

IDENTIFICATION OF MOLECULAR MARKERS ASSOCIATED WITH FRAXINUS EXCELSIOR TOLERANT TO ASH DIEBACK

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Ash dieback, caused by the invasive fungal pathogen *Hymenoscyphus fraxineus*, poses a significant threat to European ash trees (*Fraxinus excelsior*) and has led to widespread tree mortality across the continent. In the quest to mitigate the impact of this devastating disease, researchers have been investigating the potential use of molecular markers as a means to identify and predict reduced susceptibility to ash dieback [1, 2]. Several previous studies have shown that there is a low frequency of heritable resistance to ash dieback in European ash trees populations [2]. Molecular markers, which can include DNA-based techniques like genotyping and gene expression analysis, offer a promising avenue for identifying resistant or tolerant ash tree genotypes.

Molecular markers are specific segments of DNA that can be associated with particular traits or characteristics in organisms. In the context of ash dieback resistance, molecular markers are used to identify genetic variations in ash trees that are associated with either increased resistance or tolerance to the pathogen. These genetic markers can be valuable tools for tree breeders and conservationists seeking to develop ash populations with enhanced resistance to the disease [3].

Molecular markers have been used to assess the genetic diversity within ash tree populations. Understanding the genetic makeup of different ash trees can help identify genotypes that exhibit natural resistance to *Hymenoscyphus fraxineus*. This is particularly crucial for the selection of parent trees in breeding programs aimed at developing resistant ash varieties. [4]. This study explores the concept of utilizing molecular markers as valuable predictors of reduced susceptibility to ash dieback.

We used SNP markers, single nucleotide polymorphism markers to predict susceptibility to ash dieback. SNPs are single nucleotide variations in the DNA sequence of an organism and can be highly informative for identifying genetic differences associated with resistance or susceptibility to a particular pathogen, such as *Hymenoscyphus fraxineus* [2].

Identification of resistance-associated genes has been done within national program in UK using the pooled sequence data to train a genomic prediction model, cross-validated using individual whole genome sequence data generated for 75 healthy and 75 damaged trees from a single seed source [2, 5]. Molecular markers can pinpoint specific genes or genomic regions associated with resistance to ash dieback. By studying the genetic basis of resistance, we gained insights into the molecular mechanisms that underlie a tree's ability to withstand the pathogen. This knowledge is essential for breeding programs, as it allows for the targeted selection of resistant genotypes.

Molecular markers can expedite the process of breeding ash trees with improved resistance. Through molecular markers, breeders can select individuals with favourable genetic markers for resistance, thereby increasing the chances of producing offspring with enhanced resistance. This targeted approach accelerates the development of disease-resistant ash populations.

Moreover, molecular markers can also be employed for the early detection of ash dieback and monitoring its spread. By analysing the presence of pathogen-related DNA markers in environmental samples, we can track the disease's movement and assess its impact on ash tree populations. As the main aim of the study was to check for the presence of that marker in mapped and monitored visually healthy ash trees, we compared the occurrence of the marker in trees exhibiting severe ash dieback symptom.

Monitoring of healthy-looking ash trees in eastern Ukraine (2018-2021) showed that 92,5% of trees had 0-25% crown damage, thus remaining in a similar health condition. Molecular analysis of leaf tissues collected all ash trees showing the presence of the molecular marker for disease tolerance in 21 (84%) trees, while the marker was absent in 4(16%) trees.

By contrast, analysis of leaf tissues from 25 severely diseased trees with strong crown damages (75-100%) showed the presence of the SNP marker in 16 (64%) trees, but its absence in 9 (36%) trees. Therefore, our results demonstrate that monitoring of healthy-looking ash trees is a simple and reliable way for the selection of ADB-tolerant ash trees for future breeding program. In case of using SNP markers, it was revealed rather limited capacity to discriminate between presumably tolerant and susceptible ash tree genotypes and more broad marker set are needed. Additionally, the interaction between genetic and environmental factors in resistance needs further exploration.

In the future, the integration of advanced genomic technologies, such as whole-genome sequencing and CRISPR-Cas9 gene editing, may further enhance our ability to select and develop ash trees with increased resistance to ash dieback.

To conclude, the use of molecular markers as predictors of reduced susceptibility to ash dieback is a promising approach in the battle against this devastating disease. Through the identification of genetic markers associated with resistance and the application of marker-assisted selection, researchers and conservationists can work together to develop ash tree populations with increased resilience. Once the informative SNP set was identified, ash tree samples from a population are genotyped to determine the presence or absence of these specific SNPs. Genotyping can be performed using various methods, including high-throughput sequencing, DNA microarrays, or PCR-based techniques. Moreover, based on our results it's important to validate the identified SNP markers to ensure their accuracy and reliability. This can be done by testing them on a larger set of ash tree samples to confirm their association with susceptibility. The validation process helps ensure that the selected markers are robust predictors of susceptibility.

This strategy not only offers hope for the preservation of ash trees but also contributes to the broader field of forest health management, where genetic tools can be leveraged to combat emerging threats to tree species.

It's important to note that while SNP markers are a powerful tool for predicting susceptibility to ash dieback, they are most effective when used in combination with other information, such as environmental factors, pathogen variation, and phenotypic data. Additionally, the success of SNP marker-based prediction depends on the availability of a well-characterized and diverse set of ash tree samples for SNP discovery and validation.

References

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