Genetic Analysis of an Emerging Agricultural Pathogen of Turkish Hazelnut

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ABSTRACT

Erysiphe corylacearum, a type of fungus that causes powdery mildew in Turkish hazelnut, reduces its economic value and decreases its product quality. The purpose of this project was to understand the genetic structure of the powdery mildew pathogen and establish the foundation principles for developing quantitative PCR. Five selected genes from the fungus were analyzed with PCR methods for characterization of the genetic sequence of *Erysiphe corylacearum.* The optimization was made at the temperature and $MgCl_2$ concentration to attain fungus DNA in each gene. Our results propose an effective DNA extraction method to obtain fungi DNA. Fungus DNA for some selected genes was detected. The long-term future work is to clarify gene sequences of *Erysiphe corylacearum* by using the knowledge of current and next experiments.

Figure 1: Healthy hazelnut leaf sample.

Figure 2: Powdery mildew hazelnut leaf sample.

Observations

Fresh hazelnut leaves from trees cultivated at SUNUM in Sabancı University were observed qualitatively. Dry hazelnut leaves from Akçakoca, Sakarya, Cumayeri were also analyzed microscopically. The morphology of sexual and asexual spores of powdery mildew were examined (Fig. 3 and Fig. 4) to detect species of *Erysiphe corylacearum* (Braun & Cook, 2012).

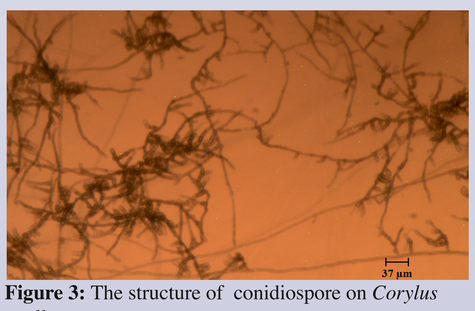

Figure 3: The structure of conidiospore on *Corylus avellana*.

Figure 4: The sexual spore structure of cleistothecium.

Data Analysis

We developed a new DNA extraction method which provides the high nucleic acid concentration from dry leaves at the different region of Black Sea. NanoDrop 2000C spectrophotometer was used to detect the numerical value of nucleic acid absorption (Table 1). The assessment of DNA concentration at A260 and A280 was high for the first sample. 1.8 for DNA purification ratio of 260/280 can be considered enough (Page, 2010).

Table 1: Nucleic acid concentrations and absorbance value of dry hazelnut leaves which collected from different area and time. **C**: Cumayeri, **S:** Sakarya, **A:** Akçakoca, **PM-:** Control group.

#	Name of Sample	N.acid(ng/ul)	A260(abs)	A280(abs)	260/280	260/230
1	09.05.18- C, P.M-	74.7	1.494	0.841	1.78	0.15
2	09.05.18- A, P.M-	52.8	1.056	0.658	1.60	0.08
3	06.06.18- A	47.6	0.952	0.682	1.40	0.11
4	09.05.18- A	39.8	0.795	0.444	1.79	0.12
5	06.08.18- C	33.0	0.661	0.470	1.41	0.05
6	09.05.18- C	23.8	0.476	0.293	1.62	0.04
7	24.05.18- A	48.7	0.975	0.484	2.02	0.11
8	24.05.18- C	27.2	0.543	0.300	1.81	0.05
9	24.05.18- S	24.1	0.481	0.290	1.66	0.06
10	11.06.18- S	31.6	0.633	0.372	1.70	0.05

We used Primer3web (version 4.1.0) to design primers for DNA amplification of *Erysiphe corylacearum* genes. Primer sequences were selected considering 200-800 bp product size, optimal 24 base length and 0 hairpin value (Table 2). By making optimizations to the temperature from 52°C to 60°C and the $MgCl_2$ concentration from 0 ul to 0.5 ul, suitable PCR amplification conditions were formed for the selected genes.

Table 2: Forward and reverse primer sequences of each selected gene is shown. Other parameters are also indicated.

	Name of Gene	Start	Length	Tm	GC%	Hairpin	Sequence (5'-3')
	Forward Primer	42	24	58.95	33.33	0	AACATCGCGATTTTGGTTCAATTA
GPI8	Reverse Primer	201	24	59.02	41.67	0	GCTATAAACAGTTCCAGGAAAAGC
	Product Size:733						
	Forward Primer	242	24	58.97	37.50	0	CAATGCGTTGTGAAAGAATCTGTA
ADE13	Reverse Primer	541	24	59.07	41.67	0	CTTGTCGAGATGCACCTTTAGATA
	Product Size: 729						
	Forward Primer	59	24	59.01	37.50	0	TACTTACGCGTTATCCATCTGTTT
GUK1	Reverse Primer	302	24	59.04	41.67	0	TCCATCTCTATATCAAGAACCGGA
	Product Size:386						
	Forward Primer	703	24	58.78	41.67	0	CATTTGAAGCACAGACCATCTAAG
RNR2	Reverse Primer	943	24	58.85	37.50	0	CTTCCCAGCCAAAGAGATATTTTC
	Product Size:241						
	Forward Primer	65	24	59.01	41.67	0	AAGAAGAGCGACTAGTTGTAATCC
DIB1	Reverse Primer	220	24	59.00	33.33	0	ACATTTGTTTGAAATCTGGCACTT
	Product Size:414						

The attained product size was lower than target product size. Two possibilities can be suggested for these data. The selected genes do not exist in the fungus DNA or DNA extraction method was not successful to obtain fungus DNA. To test assumptions, the PMITS1, PMITS2, TW14, NL1, and PM3 primers which are found fungi were used at the nested PCR to obtain directly target fungus DNA (Nilsson et al., 2008).

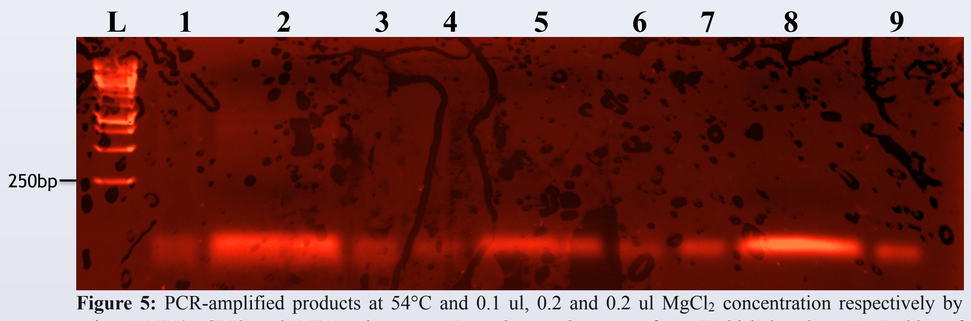

Figure 5: PCR-amplified products at 54°C and 0.1 ul, 0.2 and 0.2 ul $MgCl_2$ concentration respectively by using ADE13, GPI8 and DIB1 primers on *Erysiphe corylacearum* from Turkish hazelnut. L: Ladder of molecular size (1 kb), Bands 1, 2, 3: Primers of ADE13 and 5 ul, 48 ul samples and control group. Bands 4, 5, 6: Primers of GPI8 and 5 ul, 48 ul samples and control group. Bands 7, 8, 9: Primers of DIB1 and 5 ul, 48 ul samples and control group.

Results

Ten dry hazelnut leaves from different groves in Düzce/Sakarya were amplified by using PMITS1 and PMITS2. Fungus DNA was detected at the Cumayeri 24.05.18 sample (Fig. 6). We also observed fungus DNA from the Cumayeri 09.05.18 sample of Fig.7 by using TW14, NL1, and PM3 primers but DNA belonged to the healthy hazelnut. At one point, this can understandable because the nucleic acid concentration of the Cumayeri 09.05.18 sample was higher than other samples (Table 1). It proved that our new DNA extraction method is successful for extraction of fungus genome but the issue could be related to selected genes and their primers.

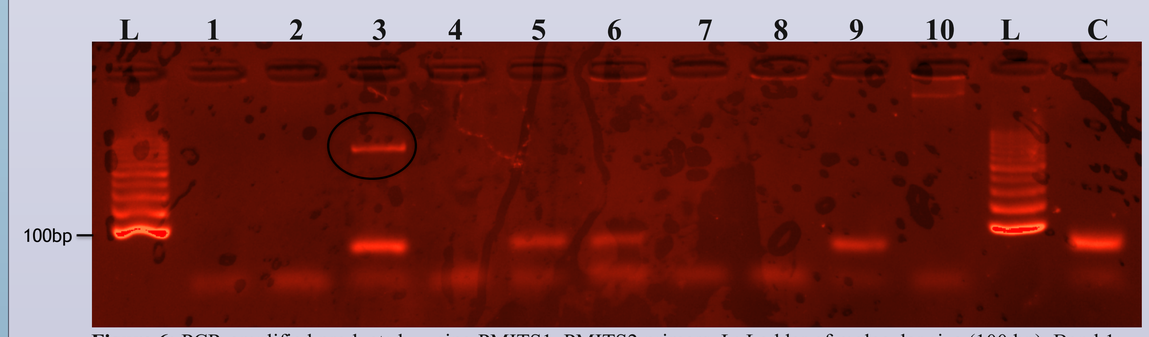

Figure 6: PCR-amplified products by using PMITS1, PMITS2 primers. L: Ladder of molecular size (100 bp), Band 1: 24.05.18-S, Band 2: 24.05.18-A, Band 3: 24.05.18-C, Band 4: 09.05.18-A, Band 5: 09.05.18-C, Band 6: 09.05.18-A, P.M-, Band 7: 09.05.18-C, P.M-, Band 8: 11.06.18-S, Band 9: 06.06.18-A, Band 10: 06.08.18-C, C: Control group.

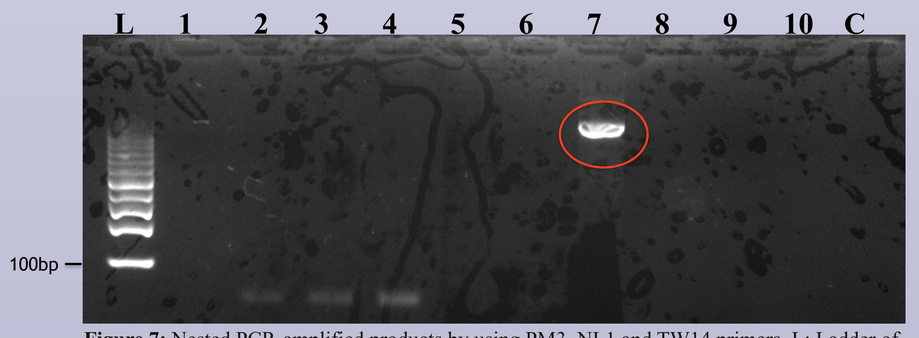

Figure 7: Nested PCR-amplified products by using PM3, NL1 and TW14 primers. L: Ladder of molecular size (100 bp), Band 1: 24.05.18-S, Band 2: 24.05.18-A, Band 3: 24.05.18-C, Band 4: 09.05.18-A, Band 5: 09.05.18-C, Band 6: 09.05.18-A, P.M-, Band 7: 09.05.18-C, P.M-, Band 8: 11.06.18-S, Band 9: 06.06.18-A, Band 10: 06.08.18-C, C: Control group.

Conclusion

The aim of this project was to collect more data about the fungus genome and confirm the existence of ADE13, GPI8, GUK1, DIB1 and RNR2 in the fungus DNA by using normal and optimised PCR conditions. It is difficult to deduce whether the selected genes are present in the fungi, from the PCR results even though ADE13 and GPI8 showed better amplification than the other genes; therefore, these PCR products will be sequenced. In this way, we will develop a DNA detection protocol for fungus monitoring. Seeing fungus DNA from the healthy leaves can indicate that the mistake was made at the identification or weighing process.

Future Work

- To develop a geographical mapping system to understand under the which environmental conditions the pathogenic fungus spreads more quickly.
- To complete the quantitative PCR method that can analyze effectively the powdery mildew disease based on *Erysiphe corylacearum*.
- Uncovering all genomic sequence of *Erysiphe corylacearum* using genetic analysis methods.

References

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