AKADÉMIAI KIADÓ

Geospatial share of fungicide resistant *Botrytis cinerea* mutations in the Tokaj and Eger wine regions according to local pest management strategies

Júlia Hegyi-Kaló, Richárd Golen, Zoltán Karácsony, Adrienn Gomba-Tóth, Thomas Cels, Ádám István Hegyi and Kálmán Zoltán Váczy* ⓘ

Food and Wine Research Institute, Eszterházy Károly Catholic University, Leányka utca 8., H-3300 Eger, Hungary

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ABSTRACT

Botrytis cinerea is one of the fungal pathogens with the widest host plant spectrum, causing serious yield losses and significant economic damage in vineyards from year to year. As an ubiquitous, polyphagous fungal pathogen, with both saprophytic and parasitic lifestyle. The sequential use of active substances belonging to the same chemical family to protect vineyards can lead to an increase in fungal chemical resistance, which is reflected in the enrichment of point mutations in the genomic regions coding proteins involved in the mechanism of action of different pesticides. The aim of our studies was to compare the sensitivity to different fungicides of *B. cinerea* populations in two wine regions with different pest management practices: the Tokaj region, where the presence of *B. cinerea* is necessary to produce noble rot wines, and the adjacent Eger Region, where a total protection against *B. cinerea* is desired. Our study is the first Hungarian report of some previously studied resistance mutations in ERG27 and SDHB protein-coding genes. We identified point mutations in ERG27 transmembrane domain that have not been previously described but may affect the emergence of resistance to certain fungicides. Our study shows that the *B. cinerea* population of the Northern Hungary region is consistently characterized by an increase in fenhexamid resistance.

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* Corresponding author. E-mail: vaczy.kalman@uni-eszterhazy.hu

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INTRODUCTION

Botrytis cinerea is a well-known plant pathogenic fungus, infecting more than 1,200 different host plants (Elad et al., 2016), resulting in significant economic damage to agriculture. In the case of grapevines, it is also known as a two-faced fungus, as it is one of the most important pathogens of grapes, but it is also responsible for noble rot, necessary for the production of unique wines like Tokaji aszú, the earliest protected wine product in the world. For a long time, cell wall-degrading enzymes and toxins secreted by the fungus were attributed to the development of the infection pathway, but a rich toolbox of *B. cinerea* pathogenicity has now been described, revealing multiple infection pathways contributing to disease development.

In addition to reducing environmental impact and focusing on precision pesticide application, technology developers in crop protection practice are also concerned with the resistance of pathogens to pesticides. Practices that use the same pesticide active ingredient over a long period can very quickly cause a loss of sensitivity or resistance in the targeted pathogens. Resistance is one of the biggest threats posed by the widespread use of pesticides today, making pest management even more difficult (Lucas et al., 2015).

However, synthetic plant protection products have an important role in achieving the objectives of the European Union on plant health and food safety. Meanwhile, their excessive or otherwise inappropriate use can have adverse effects on soil, water, and agricultural biodiversity, with an additional threat to plant, animal, and human health. The European Commission has set ambitious targets for the sustainable use of pesticides as part of its Common Agricultural Policy (CAP) (Alexoaei et al., 2022).

Over the past decades, studies investigated the efficacy of pathogen-specific pesticides. In this area, *B. cinerea* was the first pathogen found to be resistant in a significant share of the field population. It soon became evident that the emergence of resistance depends on a wide range of biotic and abiotic factors, such as the chemical mode of action, the reproductivity of the targeted pathogen, and the frequency of fungicide application. In the case of *B. cinerea*, the frequency of application is the major determinant of resistance (Hahn, 2014).

Hydroxyamide fenhexamid, a Class III sterol biosynthesis inhibitor is one of the most used fungicides against *B. cinerea* worldwide (keto-reductase inhibitor, FRAC Code 17). It acts by inhibiting the 3-ketoreductase (ERG27) enzyme in the ergosterol biosynthesis pathway. This site-specific mode of action carries the risk of resistance and several studies have been conducted on which point mutations cause reduced susceptibility or resistance in the pathogen strains and the prevalence of these resistant strains in vineyards (Fillinger et al., 2008; Grabke et al., 2013).

Several amino acid substitutions have been identified that are responsible for resistance to certain classes of pesticides. Some of these are stable in the genome of the population during the reproduction of the pathogen and can be considered dominant mutations in certain areas or even worldwide (Leroux et al., 2010; Fillinger et al., 2016). In addition to the resistance against the ketoreductase-inhibitor pesticide, resistance can easily develop to the novel 14α-demethylase-inhibitor (DMI) pesticides by the mutation of the CYP51 gene (Harper et al., 2022). CYP51 (Erg11) belongs to the cytochrome P450 monooxygenase (CYP) superfamily and mediates a crucial step of the synthesis of ergosterol. In *B. cinerea* the CYP51B paralog

was detected by Hawkins et al. (2014). The third most used group of pesticides against *B. cinerea* is SDHI (succinate-dehydrogenase inhibitor, FRAC Code 7), whose effectiveness is also reduced by a mutation, causing an amino acid substitution on the SDHB protein (Amiri et al., 2014).

In this study we investigated resistance markers in *B. cinerea* populations of two wine regions in Northern Hungary, Eger and Tokaj. To the best of our knowledge, a limited number of studies have been carried out in the last few years on such characteristics of Hungarian *B. cinerea* populations (Váczy et al., 2006; Asadollahi et al., 2010; Szojka et al., 2011). The selection of the wine regions that represent the sampling sites is an important factor. The wine regions of Eger and Tokaj were chosen due to the different pest management strategies applied by grape growers in these two regions against *B. cinerea*. In the Eger wine-growing region, *B. cinerea* is mostly considered a harmful pathogen causing serious yield losses, and therefore targeted pesticides are applied during the whole vegetation period. In contrast, in the Tokaj wine-growing region, *B. cinerea* causes the so-called noble rot on grapes in certain years. This process results in shriveled, raisin-like berries, with a very high sugar content, utilized in the making of sweet, noble rot wines. This process occurs about four to six times in ten years. Since it is not possible to determine whether a given vintage is suitable for aszú production, many aszú wine producers apply limited or no protection against *B. cinerea* during the last part of the plant protection period. This practice leads to the loss of raw material for other wines, but the tradition of aszú making and the uniqueness of the aszú wine encourages producers to take this risk.

This study aims to investigate the occurrence of pesticide resistance in *B. cinerea* populations in the two largest wine-growing regions of Northern Hungary. In addition, mutations that are possibly responsible for the detected resistant phenotypes were also examined. By comparing the resistance of the *B. cinerea* populations in the Eger and Tokaj-Hegyalja wine regions, we are not only contrasting two geographical areas, but the two above mentioned plant protection practices, which, in Tokaj sweet wine producing vineyards, contributes the development of *B. cinerea* noble rot.

MATERIAL AND METHODS

Sampling was carried out in two vineyards in the Eger wine region (Mácsalma denoted *Eger1* and Csájszél denoted *Eger2* in Szomolya village) and two vineyards in the Tokaj wine region (Betsek denoted *Tokaj1* and Szent Tamás denoted *Tokaj2* in Mád village) in Northern Hungary, in 2015, 2017 and 2020. Grape berries covered with *B. cinerea* mycelia were collected randomly from *Vitis vinifera* cv. *Furmint* in Tokaj and cv. *Olaszrizling* in Csájszél and cv. *Turán* in Mácsalma during harvest time (late September to early October) resulting in a total of 250 berry samples. Collected berries were aseptically stored in 50 mL tubes which were placed on ice and taken to the microbiology laboratory.

Conidia from the berries were directly sprinkled onto Dichloran Rose Bengal chloramphenicol (DRBC) agar medium. Cultures were incubated at 25 °C in the dark and checked daily for mycelia development. Colonies with morphology similar to *B. cinerea* were used for the preparation of conidial suspension in distilled water. Suspensions were streaked on the surface of DRBC plates and the process was repeated once with developed separate colonies to

obtain pure cultures. The isolates were subcultured on PDA medium, at 25 °C, and were preserved in 50 v/v% glycerol at −80 °C. Fungi were identified according to morphological characteristics. A total of 196 *B. cinerea* isolates were collected. Based on the morphological characters, it is not possible to distinguish *B. cinerea* from *Botrytis pseudocienrea*. Previous studies (Plesken et al., 2015) have described that *pseudocinerea* co-occurs with *cinerea*, but usually at a lower frequency. It is also mentioned that *pseudocinerea* can become dominant in certain host plants, but this phenomenon has not been described in grapes. On cultivated strawberries, it was frequently found in spring but was largely displaced by *B. cinerea* following fungicide applications. These data suggest, and our previous experience has shown, that the isolates collected are most likely to be of *cinerea* species. The isolates were tested by the gradient plate method for sensitivity against three different commercially available fungicidal agents (fenhexamid, tebuconazole and fluopyram + tebuconazole combination). The gradient plate method wascompared with the widely used agar dilution method in a previous study (Hegyi-Kaló et al., 2018), which demonstrated the accuracy of our gradient method protocol. *B. cinerea* strains were grown on toothpicks by placing them on PDA plates previously inoculated with a mycelial disk of the fungus. Cultures were incubated at 25 °C until the mycelia completely covered the medium. To prepare the fungicidal gradient medium, 15 mL of PDA medium amended with different concentrations of fungicidal solutions was poured into a tilted petri dish, followed by the addition of an equal volume of PDA in a horizontal position, after the solidification of the first layer. Thus, a medium with a linear gradient between zero and a given maximum concentration was obtained. A wide range of maximum concentrations was used (from 0.05 to 10 µg mL^{-1}) to estimate the EC50 values for each isolate and fungicide. In addition to the intoxicated media, inoculated toothpicks were also placed on PDA as a control to determine the growth rate of each *B. cinerea* isolate in the absence of fungicides. The fungicide concentration at the point of 50% growth of the control sample could be calculated from the maximal concentration of the bottom layer of the gradient plate, since its thickness was assumed to be proportional to the resulting combined concentration, i.e. a linear concentration distribution (see Fig. 1). After the determination of the EC50 values, the strains that fell in the upper quartile according to the values for each fungicide were selected for molecular studies. DNA was isolated from these samples by thermolysis method. The *B. cinerea* strains were grown on PDA plates at 25 °C, and a small amount of mycelium was suspended in 100 µl of distilled water in a 1.5 mL microcentrifuge tube. The mixture was vortexed thoroughly and then centrifuged at 10,000 g for 1 min. After carefully discarding the supernatant, 400 µl of lysis solution (5 v/v% glycerol + 1 × TAE) was added to the microcentrifuge tube. The mixture was finally incubated at 85 °C in heat plate for 30 min. The lysate was centrifugated at 10,000 g for 5 min. A 100 µl portion of the supernatant was transferred in a new microcentrifuge tube and stored at −20 °C until further use as a template in the PCR reactions. Three sequences coding the Erg27, SdhB, and Cyp51 proteins were selected as possible bearers of resistance mutations. The different loci were PCR amplified with their corresponding primers using the following settings for *erg27*, *sdhB* and *cyp51* genes case, respectively: initial preheating 95 °C – 3 min, 98 °C – 0.5 min and 95 °C – 3 min; denaturation 95 °C – 30 s, 98 °C – 5 s and 95 °C – 30 s; annealing 58 °C – 30 s, 59 °C – 5 s and 58 °C – 45 s; extending 72 °C – 1 min for each; repeat 35 × for each. The oligonucleotide sequences used for PCR amplification are listed in Table 1. Previously designed primers were applied in the reactions: the erg27 primer-pair was designed by
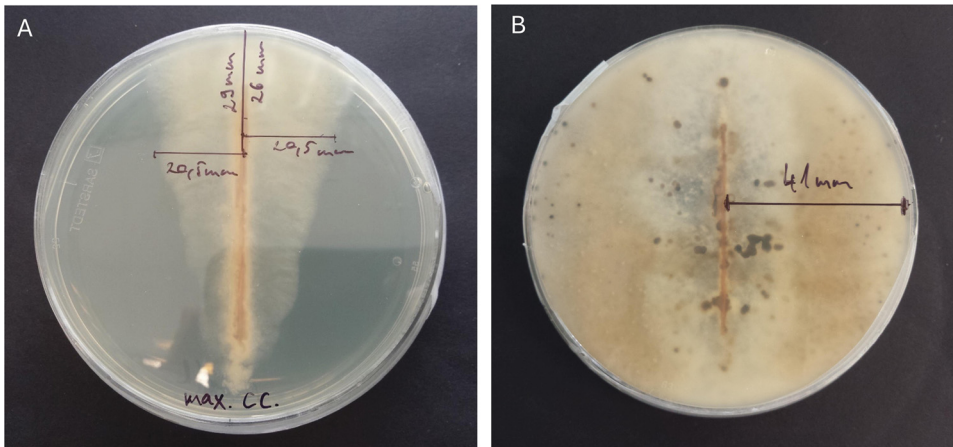
Fig. 1. Botrytis cinerea isolate growing on gradient plate (subfigure A) at 25 °C temperature 96 h post inoculation with tebuconazole was added to the bottom PDA medium of 0.2 ug mL^{-1} concentration. On the control petri dish (subfigure B) the growth was 41 mm, thus EC50 concentration can be estimated where the growth of the colony is 20.5 mm, marked on the figure. The average of the concentration value on both sides of the toothpick gives the EC50 value

Table 1. The sequences of the used oligonucleotide pairs

Primer name	Target locus		Sequence	Annealing temperature
erg27Beg	*erg27*	Forward	TGGGATTACCACCATGGGAGACAAGTG	58 °C
erg27End		Reverse	CAATGGTTCCGCATTTCTTTGCCTCCC	
Beg	*cyp51*	Forward	TGCGATGGGGATTCTTGAAC	59 °C
End		Reverse	TTATCGTCGCTCCCAAGCTAC	
IpBcBeg-F	*sdhB*	Forward	CCACTCCTCCATAATGGCTGCTCTCCGC	58 °C
IpBecEnd2-R		Reverse	CTCATCAAGCCCCCTCATTGATATC	

Fillinger et al. (2008), the sdhB by Fournier et al. (2005) and the sdhB by Leroux et al. (2010).

The amplified DNA was sequenced by Eurofins Genomics Germany Gmbh (Ebersberg, Germany) with Sanger's method result in raw ".*fasta*" files. Libraries were filtered and preprocessed by SnapGene software (www.snapgene.com). The identified nucleotide sequences were translated into amino acid sequences using Jaelview 2 software (Waterhouse et al., 2009) and the location of point mutations and amino acid substitutions were determined The sequences are deposited in the NCBI GenBank database under PP587884 – PP587936, PP587937 – PP587985 and PP587986 – PP588055 accession numbers.

For statistical analysis of the data, the vegan package (Oksansen et al., 2007) of the R programming language was used. EC50 values determined for different sampling sites were compared using ANOVA and significant differences between locations were also determined using Tukey's HSD test.

RESULTS

In the present study, the tolerance of *B. cinerea* isolates from two wine regions of Northern Hungary to three commercially available fungicides were determined. Point mutations in certain genes of the isolates that may affect the resistance were also investigated.

Using the gradient plate method, EC50 values for different fungicides were determined for each *B. cinerea* isolate. The distribution of the values is shown as boxplots in Fig. 2. It can be clearly seen, that in the case of fenhexamid, *Tokaj2* site showed an increased resistance compared to *Tokaj1* and *Eger2* with a large variability in EC50 values. Using tebuconazole as fungicide in the plate media the *Tokaj2* isolates show significantly lower EC50 values, contrary to fenhexamid. Regarding the combined fungicide media *Tokaj1* showed significantly higher sensitivity of strains than both *Eger* sites, which trend can be found about the other two fungicides with less significance (Fig. 2).

From the most tolerant 25% of the isolates we sequenced (the most tolerant 49 pcs of isolates resulted by each fungicide tests) three genes that can carry mutations resulting in the observed resistant phenotype. In Table 2 the found point mutations are listed. The amino acid substitution from phenylalanine to serine at position 412 of the Erg27 protein (F412S) was found in all sequenced isolates. We found at position 369 an asparagine to asparagic acid mutation (N369D) regarding to two isolates in both *Eger1* and *Eger2* sites (B15-Or193, B15-Or 1, B15-Tu 45, B15-Tu 173). This point mutation is the so-called HydR3− mutant showing reduced pathogenicity and other fitness parameters without selective pressure isolated from several Chilean, North American, French and German vineyards (Weber, 2010; Billard et al., 2011; Esterio et al., 2011; Moorman et al., 2012). The mutation histidine to arginine at position 272 (H272R) in the gene *sdhB* was reported from French vineyards in 2010 (Leroux et al., 2010) and Chilean table grapes in 2015 (Esterio et al., 2015) which caused boscalid resistance in *B. cinerea* strains. We identified this variant in one *Eger1* isolate (B15-Tu 260), and it was also found in Australia and Italy (Toffolatti et al., 2020; Harper et al., 2022). To our knowledge, this is the first study where N369D and H272R mutant strains have been identified in Hungarian vineyards.

In addition to the well-known mutations from other studies (reviewed by Fillinger et al., 2016), we have identified four amino acid substitutions in the Erg27 protein that may affect the fungicide susceptibility of the strains (see Table 2, last column where NAs are denoted). The V309G + L327S (valine to glycine at position 309 and leucine to serine at position 327) mutant isolate (B15-Tu 243) as well as the V367A (B15-Or 4, valine to alanine at position 367) gave a similar EC50 value for fenhexamid as the known resistant N369D mutant.
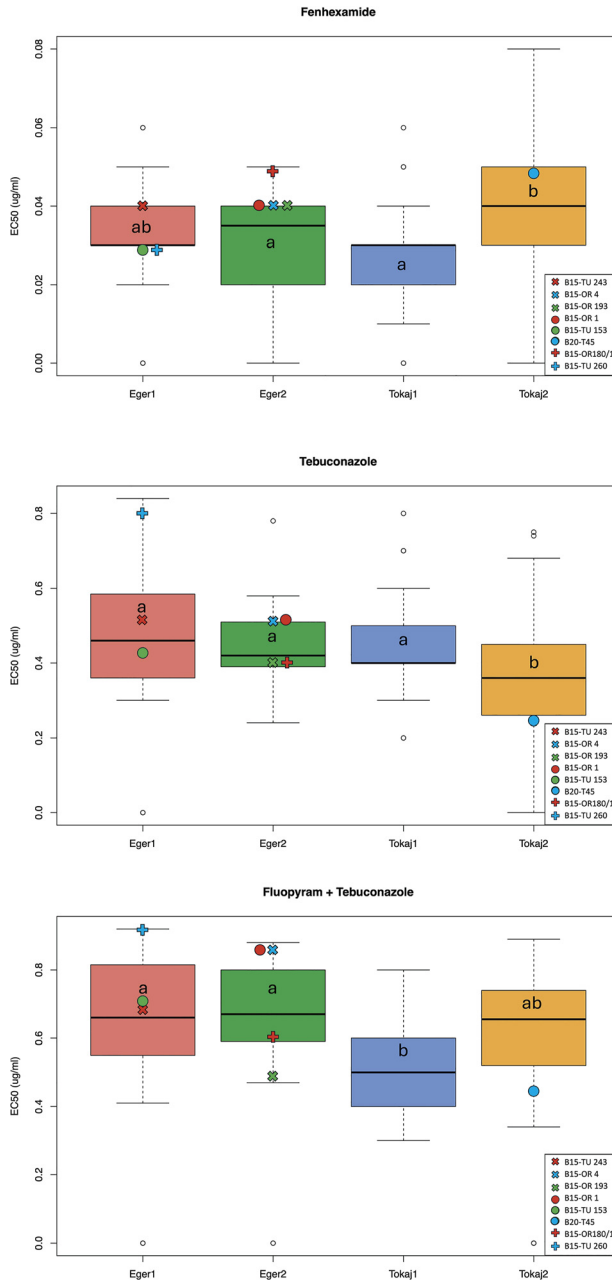
Fig. 2. Boxplots of measured EC50 values of the isolates in case of different fungicides. Letters in the boxes shows the significant differences of the Tukey HSD test. Colored, shapes are showing the EC50 values of the isolates in case of which point mutations were identified (see the legend to identify)

Table 2. The list of identified amino acid substitutions in the sequenced genes. In the table the isolates are mentioned in which we found point mutation. Mutations with previously described effects were cited. In case of Erg27 protein the EC50 values are denoted for the fenhexamid intoxiacated media, in case of SdhB for fluopyram intoxicated media

Protein	Amino Acid change	Isolate name	Effect	Reference	Location	EC50 [μg mL^{-1}]
Erg27	F412S	ALL	Mutant strains were shown to be fenhexamid-resistant, grew more slowly than the wild-type strain and displayed variations in the production of sclerotia and conidia with temperature and sensitivity to freezing.	Grabke et al. (2013)		not relevant
	V309G	B15-TU 243		NA	Eger1	0.04
	L327S	B15-TU 243		NA	Eger1	0.04
	V367A	B15-OR 4		NA	Eger2	0.04
	N369D	B15-OR 193	Several resistant strains harbored the mutation of the parental HydR3— mutant N369D, showing reduced pathogenicity and other fitness parameters without selective pressure.	Fillinger et al. (2016)	Eger2	0.04
	N369D	B15-OR 1			Eger2	0.04
	N369D	B15-TU 153			Eger1	0.03
	N369D	B20-T45			Tokaj1	0.05
	Y408N	B15-OR180/1		NA	Eger2	0.05
SdhB	H272R	B15-TU 260	Strains confer moderate resistance levels against boscalid but they do not confer resistant to others SDHIs.	Leroux et al. (2010)	Eger1	0.81

DISCUSSION

The two selected wine regions have different pest management practices. It can be noted that in the case of Tokaj, the treatments against grey mold concentrated to the late May-late June period, which corresponds to the post-flowering stage in terms of plant phenology. In the case of the Eger, more treatments against *B. cinerea* were performed generally from post-flowering until the pre-harvest. The treatment at veraison limits the amount of *B. cinerea* infections that serve as a basis for later grey or noble rot in the grape maturation period.

In the Tokaj wine region, so-called *Botrytis*-friendly plant protection technology and viticultural practices are used in certain areas to grow Aszú grapes, while in the Eger wine region, grey mold is generally controlled. These two different pest management practices may have an impact on the prevalence of point mutations associated with fungicide resistance in *B. cinerea* strains, as the spraying practice in the Eger wine region may result in long-term exposure to chemicals.

The veraison period when most *B. cinerea* colonies on bunches or stems are sporulating and present as infection sites during ripening, and the weather conditions (warm, humid) during this period are suitable for grey mold. We found all the identical point mutations in samples from the Eger wine region which suggests that continuous fungicide exposure puts more chemical pressure on the fungus, resulting in increased survival of resistant mutants despite their reduced fitness, leading to their enrichment in the population (Laleve et al., 2014). We found more variants in Erg27 protein so this protein was more diverse in the *B. cinerea* samples. An interesting coincidence is that much larger amount of fenhexamid fungicide used in Hungarian agriculture than tebuconazole or fluopyram (Medináné Lázár, 2020). It is certainly worth investigating further whether there is a possible link between greater Erg27 diversity at the population level and the evolution of potential resistance due to higher pesticide use.

The significantly higher average fenhexamid EC50 values for *Tokaj2* compared to *Tokaj1* suggest that the pesticide pressure is likely higher in the former. The possible role of other factors like microclimate, age of the vineyard, or terroir in this difference can not be determined. However, it is notable, that while *Tokaj2* is mostly producing dry white wines, *Tokaj1* is producing sweet wines made from botrytized grapes. This suggests that the *Tokaj1* area is less affected by the application of pesticides against *B. cinerea*, therefore the population of this fungus in the area is not as much evolved to overcome this chemical pressure. On the other hand, *Tokaj 2* area is probably subjected to a more intensive pest management with fenhexamide against *B. cinerea*, explaining the differences between these two areas.

In contrast, for tebuconazole, EC50 values for *Tokaj2* isolates were found to be significantly lower. This difference could be a local population characteristic, probably the result of the distance between the two areas and their different mesoclimatic conditions, as well as the different pesticide applications. While fenhexamid, can be assumed to be specific to grape grey mold, tebuconazole is also effective against powdery mildew. In the *Tokaj2* area, which produces the raw material for dry wine, it is advisable to control *B. cinerea* specifically with fenhexamid during the period of veraison, as the maximum application quantity of the other active substance may have been used up in the previous treatments. To reinforce the role of pest management practices in the observed differences between the *B. cinerea* populations at *Tokaj1* and *Tokaj2*, further field trials need to be designed.

For the combined agent, differences between sampling sites like those observed for fenhexamid were found. It is assumed that in this case too, the aszú grape growing aspect of the *Tokaj1* area and the consequent reduced use of fungicides targeting *B. cinerea* result in a higher sensitivity in its population.

The EC50 value of the fenhexamid resistant isolate, the N369D variant was not significantly different from that of wild isolates when tested by the gradient plate method. Only one newly found point mutation showed a correlation with the resistance, the Y408N variant. However, all the isolates contain the F412S amino acid substitution which is the most frequently reported

fenhexamid resistance marker. It is important to note that laboratory mutant isolates with F412S/I/V mutations grew slower than sensitive isolates and had variability in sclerotia and conidia production under different temperatures and on different media (Billard et al., 2012). In previous studies, there were statistically significant differences in growth rate for some fenhexamid-resistant isolates. They showed that overall, the resistant isolates had similar mycelial growth to the sensitive isolates however laboratory mutants of fenhexamid-resistant isolates of *B. cinerea* with F412S/I/V point mutations in *erg27* had fitness loss, reduced growing rate in mycelial development compared with the sensitive isolates (Sofianos et al., 2023) In the context of these results, we hypothesize that the sensitivity to fenhexamid of the *B. cinerea* population in Northern Hungary is mainly determined by the presence of the F412S resistance mutation. The effect of the new point mutation will be confirmed by future studies.

It is also well-studied that, the predominant mutation of *sdhB* gene is the H272R, while in the French grape population, the predominant mutation is H272Y (Veloukas et al., 2011). This is a worldwide predominant mutation while mutations conferring high levels of resistance were found in lower frequencies. Such differences in mutation frequencies may account for differences in the fitness of the mutant strains, their survival and competitive ability in the field, the fungicide spray schedules, and the application doses. Reported results suggested that, despite a certain degree of variability, the two most found mutations (H272R and H272Y) were usually associated with moderate levels of resistance to boscalid. This finding agrees with that of Leroux et al. (2010) who also found that these two mutations were correlated with moderate levels of resistance to boscalid. We found H272R mutation in one isolate from *Eger1* site, which shows higher resistance to tebuconazole and the combined pesticide (blue cross on Fig. 2). Others have also shown that this point mutation in the *sdhB* gene can cause fluopyram resistance (Harper et al., 2022). Further study of this isolate may shed light on what underlies the increased resistance.

The combined mutation of N369D and F412S causes elevated resistance to KRI-fungicides (Fillinger et al., 2008), however, in our study, isolates with a combined point mutation did not show higher resistance than those with only F412S. This also does not confirm that the specific mutations we found, which have not been previously described, directly cause this combined increased resistance. The EC50 values of N369D mutant is indicating a greatly reduced sensitivity to this KRI-fungicide in this variant. The mutant tyrosine to asparagine at position 408 (Y408N, B15-Or 180/1) showed higher EC50 values against fenhexamid than the resistant variants described in previous studies (Fillinger et al., 2016). These specific high EC50 values do not necessarily mean that a new resistance factor has been identified, as the combined presence of several point mutations in previously identified cases reveals clear fungicide resistance.

Figure 3 shows the structure of the Erg27 protein. The amino acid positions of the point mutations we have identified are indicated by an orange stripe. F412S, widely discussed in
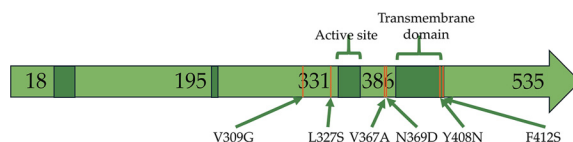

Fig. 3. The structure of ERG27 protein, where the active site and the transmembrane domain is denoted. Orange strips marks the identified amino acid substitutions' position

studies, is located in the transmembrane domain. Among the mutations we identified that have not been previously studied, Y408N is located in the same region. The active site catalyzing the formation of a hydroxyl group on position C3 of sterols is located near the transmembrane domain allowing the anchorage of the enzyme to the endoplasmic reticulum (Billard et al., 2011) which is reinforced by the fact that this isolate showed a high EC50 value for fenhexamide in a medium. Further investigation of this variant is required to understand the exact role of the identified point mutation.

CONCLUSION

In this study, we investigated the sensitivity of *B. cinerea* populations in the North Hungarian wine regions of Tokaj and Eger to different pesticide active substances used against grey rot. In all isolates, the F412S point mutation of the Erg27 protein causing fenhexamid resistance was identified, suggesting that *B. cinerea* strains infecting the vineyards of Northern Hungary have increased resistance. In addition, it was observed that the plant protection programme in the Eger wine region, which controls grey rot from flowering to veraison, causes increased resistance in the *B. cinerea* strains. To the authors' knowledge, this study is the first report to identify the N369D mutation of *erg27* and H272R mutation of *sdhB* gene in Hungarian vineyards responsible for fenhexamid and boscalid resistance respectively. We have identified four previously unstudied point mutations in the *erg27* gene, of which the Y408N amino acid substitution is located in the transmembrane domain of the protein and the mutant isolate shows increased fenhexamid resistance. Further investigation of this strain is strongly indicated.

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