T.C.

ÇANAKKALE ONSEKIZ MART UNIVERSITY

SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF MOLECULAR BIOLOGY AND GENETICS

GENOME-WIDE DISCOVERY OF STRUCTURAL VARIANTS AND THEIR POTENTIAL ROLE IN DMI-PROPICONAZOLE RESISTANCE IN *MONILINIA* SPECIES

MASTER OF SCIENCE THESIS

MUHAMMED RAŞİT DURAK

Thesis Supervisor

ASSOC. PROF. HİLAL ÖZKILINÇ

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PLAGIARISM DECLARATION PAGE

I declare that all the information and results offered in visual, audio, and, written form are obtained by myself observing the academic and ethical rules. Moreover, all other results and information referred to in the thesis but not specific to this study are cited.

Muhammed Raşit DURAK

28/06/2022

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Muhammed Raşit DURAK

Çanakkale, June 2022

ÖZET

MONILINIA TÜRLERİNDE YAPISAL VARYANTLARIN GENOM ÇAPINDA KEŞFİ VE DMI-PROPIKONAZOL DİRENCİNDEKİ POTANSİYEL ROLÜ

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Yapısal varyantlar (SV'ler), boyutları 50 baz çiftinden büyük olan ve genomik DNA'nın boyutunu, kopya numarasını, konumunu, yönünü ve dizi içeriğini değiştirebilen varyantlardır. SV'lerin çeşitli tipleri arasında insersiyonlar, delesyonlar ve duplikasyonlar (kopya numarası varyasyonu (CNV) olarak da adlandırılır), translokasyonlar, inversiyonlar ve mobil element yer değiştirmeleri bulunmaktadır. Bu varyantların hayat ağacı boyunca doğal popülasyonlarda meydana gelebildikleri ve fenotipik farklılıklarla ilişkili olabildikleri kanıtlanmıştır. Bu nedenle, bu varyantların kapsamını belirlemek, doğal varyasyonun ve potansiyel adaptif evrimsel süreçlerin genetik temelini anlamak için önem arz etmektedir.

Fungal bitki patojenlerinde fungisit direncinin ortaya çıkması patojenlerle mücadeleye zorlaştırrarak veya zayıflatarak tarımsal ürünlerde ciddi kalite ve verim kayıplarına neden olabilmektedir. Bu direncin ve/veya dirence katkı sunan genetik mekanizmaların anlaşılması hastalık yönetimi stratejilerinin geliştirilmesinde çok önemli bir adım sunmaktadır. Yapısal varyantların da fungisit direnç cevaplarını oluşturma yönünde etkileri olabileceği beklenmektedir.

Bu tez çalışması kapsamında, *Monilinia* cinsi içerisinde yer alan ve özellikle sert çekirdekli meyvelerden şeftali konukçusunda kahverengi çürüklük hastalığının en öne çıkan etmenleri olan *M. fructicola* ve *M. laxa* patojenleri için hem tür içi hem türlerarası genom kıyalsmaları SV'ler açısından değerlendirilmiştir ve bu değerlendirmelerin DMI grubu fungisitlerden porpikanazol etken maddesine verilen cevaplarla ilişkisi sorgulanmıştır.

Çalışma kapsamında Türkiye'den 108 *M. fructicola* ve 20 *M. laxa* izolatından oluşan *Monilinia* popülasyonunun Demetilasyon İnhibitör (DMI) grubu fungisiti propikonazol'e karşı duyarlılık durumu belirlenmiş, popülasyonun DMI-propikonazol'e karşı duyarlı olduğunu ortaya koyulmuştur. Fungisit fenotipleri dikkate alınarak seçilen toplam 16 izolatın tüm genom dizileri incelenmiştir. Sekiz *M. fructicola* ve sekiz *M. laxa* izolatı için karakterize edilen SV'lerin (INDEL'ler, CNV'ler, inversiyonlar ve mobil elementler (TEs)) DMI-propikonazol direncindeki potansiyel rolü, daha önce DMI direncinde rol aldığı kanıtlanmış aday genlerdeki veya yakınındaki SV durumu ile araştırılmıştır. Bu türler için tür içi ve türlerarası genom kıyaslamaları ile geniş kapsamlı olarak genom çapında SV'ler ve tek nükleotid polimorfizmleri (SNP'ler) ilk kez belirlenmiştir.

Sonuç olarak, *M. fructicola* genomlarının, referans temelli varyant çağırmasına dayalı olarak *M. laxa* türüne kıyasla daha fazla varyant içerdiği bulunmuştur (sırasıyla toplam 266.618 ve 190.599 SNP; 1540 ve 918 SV). Genomik datasetlerinde karakterize edilen toplam duplikasyon sayısı, *M. fructicola* için yedi duplikasyon ve *M. laxa* için üç duplikasyon ile her iki tür için de düşük sayıda bulunmuştur. SNP'lerin ve SV'lerin kapsamı ve dağılımı, türler arasında korunmuş ve türler arasında yüksek derecede değişken bulunmuştur. Ayrıca, her izolat için CNV'nin ayrıntılı karakterizasyonu, *M. fructicola* genomlarının yaklaşık %0,67'sinin ve *M. laxa* genomlarının yaklaşık %2,06'sının CN değişkeni olduğunu ortaya koymuştur. Ayrıca, 15'ten fazla sınıflandırılmış familyaya sahip her iki tür için de mobil element içeriği yaklaşık %9 olarak bulunmuştur. Sınıf I/Retrotranspozonlar (özellikle LTR elementleri), beklendiği gibi her iki tür için de en baskın TE'ler olarak bulunmuştur. Her iki türün referans genomlarındaki TE'lerin Kimura-2 mesafeleri, yakın zamanlı insersiyonların her iki tür için de düşük olduğunu ve mevcut TE içeriğinin çoğunun daha eski insersiyon/çoğalmalardan kaynaklandığını göstermiştir.

Sonuç olarak, SV'lerin DMI-propikonazol direncindeki potansiyel rolünün araştırılması, her iki tür için farklı duyarlılık seviyelerine sahip izolatlar için insersiyon, delesyon, duplikasyon ve inversiyon tiplerinden hiçbir SV'nin aday *CYP51* ve *ABC* taşıyıcı genlerinde veya yakınında farklılık göstermediğini ortaya koyuştur. Ancak, *M. fructicola* izolatlarında *CYP51* geninin yukarı bölgesinde Sınıf II/Sat-2-LVa ve Sınıf I/DNA-8-3_HM'den oluşan iç-içe geçmiş bir TE'nin varlığı/yokluğu, DMI'ye karşı direnç için potansiyel bir risk sunmuştur.

Anahtar Kelimeler: Yapısal Varyantlar, Mobil Elementler, Kopya Sayısı Varyasyonu, Fungisit Direnci, *Monilinia* spp.

ABSTRACT

GENOME-WIDE DISCOVERY OF STRUCTURAL VARIANTS AND THEIR POTENTIAL ROLE IN DMI-PROPICONAZOLE RESISTANCE IN *MONILINIA* SPECIES

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Structural variants (SVs) are variants with sizes bigger than 50 base pairs and capable of changing the size, copy number, location, orientation, and sequence content of genomic DNA. The diverse forms of SVs include insertions, deletions, and duplications (also referred to as copy number variation(CNV)), inversions, translocations, and mobile-element transpositions. These variants have proven to be extensive and associated with phenotypic differences in natural populations along the tree of life. Thus, determining the extent of these variants is essential to uncovering the genetic basis of natural variation and potential adaptive evolutionary processes.

The occurrence of fungicide resistance in fungal plant pathogens results in great losses of quality and yield in crops and makes the management strategies challenging. Thus, understanding the genetic basis of resistance is a crucial step in the improvement of disease management strategies. It is also expected structural variants to affect fungicide resistance responses.

In this thesis, the intra- and inter-species extent of SVs, as well as single nucleotide polymorphisms (SNPs), have been determined for two prominent species of the *Monilinia* genus (the causal agents of brown rot disease in pome and stone fruits): *M. fructicola* and *M. laxa* for the first time and their relation to the DMI group fungicide propiconazole has been investigated. In addition, the sensitivity status of the *Monilinia* population from Turkey consisting of 108 *M. fructicola* and 20 *M. laxa* isolates against Demethylation Inhibitor

(DMI) group fungicide propiconazole has presented that the population is sensitive against DMI-propiconazole. Then, whole-genome sequences of a total of 16 isolates that were selected based on their fungicide sensitivity phenotypes are investigated. The potential role of SVs (INDELs, CNVs, inversions, and mobile elements (TEs)) is then investigated for eight *M. fructicola* and eight *M. laxa* isolates in DMI-propiconazole resistance-related candidate genes that are previously proven to be involved in DMI resistance. Genome-wide SVs and single-nucleotide polymorphisms (SNPs) have been identified for the first time comprehensively by intra- and inter-species genome comparisons for these species.

As a result, the genomes of *M. fructicola* were found to be more variant rich in contrast to *M. laxa* based on the reference-based variant calling (with a total number of 266.618 and 190.599 SNPs; 1540 and 918 SVs, respectively). The total number of characterized duplications was extremely low for both species with seven duplications for *M. fructicola* and three duplications for *M. laxa* genomic datasets. The extent, as well as distribution of SVs, presented high conservation within the species and high diversity between the species. Moreover, the detailed characterization of CNV for each isolate revealed that around 0.67% of *M. fructicola* genomes and 2.06% of *M. laxa* genomes are CN variable. In addition, the transposable element content was found at approximately 9% for both species with more than 15 classified families. The Class I/Retrotransposons (especially the LTR elements) were found to be the most dominant TEs for both species as expected. Kimura-2 distances of TEs in reference genomes of both species have shown that the recent insertions were low for both species and most of the current TE content was due to older insertions/proliferations.

Finally, the investigation of the potential role of SVs in DMI-propiconazole resistance revealed that no SV in the form of insertion, deletion, duplication, and inversion differ at or near candidate *CYP51* and *ABC* transporter genes for isolates with differentiating sensitivity levels for both species. However, the presence/absence of a nested TE consisting of Class II/Sat-2-LVa and Class I/DNA-8-3_HM at the upstream region of the *CYP51* gene in *M. fructicola* isolates presented a potential risk for resistance against DMI-propiconazole.

Keywords: Structural Variants, Transposable Elements, Copy Number Variation, Fungicide Resistance, *Monilinia* spp.

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ABBREVIATIONS

BRD	Brown Rot Disease
°C	Degree Celsius
µg	Microgram
µl	Microliter
BAM	Binary Alignment Map
BLAST	Basic Local Alignment Search Tool
bp	Base-pair
CNV	Copy Number Variant
CNVR	Copy Number Variable Region
dH_2O	Distilled Water
DMI	Demethylation Inhibitor
GATK	Genome Analyses Toolkit
gVCF	Genomic Variant Call Format
GWAS	Genome-wide Association
IC_{50}	Half Maximal Inhibitory Concentration
INDEL	Insertion-Deletion
kb	Kilobase
LINE	Long Interspersed Nuclear Element
LTR	Long Terminal Repeat
Mb	Millions of Base Pairs
MOA	Mode of Action
NAHR	Non Allelic Homologous Recombination
ng	Nanogram
NGS	Next-generation Sequencing
NHEJ	Non Homologous End Joining
PAV	Presence/Absence Variation
PDA	Potato Dextrose Agar
RG	Relative Growth
RGAAT	Reference-based Genome Assembly and Annotation Tool
RL	Resistance Level
SAM	Sequence Alignment Map

SINE	Short Interspersed Nuclear Element
SNP	Single Nucleotide Polymorphism
SV	Structural Variant
TE	Transposable Element
TIR	Terminal Inverted Repeat
TRF	Tandem Repeat Finder
VCF	Variant Call Format
WGS	Whole Genome Sequencing

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CHAPTER 1
INTRODUCTION

1.1. Fungal Plant Pathogens: *Monilinia* spp. and Brown Rot Disease

Fungal plant pathogens have impacted the human communities both from the perspective of economy and crop production for centuries and, there is a constant race between new management approaches and the ability of fungal pathogens to adapt (Covo, 2020). These pathogens are representing the most diversity and economic relevance in terms of plant pathogens and are grouped in two main phyla: Ascomycota and Basidiomycota. *Monilinia* spp. is a necrotrophic phytopathogenic fungi in the Ascomycota phylum that causes brown rot disease (BRD) primarily on pome and stone fruits (e.g. peach, plum, cherry) (Byrde & Willetts, 2013). The most prevalent species of this genus include *M. fructicola*, *M. laxa*, and *M. fructigena*. The BRD symptoms caused by these species are mainly observed on fruits, twigs, leaves, and buds, and the spread of this disease is favored by high humidity, warm temperatures, and rainfalls (Abate et al., 2018). Out of the three prominent species, *M. fructicola* is predominant with its devastating impact on fruit production -especially on peach- and the economy (Ozkilinc et al., 2020) and is reported as a quarantine pathogen by the European and Mediterranean Plant Protection Organization (EPPO) (https://www.eppo.int/). On the other hand, the second most prominent species *M. laxa* is mainly observed on twigs, blossoms, and branches of stone fruits (Holb, 2004). The species belonging to the *Monilinia* genus as well as the BRD caused by these species have been reported in almost all continents including Europe (Abate et al., 2018); Asia (Hu et al., 2011); Africa (Carstens et al., 2010); Australia (Tran et al., 2017) and America (Snyder & Jones, 1999).

1.2. Fungicides

Fungicides are chemical compounds that are used to mitigate, prevent or inhibit the growth of fungi and the usage of these compounds is the most common method to prevent plant diseases caused by fungal pathogens. Fungicides have been used for over a century and have proven to be a successful technique to prevent fungal diseases for many years (Lucas,

Hawkins & Fraaije, 2015), however, constant misusage of these chemicals has resulted in resistance in natural populations. Modern fungicides act as inhibitors for several biochemical processes and bind to specific protein targets (described as single-site or site-specific) in contrast to earlier fungicides which act on multiple sites. Currently, there are more than 85 groups of fungal control agents with more than 10 biochemical modes of action (MOA) according to the Fungicide Resistance Action Committee (FRAC https://www.frac.info/). The main biosynthetic pathway of fungal plant pathogens targeted by different MOAs includes nucleic acid metabolism, cytoskeleton and motor protein, respiration, amino acid, protein synthesis, signal transduction, sterol biosynthesis in the membrane, and so on (FRAC, https://www.frac.info/). The most commonly used and well-known groups of site-specific fungicides are strobilurin (or Quinone outside Inhibitor (QoI)) (FRAC group 11), succinate dehydrogenase inhibitors (SDHIs) (FRAC group 7), and azoles (or demethylation inhibitors (DMIs) (FRAC group 3).

1.2.1. Demethylation Inhibitors and Fungicide Resistance

DMI fungicides act on the biosynthesis of ergosterol which is a major component of the membrane for fungal pathogens (Maertens & Boogaerts, 2000). More specifically, DMIs bind to haem iron of the cytochrome P450 14-α-demethylase (product of *CYP51* gene) and thus interfere with the biosynthetic pathway of ergosterol synthesis by preventing the conversion of lanosterol to 4,4-Dimethylcholesta-8,14,24-trienol (Figure1).

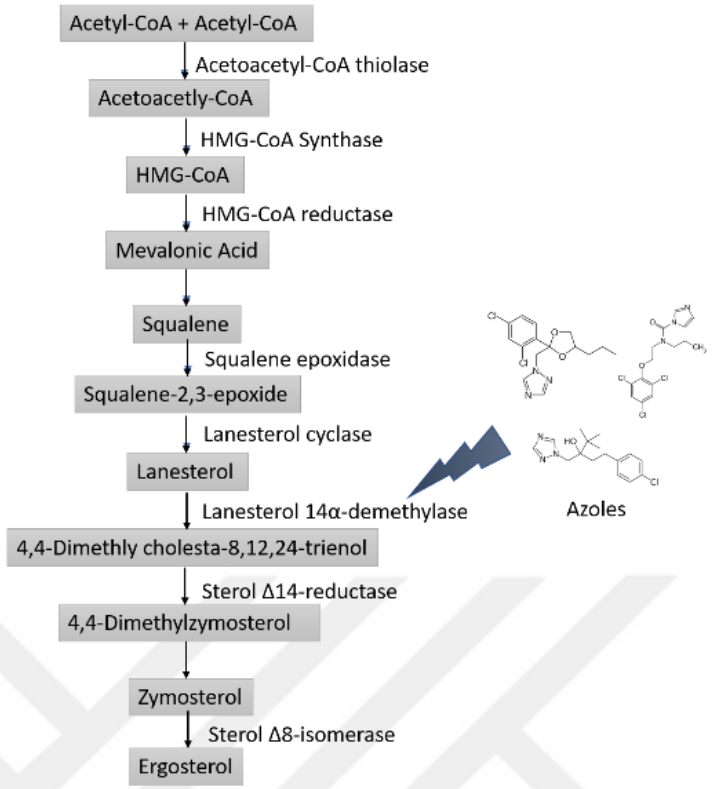

Figure 1. Ergosterol biosynthesis pathway for fungal organisms and the interference of azole group fungicides. This scheme is adopted and revised from (Maertens & Boogaerts, 2000).

These site-specific fungicides are also highly active and often distributed along the plant tissues even with low doses, thus providing efficient disease management (Hahn, 2014). In this way, strong selection acts upon resistant genotypes by the removal or inhibition of the majority of pathogen populations. However, the combinations of pathogen biology (e.g. short generation time, large population size), environmental conditions and MOA/dose of fungicide may direct the selection of resistant phenotypes resulting in resistance in natural populations. In this manner, resistance may be conferred either by the selection of variants at the target protein or the selection of variants at the non-target sites. Even the selection of a single nucleotide polymorphism (SNP) with an effect on the target protein that results in decreased efficiency between the fungicide and the target may confer a high level of resistance in site-specific fungicides (Hu & Chen, 2021). As also stated above, resistance phenotypes may also arise from alterations at multiple targets/non-target sites which are referred to as quantitative resistance. For example, many studies have revealed the association between the increased activity of efflux pumps and the fungicide resistance, underlying the crucial and common relevance of these transporters in resistance occurrence

(de Ramón-Carbonell et al., 2019). *ABC* superfamily, with their ability to transport a wide range of compounds based on their low substrate specificity, is the most attributed transporter that takes place in the fungicide resistance (Hu & Chen, 2021). Thus, the alterations that occurred at non-target sites may confer resistance to not only the fungicide used but to other fungicides that will be used in nature as well.

1.3. The Diversity of Fungal Genomes and Structural Variants

Advances in next-generation sequencing technologies made whole-genome sequences affordable thus, researchers now can easily study the genomic variations between different individuals/organisms for all kingdoms of life. Ongoing improvements in genomic assembly and comparative genomic approaches can be useful for many purposes including the determination of molecular genetic markers and conservation biology (Kumar et al., 2020). Genomic exploration of agriculturally devastating fungal pathogens by whole genome sequencing is important to understand these organisms and to control the plant diseases associated with fungal pathogens. Progress on fungal genomes has revealed many indications in terms of disease management (e.g. host adaptation, fungicide resistance, etc.,) (Mohd-Assaad, McDonald & Croll, 2016).

The size of the genome and diversity vary from species to species. One review study compared the genomic data of 172 fungal species and showed the tremendous diversity of fungal genomes which presents a variation from 8.97 Mb to 177.57 Mb in size (Mohanta & Bae, 2015). Adaptive theories of genome evolution state that adaptive needs and natural selection are the major determinants of variation in genomes (Petrov, 2001) and the dynamic nature of fungal pathogens is suitable for the diversification of genomes of these organisms. Alongside, the modern agriculture applications have resulted in genetically highly similar crops in the field and this reduced genetic diversity increases the probability of a spread of adapted fungal pathogens (Möller & Stukenbrock, 2017). Moreover, environmental factors (e.g. increased temperature, and fungicide usage) are major factors affecting the propagation and evolution thus, both managed ecosystems and agricultural applications potentially present a suitable environment for adaptation and rapid evolution of fungal pathogens. Selections of existing genetic variations and selection of new mutations are two distinct ways

for populations to adapt to novel environments (Barrett & Schluter, 2008) and the determination of these genomic variations is one of the key steps in understanding the evolutionary outcomes and phenotypic differences for microbial populations. Single nucleotide polymorphisms (SNPs), and structural variations (SVs) (copy number variations (CNVs), presence-absence of transposable elements (TEs), deletions, insertions, etc.) are the main sources of genomic variability, and SNPs were believed to be the predominant form of variation (Sachidanandam et al., 2001). On contrary, since the early 2000s, many studies have been shown that the structural variations are extensive throughout the tree of life and have associated with differences in phenotypes (Wang et al., 2020; Zhao & Gibbons, 2018).

Structural variation (SV), in general, is defined as the variants that change the size, copy number, location, orientation, and sequence content of genomic DNA and these variants have remained difficult to interpret due to their functional consequences (Fan et al., 2014). SV is classified as sequence variants of at least 50 base pairs (bp) in size, thus this classification makes these variations distinct from other forms of variants such as single-nucleotide variants and, insertions/deletions (INDELs) and the change in the copy number relative to the reference genome are the most common forms of SVs (Mills et al., 2011). The recognized class of SVs comes in many different shapes and forms and includes many different types of genomic events including deletions, duplications, novel insertions, inversions, mobile-element transpositions, and translocations (Periwal & Scaria, 2015). Main SV types relative to a given reference have been visually represented in Figure 2.

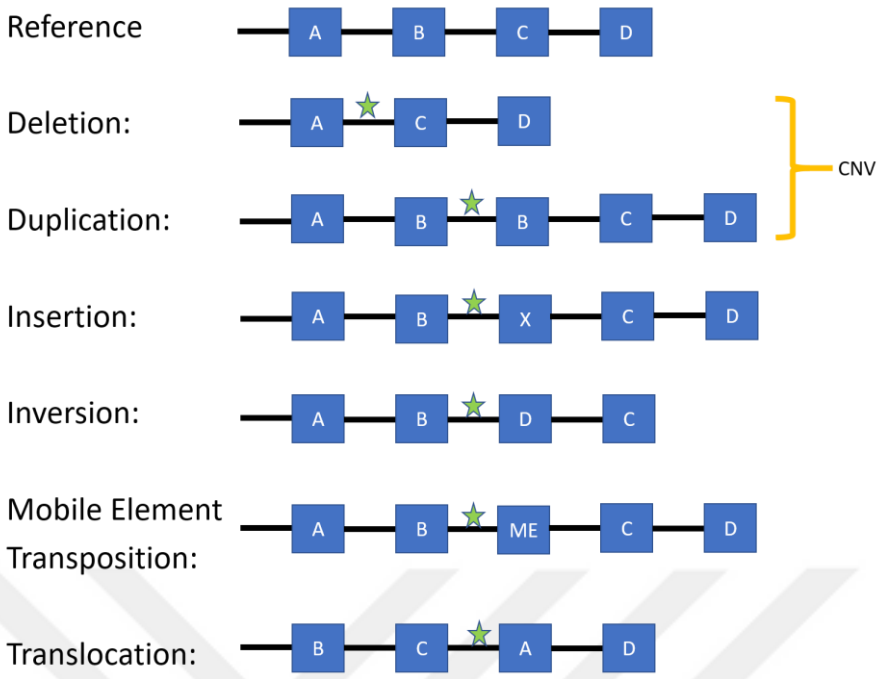

Figure 2. Visual illustration of recognized structural variant types (deletion, duplication, insertion, inversion, mobile element transposition, and translocation) relative to a given reference. CNV: Copy number variation; ME: Mobile Element.

Structural variants, in general, are subdivided into two groups based on their impact on the sequence content: balanced, where no gain or loss of genetic material occurs (i.e. inversions, translocations), and unbalanced, in which part of the genome is lost/deleted or duplicated (i.e. CNV) (Collins et al., 2017). Interpretation of SVs has been difficult in the past due to limitations on the sequencing technologies, bioinformatics approaches, and their functional consequences (Fan et al., 2014). Based on the demand for SV detection, the number of tools to detect SVs has been increasing in recent years (Lei et al., 2022).

1.3.1. Copy Number Variation

CNV, one of the most commonly occurring unbalanced SV type, is defined as the deletion and duplication of genomic sequences and is a major source of variation in many species (Bai et al., 2016). Even though these variants are accounted for as one of the main type of SV, the definition is still highly variable in different studies. Earlier studies, for example, defined these variations with the size of at least 1000 bp (Feuk, Carson & Scherer,

2006). However, later studies have expanded the size range of these variants from 50 bp to several megabases (Mb) (Alkan, Coe & Eichler, 2011).

At the molecular level, a change in the chromosome structure has the potential to yield a change in the copy number. Predominantly, non-allelic homologous recombination (NAHR) and non-homologous end joining (NHEJ) are accounted for the formation of copy number variation (Gu, Zhang & Lupski, 2008). Even though the CNVs were the first genetic variant that has been discovered (in the form of insertions and deletions) (Tice, 1914), the studies on these variants are still insufficient and highly limited for non-model organisms.

1.3.2. Transposable Elements

Transposable elements (TEs), another important type of SV, are mobile genetic elements that were first discovered by McClintock in 1950. Two major categories of these elements are classified as Class I (TEs that transposase through an RNA intermediate and Class II (TEs that transposase through a cut-paste mechanism) (Daboussi & Capy, 2003; Mc, 1950). Based on their highly dynamic nature, both classes of TEs are divided into subclasses and orders which contain various numbers of superfamilies and families (Figure 3).

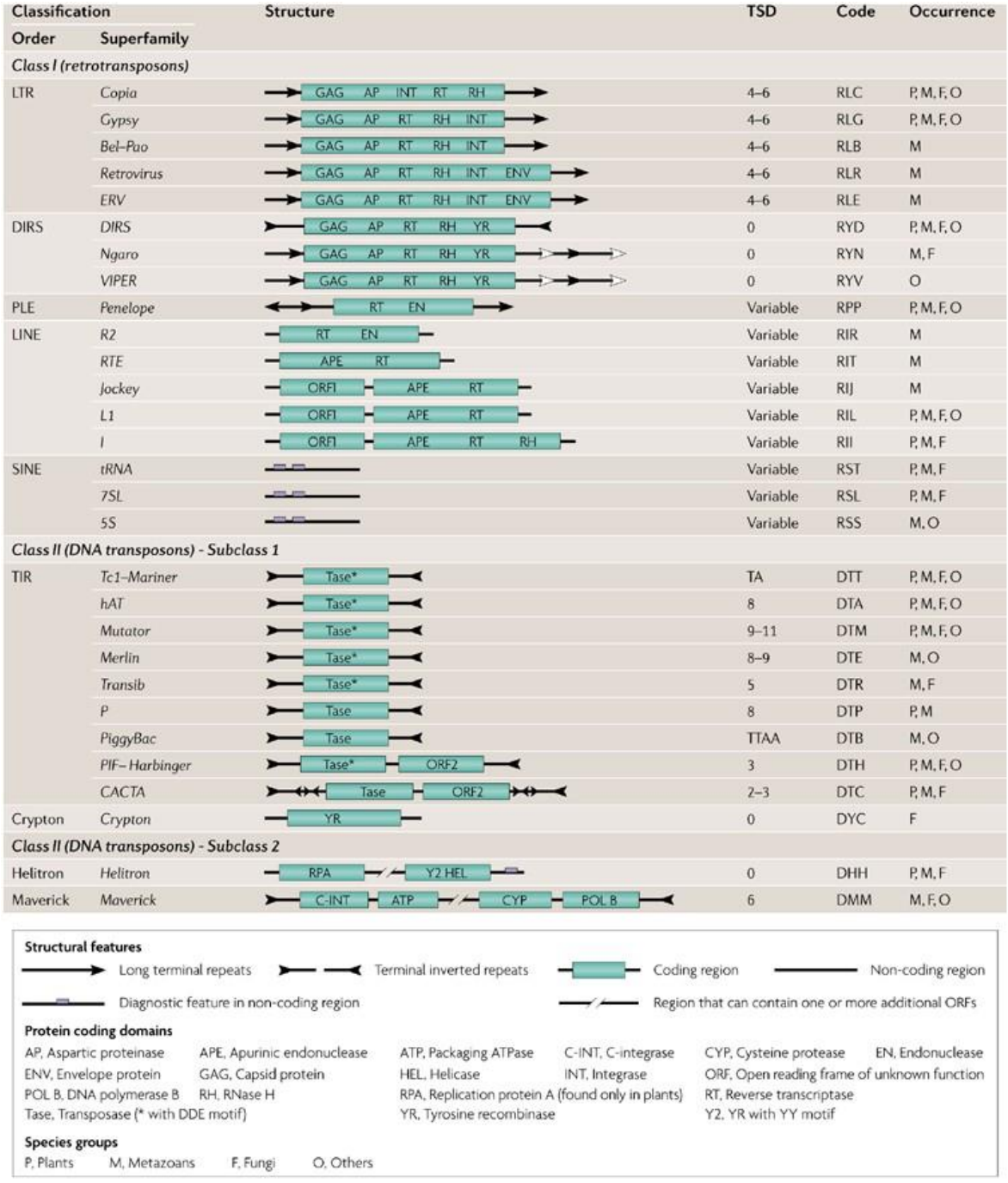

Figure 3. The Wicker classification of transposable elements into classes (Class I and Class II), orders and superfamilies with their structures, target site duplication sizes, three-letter naming codes, and the species they occur (Wicker et al., 2007).

All Class I elements yields a new copy at every transposition cycle via an RNA intermediate (Wicker et al., 2007). This transposition mechanism makes retrotransposons contribute to producing large repetitive sequences in the genome in contrast to DNA transposons. Two major subclasses of retrotransposons include the LTRs (Long Terminal Repeats) and non-LTRs (LINEs (Long Interspersed Nuclear Elements), SINEs (Short Interspersed Nuclear Elements), Penelope and DIRS Elements) which are mainly become distinct with the presence/absence of long/short repetitive sequences at their end and the coding regions they include (Wicker et al., 2007). On the other hand, DNA transposons include two main subclasses which are mainly differentiated by their transposition mechanism. In the "Subclass I", both integration and excision sites are cut, whereas in "Subclass II" a transposition occurs through replication without a double-strand cleavage (Santana et al., 2012). Two main orders of "Subclass I" are TIRs (Terminal Inverted Repeats) with nine superfamilies and Crypton which mainly differentiates from TIRs by coding tyrosine recombinase instead of transposase for their transposition. Subclass II, on the other hand, has two orders/superfamilies named *Helitron* and *Maverick* (Santana et al., 2012). Since their discovery, the presence of TEs has been constant for almost all eukaryotic species except for some lower eukaryotes like *Plasmodium falciparum* (Gardner et al., 2002). These elements provide plasticity with their ability to move and replicate and play a major role in epigenetic changes in genomes (Eichler & Sankoff, 2003). The vast majority of the activity of these elements are coming with a variety of costs to the host including disruption of functional genes (Hancks & Kazazian, 2016), cellular cost of replicating (Nuzhdin, 1999), and changing the expression profiles in the regions they have been inserted (Rebollo, Romanish & Mager, 2012). Even though the majority of the TE replication is deleterious for the host, some are proven to be beneficial to the host under stressful conditions (Chuong, Elde & Feschotte, 2017). In addition, the mutational activity of the TEs (e.g. through host defense mechanisms) may increase the genetic diversity and speed up the adaptive evolutionary processes regardless of their effects (Santana et al., 2012).

1.4. The Aim of the Study

This master's thesis aimed to extensively discover the structural variants in the *M. fructicola* and *M. laxa* species and to compare the SV content for these closely related species. Besides, it was aimed to determine the sensitivity level of the *Monilinia* population from Turkey to DMI group fungicide propiconazole and to unravel the potential role of SVs in propiconazole resistance. This thesis represents the SV content and their potential functional effects for *M. fructicola* and *M. laxa* species in detail for the first time and attempts to investigate the potential role of SVs in the fungicide resistance. Both the findings and the data generated in this thesis present fundamentals to understand the diversity and adaptive evolutionary processes in these species and contributes to our understanding of complex genetic mechanisms that underlie the fungicide resistance.

CHAPTER 2
PREVIOUS STUDIES

Defining the sensitivity level of a fungal plant pathogen population against a fungicide and elucidation of the potential genetic mechanisms that might underlie resistance in natural populations are two crucial components of disease management strategies. Firstly, knowledge of the sensitivity level of a fungal pathogen against a given fungicide provides fundamental knowledge at the global level based on the serious losses in the yield and quality of plants. Studies conducted on *Monilinia* populations from U.S.A and Brazil have reported resistance against DMI group fungicides (Lichtemberg et al., 2017; Villani & Cox, 2011). However, the definition of sensitive/resistant is highly variable even for the resistance reports given above. For example, *M. fructicola* isolates that were able to grow 50% or more relative to control at a discriminative dose (0.3 µg/ml) are defined as resistant, and the ones that did not grow at this dose are defined as sensitive in the study conducted by (Zehr et al., 1999). In another study, 100x of EC_{50} concentration have been used to differentiate the *M. fructicola* isolates as sensitive or resistant (Villani & Cox, 2011). In this thesis, sensitivity levels of the *Monilinia* population from Turkey against DMI group fungicide propiconazole have been defined based on a method developed and successfully applied to define sensitivity against respiratory fungicides for the same population by us (Durak et al., 2021). Up to date, two main resistance mechanisms against DMI group fungicides have been proposed for *Monilinia fructicola* (Figure 4) and no resistance mechanism for *M. laxa* is present to our knowledge. In one of these resistance mechanisms, a 65 bp long genetic element called "Mona" the upstream of the *CYP51* gene has been associated with the resistance by causing an increase in the *CYP51* gene when present (Luo et al., 2008). However, this element was also shown to be present in the sensitive *M. fructicola* isolates as well (Villani & Cox, 2011). In the second proposed resistance mechanism, a substitution at the position 1492 of the *CYP51* gene which causes an amino acid substitution from Glycine (G) to Serine (S) has been associated with the resistance against DMI group fungicide tebuconazole in *M. fructicola* population from Brazil (Lichtemberg et al., 2017). In addition to these resistance mechanisms, a study conducted on *M. fructicola* has shown that the expression level of the *ABC* transporter gene might also be a DMI resistance determinant based on a non-target mechanism (Schnabel, Dait & Paradkar, 2003). Up to date, no genome-wide investigation has been conducted to study the fungicide resistance mechanism for

Monilinia species, and the genome-wide studies that attempt to understand fungicide resistance are highly limited to model fungal organisms including clinically relevant *Candida albicans* (Rogers & Barker, 2003), wheat pathogen *Zymoseptoria tritici* (McDonald et al., 2019) and barley pathogen *Rhynchosporium commune* (Mohd-Assaad, McDonald & Croll, 2016). Considering fungicide resistance to be a complex trait that results from variations within multiple genes (Arslan et al., 2022, unpublished data), more studies that focus on resistance mechanisms in a genome-wide manner are needed.

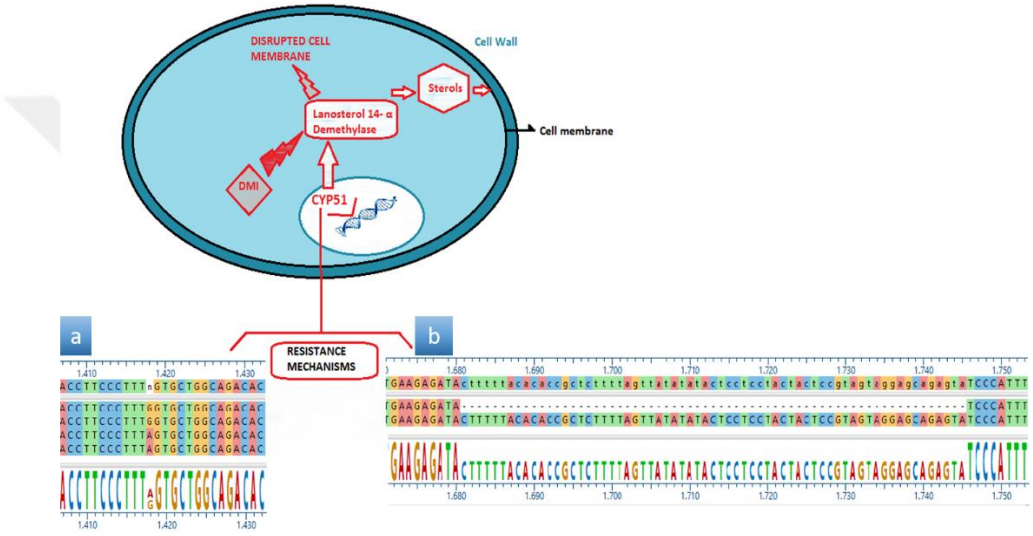

Figure 4. Two proposed resistance mechanisms against DMI group fungicides for *M. fructicola*; a. The single nucleotide substitution from A to G at position 1492 in the *CYP51* gene results in amino acid substitution from Glycine (G) to Serine (S) (Lichtemberg et al., 2017) and b. The insertion of a 65 bp long genetic element called "Mona" to the upstream region of the *CYP51* gene (Luo et al., 2008).

The studies on structural variants, on the other hand, have been increasing with respect to the increase in the sequencing data generated and the proven functional effect of these variants (e.g. (Zhao & Gibbons, 2018)). However, the studies on SVs are highly limited to the model organisms. For example, SVs have been extensively characterized and proven to have direct associations with many chronic diseases including cancer in humans (e.g. (Feuk et al., 2006)). In addition, most studies focus on a specific form of SVs (i.e. copy number variation, transposable element) based on their challenging discovery and diverse

shapes and forms. For instance, several disorders such as autism in humans have been directly correlated with variability in the copy number of genes (Marshall et al., 2008). As all these studies show, CNV is an important source for differences in phenotypes and these regions may cover a large portion of the genome in different species. On the contrary, the number of studies that investigates the CNV in fungal pathogens is relatively much small than in other organisms. As an example, the contribution of CNV to genetic diversity in 71 *Aspergillus nidulans* isolates has been characterized and comparative genomic approaches have been used to present the CNV profile (Zhao & Gibbons, 2018). This study has shown that approximately 10% of the genome is CN variable and these CNVs are non-randomly distributed around the genes related to transposable elements and secondary metabolism functions. In another study conducted on powdery mildew pathogen, *Erysiphe necator* has been shown that the isolates collected from fungicide exposed vineyards show CNV on the target region of azole fungicides (*CYP51* gene) (Jones et al., 2014).

Similar to CNVs, TEs also have been proven to be associated with phenotypic changes (Van't Hof et al., 2016). For example, a study conducted by (Chen et al., 2015) found that continuous exposure to fungicides in *M. fructicola* isolates caused a transposon transposition named *Mftc1*. The TE composition of fungal genomes varies from less than 1% (for *Fusarium graminearum*) to more than 90% (for *Blumeria graminis*) (Cuomo et al., 2007; Frantzeskakis et al., 2018). Even though most of these elements are shown to be inactivated/repressed by the host via the mechanisms stated in the introduction section, studies have shown that the de-repression of these elements is possible and common (Fouché et al., 2020). The dynamics/ distributions of TEs and how these elements are shaping the evolution are well characterized in plant fungal pathogens such as *Zymoseptoria tritici* (Oggenfuss et al., 2021) and *Mycosphaerella fijensis* (Tsushima et al., 2019) but the studies on other fungal plant pathogens are highly limited.

CHAPTER 3
MATERIALS AND METHODS

3.1. Materials

3.1.1. Fungal Isolates

A total of 128 isolates which consist of 108 *M. fructicola* and 20 *M. laxa* isolates collected from peach orchards in six provinces located in 5 different geographical locations of Turkey were used for fungicide sensitivity assays. In addition, nine *M. fructicola* isolates (BG-B3-A1, SC-B2-A3, SC-B2-A4, B5-A4, T-B1-A5, YK-1, BG-B1-A8, BO-B3-A1, and Ti-B3-A3-2) and nine *M. laxa* isolates (2B1A2-2, Ni-B3-A2, MM-B4-A4, MT-B1-A3-1, T-B1-A4-2, MM-B2-A2, 2B1-A5, MM-B4-A3 and Yıldırım-2) were used for all genomic analyses. List and detailed characteristics for all isolate collection were provided in the publication by Ozkilinc et al., (2020). Stored isolates on Whatman filter paper no 1 at -20˚C were grown on fresh potato dextrose agar (PDA) at 23˚C in dark. Ten days old cultures were used as starting material for all *in vitro* fungicide sensitivity assays.

3.1.2. Fungicide

Technical grade of demethylation Inhibitor (DMI) group fungicide propiconazole (≥98%, Sigma Aldrich Co.) was used. Stock solutions were stored at 4˚C and acetone was used as a solvent for propiconazole.

3.2. Methods

3.2.1. *In vitro* Mycelium Growth Inhibition Assay

PDA was used for *in vitro* mycelium growth-based fungicide sensitivity assays. The method described in (Durak et al., 2021) was used for all *in vitro* assays. In short, the mean concentration required for 50% inhibition (IC_{50}) for representative 20 isolates (consists of 11 *M. fructicola* and 9 *M. laxa* based on random selection that contains isolates from each province sampled) was calculated for each species separately. Then, the whole collection has

been scanned at the corresponding mean IC_{50} concentration (defined as the discriminatory concentration) for each species. Relative growth (RG) value for each isolate considering its growth on the control in respect to its growth on discriminatory concentration was calculated (Average growth value on discriminatory concentration x 100)/Average growth value on control).

Concentrations of 0.05, 0.1, 0.3, 0.5, 0.7 and 1 µg/ml were assessed in the *in vitro* sensitivity assay for representative collection. The IC_{50} value for each isolate in the representative group was calculated by using GraphPad Prism version 6.00 (Swift, 1997). Mean IC_{50} values are evaluated for *M. fructicola* and *M. laxa* separately based on the representative collection. IC_{50} values, confidence intervals and mean IC_{50} concentrations against propiconazole for the representative collection were presented in Table 1. In *in vitro* assays, 20 mL of medium was poured equally to each Petri and mycelial plugs (1 cm in diameter) from 10 days old cultures were transferred to fungicide amended PDA. Fungicide unamended media was used as the control for all *in vitro* assays and each isolate/concentration was tested three times. Cultures were kept at 23 ˚C for 7 days in the dark and mean colony diameters (excluding the size of the initial 1 cm inoculum) were measured in two perpendicular directions at the end of the 7th day. No significant difference was found ($P > 0.05$) between the replicates of *in vitro* mycelial growth inhibition assays based on one-way ANOVA test thus, the mean values of the replicates were used for IC_{50} and relative growth (RG) calculation. All calculations for *in vitro* assays were performed as described in (Durak et al., 2021).

3.2.2. Fungal Genomic DNA Extraction, Whole Genome Sequencing, and Quality Filtering of Short Reads

DNA from four *M. fructicola* and six *M. laxa* selected isolates were extracted and sequenced in (Yildiz & Ozkilinc, 2020, 2021). In addition, the DNA of the four *M. fructicola* isolates (BG-B3-A1, BG-B1-A8, BO-B3-A1, SC-B2-A3) and the two *M. laxa* (2B1-A2-2 and MM-B4-A3) isolates was extracted. Total genomic DNA extraction was carried out from mycelium grown in 50 mL of Potato Dextrose Broth (PDB) in a rotary shaker at 150 rpm at room temperature (RT) by Norgen Plant/Fungi DNA Isolation Kit (Norgen, Canada),

following the manufacturer's protocol. DNA concentrations have been quantified by using Qubit 3.0 fluorometer (Thermo Fisher Scientific, U.S.A.). Next-generation sequencing was performed by Macrogen Inc., Sequencing Service using the Illumina TruSeq Nano Library construction kit with 350 bp insert size followed by Illumina NovaSeq 6000 platform with 2x150 bp paired-end sequencing.

Trimmed Illumina reads (paired-end 2×151 bp) that belong to selected sixteen isolates (eight *M. fructicola*; eight *M. laxa*) were used for all downstream analyses. The low-quality read and adapter removal was performed by Trimmomatic version 0.36 (Bolger, Lohse & Usadel, 2014) by setting the parameters as "ILLUMINACLIP:TruseqHT.fa:2:30:10 LEADING:10 TRAILING:10 (remove the bases that have quality lower than "10") SLIDINGWINDOW:5:20 (scan the read in 5 base-wide sliding windows and cut when average quality per base drops below "20") MINLEN:151 (drop reads when smaller than 151 bp)". The quality statistics of the "*.fastq" files were checked with FastQC before and following the trimming (Andrews, Gilley & Coleman, 2010). Then the total quality statistic report was formed and checked by MultiQC (Ewels et al., 2016).

3.2.3. Reference-based Genome Assembly for Mapping-Based Variant Discovery

Reference-based genome assembly and annotation have been performed by the Reference-based Genome Assembly and Annotation Tool (RGAAT) (Liu et al., 2018) for Ti-B3-A3-2 (*M. fructicola*) and Yildirim-1 (*M. laxa*) isolates. Previously assembled genomes for *M. fructicola* (De Miccolis Angelini et al., 2019) and *M. laxa* (Landi et al., 2020) were used as references. RGAAT uses binary alignment map files (bam) and reference genome in the FASTA format and updates the reference genome file by performing coordinate conversion based on variant calling results the tool itself performs. The sequence alignment map (sam) files required for the tool is obtained by mapping the sequence reads of Ti-B3-A3-2 (*M. fructicola*) and Yildirim-1 (*M. laxa*) to previously published reference genomes under GenBank accessions "GCA_008692225.1" for *M. fructicola*; "GCA_009299455.1" for *M. laxa* with BWA MEM version 0.7.17 (Li & Durbin, 2009). The

SAM files were then converted to binary alignment map (BAM) files by samtools version 1.12 (Li et al., 2009), and remapping of the reads was performed by Stampy version 1.0.32 (Lunter & Goodson, 2011) with –bamkeepgoodreads flag.

3.2.4. *de novo* Genome Assemblies for Assembly Based Discovery of Variants

de novo genome assemblies for all isolates were generated by SPADES version 3.11.1 (Bankevich et al., 2012) with –careful parameter and k-mer range of "21,33,55,77,99,127". Following the reference-based and *de novo* assembly, the quality assessments of the genome assemblies for both species have been checked by Quast version 5.0.2 (Gurevich et al., 2013).

3.2.5. Mapping of Short Reads to Reference Genomes

Reference genomes of "Ti-B3-A3-2" for *M. fructicola* (44.02 Mb in size) and "Yıldırım-1" for *M. laxa* (42.80 Mb in size) strains obtained by RGAAT (Liu et al., 2018) was used as references for all downstream analyses.

Illumina reads were aligned to the corresponding species' reference genomes obtained by RGAAT using BWA MEM version 0.7.17 (Li & Durbin, 2009) to generate sequence alignment map (SAM) files. All SAM files were converted to binary (BAM) files and sorted by PICARD tools version 2.23.6 (https://broadinstitute.github.io/picard/). PICARD TOOLS version 2.23.6 was used for marking and removing the duplicates from the BAM files. Qualimap version 2.2.1 was then used to generate summary statistics of each alignment file (García-Alcalde et al., 2012).

3.2.6. Single Nucleotide Polymorphism, Structural Variant and Copy Number Variation Calling, Selection of Variants, and Quality Filtering

Following the alignment, the Genome Analysis Toolkit (GATK) version 4.2-0 (McKenna et al., 2010) was used to call SNPs between Illumina reads and the given references. First, the module "HaplotypeCaller" with the "-ploidy 1" option was used to create genome variant call format (gVCF) files and gVCFs were combined by "CombineGVCFs". Then the combined gvcf file is genotyped by the "GenotypeGVCFs" module. Then the module "SelectVariants" were used to select SNPs only. The module "VariantFiltration" was used to quality filter the SNPs (based on QD < 2.0 || FS > 60.0 || MQ < 40.0 || MQRankSum < -12.5 || ReadPosRankSum < -8.0) individually.

The combination of LUMPY Express version 0.2.13 (Layer et al., 2014) and GATK version 4.2-0 (McKenna et al., 2010) were used to call structural variations. First, the "SelectVariants" module of GATK was used on the genotyped vcf obtained above to select INDELs with sizes >50 bp. Then the "VariantFiltration" was used to quality filter the INDELs (>50 bp) with "QD < 2.0 || FS > 200.0 || ReadPosRankSum < -20.0". Then the LUMPY Express was used to call large structural variants (deletions, duplications, and inversions) for each isolate using the alignment (bam) files obtained above following the workflow on Github (https://github.com/arq5x/lumpy-sv). In summary, discordant paired-end alignments were extracted with samtools view, filtering out reads by flag 1294 and split-read alignments were extracted by extractSplitReads_BwaMem script implemented in LUMPY (Layer et al., 2014). Both extracted alignment reads were sorted and then provided as input to the lumpyexpress utility along with the original bam file to obtain structural variants. "vcffilter" tool of vcflib version 1.0.2 (Garrison et al., 2021) was used to filter out the calls if SVTYPE is not "DEL" "DUP" or "INV" with PE<3 and "IMPRECISE" with a negative confidence interval (CIPOS95) value. The resulting vcf outputs were merged with the vcf-merge of vcftools version 0.1.17 (Danecek et al., 2011) for each species individually. Then bcftools isec of the bedtools suite (Quinlan & Hall, 2010) was used to find intersects of SVs called by GATK and LUMPY, and one of the variants present in both vcf output was kept for downstream analyses.

In addition, the CNVs are obtained as deletions and duplications with PE>3 following the additional filtration on "IMPRECISE" calls with a negative confidence interval value (CIPOS95) from each vcf output of lumpyexpress with vcffilter tool of vcflib version 1.0.2 again (Garrison et al., 2021). The resulting vcf files were merged with the vcf-merge of vcftools version 0.1.17 (Danecek et al., 2011) for each species.

3.2.7. The Distribution and Prediction of Functional Effects of Variants Along the Genomes of *M. fructicola* and *M. laxa*

The variant annotation, distribution based on the scaffolds, and functional effects of final VCFs (SNPs and SVs) for each species were predicted by SnpEff version 5.0 (Danecek et al., 2011). The reference genomes for both *M. fructicola* and *M. laxa* were not present in the database of SnpEff version 5.0 thus the reference genomes (under GenBank accessions "GCA_008692225.1" for *M. fructicola*; "GCA_009299455.1" for *M. laxa*) were built manually.

3.2.8. Transposable Element Consensus Identification, Classification, and Annotation

Transposable elements (TEs) and other repeats were identified with individual runs of RepeatModeler and RepeatMasker pipelines on reference genomes of *M. fructicola* (isolate Ti-B3-A3-2) and *M. laxa* (isolate Yildirim-1). First, a de novo repeat library was generated for these two reference genomes by RepeatModeler version 2.0.2 (Flynn et al., 2020) which is a program that uses RECON (Bao & Eddy, 2002), RepeatScout (Price, Jones & Pevzner, 2005), and Tandem Repeat Finder (TRF) (Benson, 1999) to build the library. The repeats obtained from the RepeatModeler consensus file were further processed by checking the duplicates and combined with the repeat library from Repbase (Bao, Kojima & Kohany, 2015). Then the RepeatClassifier implemented in the RepeatModeler was used to classify the repeats on the custom library. Repeats and TEs were then annotated by RepeatMasker version 4.1.2 (Smit, Hubley & Green, 2015) based on the custom library by using the -no_is (to skip bacterial insertion element check), -a (to produce an alignment file of the repeats), and -s (for slow search to increase the sensitivity) parameters with the "RM_BLAST" as the search model. The "calcdivergencefromalign.pl" and

"createrepeatlandscape.pl" scripts of RepeatMasker were used to calculate the Kimura divergence values and plot the repeat landscape for both species.

Then repeat annotation was performed for all other isolates using the draft genome assemblies obtained from the SPADES version 3.11.1 (Bankevich et al., 2012) based on the custom libraries mentioned above with the same parameters. The "Simple repeats" and "Low Complexity" matches were then removed to select only interspersed repeats and the output file is further processed with the Perl script "One code to find them all" (Bailly-Bechet, Haudry & Lerat, 2014).

3.2.9. Investigating the Potential Role of Structural Variants and Transposable Elements in DMI-Propiconazole Resistance

The structural variation and possible presence/absence of any transposable element of the propiconazole target *CYP51* and non-target *ABC* transporter genes were manually checked for each isolate to investigate the potential role of these SVs to DMI group fungicide propiconazole resistance in selected isolates.

CYP51 and *ABC* genes were retrieved from NCBI (with GenBank Accession Numbers MT724702.1 (*M. fructicola, CYP51* gene); LT615209 (*M. laxa, CYP51* gene); AY077839.1 (*M. fructicola, ABC* transporter gene) and mapped to reference genomes to find the scaffold/location of these genes. Finally, the copy number variation status of these genes was checked from vcf outputs of CNVs obtained from the Lumpy pipeline (Layer et al., 2014) as well as manually from *de novo* genome assemblies based on manual inspections of pair-wise alignments for each isolate separately.

Finally, the neighboring regions of *CYP51* and *ABC* transporter genes were manually inspected on *de novo* genome assemblies based on the outputs of "onecodetofindthemall" perl script for each isolate separately to investigate the presence/absence of any transposable elements.

CHAPTER 4
RESULTS AND DISCUSSION

4.1. *In vitro* Mycelium Growth Inhibition Assay

The IC_{50} values of *M. fructicola* isolates in the representative population ranged between 0.15 and 0.29. Besides, the range of IC_{50} values was between 0.10-0.19 for *M. laxa* isolates (Table 1). The discriminatory doses (mean IC_{50} values) based on the representative collection were 0.22 and 0.15 µg/ml for *M. fructicola* and *M. laxa*, respectively, and these concentrations were used as discriminatory doses to scan the corresponding species' whole collection.

Table 1

IC_{50} values, confidence intervals and mean IC_{50} values against propiconazole for the isolates of representative collection based on mycelial growth assays for both species: *M. fructicola*, *M. laxa*.

Species	**Isolate Code**	**City**	**Propiconazole**	
			IC_{50} (µg/ml)	Confidence Interval (CI)
M. fructicola	SC-B3-A2	Samsun	0.19	0.16-0.22
	BG-B1-A13	Bursa	0.15	0.14-0.17
	SC-B2-A4	Samsun	0.18	0.14-0.23
	B5-A4	Canakkale	0.21	0.17-0.26
	BG-B1-A17	Bursa	0.29	0.27-0.31
	T-B2-A6	Izmir	0.18	0.16-0.21
	T-B4-A2-3	Izmir	0.23	0.20-0.25
	B1-A3	Canakkale	0.29	0.26-0.31
	Ti-B3-A2	Izmir	0.22	0.16-0.28
	Ni-B3-A1	Nigde	0.23	0.18-0.28
	MM-B4-A2	Mersin	0.23	0.20-0.27
	Mean		**0.22**	

Table 1 (continued).

M. laxa	2B2-A1	Canakkale	0.17	0.14-0.21
	Yıldırım-2	Bursa	0.17	0.14-0.22
	T-B1-A6	Izmir	0.15	0.13-0.18
	2B1-A1	Canakkale	0.11	0.09-0.15
	T-B1-A4-2	Izmir	0.17	0.15-0.2
	SHD-3	Bursa	0.19	0.15-0.25
	MM-B4-A10	Mersin	0.16	0.10-0.27
	MT-B1-A3-1	Mersin	0.10	Very Wide
	Ni-B3-A2	Nigde	0.18	0.15-0.22
	Mean		**0.15**	

The sensitivity level of the total of 128 *Monilinia* isolates from peach fruits in Turkey was determined and the population against propiconazole was found to be sensitive since >90% of the isolates had an RG value <50 for both species. The RG values ranged between 3.33 to 69.61 for 108 *M. fructicola* and 5 to 33.33 for 20 *M. laxa* isolates. The median of the relative growth (RG) values was 24.67 and 25.85 for *M. fructicola* and *M. laxa,* respectively. The isolates with RG value higher than the median were classified as "Sensitive" and isolates with RG value lower than the median were classified as "Highly Sensitive". Based on the discrimination of the isolates, 50 isolates were "Highly Sensitive" and 58 isolates were "Sensitive" for *M. fructicola*. *Monilinia laxa* isolates were distributed equally as 10 isolates being "Sensitive" and 10 isolates being "Highly Sensitive". The RG values and resistance levels (RL) of the whole collection were presented in Appendix Table 1.

Eight isolates per species were then chosen based on the RG value and resistance level of the isolates. The selection was carried out to represent the resistance status equally for both species (four Sensitive isolates with high RG value and four Highly sensitive isolates with low RG value). The RG values and RL status of the selected isolates were presented in Table 2.

Table 2

Relative growth values and resistance levels of selected isolates based on *in vitro* mycelial growth inhibition assay against propiconazole for both species.

Species	Isolate Code	Relative Growth	Resistance Level
M. fructicola	BG-B3-A1	3.33	HS*
	SC-B2-A3	12.27	HS
	SC-B2-A4	18.94	HS
	B5-A4	21.81	HS
	T-B1-A5	28.00	S*
	YK-1	29.13	S
	BG-B1-A8	39.59	S
	BO-B3-A1	47.67	S
M. laxa	2B1-A2-2	5.00	HS
	Ni-B3-A2	18.46	HS
	MM-B4-A4	20.90	HS
	MT-B1-A3-1	21.73	HS
	T-B1-A4-2	28.33	S
	MM-B2-A2	30.47	S
	2B1-A5	32.25	S
	MM-B4-A3	33.33	S

*HS: Highly Sensitive; S: Sensitive.

4.2. DNA Extraction, Raw Data Statistics, and Quality of Filtered Short Reads

The DNA extraction of samples resulted in high DNA concentrations ranging from 80 to 250 ng/µl (3.2 to 10 µg in quantity in a total of 40 µl) which exceeds the minimum requirement of 0.1 µg/mL in quantity for further library preparation and sequencing. In addition, the quantity, as well as quality of the samples, were further checked by a fluorescence-based quantification and by gel electrophoresis, respectively by Macrogen Inc., Sequencing Service.

Once the raw reads in FASTQ format were provided by the Sequencing service, quality check controls were obtained. The total read bases, number of reads, and GC(%) content of the sequenced samples were highly similar with average values of 7.30 billion bases, 48 million reads, and 40% GC content in the respective order. The overall ratio of bases with phred quality over 30 was 91. The raw data statistics for all sequence samples were presented in Table 3 in detail.

Table 3

The raw data statistics and the average values of the total read bases, the number of reads, GC/AT contents (%), and the ratio of bases with phred quality scores over 20 and 30 for each isolate.

Isolate Code	**Species**	**Total read bases (bp)**	**Total reads**	**GC (%)**	**AT(%)**	**Q20 (%)**	**Q30 (%)**
BG-B3-A1	*M. fructicola*	7.934.656.796	52.547.396	40.06	59.94	96.73	91.25
BO-B3-A1	*M. fructicola*	7.359.764.160	48.740.160	39.42	60.58	96.61	90.94
SC-B2-A3	*M. fructicola*	7.934.272.048	52.544.848	39.93	60.07	96.44	90.75
BG-B1-A8	*M. fructicola*	5.459.641.768	36.156.568	39.82	60.18	96.42	90.81
2B1-A2-2	*M. laxa*	7.689.314.714	50.922.614	40.17	59.83	96.4	90.54
MM-B4-A3	*M. laxa*	7.450.487.980	49.340.980	40.06	59.94	96.28	90.21
Average		7.304.689.578	48.375.428	40	60	96	91

In addition, the per base and per sequence quality score plots belonging to the trimmed/quality filtered NGS reads generated in this thesis were presented in Figure 5.

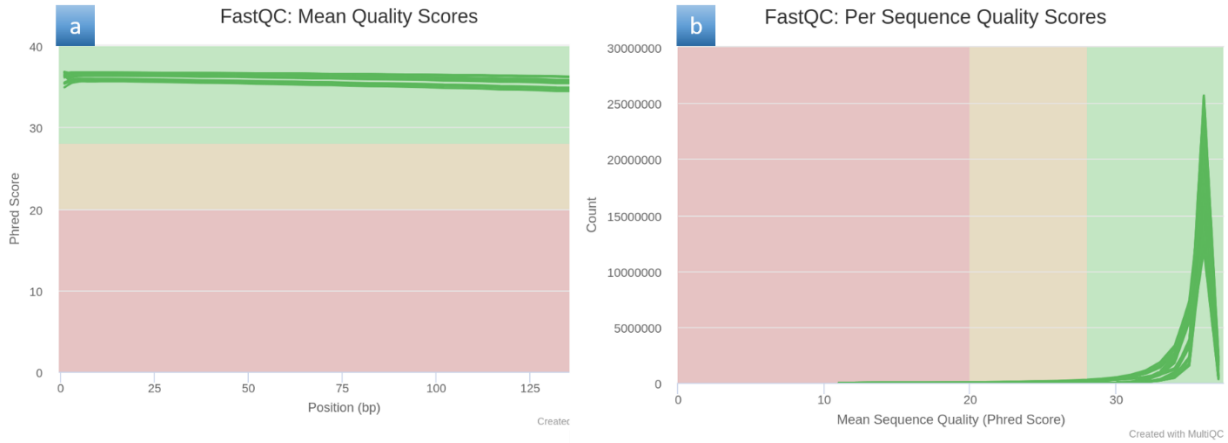

Figure 5. The collective a) per base and b) per sequence quality score plots for next-generations sequencing reads of four *M. fructicola* isolates (BG-B3-A1, BG-B1-A8, BO-B3-A1, SC-B2-A3) and two *M. laxa* (2B1-A2-2 and MM-B4-A3) isolates) generated in this thesis.

4.3. Genome Assembly Statistics

4.3.1. Reference-based Genome Assembly Statistics

Reference-based genome assembly was performed on one isolate per species (Ti-B3-A3-3 for *M. fructicola*; Yıldırım-1 for *M. laxa*) based on the available well-assembled reference genomes available on NCBI (with GenBank accession numbers "GCA_008692225.1" and "GCA_009299455.1" for *M. fructicola* and *M. laxa,* respectively) to discover the SNPs and structural variants of the selected isolates based on bioinformatics pipelines that use mapping strategies. The assembly statistics of reference-based genome assembly were found to be the same as the references used with 20 and 49 contig numbers; 2.5 and 2.4 Mb N50 size; 44 and 42 Mb in size for *M. fructicola* and *M. laxa,* respectively.

4.3.2. *de novo* Genome Assembly Statistics

de novo genome assemblies for each selected isolate were generated by SPADES version 3.11.1 (Bankevich et al., 2012) to further confirm the results of structural variant discoveries and to identify, classify and annotate the transposable elements for both species. Based on the *de novo* genome assemblies, the genomes of *M. laxa* presented better quality

statistics with an average of 155 contigs and 552 kilobases (kb) N50 size. On the other hand, the genomes of *M. fructicola* isolates presented an average of 679 contigs and 131 kb N50 size. In addition, the total lengths of the assembled *M. laxa* genomes were more similar to each other with sizes ranging from 41.99 to 42.16 Mb in length. The total length of the *M. fructicola* isolates ranged between 43.84 and 44.51 Mb. Genome assembly quality statistics of each isolate based on the Quast version 5.0.2 (Gurevich et al., 2013) have been presented in Table 4.

Table 4

Basic assembly statistics and averages of the number of contigs, N50, and length for the *de novo* assembled genomes of each isolate.

Species	**Isolate Code**	**Number of Contigs**	**N50 (bp)**	**Total Length (Mb)**
M. fructicola	BG-B3-A1	691	120683	43.84
	SC-B2-A3	828	111215	44.18
	SC-B2-A4	586	146461	44.20
	B5-A4	595	154409	43.91
	T-B1-A5	610	140742	44.23
	YK-1	666	129314	43.88
	BG-B1-A8	760	121744	44.51
	BO-B3-A1	701	130119	44.18
	Average	**679**	**131835**	**44.11**
M. laxa	2B1-A2-2	226	412408	42.01
	Ni-B3-A2	181	653047	42.06
	MM-B4-A4	117	631930	42.00
	MT-B1-A3-1	103	673182	42.01
	T-B1-A4-2	124	633580	41.99
	MM-B2-A2	161	539125	42.02
	2B1-A5	163	465265	42.16
	MM-B4-A3	168	411621	42.11
	Average	**155**	**552519**	**42.04**

4.4. Mapped Short Reads

The Illumina reads of each selected isolate were mapped to the reference genomes (Ti-B3-A3-2 for *M. fructicola*, Yildirim-1 for *M. laxa* isolates) obtained from reference-based genome assembly. The global summary statistics of the final bam files were inferred from the outputs of Qualimap version 2.2.1 (García-Alcalde et al., 2012). Overall, more reads were mapped for *M. laxa* isolates with percentages varying from 96.69% to 99.01% where these percentages were between 83.70% and 92.17% for *M. fructicola* isolates. The average mean coverages were 118 and 154 for *M. fructicola* and *M. laxa,* respectively. These high average coverages allowed the precise identification of variants for each isolate. Main global summary statistics based on the final bam files that are used in variant discovery for each isolate have been presented in Table 5.

Table 5

Number of reads (in millions), overall properly paired read percentages, and mean coverages with average values based on bam statistics for each isolate based on *M. fructicola* (Ti-B3-A3-2 as reference) and *M. laxa* (Yildirim-1 as reference).

Species	**Isolate Code**	**Number of Reads (million)**	**Overall Mapped Reads (%)**	**Mean Coverage**
M. fructicola	BG-B3-A1	38.43	84.77	110.70
	SC-B2-A3	37.81	84.99	109.28
	SC-B2-A4	36.84	92.17	115.38
	B5-A4	32.22	87.14	95.09
	T-B1-A5	54.61	90.36	167.61
	YK-1	54.98	90.07	169.46
	BG-B1-A8	26.61	85.00	76.54
	BO-B3-A1	35.15	83.70	100.05
	Average		**87.27**	**118.01**
M. laxa	2B1-A2-2	36.54	98.61	216.49
	Ni-B3-A2	33.31	98.92	115.77

Table 5 (continued).

	MM-B4-A4	50.90	99.01	176.91
	MT-B1-A3-1	38.28	98.28	132.14
	T-B1-A4-2	37.83	98.52	130.81
	MM-B2-A2	51.01	98.81	176.94
	2B1-A5	48.28	98.01	165.99
	MM-B4-A3	35.02	96.69	118.87
	Average		**98.35**	**154.24**

4.5. The Abundance, Distribution, and Prediction of Functional Effects of SNPs and Structural Variants Along the Genomes of *M. fructicola* and *M. laxa* Based on Reference-based Variant Calling

The distribution and the density of the variants were highly variable among scaffolds and species. Especially the representation of SVs along the genomes was highly variable between species. SVs were present along the genome of *M. fructicola* but distinct regions where SVs were absent along the genome of *M. laxa* were observed (Figure 6). The circular plots showing the abundance and distribution of discovered SVs along the genome in the genomic dataset have been presented in Figure 6.

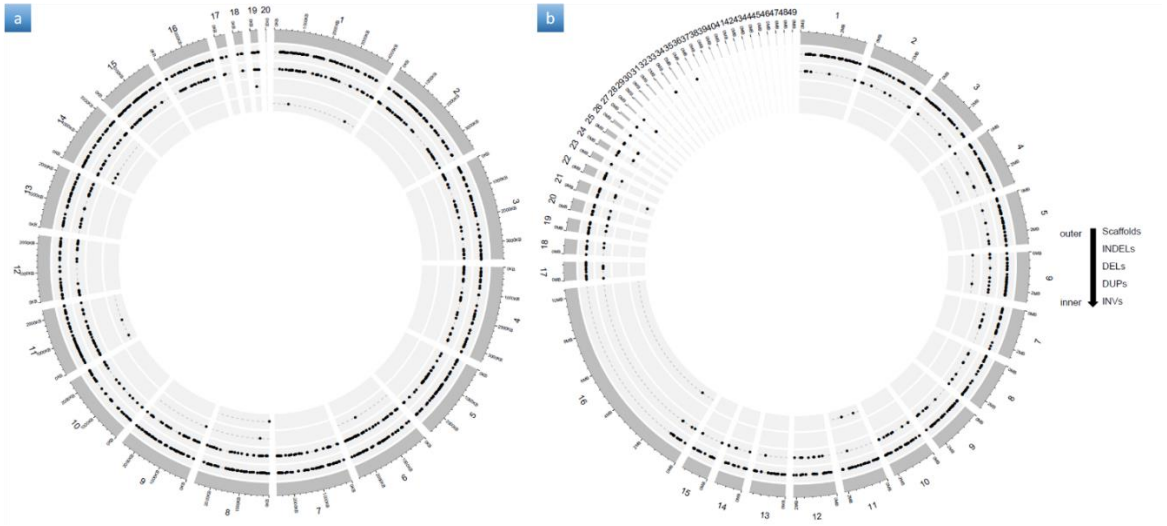

Figure 6. The circular plots showing the distribution of discovered structural variations (from outer to inner circle as named in the legend) based on variant calling from all isolates along the genome of a) *M. fructicola*, b) *M. laxa*.

Even though the genome sizes of isolates on average are highly similar (44 Mb for *M. fructicola*; 42 Mb for *M. laxa*), the number of both SNPs and SVs were around 1.5 times higher for *M. fructicola* genomic dataset (Table 6). The number of duplication and inversion events was extremely low for both species (Table 6), however, this result needs to be taken cautiously based on the filtering of the SVs to eliminate the false-positive variants.

Table 6

Total number of SNPs, structural variants, number of structural variants by type (INDELs, Deletions, Duplications, and Inversions), and the variant ratio between species.

	M. fructicola	***M. laxa***	
Type of Variant	Number of Variants		Variant Ratio (MF/ML)*
SNPs	266.618	190.599	1.40
Total SV	1.540	918	1.67
INDELs	964	740	
DELETIONS	564	171	
DUPLICATIONS	7	3	
INVERSIONS	5	4	

*Variant ratio: Number of variants in *M. fructicola* /Number of variants in *M. laxa*

Based on the SNPs, the transition (Ts) / Transversion (Tv) ratio was higher in *M. laxa* (5.93 for *M. laxa*; 2.53 for *M. fructicola*). As expected, the substitution rates of transitions were found to be higher than transversions for both species. Even though the genomic datasets belonging to two species consist of a low number of individuals in them, the difference between the Ts/Tv ratio might be due to the divergent evolutionary processes of these species.

Variant distribution for both species was investigated in detail after normalizing the dataset considering the number of variants based on the corresponding scaffold's length (by Relative Abundance (%) = (100*Number of variants on the scaffold) / The length of the scaffold). In general, the variants were more evenly distributed along the genomic scaffolds of *M. fructicola* (Figure 7). The relative abundance of SNPs was the lowest at Scaffold 18 (2.8%) and 20 (0.03%) (with scaffold names VICG010000018/20.1) for *M. fructicola*. The relative abundance of both SNPs and SVs was the highest at scaffold 19 (with scaffold name

VICG010000019.1) with 7219 SNPs and 27 SVs. Interestingly, there was only one SNP at scaffold 20 (VICG010000020.1) and no SV was present at this scaffold. Further investigation of this one SNP revealed that this variant is only present in BG-B1-A8 isolate and other isolates do not contain any SNPs in this scaffold.

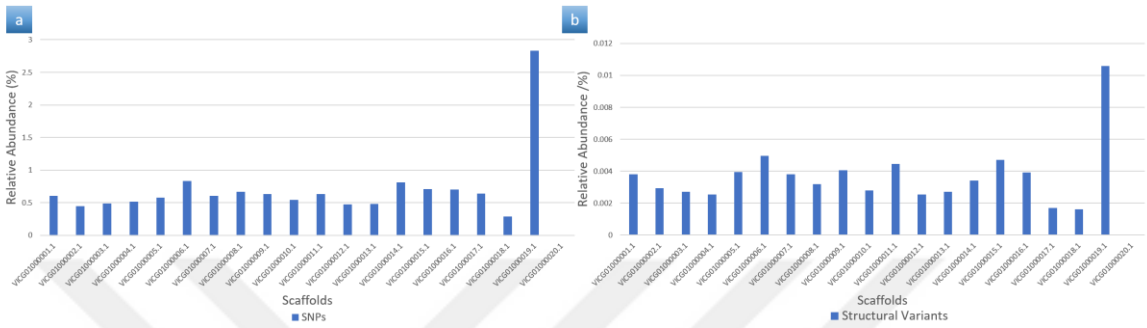

Figure 7. The relative abundances of a) SNPs and b) Structural variants along the twenty scaffolds for *M. fructicola* genomic dataset.

The distribution and the abundance of the variants along the genome of *M. laxa* were more variable in comparison to the *M. fructicola* (Figure 8). Scaffold 33 (with 62.38 kb size) did not contain any SNPs and many other scaffolds (scaffolds 29,30,34,35 and 38) with sizes varying between 70 to 24 kb had a highly low number of SNPs. The relative abundance of SNPs peaked at Scaffold 49 and no SV was present at this scaffold. Besides, eighteen scaffolds with sizes varying between 70 to 5 kb did not contain any SV. Moreover, three scaffolds (30, 35, 38 with 69 kb, 49 kb, and 24 kb length respectively) had only one SNP in them. When these scaffolds are further investigated, it has been seen that only one isolate out of eight carries the corresponding SNP (isolates Ni-B3-A2, MM-B4-A3, and MM-B2-A2 respectively).

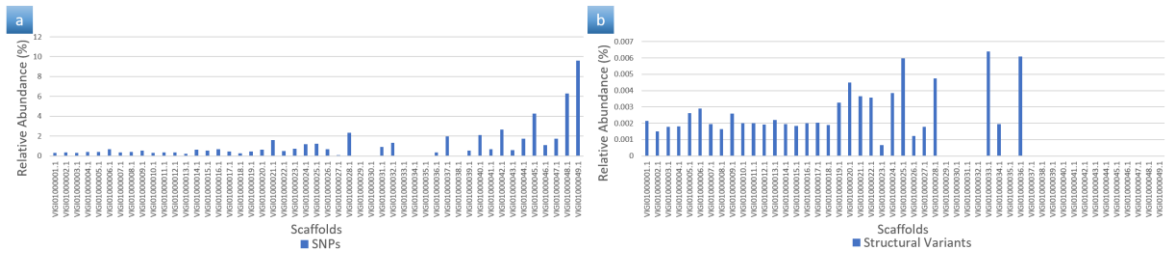

Figure 8. The relative abundances of a) SNPs and b) Structural variants along the forty-nine scaffolds for *M. laxa* genomic dataset.

The length of the scaffolds with the number and relative abundance of the SNPs and structural variants for both species have been presented in Appendix-Tables 2&3.

Moreover, the prediction of functional effects of variants for the *M. fructicola* genomic dataset by SnpEff version 5.0 (Cingolani et al., 2012) revealed that intergenic regions contain the most SNPs (47.36% of all SNPs). This result is followed by down- and upstream regions of genes with 24.02% and 23.05% of all SNPs located in these regions respectively. The results were similar for *M. laxa* as most of the SNPs (41.02%) were located in the intergenic regions along the genome and 27.87% and 25.59% of the SNPs were located at down- and upstream of genes. Since many of the SNPs were not located in genic regions, only a small proportion of the SNPs were resulting in functional effects (e.g. lost/gain of a start codon). The number and percentages of SNPs by type were presented in Table 7 for both species.

Table 7

The count and percent of SNPs based on the prediction of functional effects by main types in alphabetical order for *M. fructicola* and *M. laxa*.

	M. fructicola		***M. laxa***	
Annotation	**Count**	**Percent (%)**	**Count**	**Percent (%)**
Downstream Gene Variant	122.811	24.022	115.828	27.877
Intergenic Region	242.133	47.361	170.458	41.026
Intron Variant	5.785	1.149	4.332	1.043
Missense Variant	11.685	2.286	9.653	2.323
Splice Region Variant	922	0.180	630	0.152
Synonymous Variant	8.941	1.749	7.430	1.788
Upstream Gene Variant	117.874	23.056	106.345	25.595

In addition, the prediction of functional effects of SVs for *M. fructicola* showed that 57.79% of the SVs were found in regions with transcript sequences and only 10.95% of these variants were in intergenic regions. Similar to the locations of SNPs, many SVs were also found to be at up-and downstream regions of genes. *M. laxa* genomic dataset presented highly similar results in which most SVs were corresponding to transcript sequences and many SVs were present up up-and downstream of genes. The number and percentages of SVs by main types were presented in Table 8 for both species.

Table 8

The count and percent of SVs based on the prediction of functional effects by main types in alphabetical order for *M. fructicola* and *M. laxa*.

	M. fructicola		***M. laxa***	
Annotation	**Count**	**Percent (%)**	**Count**	**Percent (%)**
Downstream Gene Variant	772	5.859	1.195	5.347
Intergenic Region	1.444	10.959	885	3.960
Intron Variant	41	0.311	70	0.313
Transcript Ablation	7.615	57.794	13.742	61.488
Upstream Gene Variant	786	5.965	1.306	5.844

4.6. Genome-wide Copy Number Variation Status of *M. fructicola* and *M. laxa*

In addition to the total structural variants, the copy number variations are retrieved as deleted and duplicated regions from the SVs for each isolate. The number of copy number variable regions (CNVRs) was highly similar within the species. On the other hand, the genome size affected by CNV was highly similar for *M. fructicola* isolates (with sizes ranging between 237 kb and 373 kb) but variable for *M. laxa* isolates (with sizes ranging from 406 kb to 1.098 Mb). The detailed number of CNVRs, base pairs affected and percent of CNVRs for each isolate has been presented in Table 9.

Table 9

The number of copy number variable regions, base pairs affected, and the percent (%) of copy number variable regions for each isolate.

Species	Isolate Code	Number of CNVRs	Base pairs affected (bp)	Genome Size (Mb)	% of the Genome
M. fructicola	B5-A4	160	354.560	43.91	0.80
	BG-B1-A8	136	237.546	44.51	0.53
	BG-B3-A1	162	276.370	43.84	0.63
	BO-B3-A1	132	229.826	44.18	0.52
	SC-B2-A3	142	273.663	44.18	0.61
	SC-B2-A4	164	332.562	44.20	0.75
	TB1-A5	178	329.094	44.23	0.74
	YK-1	210	373.390	43.88	0.85
	Average	**160.5**	**300.876**	**44.11**	**0.67**
M. laxa	2B1-A5	71	406.513	42.16	0.96
	2B1-A2-2	74	420.554	42.01	1.00
	MM-B2-A2	78	1.077.540	42.02	2.56
	MM-B4-A3	60	574.503	42.11	1.36
	MM-B4-A4	88	1.146.113	42.00	2.72
	MT-B1-A3-1	76	1.097.739	42.01	2.61
	NI-B3-A2	75	1.098.551	42.06	2.61
	TB1-A4-2	84	1.127.632	41.99	2.68
	Average	**75.75**	**747.419**	**42.04**	**2.06**

Similar to the SVs, the number of CNVRs was approximately 2 times higher in *M. fructicola* isolates. However, when it comes to the base pairs affected by the copy number variation along the genome, the CNVRs of *M. laxa* isolates were consisting of bigger deletions/duplications in size. The size distribution of the CNVRs for both species has been presented in Figure 9. On average, 0.67% of *M. fructicola* genomes and 2.06% of *M. laxa* genomes were showing copy number variation.

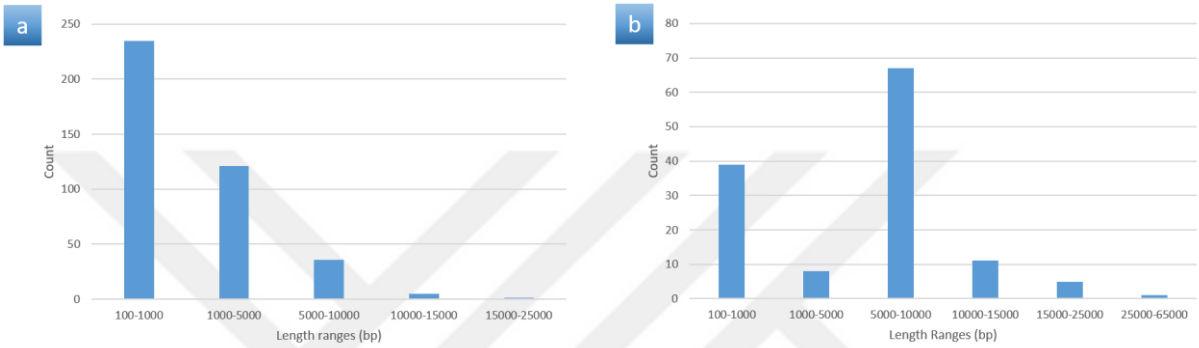

Figure 9. The size distribution of deletions and duplications for a) *M. fructicola*, and b) *M. laxa* isolates.

4.7. Transposable Element Content and Their Dynamics in *Monilinia* species

On reference genomes used, the TE content was comprising 3.94 Mb in *M. fructicola* (8.97% of the genome) and 4.24 Mb in *M. laxa* (9.92% of the genome). A total of 15327 and 10710 elements were found for *M. fructicola* and *M. laxa* respectively. Following the trend in the literature, the number of Class I elements was higher for both species (Table 10).

Table 10

The class and number of transposable elements, the length they occupy, and the relative represented percent (%) of the genome for reference genomes of *M. fructicola* and *M. laxa.*

	M. fructicola			***M. laxa***		
Class	Number of Elements	Length Occupied (bp)	% of the Genome	Number of Elements	Length Occupied (bp)	% of the Genome
Class I (Retrotransposons)	4642	647936	1.47	4627	2288342	5.35
Class II (DNA Transposons)	3508	247489	0.56	2676	159784	0.38
Unknown	7177	3065317	6.96	3407	1807607	4.22
TOTAL	**15327**	**3948425**	**8.97**	**10710**	**4244622**	**9.92**

The detailed TE classification revealed that most of the TE content were *LTR* retrotransposons for both species. Respectively, 2600 and 2749 LTR elements which comprise 1.18% and 3.96% of the genomes of *M. fructicola* and *M. laxa* were found. Among the LTRs, *Bel-Pao, Ty1-Copia*, *Gypsy-Dırs1,* and Retroviral families were found. *Gypsy* was the most represented family (with 1284 and 1566 copies) followed by *Copia* (863 and 810 copies) and *LINE L2-CR1-Rex* (637 and 540 copies) families for *M. fructicola* and *M. laxa* species respectively. Classified Class II elements were represented at a low rate for both species (0.56% and 0.38% of the genome for *M. fructicola* and *M. laxa* respectively). The most represented family of the Class II/DNA transposons was the *hobo-Activator* for both species. Moreover, the number of *SINE* elements was extremely low for both species with 30 (0.004% of the genome) and 35 (0.005% of the genome) elements for *M. fructicola* and *M. laxa* respectively. The detailed TE content based on the families for both species were presented in Tables 11 and 12.

Table 11

The detailed transposable element content based on class, order, number, and total length occupied for the reference genome of *M. fructicola*.

Class	Order	Superfamily/Family	Number of Elements	Total Length
Class I	SINE	-	30	2186
	Penelope		268	15931
	LINE	CRE/SLACS	44	2496
		L2/CR1/Rex	637	40114
		R1/LOA/Jockey	97	6179
		R2/R4/NeSL	92	4997
		RTE/Bov-B	117	6264
		L1/CIN4	419	24254
	LTR	BEL/Pao	168	10407
		Ty1/Copia	863	109555
		Gypsy/DIRS1	1284	387094
		Retroviral	285	14934
Class II		hobo-Activator	482	26369
		Tc1-IS630-Pogo	366	67234
		PiggyBac	38	2171
		Tourist/Harbinger	115	7145
		Other (Mirage, P Element, Transib)	55	3315
	Rolling Circles		241	12317
Unclassified			7177	3065317
		TOTAL	**15327**	**3948425**

Table 12

The detailed transposable element content based on class, order, number, and total length occupied for the reference genome of *M. laxa*.

Class	Order	Superfamily/Family	Number of Elements	Total Length
Class I	SINE	-	35	2368
	Penelope		248	13301
	LINE	CRE/SLACS	31	1464
		L2/CR1/Rex	540	32711
		R1/LOA/Jockey	78	4808
		R2/R4/NeSL	66	4141
		RTE/Bov-B	70	3599
		L1/CIN4	350	21246
	LTR	BEL/Pao	140	9062
		Ty1/Copia	810	429826
		Gypsy/DIRS1	1566	1245803
		Retroviral	233	12857
Class II		hobo-Activator	336	16414
		Tc1-IS630-Pogo	223	18119
		PiggyBac	31	1863
		Tourist/Harbinger	100	6265
		Other (Mirage, P Element, Transib)	54	2955
	Rolling Circles		206	11111
Unclassified			3407	1807607
		TOTAL	**10710**	**4244622**

The TE content within and between the species was highly similar to each other where on average 9.29% (with 15809 elements) and 8.59% (with 10401 elements) of the genomes of *M. fructicola* and *M. laxa* were consisting of TEs. The content of different TE families for each isolate was similar to the reference genomes. The detailed TE content for each isolate used in this thesis has been presented in Table 13.

Table 13

The transposable element content based on the number and percent of the genome occupied for all *M. fructicola* and *M. laxa* isolates used in this thesis.

Species	**Isolate Code**	**Retrotransposon**		**DNA Transposon**			
		Number of Elements	Percent of Genome	Number of Elements	Percent of Genome	Total Number of Elements	Percent of Genome
M. fructicola	B5-A4	4744	1.40	3424	0.53	15657	9.20
	BG-B1-A8	4778	1.59	3347	0.52	16119	9.67
	BG-B3-A1	4768	1.45	3353	0.54	15624	9.09
	BO-B3-A1	4747	1.56	3366	0.54	15684	9.28
	SC-B2-A3	5044	1.70	3386	0.52	16099	9.45
	SC-B2-A4	4741	1.52	3318	0.51	15833	9.31
	TB1-A5	4816	1.55	3353	0.52	15819	9.33
	YK-1	4810	1.50	3443	0.55	15637	9.02
	Average	**4806**	**1.53**	**3373**	**0.52**	**15809**	**9.29**
M. laxa	2B1-A5	4569	4.62	2507	0.35	10412	8.88
	2B1-A2-2	4512	4.48	2492	0.35	10311	8.51

Table 13 (continued).

	MM-B2-A2	4532	4.25	2548	0.36	10394	8.56
	MM-B4-A3	4504	4.41	2524	0.35	10341	8.67
	MM-B4-A4	4546	4.39	2588	0.36	10457	8.58
	MT-B1-A3-1	4519	4.35	2556	0.36	10389	8.53
	NI-B3-A2	4557	4.29	2553	0.36	10442	8.50
	TB1-A4-2	4604	4.32	2545	0.36	10469	8.54
	Average	**4542**	**4.38**	**2539**	**0.35**	**10401**	**8.59**

In addition, divergence landscapes of TEs based on the reference genomes have been generated for both species (Figure 10). Assuming the neutral evolution of new inserted TE copies into the genomes, the amount of divergence of each copy is a proxy for the time since its duplication, with older copies accumulating more substitutions. Thus, TE landscapes allowed us to see the sequence divergence of each detected TE with Kimura-2 distances from the consensus sequence (inferred ancestral copy) and the evolutionary history of TE insertions along with the evolution of these species.

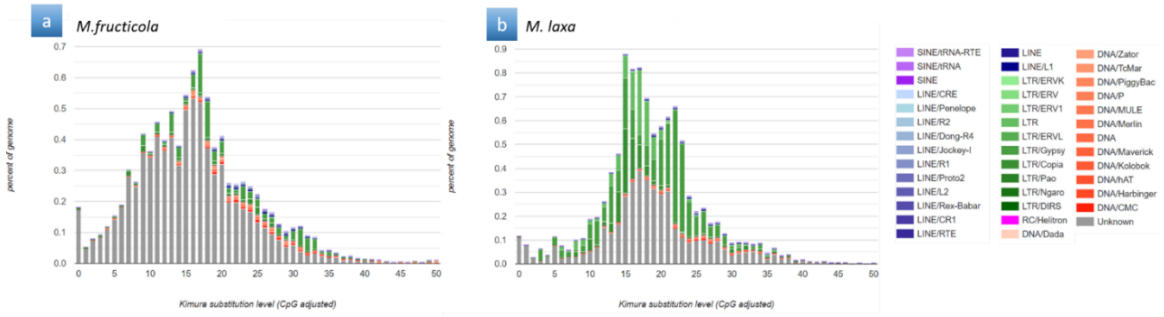

Figure 10. Repeat Landscape plots and the TE burst events for a)*M. fructicola* and b)*M. laxa*. Sequence divergence of each TE copy from the consensus measured by Kimura-2 distance. The further to the left, the younger the insertion of the corresponding TE.

Most of the TE burst events for both species were old with Kimura-2 distance values between 10-30 and the recent insertions were low for both species. However, *M. fructicola* presented relatively younger TE burst events and in general, the TE insertion events were more dynamic when compared to the *M. laxa*. The majority of TE burst events were mainly consisting of *LTR* elements. Moreover, the recent insertions (TE copies with zero divergences) was consisting of *LTR-Copia* and *Pao*, *LINE-Jockey* elements for both species. The oldest insertions into the genomes were belonging to the Class I/*SINE* and Class II/*Tc-Mariner* elements for both species.

4.8. The Potential Role of Structural Variants in DMI Resistance

The potential role of structural variation in DMI resistance for both species was investigated by manually checking the *CYP51* and *ABC* transporter genes. *CYP51* is a gene that belongs to the cytochrome P450 monooxygenase superfamily and mediated a crucial step in the biosynthesis of ergosterol (Zhang et al., 2019). *ABC* transporters, on the other hand, are responsible for the import and export of substrates including fungicides (Sipos & Kuchler, 2006). Both genes based on their function have a direct impact on the fungicide/DMI resistance. Based on the reference-based variant calling, no SV (including CNV) was found at or near *CYP51* and *ABC* transporter genes for each species. Moreover, regardless of their differentiating sensitivity levels against propiconazole, the SV status at/near these genes was the same as all isolates.

In addition, investigations of TE at/near the *CYP51* gene revealed a TE insertion at the upstream region of the *CYP51* gene for three out of eight *M. fructicola* isolates and no TE was present at/near the *CYP51* gene for *M. laxa* isolates. The movement of a TE into a pre-existing TE within the genome results in a nested TE (Gao et al., 2012). Intriguingly, a nested TE at the 223 bp upstream region of the *CYP51* gene consisting of Class I/*Sat-2_LVa* and Class II/*LINE-CR1-DNA-8-3_HM* elements was found in two *M. fructicola* isolates (T-B1-A5 and YK-1) classified as propiconazole-sensitive (Figure 11).

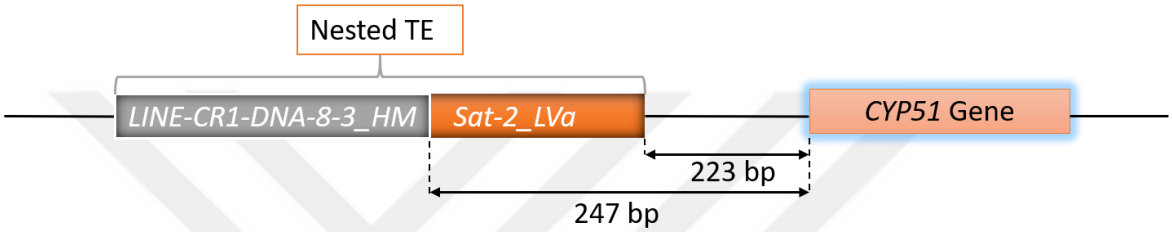

Figure 11. Visual representation of the detected nested TE (Class I/*Sat-2_LVa* and Class II/*LINE-CR1-DNA-8-3_HM*) and its relative distance to the upstream region of the *CYP51* gene in *M. fructicola*.

However, the same nested TE was also present in one of the highly sensitive *M. fructicola* isolate (SC-B2-A4) and absent in one of the sensitive *M. fructicola* isolate (BO-B3-A1).

Furthermore, since the detected TE is nested, the Kimura-2 distances of *LINE-CR1-DNA-8-3_HM* and *Sat-2_LVa* elements were investigated to predict the order of insertions. Class II/ *LINE-CR1-DNA-8-3_HM* had relatively less Kimura-2 distance to its consensus sequence (19.98) when compared to the Class I/*Sat-2_LVa* element (23) which indicates the later insertion of the *LINE* element to the region. In addition, no TE was present near the *CYP51* gene for other highly sensitive *M. fructicola* isolates B5-A4, BG-B3-A1, and SC-B2-A3. Moreover, no TE was present at or near the *ABC* transporter gene for both species.

4.9. Discussion

Characterization of the structural variations in a given species is a crucial step in understanding the adaptive evolutionary processes (Lowry & Willis, 2010) but these variants remain unexplored for many phytopathogenic fungi. In many other organisms, it has been now realized that these variants are not only abundant in the genomes but they are also responsible for many phenotypic changes (Wang et al., 2020; Zhao & Gibbons, 2018). To date, the definition, as well as detection of these variants, have been a challenge. Originally, these variants were defined as INDELs and inversions with sizes greater than 1 kb (Feuk, Carson & Scherer, 2006). Later on, with the advancement in sequencing technologies, and with researchers routinely sequencing many genomes of model and non-model organisms, the definition of SVs have been evolved as well. Currently, the definition of the SVs has been elaborated as variants that change the size, copy number, location, orientation, and sequence content with a size as small as >50 bp (Escaramís, Docampo & Rabionet, 2015). The challenge, now, has been turned into the discovery of the extent of SVs and their role in phenotypic changes and evolutionary processes along the tree of life. The number of tools to detect SVs has been increasing (Lei et al., 2022), however, the precise detection of SVs is highly challenging based on the diverse forms of these variants. For example, mobile element transpositions (e.g. TEs) are also considered SVs based on their characteristics, and the common bioinformatics approaches to detect genome-wide SVs are not sufficient enough the detect the highly dynamic nature of TEs. Thus, combinational and/or separate tools need to be used to characterize the genome-wide SVs for a given genomic dataset.

Fungicides are the key factors for controlling the plant diseases caused by plant fungal pathogens for decades and the knowledge of the resistance status of a fungal population against a fungicide is crucial to understanding the current and prospects of the disease management strategies. Furthermore, understanding the genetic mechanisms that underlie the resistance in a given population gives valuable information on the resistance evolution in both continental and global manner. In general, studies conducted so far to elucidate the genetic mechanisms of fungicide resistance have been mainly focused on the genome-wide SNPs so far (e.g. (Pereira, McDonald & Croll, 2020)). However, no study for phytopathogenic fungi has been conducted to associate the genome-wide SVs with fungicide

resistance. The studies that correlate the SVs with fungicide resistance solely focus on the specific type of SV (e.g. (Steinhauer et al., 2019)) rather than looking for an association between total SVs and fungicide resistance.

In this thesis, the detailed SNP and SV content for two prominent species of the *Monilinia* genus, *M. fructicola* and *M. laxa* have been investigated for the first time. Also, the sensitivity level of our population consisting of 109 *M. fructicola* and 20 *M. laxa* isolates collected from five geographical regions in Turkey against DMI group fungicide propiconazole has been defined for the first time, as well. The determination of the sensitivity level of the population has been performed based on a previously developed novel method by us (Durak et al., 2021). Overall, the population is defined as sensitive to the tested DMI group fungicide propiconazole. Considering these pathogens to have devastating effects on crops (especially peaches) in Turkey (Ozkilinc et al., 2020), this fungicide can be considered a promising candidate for controlling the brown rot disease in the close future. Overall, isolates were differentiated either as Highly Sensitive (HS) or Sensitive (S) for both species. The IC_{50} values were slightly higher in the representative collection of *M. fructicola* in comparison to *M. laxa* isolates (with average values of 0.22 and 0.15 µg/ml, respectively). In addition to determination of *in vitro* sensitivity levels of the population and genome-wide variant characterization, the potential role of structural variants at/near the selected genes in DMI resistance has been inspected for both species for the selected isolates with differentiating sensitivity levels.

Here, a total of 266.618 and 190.599 SNPs; 1540 and 918 SV based on variant calling via combinational bioinformatics approaches have been detected for *M. fructicola* and *M. laxa* isolates, respectively. The number of both SNPs and SVs were approximately 1.5 times higher in *M. fructicola* genomic dataset. The extent, as well as distribution of SNPs and SVs, were highly conserved within the species but highly diverse between the species. The INDELs were the most commonly occurring SV type for both species and the number of duplication and inversion events was extremely low for both species. Moreover, in some cases, only one isolate was carrying an SNP or SV in the total genomic datasets (e.g. SNP at scaffold 20 carried by *M. fructicola* BG-B1-A8 isolate). Plant fungal pathogens may harbor

so-called “accessory chromosomes” which carry genes that are not essential to survival (e.g. pathogenicity and virulence genes) and these chromosomes may show presence/absence variation (PAV) within the individuals of the same species (Witte et al., 2021). Thus, the sole variants coming from a single isolate raised to question of whether other isolates carry the corresponding scaffold/region or not, in other words, do these regions show PAV for different isolates? However, the genome sizes based on *de novo* genome assemblies (Table 4), as well as manual inspections of alignment files have revealed that these region/s are present in each isolate for both species and the reason for the presence of one variant at the corresponding regions is that other isolates do not contain any variants in these regions. On the other hand, the knowledge of the chromosome number for *Monilinia* species does not exist. Considering the variation discovered within this thesis and the diversity of fungal pathogens present, more studies with a high number of isolates around the world are required to unravel the detailed genomic properties of these species.

Interestingly, no SVs were present from a large proportion of the scaffold 16 (with a size of 1.04 Mb) as well as in many other scaffolds (e.g. scaffolds from 37 to 49 with sizes ranging from 25 to 5 kb) for *M. laxa* isolates. On the other hand, SVs were present along with the genomic dataset of *M. fructicola* species. The extensive genomic studies provided us with many insights into genome-wide adaptive evolutionary processes and many new hypotheses have been derived. One of the hypotheses states that the repeat-rich/gene-sparse regions in the genome evolve faster in contrast to repeat-poor/gene-rich regions with a so-called “two-speed genome model” to describe the evolution of plant pathogenic fungi (Dong, Raffaele & Kamoun, 2015). *M. laxa* genome might suit this “two-speed genome model” considering the highly variable variant rate at different compartments of the genomes.

Furthermore, predictions of functional annotations of the detected variants revealed the potential relevance of both SNPs and SVs based on their locations and types. In this context, most of the SNPs were found in intergenic regions. On the other hand, most of the SVs (57.79% and 61.48% of all SVs for *M. fructicola* and *M. laxa*, respectively) were corresponding to transcript sequences that have a high potential functional effect that might take place in adaptive evolutionary processes. These results show the direct potential effects of SVs in comparison to the SVs and underlie the potential importance of these variants in

any given phenotype considering their abundance and effects along the genome of both species.

In addition, the CNVs were retrieved from the total SVs as deleted and duplicated sequences to further investigate the CNV status for each isolate separately. The number of CNVRs and the length they affect in the genomes were highly similar within the species (Table 9). On the other hand, the results revealed that the size of the CNV matters, and even though *M. laxa* isolates consisted of a lower number of CNVRs, the total base pairs in length they affect in the genomes were more than two times larger in comparison the *M. fructicola* (with averages of 2.06% and 0.67, respectively) (Table 9). The number of deletion events was considerably higher in comparison to the duplication events for both species (in total 564 deletions and 7 duplications for *M. fructicola*; 171 deletions and 3 duplications for *M. laxa*). At the evolutionary level, duplication or deletions are common processes that have the potential to change the gene dosage when the events occur with duplication/deletions of genes (Rice & McLysaght, 2017). The "gene dosage effect hypothesis" proposes the direct effect of an imbalanced copy number of genes to a given phenotype with high potential in the change of the product of the given gene (Pritchard & Kola, 1999). But the duplicability and the fixation of a gene are highly dependent on many factors including the importance, complexity, and number of alternative spliced forms of the gene (Qian & Zhang, 2008). It has been shown that the fixation of gene duplication is mainly driven by positive selection based on the effect of the change in gene dosage (Kondrashov & Kondrashov, 2006). Thus, the low number of duplication events might be due to non-favorable selection processes of the duplicated regions for these species. However, it should also be noted that the filtering parameters for the variant calling were strict to only obtain the true positive variants and these low numbers might also be related to the filtering applied.

To further characterize the SVs for these species extensively, the TE content and their dynamics are further investigated via a comprehensive TE detection/annotation bioinformatics pipeline. A combinational bioinformatics approach was used to detect TEs in the genomes of *M. fructicola* and *M. laxa*. First, RepeatModeler version 2.0.2 (Flynn et al., 2020), was used to build a *de novo* library for the given assembly. RECON implemented in RepeatModeler applies whole-genome alignments to detect/classify the repeats *de novo*.

On the other hand, RepeatScout implemented in RepeatModeler uses a k-mer-based approach to describe the repetitive sequences in the genome. Since both alignment and k-mer-based approaches do not use a library, the novel repeats are also discovered through the RepeatModeler. Secondly, a custom library is formed by combining the *de novo* repeat library and Repbase library, and the elements in this custom library are classified by RepeatClassifier which is a program implemented in RepeatModeler. Then the RepeatMasker version 4.1.2 (Smit, Hubley & Green, 2015) which uses a library of previously characterized repetitive elements in a similarity-based manner was used. Even though RepeatClassifier uses an extensive homology-based approach that compares the repeat families to Repeat Protein Database and provided a repeat library, a big proportion of the detected elements remained unclassified (7177 and 3407 elements for *M. fructicola* and *M. laxa* reference genomes, respectively). There could be several reasons for many of the TEs to stay as unclassified for both species. One of them would be due to insufficient representation of the fungal TEs in the RepBase library used. On the other hand, this might be due to novel repetitive sequences in both *M. fructicola* and *M. laxa*. Even though many other approaches are present for manual curation and classification of TEs, this requires expertise and a high level of knowledge in TE biology. Nevertheless, more than 15 TE families which in total represent approximately 9% of the genome were successfully curated for both species. As Class I/Retrotransposons follow the copy-paste mechanism for replication, these elements are considered major contributors to the repetitive sequences along the tree of life and these elements (especially LTR elements) were composing the largest fraction of the genomes for both *M. fructicola* and *M. laxa* isolates. Class I elements have been correlated with the increase in the genome size for other fungal pathogens. For example, it has been shown that the genome of *Blumeria graminis* is four-time larger than an average ascomycete genome with the major contribution of LTR elements (Spanu et al., 2010). In another study, interestingly, only two LTR families were composing 29% of the *Phytophthora infestans* genome (Haas et al., 2009). Furthermore, Kimura-2 distances of TEs in reference genomes of both species have shown that most of the insertions/proliferations are relatively old and the number of recent insertions is relatively low. And following the trend in the literature explained above, almost all TE burst events (the sudden increase in the proportion of TEs along with the evolution of species) were mainly consisting of LTR elements. Thus, LTRs might potentially take place in the future evolutionary processes for both species.

Finally, the investigation of the potential role of SVs at/near *CYP51* and *ABC* genes revealed that these genes do not show CNV for both species and no SV discovered via variant calling is present at/near these genes. It should be noted that, even though the isolates with extreme sensitivity phenotypes are chosen to potentially discover the role of SVs in DMI resistance, the population, overall, is sensitive against the tested fungicide propiconazole. So, the inclusion of resistant isolates as well as a genome-wide association (GWAS) at the population level might present more precise results for the contribution of SVs against fungicide resistance. However, we did not have resistant isolation in our population and eight isolates per species were insufficient for the statistical requirements of the GWAS. Thus, in addition to creating an extensive genome-wide variant catalog for both species for the first time, we manually investigated the variants at/near the most probable genes that can take a role in the DMI resistance. Yet, we were able to detect a nested TE at the upstream region of the *CYP51* gene that might take a role in DMI resistance in *M. fructicola*. This detected TE was present in two of the isolates showing a less sensitive phenotype and absent in three highly sensitive isolates. Even though the same TE was present at one of the less sensitive isolates as well, the fungicide resistance is considered to be a complex trait (Arslan et al., 2022, Unpublished Data). Moreover, considering TEs provide additional promoters and cis-regulatory elements and regional chromatin changes caused by these elements into the region they have been inserted in, they might have a direct effect on the expression levels of nearby genes (Sundaram & Wysocka, 2020). Thus, further investigation of the change in the expression level for the isolates with and without this nested TE is needed to see the potential effect of this discovery at the transcription level.

CHAPTER 5
CONCLUSION

Characterizing the genome-wide variants and investigating the effect of variants along the tree of life is a crucial task in evolutionary biology. Thus, the determination of the genome-wide variants is the first step to understanding the evolution of a given species. In this thesis, in addition to the determination of the sensitivity status of the population consisting of the two prominent species of the *Monilinia* genus (*M. fructicola* and *M. laxa*) from Turkey against the DMI group fungicide propiconazole, an extensive SNP and SV characterization have been determined for the first time. In addition, the potential role of the characterized SVs (INDELs, CNVs, inversions, and TE) in the propiconazole resistance has been investigated.

The whole population was defined as sensitive to propiconazole which makes this group of fungicides promising in terms of disease management in Turkey. Overall, *M. fructicola* isolates were more SV rich in contrast to *M. laxa* isolates, and SVs in *M. laxa* isolates were absent in large proportions of the genomes which suggests potential differing evolutionary processes for these two closely related species. On the other hand, the TE content for these species was highly similar with approximately 9% of the genomes filled with TEs, and most of the insertions of TEs were relatively old. Class I (especially the LTR elements) comprised the largest proportion of the genomes for both species. In addition, no SV (detected via variant calling) were found at or near the selected candidate *CYP51* and *ABC* genes in the selected isolates with differentiating sensitivity levels for both species. However, a nested TE at the upstream *CYP51* gene in *M. fructicola* which is present in two of the less sensitive isolates and absent in three highly sensitive isolates has been discovered. Thus, the TEs might potentially take a role in DMI resistance in the quarantine pathogen *M. fructicola* population in Turkey. Further investigation of this detected TE on the DMI resistance (e.g. its potential effect on *CYP51* gene expression) and association studies of the SVs with a higher number of isolates would be highly informative to unravel the effects of these variants.

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APPENDIX

Table 1. Resistance levels and relative growth values of the whole collection to propiconazole based on *in vitro* mycelial growth inhibition assay. (S: Sensitive, HS : Highly Sensitive)

Species	Isolate Code	Resistance Level	Relative Growth
M. fructicola	BG-B1-A9	S	38.94
M. fructicola	BO-B3-A1	S	47.67
M. fructicola	BO-B3-A2	S	26.52
M. fructicola	BO-B1-A5	S	25.80
M. fructicola	BG-B1-A11	S	27.50
M. fructicola	BO-B1-A3	HS	18.46
M. fructicola	BG-B1-A14	HS	23.07
M. fructicola	BO-B1-A2	HS	17.35
M. fructicola	BO-B1-A1	S	33.04
M. fructicola	BO-B2-A2	S	30.95
M. fructicola	BO-B3-A3	S	28.37
M. fructicola	BO-B1-A7	HS	17.55
M. fructicola	BO-B1-A6	S	24.61
M. fructicola	BG-B1-A1	HS	22.93
M. fructicola	BG-B1-A8	S	39.59
M. fructicola	BG-B1-A16	S	24.45
M. fructicola	BO-B2-A1	HS	14.81
M. fructicola	BO-B1-A4	R	69.61
M. fructicola	BO-B4-A2	HS	20.86
M. fructicola	BG-B4-A3	HS	22.30
M. fructicola	BG-B1-A2	HS	23.41
M. fructicola	BG-B3-A1	HS	3.33
M. fructicola	BG-B1-A7	S	30.95
M. fructicola	BG-B1-A12	S	32.14
M. fructicola	BG-B3-A2	HS	17.14
M. fructicola	BG-B1-A15	HS	16.73

Table 1 (continued).

M. fructicola	BG-B1-A5	HS	16.40
M. fructicola	BG-B1-A6	HS	18.26
M. fructicola	BG-B1-A4	HS	14.21
M. fructicola	SHD-5	HS	18.03
M. fructicola	SHD-2	HS	17.30
M. fructicola	SHD-1	HS	15.20
M. fructicola	YILDIRIM-4	HS	19.35
M. fructicola	YILDIRIM-3	HS	19.14
M. fructicola	TOPLAMA YERİ-1	S	27.27
M. fructicola	BG-B1-A13	HS	18.60
M. fructicola	BG-B1-A17	S	30.00
M. fructicola	BG-B1-A3	S	27.22
M. fructicola	Nİ-B3-A1	S	31.00
M. fructicola	MT-B1-A3	HS	24.00
M. fructicola	B5-A7-1	HS	18.93
M. fructicola	B5-A6-2	HS	19.58
M. fructicola	B1-A1	S	28.98
M. fructicola	B1-A3	S	30.90
M. fructicola	B1-A4	HS	23.07
M. fructicola	B5-A5	S	26.60
M. fructicola	B1-A5	S	25.30
M. fructicola	B5-A3-2	S	28.88
M. fructicola	B5-A6-1	HS	19.18
M. fructicola	B5-A4	HS	21.81
M. fructicola	B5-A2	S	30.80
M. fructicola	B5-A6-3	S	25.10
M. fructicola	B5-A3-1	S	31.57
M. fructicola	B5-A1	S	24.23
M. fructicola	B5-A7-2	HS	18.57
M. fructicola	SÇ-B4-A1	S	35.26

Table 1 (continued).

M. fructicola	SÇ-B5-A7-1	S	26.36
M. fructicola	SÇ-B2-A1	S	36.42
M. fructicola	SÇ-B1-A1	S	33.33
M. fructicola	SÇ-B4-A5-1	HS	19.56
M. fructicola	SÇ-B5-A1	HS	20.00
M. fructicola	SÇ-B2-A3	HS	12.27
M. fructicola	SÇ-B4-A7-1	HS	23.84
M. fructicola	SÇ-B2-A2	S	24.73
M. fructicola	SÇ-B3-A1	S	24.23
M. fructicola	SÇ-B5-A2	S	24.73
M. fructicola	SÇ-B4-A4	S	26.00
M. fructicola	SÇ-B5-A5	HS	22.08
M. fructicola	SÇ-B4-A6-2	S	26.66
M. fructicola	SÇ-B5-A4-1	HS	23.84
M. fructicola	SÇ-B5-A4-2	HS	24.04
M. fructicola	SÇ-B4-A8-1	HS	22.35
M. fructicola	SÇ-B3-A4	S	30.00
M. fructicola	SÇ-B4-A3	HS	22.26
M. fructicola	SÇ-B4-A2-2	S	26.11
M. fructicola	SÇ-B5-A3	S	35.71
M. fructicola	SÇ-B4-A8-2	HS	22.22
M. fructicola	SÇ-B3-A2	HS	20.38
M. fructicola	SÇ-B2-A4	HS	18.94
M. fructicola	BG-B1-A10	HS	16.19
M. fructicola	T-B1-A8	HS	23.00
M. fructicola	YOL KENARI-5	S	28.40
M. fructicola	T-B1-A5	S	28.00
M. fructicola	Tİ-B3-A3-1	S	27.38
M. fructicola	Ti-B6-A1	S	25.09
M. fructicola	T-B1-A1	S	32.60
M. fructicola	T-B2-A3	S	30.36

Table 1 (continued).

M. fructicola	SB1-A1	S	27.20
M. fructicola	T-B1-A7	HS	21.30
M. fructicola	T-B2-A1	HS	16.73
M. fructicola	T-B2-A5	S	32.67
M. fructicola	T-B4-A2-3	S	27.50
M. fructicola	Ti-B4-A2-2	HS	22.22
M. fructicola	Tİ-B3-A3-2	S	42.66
M. fructicola	T-B3-A4	S	25.18
M. fructicola	T-B1-A4-1	S	27.60
M. fructicola	YOL KENARI-2	S	28.50
M. fructicola	T-B1-A2	S	28.80
M. fructicola	Tİ-B3-A2	S	38.80
M. fructicola	Tİ-B5-A1	HS	21.92
M. fructicola	YOL-KENARI-1	S	29.13
M. fructicola	T-B2-A4	HS	20.83
M. fructicola	2B3-A1	HS	22.50
M. fructicola	T-B2-A7	S	33.47
M. fructicola	2B1-A6	HS	20.38
M. fructicola	2B3-A3	S	30.76
M. fructicola	T-B2-A6	R	55.00
M. fructicola	MM-B4-A2	HS	20.86
M. laxa	2B1-A2-1	S	29.70
M. laxa	2B1-A5	S	32.25
M. laxa	2B2-A4-1	HS	18.28
M. laxa	2B2-A1	S	28.47
M. laxa	MM-B2-A2	S	30.47
M. laxa	Yıldırım-2	S	32.11
M. laxa	T-B1-A6	HS	15.69
M. laxa	T-B1-A4-2	S	28.33
M. laxa	SHD-3	S	32.50
M. laxa	Ni-B3-A2	HS	18.46

Table 1 (continued).

M. laxa	MM-B4-A5	S	28.57
M. laxa	MM-B4-A4	HS	20.90
M. laxa	2B1-A2-2	HS	5.00
M. laxa	2B1-A1	HS	25.71
M. laxa	Yıldırım-1	HS	19.44
M. laxa	MM-B4-A1	HS	24.16
M. laxa	Ni-B4-A1	S	26.00
M. laxa	MM-B4-A10	HS	5.00
M. laxa	MT-B1-A3-1	HS	21.73
M. laxa	MM-B4-A3	S	33.33

Table 2. The length of each scaffold, number of variants, and relative abundance of each variant type for *M. fructicola*.

		Number of Variants		**Relative Abundance of Variants (%)***	
Scaffold Name	Length (bp)	SNPs	Structural Variants	SNPs	Structural Variants
VICG01000001.1	4,238,823	25,566	162	0.603139	0.003822
VICG01000002.1	3,728,272	16,705	110	0.448063	0.00295
VICG01000003.1	3,609,855	17,651	98	0.488967	0.002715
VICG01000004.1	3,332,839	17,271	85	0.518207	0.00255
VICG01000005.1	2,656,653	15,268	105	0.574708	0.003952
VICG01000006.1	2,602,481	21,574	129	0.828978	0.004957
VICG01000007.1	2,592,823	15,607	99	0.601931	0.003818
VICG01000008.1	2,576,762	17,134	82	0.664943	0.003182
VICG01000009.1	2,530,796	15,957	103	0.630513	0.00407
VICG01000010.1	2,442,597	13,231	68	0.541678	0.002784
VICG01000011.1	2,335,009	14,766	104	0.632374	0.004454
VICG01000012.1	2,285,153	10,758	58	0.470778	0.002538
VICG01000013.1	2,201,684	10,515	60	0.477589	0.002725
VICG01000014.1	2,106,460	17,026	72	0.808275	0.003418
VICG01000015.1	1,968,927	13,945	93	0.708254	0.004723
VICG01000016.1	1,888,073	13,253	74	0.701933	0.003919
VICG01000017.1	355,621	2,278	6	0.64057	0.001687
VICG01000018.1	311,593	893	5	0.286592	0.001605
VICG01000019.1	255,115	7,219	27	2.829704	0.010583
VICG01000020.1	28,364	1	0	0.003526	0

* Relative Abundance = (100* “Number of Variant Type”)/ “Length of the Scaffold”.

Table 3. The length of each scaffold, number of variants, and relative abundance of each variant type for *M. laxa*.

		Number of Variants		**Relative Abundance of Variants (%)***	
Scaffold Name	Length (bp)	SNPs	Structural Variants	SNPs	Structural Variants
VIGI01000001.1	3,307,454	10,489	71	0.317132	0.002147
VIGI01000002.1	3,224,414	11,520	48	0.357274	0.001489
VIGI01000003.1	3,034,541	8,984	54	0.296058	0.00178
VIGI01000004.1	2,895,099	12,070	52	0.416911	0.001796
VIGI01000005.1	2,663,264	10,711	70	0.402176	0.002628
VIGI01000006.1	2,508,015	17,360	73	0.692181	0.002911
VIGI01000007.1	2,477,070	8,438	48	0.340644	0.001938
VIGI01000008.1	2,449,422	9,495	40	0.387642	0.001633
VIGI01000009.1	2,386,254	12,677	62	0.531251	0.002598
VIGI01000010.1	2,193,015	6,921	44	0.315593	0.002006
VIGI01000011.1	2,186,364	7,222	44	0.33032	0.002012
VIGI01000012.1	2,137,041	7,195	41	0.33668	0.001919
VIGI01000013.1	1,775,491	3,770	39	0.212336	0.002197
VIGI01000014.1	1,383,779	8,486	27	0.613248	0.001951
VIGI01000015.1	1,359,562	7,420	25	0.545764	0.001839
VIGI01000016.1	1,047,848	7,069	21	0.674621	0.002004
VIGI01000017.1	933,894	4,165	19	0.445982	0.002034
VIGI01000018.1	743,087	1,850	14	0.248961	0.001884
VIGI01000019.1	643,592	2,970	21	0.461472	0.003263
VIGI01000020.1	601,272	3,666	27	0.609707	0.00449
VIGI01000021.1	547,662	8,686	20	1.586015	0.003652
VIGI01000022.1	446,339	2,084	16	0.46691	0.003585
VIGI01000023.1	302,711	2,216	2	0.732051	0.000661
VIGI01000024.1	286,346	3,324	11	1.160833	0.003842
VIGI01000025.1	233,785	2,885	14	1.23404	0.005988
VIGI01000026.1	165,609	1,118	2	0.675084	0.001208

Table 3 (continued).

VIGI01000027.1	113,273	79	2	0.069743	0.001766
VIGI01000028.1	84,137	1,966	4	2.336665	0.004754
VIGI01000029.1	70,559	3	0	0.004252	0
VIGI01000030.1	69,030	1	0	0.001449	0
VIGI01000031.1	62,789	569	0	0.90621	0
VIGI01000032.1	58,386	766	0	1.311958	0
VIGI01000033.1	62,389	0	4	0	0.006411
VIGI01000034.1	51,566	4	1	0.007757	0.001939
VIGI01000035.1	46,913	1	0	0.002132	0
VIGI01000036.1	32,905	111	2	0.337335	0.006078
VIGI01000037.1	25,198	496	0	1.96841	0
VIGI01000038.1	24,684	1	0	0.004051	0
VIGI01000039.1	23,191	121	0	0.521754	0
VIGI01000040.1	23,152	491	0	2.120767	0
VIGI01000041.1	20,823	143	0	0.686741	0
VIGI01000042.1	20,791	554	0	2.664614	0
VIGI01000043.1	17,556	105	0	0.598086	0
VIGI01000044.1	16,968	295	0	1.738567	0
VIGI01000045.1	16,452	700	0	4.254802	0
VIGI01000046.1	14,865	162	0	1.089808	0
VIGI01000047.1	12,908	221	0	1.712117	0
VIGI01000048.1	8,084	510	0	6.308758	0
VIGI01000049.1	5,295	509	0	9.612842	0