ensuring food security (Lucas et al., 2011).

The whitefly, *B. tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is one of the most important agricultural pests (Stansly et al., 2010; Inoue-Nagata et al., 2016; Czosnek et al., 2017). It affects crops through direct feeding or transmission of viruses (Brown et al., 1995; Navas-Castillo et al., 2011). More than 350 viral species, including Begomovirus, Carlavirus, Crinivirus, Ipomovirus, and Torradovirus, have been documented to infect over 1000 plants (Jones et al., 2003; Abd-Rabou et al., 2010; Götz et al., 2016; Rodríguez et al., 2019; Lu et al., 2019). Yellowing, leaf folding, reduced growth, and malformed fruit can be seen in affected plants (Khan et al. 2018). *B. tabaci* Middle East-Asia Minor1 (MEAM1) and *B. tabaci* Mediterranean (MED) species are highly invasive and have inflicted significant economic losses (Luo et al., 2002; Chu et al., 2006).

It has been proved that controlling whiteflies is difficult. Insecticides are used to manage this pest because of their convenience and efficacy. However, targeting whiteflies with insecticides is problematic due to their feeding habits on the underside of the leaves (Simmons et al., 2000; Simmons et al.,2005; Simmons et al., 2011; Horowitz et al., 2020). Therefore, whitefly management can be accomplished through a combination of physical and mechanical methods, biological control, plant-based products and/or biotechnical strategies (Perring et al., 2018; Razze et al., 2016; Ibrahim et al., 2017; Shejulpatil et al., 2019). Furthermore, biotechnological strategies for *B. tabaci* include transgenesis and RNA interference (RNAi) (Pan et al., 2012; Hunter et al., 2021).

The ability to perceive chemicals and detect odors is vital for the normal life cycle of insects, playing a crucial role in numerous physiological behaviors, including mating, host-seeking, and distinguishing food sources (Leal et al., 2013; French et al., 2015). Proteins associated with insect olfaction primarily comprise odorant binding proteins (*OBP*s), chemosensory proteins (*CSP*s), odorant receptors (ORs), and sensory neuron membrane proteins (Liu et al., 2012; Liu et al., 2015). *OBPs* and *CSPs* within this group are small soluble proteins found in high concentrations in the sensillum lymph of insects, particularly in the head region (Honson et al., 2005; Pelosi et al., 2006; Zhou et al., 2010). These proteins play vital roles in aiding insects to detect chemical signals, thereby significantly influencing their behaviors (Tunstall et al., 2012). Consequently, they represent important molecular targets for designing and developing new pest management systems (Qiao et al., 2009; Sun et al., 2012). Our aim was to determine the targets of whitefly chemical and odor genes in specific plants and to target *B. tabaci* by using *in silico* methods.

Materials and Methods

Retrieval of Tobacco, Cotton, Tomato and Linen

Mature miRNAs belonging to tobacco, cotton, tomato and linen miRNAs were retrieved from miRBase (https://www.mirbase.org). miRBase is a public repository and standard online reference source for all documented miRNA sequences, offering textual annotations and gene nomenclature (Kozomara et al. 2019). We obtained 60 tobacco, 50 cotton, 101 tomato and 16 linen mature miRNAs from miRBase and examined them in this study.

Obtaining from CSP and OBP genes

The sequence of 13 *CSP* and 8 *OBP* genes of *B. tabaci* were downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). These genes and accession numbers were shown in Table 1.

Table 1. *CSP* and *OBP* sequences

Gene Name	Accession Number
CSP1	KT694344
CSP2	KT694345
CSP3	KT694346
CSP4	KT694347
CSP5	KT694348
CSP6	KY305449
CSP7	KY305450
CSP8	KY305451
CSP9	KY305452
CSP10	KY305453
CSP11	KY305454
CSP12	KY305455
CSP13	KY305456
OBP1	KY305457
OBP2	KY305458
OBP3	KY305459
OBP4	KY305460
OBP5	KY305461
OBP6	KY305462
OBP7	KY305463
OBP8	KT358500

Target Prediction

RNAhybrid tool was used (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid) to target predictions. For this purpose, a total of 227 miRNAs belonging to tobacco, cotton, tomato and linen were investigated whether they target *OBP* and *CSP* genes or not. The results were evaluated according to Marakli (2020). The positions and MFE values of miRNAs were recorded and the graph was drawn using MATLAB (MATLAB , R2023b MathWorks, Natick, MA, USA).

Phylogenetic Tree

After the detection of miRNA that targets *CSP* and *OBP* genes, a phylogenetic tree was constructed to examine evolutionary relationships. For this purpose, the MEGA X (Kumar et al., 2018) program was used with the following criteria: Neighbor-Joining method (Saitou et al., 1987), genetic distances computed using the p-distance model (Nei and Kumar,2000) and even bootstrap resampling using 1000 replicates (Felsenstein, 1985). After the phylogenetic tree was created in MEGA X, Interactive Tree of Life (iTOL) (https://itol.embl.de) was used to manage and display the phylogenetic tree (Letunic and Bork, 2024).

Results

According to the rules for target prediction, we identified 23 and 21 tobacco miRNAs that target *CSP* and *OBP* genes, respectively. For cotton, the numbers of miRNAs were 10 for *CSP* and 12 for *OBP*. Moreover, tomato miRNAs were 34 and 21 for *CSP* and *OBP* genes, respectively while 7 and 9 linen miRNAs were detected. We detected that some miRNAs target both *CSP* and *OBP* genes. The table containing plant genes, their interacting miRNAs, the corresponding interaction positions, and the MFE values were shown in Table 2a,b,c. The positions and MFE values of miRNAs were indicated in Figure 1.

The evolutionary relationships among a total of 100 miRNAs belonging to tobacco, tomato, linen and cotton which target *CSP* and *OBP* genes were evaluated. We observed that all sequences were divided into 3 groups in the phylogenetic tree. Furthermore, miRNAs found in 4 different plants were detected in each clade (Figure 2). Our analysis revealed that some of the miRNAs are classified as sister taxa. For example, sly-miR10535a and sly-miR10535b were found in sister clade that were predicted to interact with *CSP* 11, while nta-miR479a was a sister group to nta-miR479b which targeted *OBP* 8. Furthermore, sly-miR171d shared high sequence homology with sly-miR171e, targeting *CSP* 7. These miRNAs are predicted to interact with the same genes, supporting the notion that similar sequences may target similar genes (Friedman et al., 2009).

Nevertheless, the sequences appear to target the same gene even if they are not similar. For instance, nta-miR1919 and sly-miR1919a were found in sister clades and both of them were predicted to target *CSP* 5. Similarly, nta-miR399a and ghr-miR399a form another pair of sister clades, targeting *CSP* 6. Furthermore, there was another sister clade including sly-miR397-5p and lus-miR397a which targeted *CSP* 10.

Table 2a. Detail information for miRNA and their targets

Gene	Plant	miRNA	Position	MFE (kcal/mol)
CSP1	Tobacco	nta-miR168a	197	-34.2
	Cotton	ghr-miR3476-5p	55	-33.2
		ghr-miR7496a	1	-22.4
	Tomato	sly-miR394-3p	18	-24.2
		sly-miR477-5p	140	-24.3
	Linen	lus-miR395e	268	-23.8
CSP2	Tobacco	nta-miR477b	307	-21.6
	Cotton	ghr-miR482b	318	-22.2
	Tomato	sly-miR9470-3p	318	-21.4
		sly-miR166c-5p	99	-23.7
		sly-miR9476-5p	248	-22.6
		sly-miR9479-5p	265	-21.8
		sly-miR399b	316	-21.5
CSP3	Tomato	sly-miR482e-5p	221	-25
		sly-miR164a-3p	326	-24.2
		sly-miR403-5p	175	-21.2
		sly-miR482d-5p	40	-24.3
		sly-miR10537	326	-20.7
		sly-miR7981f	172	-23.6
CSP4	Tobacco	nta-miR5303c	5	-24.4
	Cotton	ghr-miR7497	3	-24.1
		ghr-miR7504a	9	-22.6
	Tomato	sly-miR156e-3p	23	-24.6
		sly-miR477-3p	108	-26.3
		sly-miR10531	139	-28.2
	Linen	lus-miR167a	7	-25.7
CSP5	Tobacco	nta-miR1919	46	-24.3
		nta-miR6148b	337	-21.3
	Tomato	sly-miR1919a	46	-24.9
		sly-miR1919c-5p	254	-23.3
		sly-miR9471a-3p	58	-24
CSP6	Tobacco	nta-miR399a	205	-25.4
		nta-miR477b	183	-26.7
		nta-miR1919	29	-26.7
		nta-miR6025c	179	-21.7
		nta-miR6150	457	-23.5
		nta-miR5303c	180	-27.9
		nta-miR6161d	168	-22.9
	Cotton	ghr-miR399a	554	-23.7
		ghr-miR3476-5p	420	-26.7
		ghr-miR7489	222	-28
	Tomato	sly-miR6023	552	-20.9
		sly-miR482c	218	-21.7
		sly-miR6026	323	-22.4
		sly-miR319c-5p	546	-27.3
		sly-miR9469-3p	599	-27.5
		sly-miR167b-3p	215	-22.7
		sly-miR10536	161	-21.9
	Linen	lus-miR398f	686	-28.5
CSP7	Cotton	ghr-miR7489	151	-22.2
	Tomato	sly-miR171d	172	-27.1
		sly-miR395a	50	-25.5
		sly-miR171e	164	-25.8
	Linen	lus-miR398f	164	-25.5
CSP8	Tobacco	nta-miR6148a	279	-22.2
		nta-miR6150	288	-20.2
	Cotton	ghr-miR2948-5p	18	-20.9
	Tomato	sly-miR1918	114	-22.6
		sly-miR394-3p	1	-21.7
	Linen	lus-miR398f	149	-20.5

Table 2b. Detail information for miRNA and their targets

Gene	Plant	miRNA	Position	MFE (kcal/mol)
CSP9	Tobacco	nta-miR6020b	251	-20.9
		nta-miR6144	235	-22.1
		nta-miR5303b	44	-23
		nta-miR6161c	161	-22.8
	Cotton	ghr-miR2950	20	-26
	Tomato	sly-miR1918	177	-26.4
		sly-miR9469-3p	234	-21.4
CSP10	Tobacco	nta-miR6154b	77	-22.7
	Cotton	ghr-miR3476-5p	54	-23.7
	Tomato	sly-miR397-5p	151	-23.3
		sly-miR9471a-3p	119	-23.9
	Linen	lus-miR397a	151	-24.1
CSP11	Tobacco	nta-miR477b	176	-24.9
		nta-miR6145a	163	-23
	Cotton	nta-miR6163	22	-20.4
		ghr-miR7506	279	-33.3
	Tomato	sly-miR1918	75	-21.8
		sly-miR6022	323	-22.8
		sly-miR403-5p	271	-21
		sly-miR10531	141	-25.5
		sly-miR10535a	99	-22.5
		sly-miR10535b	99	-21.6
	Linen	lus-miR395e	156	-22.1
		lus-miR398c	199	-25.7
		lus-miR398e	198	-28
		lus-miR398f	199	-24.6
CSP12	Tobacco	nta-miR397	66	-23.8
		nta-miR479a	38	-20.1
		nta-miR6149b	285	-25.5
	Linen	lus-miR169d	1	-23
CSP13	Tobacco	nta-miR1919	281	-21.8
		nta-miR6150	262	-7.2
		nta-miR6155	159	-23.8
	Cotton	nta-miR6157	95	-21.3
		ghr-miR482b	40	-23.6
		ghr-miR2948-5p	18	-26.8
	Tomato	sly-miR6022	101	-20.4
		sly-miR482c	60	-25.4
OBP1	Tobacco	nta-miR479b	182	-21.5
	Cotton	ghr-miR169b	37	-23.5
	Tomato	sly-miR6027-5p	54	-21.4
		sly-miR9471b-3p	144	-24.1
		sly-miR9471a-3p	247	-22.6
		sly-miR156e-5p	22	-26.6
		sly-miR156e-3p	135	-24.2
		sly-miR399b	138	-27.8
		sly-miR10536	185	-22.3
	Linen	lus-miR159c	149	-24.1
		lus-miR396d	130	-21.5
		lus-miR398e	249	-20.7
OBP2	Tobacco	nta-miR168a	637	-27.9
		nta-miR6158a	39	-25.3
	Cotton	ghr-miR160	6	-25.3
		ghr-miR7503	108	-20
	Tomato	sly-miR1919a	585	-20.3
		sly-miR9476-5p	95	-20.8

Table 2c. Detail information for miRNA and their targets

Gene	Plant	miRNA	Position	MFE (kcal/mol)
OBP3	Tobacco	nta-miR171a	623	-21.9
		nta-miR6154b	266	-23.5
	Cotton	ghr-miR160	20	-26.6
		ghr-miR7510a	90	-30.8
	Tomato	sly-miR1918	706	-23.8
		sly-miR6023	84	-21.4
		sly-miR397-3p	29	-22.4
	Linen	lus-miR167a	211	-23.4
		lus-miR159b	87	-25.2
		lus-miR172j	117	-26.6
		lus-miR159c	88	-25.5
		lus-miR398f	67	-29
OBP4	Tobacco	nta-miR6144	250	-24.6
		nta-miR6154b	42	-26.9
		nta-miR6155	392	-26.3
	Tomato	sly-miR395a	353	-24.3
		sly-miR10531	234	-21.3
OBP5	Tobacco	nta-miR6021	307	-24.6
		nta-miR6024	485	-20
		nta-miR6145e	340	-20.5
	Cotton	ghr-miR172	415	-23
	Tomato	sly-miR9478-3p	145	-23.4
	Linen	lus-miR166b	386	-28.4
		lus-miR172j	214	-20.9
OBP6	Tobacco	nta-miR482b-5p	13	-22.5
		nta-miR169t	9	-21
		nta-miR477a	152	-22.8
		nta-miR477b	152	-21.2
		nta-miR6144	141	-22
		nta-miR6145e	318	-25.2
	Cotton	ghr-miR2949a-5p	388	25.9
		ghr-miR2949c	388	-23.7
	Tomato	sly-miR482e-5p	14	-23.5
		sly-miR6026	127	-23
		sly-miR169e-5p	8	-21.7
		sly-miR398a	352	-30.4
		sly-miR10533	152	-20.3
	Linen	lus-miR398c	353	-27.3
		lus-miR172j	6	-22.8
		lus-miR398e	353	-27.4
		lus-miR398f	352	31.6
OBP7	Tobacco	nta-miR477a	690	-25.4
		nta-miR477b	690	-25.3
		nta-miR6145a	227	22.3
	Cotton	nta-miR6163	267	-25.6
		ghr-miR7485	586	-28.7
	Tomato	sly-miR9469-3p	123	-24.4
		sly-miR9476-5p	621	-24.2
		sly-miR10533	563	-20.1
	Linen	lus-miR398c	9	-30.6
		lus-miR398e	7	-31
OBP8	Tobacco	nta-miR168a	28	-25.3
		nta-miR479a	285	-22
		nta-miR479b	285	-21.3
		nta-miR6025e	14	-22
		nta-miR6156	162	-21.1
		nta-miR6145d	354	-21.3
		nta-miR6145e	354	-21.6
		nta-miR5303c	5	-22.3
	Cotton	ghr-miR399c	120	-20.4
		ghr-miR7497	325	-22.4
		ghr-miR7505	410	-21.4
		ghr-miR7512	398	-23.7
	Linen	lus-miR398f	218	-25.5

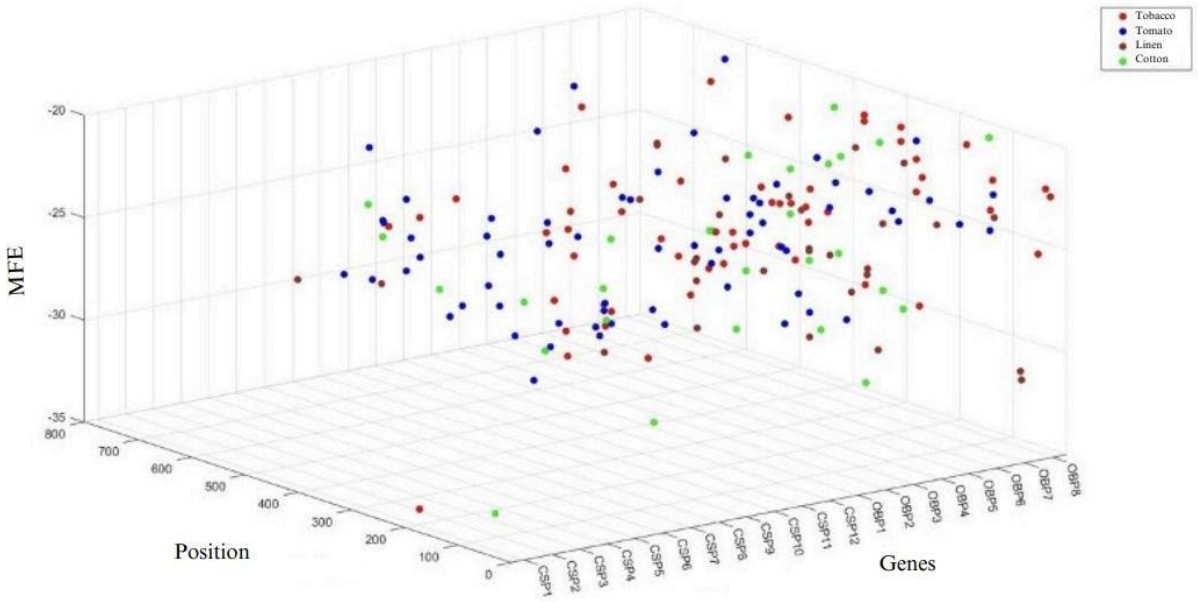

Figure 1. The 3D graphics of miRNAs. Red, blue, brown and green dots indicated tobacco, tomato, linen and cotton, respectively.

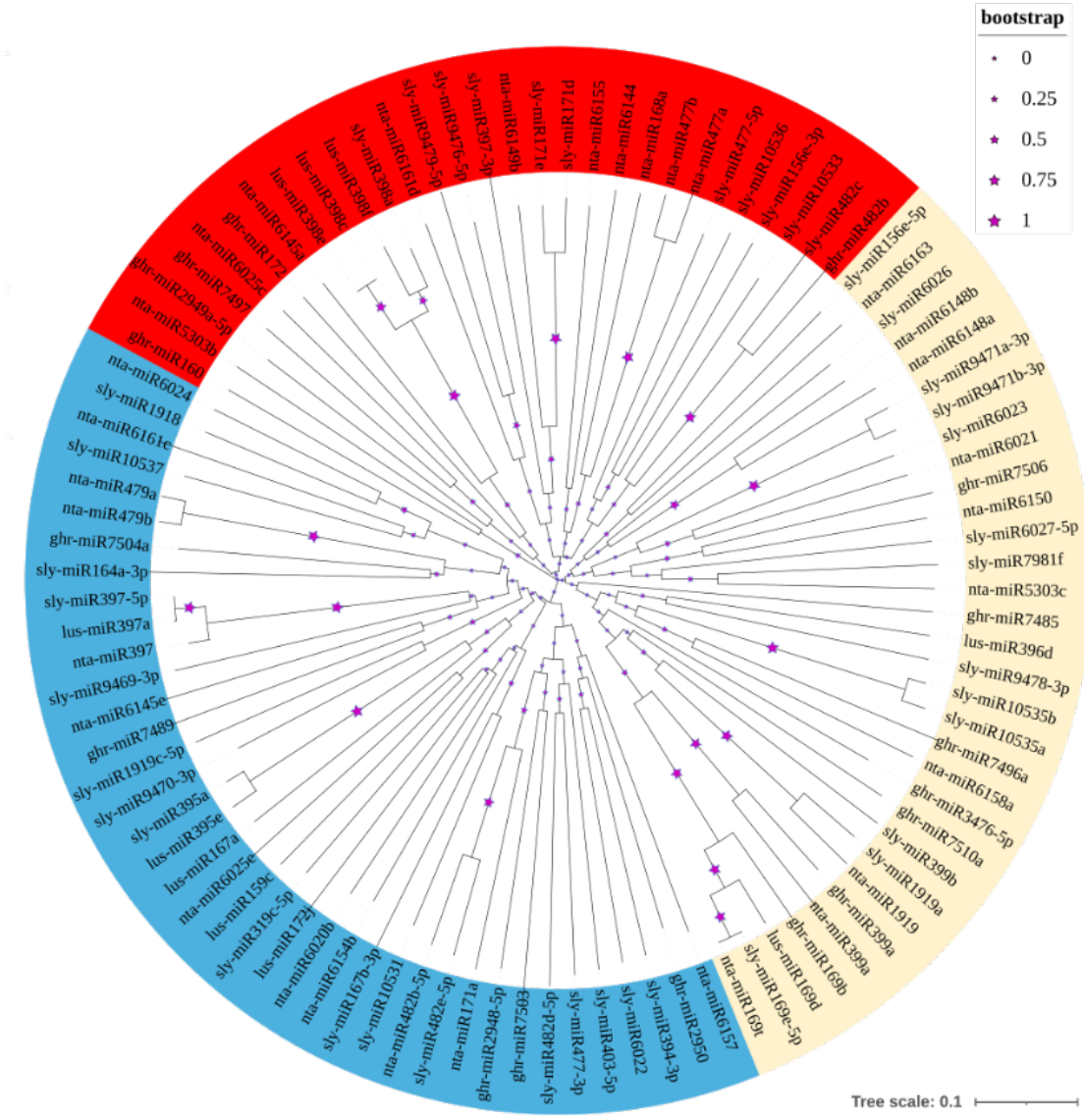

Figure 2. Phylogenetic tree generated using miRNAs belonging to tobacco, tomato, linen and cotton.

On the other hand, nta-miR482b-5p and sly-miR482e-5p were sister groups to nta-miR169t and sly-miR169e-5p, and targeted *OBP* 6. Similarly, lus-miR172j and ghr-miR172 were found as a sister group, targeting *OBP* 5. This observation suggests that while sequence similarity may often correlate with similar gene targets, this relationship is not universally applicable (Affonso-Grunz and Müller, 2015).

Discussion

The whitefly is an important pest that causes serious damage to various plants, and it is crucial to improve new management practices. The use of RNAi technology among applications has been gaining increasing interest in recent years (Animasaun et al., 2023). In these studies, RNAi-mediated gene silencing has been successfully applied to different genes. In this study, we analyzed the relationships between miRNAs - *CSP*s and miRNAs – *OBP*s to determine the potential of candidate miRNAs to be used in the management of this pest. *CSP*s and *OBP*s are responsible for capturing external odorants and transporting them to olfactory receptors which are crucial for the development of the insect olfactory system (Leal et al., 2013; Li et al., 2014; Pelosi et al., 2014). In insects, the number of *CSP* genes varies widely, with nearly 70 in *Locusta migratoria* (Zhou et al., 2013). Similarly, the number of *OBP*-coding genes is different across insect species ranging from 13 in some ants (McKenzie et al., 2014) to over 100 in certain mosquitoes (Manoharan et al., 2013).

In previous studies, *CSP*s and *OBP*s were studied in RNAi studies. Waris et al. (2018) showed that the silencing of a chemosensory protein of *Nilaparvata lugens* changed the behavior of ligand-binding specificity and decreased the behavioral responses. Rebijith et al. (2016) reported that partially silenced *OBP* from *Aphis gossypi* affected antennal response to compounds. Moreover, five sensory-related genes were screened in another pest, *Agrilus planipennis*, for RNAi and they focused on three *OBP*s (Fan et al., 2022). These genes showed significant expression differences between newly emerged and mature emerald ash borers. Following dsRNA injection, gene expression was notably down-regulated. Gong et al. (2012) revealed that silencing *SexiCSP3* in *Spodoptera exigua* females reduced survival, oviposition, and egg hatching, highlighting RNAi's role in studying reproductive genes. These results showed that RNAi technology can be used for insecticides. On the other hand, there is no study about the relationship between plant miRNAs, *CSP*s and *OBP*s genes in whiteflies. We evaluate the interactions between 4 different plants (tobacco, cotton, tomato and linen) that are affected by this pest and 13 *CSP* and 8 *OBP* genes in *B. tabaci*. Recent studies have increasingly focused on understanding the molecular mechanisms underlying the interactions between *B. tabaci* and miRNAs. These small RNAs play a crucial role in regulating gene expression, impacting various physiological processes (Hasegawa et al., 2020; Wang et al., 2020). Researchers are particularly interested in how miRNAs influence the insect's development, reproduction, and adaptability to environmental stressors, as well as how they might contribute to its resistance to insecticides. A plant-mediated strategy using artificial miRNA (amiRNA) targeting three important whitefly genes (*Sxl, AChE,* and *Orc*) was transferred to tobacco plants. They reported decreased whitefly populations with fewer eggs hatching and slower development (Zubair et al., 2020). Furthermore, Gong et al. (2023) provided basic data on whitefly miRNA patterns, highlighting novel_miR-1517 as a key miRNA that regulates *CYP6CM1* gene. This gene is involved in the response to imidacloprid, a pesticide, suggesting that miRNAs could be potential targets for managing imidacloprid resistance in whiteflies. In another study, Shen et al. (2024) identified Bta-miR-998 and Bta-miR-129 which were associated with temperature tolerance.

Conclusion

Previous studies have demonstrated that silencing certain *CSP* (Waris et al., 2018) and *OBP* (Rebijith et al., 2016) genes can alter insect behavior. Considering this, we aimed to identify miRNAs in tomato, tobacco, linen, and cotton, which are commercially significant crops commonly affected by *B. tabaci*. Our analysis revealed a total of 115 miRNAs and 21 genes, which were subsequently matched *in silico*. The results indicated that these genes were compatible with the identified miRNAs. Based on these findings, future studies can focus on targeting these genes using the identified miRNAs to protect these plants against whiteflies. This approach offers a sustainable and environmentally friendly alternative to pest control, reducing reliance on pesticides and chemicals.

Declarations

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the research.

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