ABSTRACT

***Keywords*:** grapevine, phenophases, phytosanitary treatments, isolation yeasts, morphological analysis, colonies, taxonomic identification, DNA sequencing.

In oenological practice, the basic link is the quality of the fermentative process that ensures the obtaining of high-class wines that reflect the personality and typicality of the viticulture area. Study of indigenous yeasts and their use in technology represent a permanent concern in field of research. In order to explain the quality of the fermentative process and yeasts activity from the spontaneous flora it is considered necessary to study the effects of external factors on the diversity and distribution of yeast microbiota within the vineyard.

The thesis "Studies regarding the influence of phytosanitary treatments on the diversity and distribution of yeasts in Copou Iasi Vineyard" includes the results of the research activity carried out in the period 2021-2022.

The experience was carried out at the Research Station - "Vasile Adamachi" Farm Iași. Most of the analyzes were carried out in the Phytopathology laboratory of the Faculty of Agriculture, from "Ion Ionescu de la Brad" University of Life Sciences, Iași, and the taxonomic identification of the isolated yeast strains was carried out in the BMR Genomics Laboratory in Padua, Italy, laboratory specialized in Sanger sequencing.

The purpose of the doctoral thesis is to establish the influence of phytosanitary treatments on the diversity and distribution of yeasts in Iași viticultural center. Studying the diversity and distribution of yeasts in the viticultural field is in the scientific research trends, both at the national and international level.

In order to fullfil the aim of this doctoral thesis, the following objectives were established:

- the application of phytosanitary treatments to vine culture correlated with the plant development phenophases;
- isolation and selection of yeast strains from soil samples and plant samples in different plant development phases;
- establishing the influence of experimental factors on the diversity and distribution of yeasts in the studied wine-growing area;
- establishing the morphological and physiological characteristics of isolated yeast strains;
- taxonomic identification of yeasts selected by DNA extraction and sequencing (PCR) methods.

To achieve the objectives proposed, the research activity was organized in three directions:

- the application of phytosanitary treatments according to the treatment depending on the phenophase of the plant's development.
- sampling of soil and plants in different phenophases and soil depths.
- testing of yeast strains isolated from soil and from plants in order to characterize them morphologically and taxonomically.

In the research activity, were chosen four grape varieties Fetească Neagră, Fetească Albă, Busuioacă de Bohotin and Fetească Neagră from the ampelographic collection of "Vasile Adamachi" Farm, Iași. The varieties were selected with the aim of increasing the diversity of the study and based on their fairly wide spread in Romanian vineyards.

The experimental factors studied were:
o Factor A: grape variety:
a1 – Fetească Albă;
a2 – Busuioaca de Bohotin;
a3 – Fetească Neagră;
a4 – Fetească Neagra collection;
o Factor B: source of yeasts:
b1 – grain/plant;
b2 – soil
o Factor C: the phenophase of yeast sampling:
c1 – shoot 5-7 cm;
c2 – the end of blooming;
c3 – full maturity.

Soil and plant samples were collected in three different development phenophases: shoot 5-7 cm, end of flowering and full maturity (before harvesting). These phenophases were chosen by their importance on the application of treatments as well as the dynamics process of yeast loading.

In order to analyze the soil microbiota, the samples were taken at 3 depths: 5-10 cm, 10-15 cm, 15-20 cm. To determine the influence of phytosanitary treatments on the diversity and distribution of yeasts, 2 soil samples were taken in the 5-7 cm shoot phenophase at 10-day intervals as follows:

- a series of samples taken 3 days before the treatment with the commercial product Dithane ® M-45 using a dose of 0.2% (2021) and Acrobat® MZ 69 WG (2022) which shows action on the pathogens *Plasmopara viticola* and *Botrytis cinerea* that causes downy mildew and gray mold of grape. It should be noted that both Dithane ® M-45 and Acrobat ® MZ 69 WG contain 60-80% mancozeb as active substance.
- the second sampling after 7 days from the treatment against downy mildew and gray mold.

Subsequently, 2 more samples were taken, both from soil and from plant, at the end of blooming and at full maturity before harvesting.

In order to isolate the yeasts, the samples were processed according to the standard methodology and the experimental protocol, thus resulting as sources of isolation:

- soil cleaned of impurities and plastered
- inflorescence washing water (at the end of flowering)
- berry washing water (when fully ripe).

In this study, 18 strains were isolated from soil, 5 strains were obtained from the washing water of the inflorescences and 11 strains were selected from the washing water of the grapes.

Both in 2021 and in 2022, the isolated strains were macro- and micromorphological analyzed, establishing the specific characteristics of the colonies (shape, profile, surface and color) and the appearance of the cells.

In the next step, the isolated strains were taxonomically analyzed by Sanger sequencing. Following the genomic processing, 6 yeast strains were identified that showed diversity both in terms of grape varieties and in terms of the source, the phenophase and the year of isolation.

Structurally, the doctoral thesis comprises 177 pages, 53 figures and 27 tables.

The first part, "The current stage of research" includes 4 chapters, summing up 40 pages, which incribe the history of the grapevine, the systematic importance and ecology of wine plantations, the situation of

grapevine culture on a global and national level, and the importance and taxonomy of yeasts in the wine industry.

The second part, "Personal contributions" is structured in 5 chapters summing 115 pages. This part highlights the climatic conditions in which the research activity was carried out, the aim and objectives proposed in the thesis, the research material and methods used, results regarding the phenological observations and biometric measurements, results regarding the quantitative and qualitative analyzes of isolated strains as well as their taxonomic identification.

In **the first chapter**, the history of the grapevine is briefly presented, both at national and international level.

The second chapter presents the systematics of the vine, its importance and the ecology of the plant. In this chapter, the ways of classifying the varieties and the requirements of the vine for adaptability in different wine-growing areas are briefly described.

The third chapter presents the situation of the grapevine in the last 21 years at global, European and national level.

In **the fourth chapter**, are presented aspects regarding the importance of yeasts in the winemaking industry, the taxonomy of oenological yeasts, the presentation of the genomic characteristics of yeasts found in winemaking. Aspects of yeast metabolism are also described.

In **the fifth chapter**, the experimental field is described, referring to:

- Geographical location of the experimental field;
- Geomorphology, hydrography and hydrology of the area;
- The climatic conditions, with reference to the thermal regime and the rainfall regime recorded in the experimental years.

In **the sixth chapter**, the aim and objectives of the research thesis, the biological material used, and the methods used are presented. In this chapter, the research methods were divided into field analysis methods and laboratory research methods.

Laboratory research methods included:

- isolation of yeast strains from the soil
- isolation of yeast strains from plants
- quantitative analysis of yeast colonies determination of macro- and micromorphological characteristics of selected yeast strains
- taxonomic identification of yeast strains by the PCR method.

The seventh chapter includes results regarding phenological observations and biometric measurements in case of the 4 grape varieties studied. Results are presented such as:

- phenological observations
- biometric measurements (fertility and shoot productivity, shoot growth)

These parameters were studied throughout the vegetation period in the years of experimentation, showing the results obtained for each experimental year separately, as well as the average of the years.

In **the eighth chapter**, the results regarding the yeast load of the samples both from soil and from inflorescences and fully ripped grapes, are presented.

The samples microbiota showed diversity by analyzing the phenophase of sampling and the source of isolation.

Comparing the 4 varieties studied, the yeast load of soil samples was higher both before treatment and after treatment in the case of Fetească Neagră Collection variety, which recorded a maximum threshold of 26 x 10 6 CFU/g soil before treatment respectively 24 x 10 6 CFU/g soil after treatment.

In the vertical division of the yeasts in the soil, the number of microorganisms shows a downward slope proportional to the sampling depth, so it can be deduced that the constant decrease in the activity of the microbiota in the lower layers is characterized by the lack of oxygen, the decrease in the amount of nutrients and the alkalinization of the soil.

The variations regarding the microbial load between the 4 grape varieties in different phenophases of plant development indicate that the microbiota of the wine plantations is dependent on the rhythm and dynamics of the development of each vine variety, correlated of course with the dynamics of the transfer and development of yeasts from the soil and the climatic conditions of the research year.

These results confirm the studies published in the literature according to which the amount of yeasts in the wine plantations increases proportionally with the accumulation of fermentable sugars in the berries, thus positively influencing the process of wine microbiota development.

Chapter nine includes results regarding the taxonomic identification of isolated yeasts and their morphological characterization.

Following the genomic processing, 6 strains of yeasts were identified that showed diversity both in terms of the grape varieties from which they were taken and in terms of the source, the phenophase and the year of isolation. The isolated strains belong to *Solicoccozyma, Hanseniaspora, Rhodotorula, Filobasidium, Cystobasidium* and *Cryptococcus* genera. The largest share is held by *Solicoccozyma aeria* species with 47.22% of the total, being found in all sampling phenophases both in soil and on plants, regardless of the year of study. The species *Hanseniospora uvarum* was found in the soil only in the phenophase of full maturity and on the plant it was present both at the end of flowering and at full maturity.

The species *Rhodotorula glutinis* was isolated both from soil in the phenophase of full maturity and on inflorescences and berries.

Filobasidium floriforme species had a percentage of 11.7 from total species selected, being found both in the soil and on the grapes at their full maturity. The species *Cystobasidium pinicola* and *Cryptococcus aeris* were found in the lowest percentage in the vineyard area studied. While the species of the genus *Cryptococcus* was present in the soil at the depth of 15-20 cm in the shoot phenophase 5-7 cm, the species *Cystobasidium pinicola* was found at the end of flowering on the inflorescences. Comparing the 6 selected species, we notice that *Solicoccozyma aeria* is present both in case of all sampling depths and on the vegetative organs of plants, while the other 5 species are preferentially found on sources and harvesting depths.

On a morphological basis, the colonies of the isolated strains presented a convex and flat profile, a glossy, matte surface and in some cases a mucoid appearance, the colors varying from white to cream and orange.

The doctoral thesis ends with a chapter assigned to the conclusions drawn based on the results and their interpretation.

The **bibliography** includes 172 titles from national and international literature.