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# ECOLOGICAL CONDITIONS OF VINEYARDS AND THE POLYMORPHISM OF *SACCHAROMYCES CEREVISIAE* STRAINS AS A PREREQUISITE OF TERROIR IDENTIFICATION

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**Aim.** The aim of the study was to assess whether the ecological conditions for vineyards and the phylogenetic analysis of *Saccharomyces cerevisiae* wine yeast strains isolated from several locations in the Odesa and Mykolayiv regions could be used for terroir identification. **Methods.** The study employed microbiological, analytical, expedition, cartographic, and comparative assessment methods. Phylogenetic analysis was conducted using the MEGA software program, and VIN-CAD-UKR software was used for the vineyard cadastre and the ampeleological characteristics data management. **Results.** The study highlighted differences in the ecological conditions of vineyards in the Odesa and Mykolayiv regions and within the Tairovo and Izmail territorial communities in the Odesa region, focusing on soil and microclimatic characteristics. *Saccharomyces cerevisiae* strains isolated in 2012 and 2013, years with varying weather conditions in temperature and precipitation, were primarily clustered by the year of isolation. Two notable exceptions included a strain isolated from the Isabela variety belonging to a hybrid group (direct producer, contrary to other varieties, which mainly represent *Vitis vinifera* or its hybrids with *Vitis riparia*, *Vitis amurensis*, *Vitis berlandieri*) and a strain isolated from the Odesa Black variety inoculated at the beginning of fermentation with a commercial strain of wine yeast. The 2013 isolates, collected under conditions of higher precipitation and lower temperature in the first half of the growing season, showed a more differentiated clustering pattern, with two strains isolated from the Sukholymansky White variety forming a distinct cluster, while the rest grouped together, irrespective of the region, formed of isolation. **Conclusions.** Phylogenetic analysis of 64 strains of *Saccharomyces cerevisiae* isolated from 36 technical grape varieties based on the ITS1\_5.8S ITS2 nucleotide sequence demonstrated that the year of isolation (reflecting agroclimatic factors) was the primary influence on sample clustering. These factors, as components of the ecological conditions of terroir, form the foundation for terroir identification.

**Key words:** terroir, ecological conditions, *Saccharomyces cerevisiae* strains, sequencing, phylogenetic analysis.

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## INTRODUCTION

The terroir identification is critical for wine production with protected designation of origin (in French:

Appellation d'origine contrôlée (AOC)). It envisages the application of methodological approaches to determining the sites of vineyards with the purpose of wine

production with protected designation of origin based on the comprehensive analysis of the ecological conditions of the territory and the systemic approach to assessing the state of the current vineyards. Recently, assessing the microbiome of grape plants and vineyard soil has been considered promising in achieving this goal. Even though that the study of the microbiomes of grape terroirs has started relatively recently, significant results have already been obtained in this direction, as nucleotide sequences have been determined on a wide range of different microorganisms in various regions and different grape varieties.

The data of molecular genetic studies of the yeast component of the terroir microbiome indicate that the genus, species composition, and strains of not only the cultivated *Saccharomyces cerevisiae* yeasts but also their wild relatives are the basis for wine quality. It is the species composition of wine yeasts that can become the basis for the specific characteristics of terroir wines. To do this, first of all, it is necessary to carry out a rather painstaking molecular genetic work, which should be based on the analysis of the species composition of the isolated wine yeast species and the study of intraspecific variability within each species (at the strain level).

The presence of wine yeast strains isolated from different parts of the Odesa region, their isolation from various grape varieties across different years, and, most importantly, the availability of sequenced ribosomal RNA gene sequences for each deposited strain, allow for a detailed analysis of the microbiological specificities of terroirs.

The role of *Saccharomyces* yeasts in fermentation processes across the global wine industry is hard to overestimate. As established in 2003, this genus includes six main species – *S. cerevisiae*, *S. bayanus*, *S. cariocanus*, *S. kudriavzevii*, *S. mikatae*, and *S. paradoxus* (Kurtzman, 2003; Naumov, 1996; Naumov et al, 2000). The European project on the sequencing and functional analysis of the *S. cerevisiae* genome (Goffeau et al, 1996) laid the foundation for studying the genetics of various *Saccharomyces* species. However, much remains unknown about the comparative organization of wild and cultivated yeasts (Naumov, 1996; Naumov et al, 2000; Naumov et al, 2003; Naumov, 2000; Vaughan-Martini et al, 1998).

In general, yeasts can be divided into two groups: *Saccharomyces* and non-*Saccharomyces* (wild yeasts). While *S. cerevisiae* may be present on grape berry skins, much larger populations are typically found on winery equipment (Fleet et al, 2002; Ribéreau-Gayo-

netal et al, 2006). Non-*Saccharomyces* genera, often found on grapes and must, include *Hanseniaspora* (*Kloeckera*), *Candida*, *Metschnikowia*, *Brettanomyces*, *Kluyveromyces*, *Schizosaccharomyces*, *Torulaspora*, *Rhodotorula*, *Zygosaccharomyces*, *Cryptococcus* and black-pigmented yeast-like fungus *Aureobasidium pullulans* (Fleet, 2002; Ribéreau-Gayon et al, 2006; Jolly et al, 2003; Romano et al, 2006; Jolly et al, 2014; Alesandria et al, 2015; Capozzi et al, 2015).

Yeasts accumulate as grape berries develop. The species diversity of yeasts and their relative abundance change according to the stage of grape ripening. At the onset of ripening, berries are primarily colonized by basidial yeasts (*Cryptococcus* spp., *Rhodotorula* spp., *Sporobolomyces* spp.), yeast-like fungi (*Aureobasidium pullulans*). As the ripening process progress, they are gradually inhabited by Ascomycetes (*Hanseniaspora* spp., *Metschnikowia* spp., *Pichia* spp., *Candida* spp.). Overripe and damaged grapes tend to support the growth of yeasts with high fermentation capacities (Chalvanti et al, 2021).

While *S. cerevisiae* strains play the primary role during fermentation, the initial stages are typically carried out by non-*Saccharomyces* species. For instance, *Hanseniaspora uvarum* dominates in the early stages of spontaneous fermentation, along with *Candida* spp. It has also been reported that during the initial phases of fermentation, *Kloeckera* and *Candida* genera tend to dominate (Ribéreau-Gayon et al, 2006; Romano et al, 2006). As ethanol levels increase, more resistant yeast strains, such as *Saccharomyces*, become dominant.

The species and strains of yeasts present during must fermentation are known to influence the taste and aroma of the wine, as they affect the must by producing different metabolites in varying quantities. The biodiversity of yeasts in vineyards is largely influenced by factors such as annual weather conditions, grape variety, agronomic practices, macro- and microclimatic conditions, and the geographical location of the vineyard.

Thanks to next-generation sequencing (NGS) methods, the microbial populations of vineyard, grape plant, must, and wine have been studied in detail by researchers worldwide. The association between microbial populations and the geographical location of vineyards has introduced the concept of microbial terroir.

Using short amplicon sequencing, Bokulich et al (2016) investigated the existence of non-random regional distributions of grape microbiota across large geographical scales. Their findings revealed that vineyards from different regions could be distinguished

based on the genus and species profiles of their bacteria and fungi. They also provided evidence that grape variety and climatic factors play a role in shaping the microbiome, thereby supporting the concept of “microbiome terroir”. Since then, many authors have argued that geographical location is a key factor influencing the microbial community within a vineyard.

The next step was to explore specific regional microbiomes that contribute to the characteristics of wine and to identify the association between the chemical composition of wine and the regional microbial community of grapes. Scientists have demonstrated that the wine microbiota correlates with the chemical composition of wine (Bokulich et al, 2016). The authors suggest that the grape microbiome may influence the quality of terroir products.

Almost all studies of the grape microbiome confirm the existence of regionally specific microbial populations. Therefore, future research in this area should consider the origin of strains, as it has been shown that strains involved in spontaneous fermentation partially originate from both the vineyard and winery.

Current trends in the wine market have revived the popularity of spontaneous fermentation using autochthonous wine yeast strains. This practice enhances the specificity and quality of terroir products and meets consumer demands. (Cravero, 2019; OIV, 2020; Vaudano, 2019; Feng et al, 2019; Romano et al, 2008). Similar studies are being conducted in many grape-cultivating countries, enabling the production of wines that align with ecological safety principals and feature a more complex taste and refined aroma (Capozzi et al, 2017; Feghali et al, 2020). The discovery of new yeast strains is driven by the need to identify new genotypes capable of producing a different profile of secondary metabolites due to genetic variability, while still ensuring high wine quality (Vaudano et al, 2019).

Based on these principals, producers are increasingly adopting the use of the autochthonous yeasts for wine production. Terroir yeasts help create more complex wines with distinctive stylistic characteristics and superior quality (Romano et al, 2008; Morrison-Whittle et al, 2018).

The impact of terroir on wine quality is primarily attributed to the effect of soils and climate. These factors define terroir and have been extensively studied in wine-producing regions around the world, including Italy, Canada, Portugal, China, and other countries (Fayolle E et al, 2019; Priori et al, 2019; Kotsaki et al, 2019; Prata-Sena et al, 2018; Wang et al, 2015).

Regarding the influence of terroir conditions on wine quality through soil, the most significant factor is water availability for the grapevine (Van Leeuwen et al, 2009; Swinchatt et al, 2018). Recently, due to climate change, researches have been increasingly focused on the challenge of preserving the typicality of terroir wines (Costantini, 2021).

As the term “terroir” encompasses the interaction between biotic and abiotic environmental factors as well as viticultural and enological practices, terroir wines are defined by both their geographical origin and their biodiversity.

The yeast *S. cerevisiae* is a natural component of the microbial communities found on grapes. Numerous studies have investigated its biodiversity, exploring the distribution, stability, and diversity of *S. cerevisiae* strains both in vineyards (De Celis et al, 2019; Santamaria et al, 2019; Cordero-Bueso et al, 2011) and during spontaneous wine fermentations (Vigentini et al, 2015). Studies have also analyzed metapopulations to determine the biogeographic spread of *S. cerevisiae* (Gayevskiy et al, 2012; Knight et al, 2015).

Studies on the well-known Malbec variety (Argentina) demonstrated that the vineyard ecosystem and harvest year conditions significantly influenced the composition of *S. cerevisiae* populations in each year of the study. Genetic analysis of *S. cerevisiae* populations revealed a high level of differentiation between vineyards located only 19 km apart. One vineyard was characterized by highly polymorphic *S. cerevisiae* populations, while the other exhibited low yeast polymorphism (Gonzales et al, 2023).

Despite the annual variations in the composition of *S. cerevisiae* strains in the wine yeast populations of the experimental vineyards, the diversity indices remained stable over time. The authors thus highlighted both the differences in the biodiversity of the wine yeast population between two Malbec vineyards and the temporal stability of this biodiversity (Gonzales et al, 2023).

The aim of this study was to assess the potential for using data ecological conditions of vineyards and the phylogenetic analysis of *S. cerevisiae* strains isolated in the Odesa and Mykolayiv regions to identify the terroir.

## MATERIALS AND METHODS

The subjects of this study were vineyards and strains of wine yeasts. The yeast strains were isolated from vineyards located in the Odesa (SE RF Tairovske, NSC IVW n.a. V.Ye. Tairov, Prydunaiskyi OJSC) and Mykolayiv regions (Kobleve OJSC), which justified the need

for a detailed analysis of the landscape, soils composition, climate, and weather conditions in the studied areas during the research period. The strains were specifically collected from the experimental vineyard of SE RF Tairovske and selected areas of NSC IVW n.a. V.Ye. Tairov, using grape varieties such as Sukholy-mansky White, Odeskyi Black, Cabernet Sauvignon, among others.

According to the medium-scale ampelo-ecological zoning of the Northern Black Sea region, the territory of the SE RF Tairovske, located in Odesa district, Odesa region, belongs to the 8<sup>th</sup> ampelo-ecological district – Dnistrovsko-Khadzhybeyskyi. In terms of the general natural zoning, this area is situated in the southeastern part of the East European plane, within the Steppe natural zone (Middle Steppe subzone), specifically in the Dniester-Bug Black Sea lowland region of the Odesa Prymorskyi physical-geographical district (Vlasov, 2013; Vlasov et al, 2015).

The climatic conditions in the investigated area were characterized using data from reference books on the agroclimatic resources in the Odesa and Mykolayiv regions, as well as the nearest meteorological stations (MS) in Reni, Odesa (including the meteorological site on the territory of the NSC V.Ye. Tairov Institute of Viticulture and Winemaking), and Berezan (Sytova and Adamenko, 2011; Duranik and Adamenko, 2011).

64 strains of *S. cerevisiae* isolated from 36 technical grape varieties during the ripening period (2012–2013) were used in this study. The strains were cultured on potato-dextrose agar (boiled potatoes 200 g, glucose 20 g, agar 17–20 g, distilled water up to 1,000 ml). The culture medium was prepared by adjusting the pH, then poured into vials and sterilized under 1 atmosphere of pressure (hot steam) for 15 min. After cooling the medium to 45 °C, it was poured into sterile Petri dishes under sterile conditions and left to solidify at room temperature. The dishes were then placed in a thermostat at 28–30 °C to check the agar sterility before inoculating them with grape must samples in the fermentation stage (Birger, 1973).

The nucleotide sequence analysis and dendrogram construction, MEGA phylogenetic analysis software version 5.2 (<https://www.megasoftware.net/>) was used, along with nucleotide sequences of the ITS1\_5.8S ITS2 of ribosomal DNA obtained from the NARO Genebank ([https://www.gene.affrc.go.jp/databases-micro\\_search\\_en.php](https://www.gene.affrc.go.jp/databases-micro_search_en.php)). The unweighted pair group method with arithmetic mean (UPGMA) was applied to construct the phylogenetic tree.

The study employed methodological approaches aimed at identifying vineyard sites for wine production with protected designation of origin based on a comprehensive analysis of the ecological conditions of the territory (Vlasov, 2013). Analytical, expedition, cartographic, and comparative assessment methods were utilized. The research involved the use of topographic and soil maps, materials from soil surveys, meteorological observation data, and VIN-CAD-UKR software product (registration number 4830, 04.02.2020, bull. No. 57).

## RESULTS

As mentioned earlier, and supported by the findings of numerous studies (De Celis et al, 2019; Santamaría et al, 2019; Cordero-Bueso et al, 2011; Gonzales et al, 2023), the genetic diversity of wine yeast strains is influenced not only by grape variety but also by ecological conditions of the vineyard's territory, such as landscape position, soil type, and weather and climate conditions. The strains used in this study were isolated from vineyards of the Odesa (SE RF Tairovske, NSC IVW n.a. V.Ye. Tairov, Prydunaiskyi OJSC), and Mykolaiv regions (Kobleve OJSC). This justified the need for a detailed analysis of the landscape, soils, climate, and weather conditions of the investigated areas during the research period. Specifically, the strains were isolated from the experimental vineyard of SE RF Tairovske and selected areas of NSC IVW n.a. V.Ye. Tairov, using grape varieties such as Sukholy-mansky White, Odeskyi Black, Cabernet Sauvignon, etc.

Since the vineyard sites where strains were isolated were in close proximity to each other, one site was chosen as a representative example. This was the experimental site with Sukholy-mansky White variety, covering an area of 7.2 ha (**Fig. 1**). In the vineyard under study, the slopes ranged from 0 to 3 ° (**Table 1**).

According to the soil cover zonation (Vlasov, 2009), the enterprise's territory lies within the Southern Steppe zone, where southern low-humus medium- and heavy loamy chernozem, with a loess-based granulometric composition and a humus content of up to 3 %, predominates.

A distinguishing feature of the southern chernozem zone landscape is the flatness of the watersheds. Most areas lack surface runoff and have an underdeveloped hydrographic network. The zone is characterized by a shallow occurrence of gypsum and salts: 3–4 m deep in the northern part and about 2 m in the southern part. In drained areas, salinization begins at a depth of 3–

4 meters. The thickness of the humified part of the soil profile depends largely on the geographical location and the granulometric composition of the parental bedrock. In the Transnistrian region, the profile’s thickness ranges from 65 to 85 cm, and in areas with light clay and heavy loam, it increases from east to west.

The humus profile thickness ranges from 50 to 85 cm. The soil-forming layer consists of carbonate loess, pale yellow in color. In the depressions located in the southern part of the enterprise, the loess is replaced by brownish-red loam with gypsum inclusions. Groundwater is found at depths of 20–25 m, and artesian water lies at 80–120 m. The water has an alkaline reaction, and long time use for irrigation can salinize the soil with sodium and magnesium salts (Vlasov, 2009). The soils in the vineyard territory are classified as southern medium loamy chernozem, including weak and residual saline varieties (71d) (Table 1).

The territory of Prydunaiskyi OJSC, located in the Izmail district of Odesa region, is situated in the western part of the Black Sea Middle Steppe province, within the Zadnistrovska lowland steppe. The landscape of the enterprise’s territory is predominantly flat, with a slight slope towards the southwestern side of the Danube River and a more gentle slope towards Lake Kagul. The main area of the enterprise features a terraced plain with shallow, saucer-shaped depressions. The steepness of most slopes does not exceed 3 °, and only 10 % of the slopes ranging from 3 to 5 °.

Soil-forming layers on the plateau and slopes consist of Quaternary rocks and loess. Alluvial deposits serve as the parental material for floodplain soils. On the plateau, groundwater is located at a depth of approximately 10 m and does not participate in the soil formation process. Soils of the automorphic moisture series, specifically southern chernozem, have formed here. Along



**Fig. 1.** The experimental site in the territory of SE RF Tairovske

the bottoms of the gullies, where the water table is at the depth of 3–5 m, meadow chernozem soils develop. In the Danube floodplain areas, groundwater lies at a depth of 0.5–1.5 m and, in some places, reaches the surface, leading to waterlogging.

The territory of OJSC Koblevo belongs to the Prymorska lowland steppe landscape region. The Tylihul Estuary is located to the west of the enterprise’s land use area, while the Black Sea is to the south. The proximity of these large water bodies significantly impacts the microclimate of the area. Most of the territory is flat, with slopes of up to 3 °.

The predominant soils in this region are southern chernozem with medium- and heavy loamy granulometric composition. In the northern part of the enterprise, southern heavy loamy and slightly saline chor-

**Table 1.** The characteristics of the experimental site of Sukholymansky White variety

Variety	Year of planting	Area, ha	Root stock	Scheme of planting, m	Formation type	Origin of seedlings	Slope steepness	Frost danger in the territory, °C	Soil code
<i>SE RF Tairovske</i>									
Sukholymansky White	2006	7.2	RKhR 101-14	3 × 1.25	Medium standard limit	Ukraine	0–3	–17.6 –20.0	71d

nozem are present. Along the estuary, soils exhibit varying degrees of alkalinity and salinity.

As an example, we describe the agroclimatic conditions of Prydunaiskyi OJSC territory in the Izmail district of the Odesa region. According to data from the Izmail Hydrometeorological Station, the average monthly air temperature varies from  $-0.6^{\circ}\text{C}$  in January to  $22.8^{\circ}\text{C}$  in July throughout the year. The absolute maximum temperature recorded in July–August over the past 20 years has reached  $39^{\circ}\text{C}$  in 2002, while the absolute minimum observed in December–February was  $-21^{\circ}\text{C}$  in 1987. These temperature fluctuations can significantly impact the grape microbiome. Thermal resources, estimated by the sum of active temperatures above  $10^{\circ}\text{C}$ , range from  $3,200^{\circ}\text{C}$  to  $3,400^{\circ}\text{C}$ . The sums of air temperatures above  $10^{\circ}\text{C}$  during the frost-free period, averaged over many years, range from  $3,000$  to  $3,450^{\circ}\text{C}$ . The growing season lasts an average 230–240 days, with the active growing season spanning 180–185 days. The winter period lasts 80–85 days, while the frost-free period is typically 190–200 days in the air and 175–185 days on the soil surface.

The amount of precipitation throughout the year ranges from 28 mm in April to 54 mm in June. Generally, the total precipitation for the warm period averages between 220 mm and 250 mm, while the annual total ranges from 350 mm to 400 mm. The hydrothermal Selianinov coefficient (HTC) is 0.7–0.8, characterizing the conditions as arid. Dry winds occur annually, with particularly intense winds observed on average four years out of ten years. The total number of days with dry winds typically ranges from 50 to 55 days.

**Table 2** summarizes the climatic conditions for the enterprises. The average perennial air temperature during the growing season of grapes was recorded at 19.7, 19.1, and  $19.4^{\circ}\text{C}$ , respectively. The duration of sunshine varied, with averages of 1,356, 1,418, and 1,298 hours, while the sum of active temperatures reached

3,300, 3,200, and  $3,000^{\circ}\text{C}$ . The amount of precipitation during the warm season was measured at 243, 286, and 245 mm, respectively. Additionally, the lowest moisture capacity in the one-meter-deep soil layer was recorded at 137, 155, and 152 mm.

It should be noted that in 2012 and 2013, the weather conditions during the summer and autumn months (the years in which the strains were isolated) deviated from the climate norms and varied among the investigated territories. **Fig. 2, a, b** illustrate the dynamics of the average daily air temperatures and precipitation amounts during the summer and autumn months. In 2012, the average temperatures for June, July, August, and September were recorded at 23.1, 26.6, 24.1  $^{\circ}\text{C}$ , respectively, exceeding the norm by 3.3, 4.8, and 2.8  $^{\circ}\text{C}$ . The sums of active temperatures during the entire growing season were above the norm, ranging from  $250^{\circ}\text{C}$  in May to  $500^{\circ}\text{C}$  in late August. Precipitation during these months measured 26, 51, and 52 mm, representing 53, 100, and 148 % of the monthly norm, respectively. The autumn of 2012 was characterized by warm and dry weather; in September, the average temperature reached  $19.5^{\circ}\text{C}$ , which was 2.8  $^{\circ}\text{C}$  above normal, while precipitation was only 3 mm, accounting for just 9 % of the normal range. By the end of the second decade of October, the sum of active air temperatures reached  $4,037^{\circ}\text{C}$ , exceeding the norm by 788  $^{\circ}\text{C}$ . In October, average temperatures were several degrees above normal, and during the second decade, heavy rains (over 50 mm) replenished soil moisture reserves. In contrast, during the summer and autumn of 2013, the heat conditions were somewhat closer to normal, although moisture levels fluctuated by month.

The analysis of the environmental conditions of the experimental plots revealed significant differences in weather and climate conditions, soils, and landscapes. Variations in weather and climatic conditions directly influence the genetic diversity of wine yeast strains. In contrast, differences in soil and landscape characteris-

**Table 2.** The characteristics of the main climate indices in the investigated territory

Territorial community	Average air temperature during the growing season, $^{\circ}\text{C}$	Duration of sunshine during the growing season, h	Sum of temperatures for the grapes growing season, $^{\circ}\text{C}$	Amount of precipitation in the growing season*, mm	The least moisture capacity, in the soil layer of 0–100 cm, mm
MS, Reni	19.7	1356	3300	243	137
MS, Odesa	19.1	1418	3200	286	155
MS, Berezan	19.4	1298	3000	245	152

tics, particularly slope steepness, likely have an indirect effect by impacting the grapevine characteristics such as ripening time, sugar content of berries, pathogen infestation and berry integrity, etc.

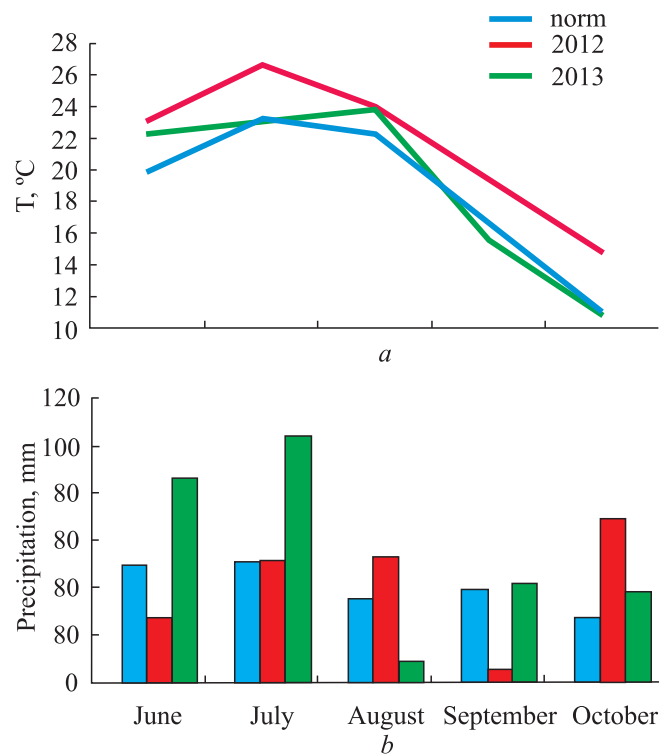
In the second stage of the study, we analyzed the species identity and nucleotide sequence data of 64 wine yeast strains isolated from the aforementioned regions. These strains were deposited in the Japanese genebank (NARO Genebank ([https://www.gene.affrc.go.jp/databases-micro\\_search\\_en.php](https://www.gene.affrc.go.jp/databases-micro_search_en.php))).

According to the data from the Genebank, all deposited strains belong to the species *Saccharomyces cerevisiae*. The sources of strain isolation are summarized in **Table 3**.

As previously mentioned, we processed the data on the nucleotide sequences of the ITS1-5.8S-ITS2 region of the ribosomal DNA using MEGA 5.2 program (<https://www.megasoftware.net/>).

To construct the phylogenetic tree (**Fig. 3**), we employed the unweighted pair group method with arithmetic mean (UPGMA).

Our assessment of the potential influence of environmental and genetic factors for terroir identification revealed significant differences between the environmen-



**Fig. 2.** The dynamics of the average monthly air temperatures (a) and precipitation amount (b). The meteorological site of the NSC IVW n.a. V.Ye. Tairov

**Table 3.** The sources of wine yeast *Saccharomyces cerevisiae* strains isolation

Code in Japan's Genebank	Grape variety	Plot, where a yeast strain was isolated	Year of isolation	Source
MAFF – 230170	Sukholymanskyi white	SE RF Tairovske 10 ha	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230171	Sukholymanskyi white	SE RF Tairovske 10 ha	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230172	Aligote	SE RF Tairovske	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230173	Odesa black	SE RF Tairovske	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230223	Rubin yuvileinyi	NSC IVW n.a. V.Ye. Tairov, selection plots	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230139	Iskorka	NSC IVW n.a. V.Ye. Tairov, selection plots	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230143	Illichivskyi rannii	NSC IVW n.a. V.Ye. Tairov, selection plots	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230140	Holubok 4556	NSC IVW n.a. V.Ye. Tairov, selection plots	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230142	Yarylo	NSC IVW n.a. V.Ye. Tairov, selection plots	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230120	Rhine Riesling	Koblevo OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230122	Sauvignon	Koblevo OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>

Code in Japan's Genebank	Grape variety	Plot, where a yeast strain was isolated	Year of isolation	Source
MAFF – 230123	Traminer	Koblevo OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230126	Cabernet Sauvignon	Koblevo OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230129	Merlot	Koblevo OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230131	Odesa black	Koblevo OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230155	Pinot Meunier	Prydunaiskyi OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230158	Kesha	Prydunaiskyi OJSC	2012	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230248	Muskat odeskyi	SE RF Tairovske	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230249	Odesa black	SE RF Tairovske	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230250	Cabernet Sauvignon	SE RF Tairovske	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230252	Cabernet Sauvignon	SE RF Tairovske	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230254	Cabernet Sauvignon Clon 1473	SE RF Tairovske	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230253	Odeskyi black (starter culture – a commercial strain)	SE RF Tairovske	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230225	Rubin tairovskyi	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230230	Yarylo	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230232	Opalovyi	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230234	Form 56-7-41 (white)	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230236	Form 56-13-28 (white)	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230226	Form 56-13-1 (red)	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230226	Lehenda	NSC IVW n.a. V.Ye. Tairov, selection plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230208	Sukholymanskyi white clone 1632	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230209	Telti-Kuruk Clone 7102	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230213	Traminer Clone FR46-106	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>

Code in Japan's Genebank	Grape variety	Plot, where a yeast strain was isolated	Year of isolation	Source
MAFF – 230200	Rhine Riesling clone B-68	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230202	Rhine Riesling clone 49	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230203	Rkatsiteli clone 4132	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230205	Sauvignon green Clone 648	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230206	Sauvignon green Clone 3873	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230256	Cabernet Sauvignon Clone 441	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230257	Cabernet Sauvignon Clone 143141	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230258	Cabernet Sauvignon Clone 2043	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230183	Cabernet Sauvignon Clone Fran VCR-10	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230185	Cabernet Sauvignon Clone Fran ICV-101	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230187	Merlot Clone VCR-13	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230191	Pinot Gris Clone VCR-1	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230198	Pinot Gris Clone VCR-5	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230255	Marseilles black early	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230197	Pinot Gris Clone 52	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230193	Pinot Black Clone 1-84	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230194	Pinot Black Clone 872	NSC IVW n.a. V.Ye. Tairov, clone experiment plots		<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>

Code in Japan's Genebank	Grape variety	Plot, where a yeast strain was isolated	Year of isolation	Source
MAFF – 230195	Pinot Black Clone VCR-9	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230188	Merlot Clone 347	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230189	Muskat odeskyi Clone 349	NSC IVW n.a. V.Ye. Tairov, clone experiment plots	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230238	Isabela	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230237	Odesa black	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230239	Merlot	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230240	Bastardo maharatskyi	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230241	Cabernet Sauvignon	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230242	Muskat hamburzkyi	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230243	Aligote	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230244	Rhine Riesling	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230245	Traminer	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
MAFF – 230246	Sauvignon	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>
AFF – 230247	Rkatsiteli	Koblevo OJSC	2013	<a href="https://www.gene.affrc.go.jp/databases-micro_search_en.php">https://www.gene.affrc.go.jp/databases-micro_search_en.php</a>

Note. MAFF – a strain code in the Japanese Genebank (MAFF – Culture Collection of Microorganisms of the Ministry of Agriculture, Forestry and Fisheries. The National Institute of Agrobiological Sciences (NIAS), Gene Bank, Tsukuba, Ibaraki, Japan).

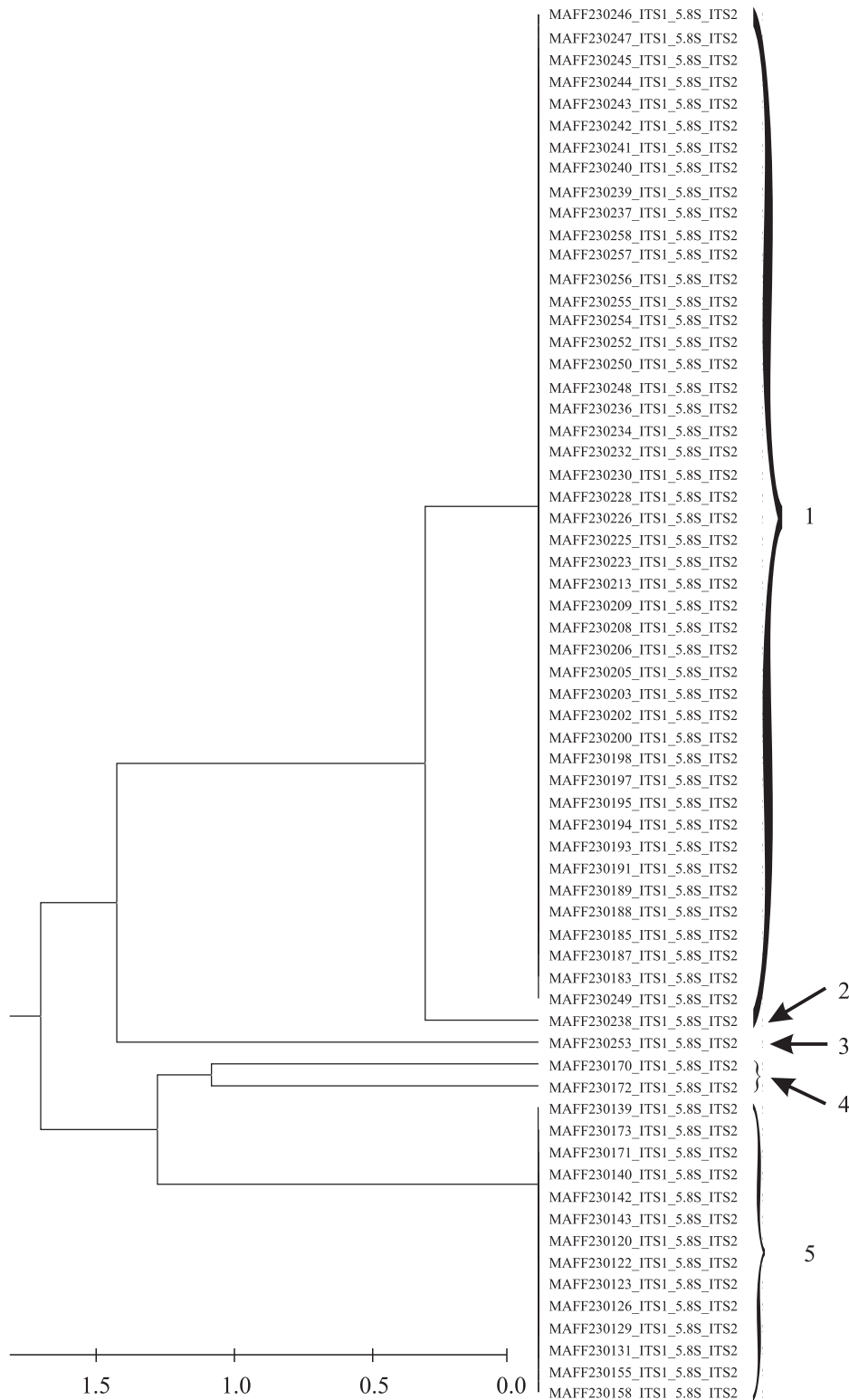
tal conditions of the vineyards located in the Odesa and Mykolayiv regions, as well as between the Tairovo and Izmail territorial communities within the Odesa region. These differences were primarily attributed to the varying characteristics of the soil cover and microclimatic conditions, which likely contribute to changes in the genetic diversity of wine yeasts.

## DISCUSSION

The study of the yeast microbiome in grapes represents a novel approach to terroir identification. However, it has yet to provide a definitive answer to whether

the strain composition of wine yeast can serve as reliable component in terroir assessment. Although efforts to identify terroirs in Ukraine have been underway for over 20 years, this research marks the first exploration of the grape microbiome as a facet of terroir analysis in Ukraine.

Research on the genetic diversity of wine yeasts is conducted directly in vineyards, during fermentation, and in both old and new wineries. The objectives of these studies range from practical application, such as improving wine quality, to more fundamental inquiries



**Fig. 3.** The phylogenetic relations between the strains of *Saccharomyces cerevisiae*, isolated from grape varieties of the selection plots (NSC IVW n.a. V.Ye. Tairov), industrial vineyards of SE RF Tairovske and Prydunaiskyi OJSC (Odessa region) and industrial vineyards of Koblevo OJSC (Mykolaiv region): 1) 2013, isolation, 46 samples, 33 varieties (including 24 clones of 12 varieties – 17 clones, bred by NSC IVW n.a. V.Ye. Tairov, 7 – Raushedo breeding (Italy), SE RF Tairovske, NSC IVW n.a. V.Ye. Tairov, Koblevo OJSC; 2) 2013, Koblevo OJSC, Isabela; 3) 2013, commercial strain; 4) 2012, SE RF Tairovske, varieties Sukholymanskyi white and Aligote; 5) 2012, NSC IVW n.a. V.Ye. Tairov – 6 samples, Prydunaiskyi OJSC – 2 samples, Koblevo OJSC – 6 samples, a total of 14 varieties, including Sukholymanskyi white

aimed at identifying grape terroir through the vineyard microbiome, particularly its yeast component (Pulchini et al, 2022; Agarbati et al, 2020, 2022; Lappa et al, 2020; Gao et al, 2022; Conacher et al, 2021; Carrau et al, 2020; Castrillo et al, 2023).

The ecosystemic role of the microbiome in winemaking is increasingly recognized by researchers (Ayogu et al, 2024). Successful attempts have already been made to differentiate terroirs by sequencing the genomes of indicator species (Cruz-Silva et al, 2023). Furthermore, the microbiome is acknowledged as a crucial component of terroir that significantly influence grape health, yield, and wine quality (Belda et al, 2020; Kamilari et al, 2020).

The composition of the vineyard microbiome, including the yeast microbiome, has been found to correlate with the geographical location of vineyards on various scales, including continents, countries, and regions (Gobbi et al, 2022). Biogeographical studies of wine yeasts suggest that mixed yeast communities are an integral component of wine region attribution (Hawkins et al, 2023). Although it has been established that wine yeast communities can contribute to regional differentiation, the precise extent of their contribution remains uncertain (Sumby et al, 2021). In fact, the diversity between geographical regions has not yet been fully explored, and some studies indicate that yeast communities can vary from year to year (Onetto et al, 2024). Nevertheless, despite the variability in the data, the potential of the microbiome in defining terroir is clearly acknowledged.

Several studies conducted in prominent wine-growing countries have demonstrated the diversity of *S. cerevisiae* strains within vineyards (Cordero-Bueso et al, 2011; Vigentini et al, 2015; Gayevskiy et al, 2012; Knight and Goddard, 2015; Gonzales et al, 2023; Liu et al, 2019), with researches attempting to assess the stability of genetic diversity levels.

For example, research in Greece, involving 4 wine regions and 34 vineyards, identified what the authors described as “temporarily stable communities” that could be used to differentiate wine regions (Chalvantzi et al, 2021). However, this differentiation is only partially explained by environmental factors such as maximum temperature, altitude, and precipitation (Chalvantzi et al, 2021; Ohwofasa et al, 2024; Awad et al, 2021). Similarly, Spanish studies found that the differences in wine yeast strains isolated from various sites were more strongly influenced by the year of harvest than by the agronomic practices used in cultivation (Castrillo and Bianco, 2022).

The evaluation of the genetic diversity of wine yeast strains isolated from a wide range of both ancient European and modern Ukrainian varieties revealed that the most influential factors on the genetic diversity of these strains were the geographical location (environmental conditions), the size of the vineyard, the year of isolation, and the grape variety from which the strains were isolated.

In our study, the strains isolated during different years (2012 and 2013), which experienced distinct weather conditions, tended to cluster together. This pattern was observed for strains from different regions as well. However, there were exceptions: a strain isolated from the Isabela variety, which belongs to a group of direct progenitor hybrids (unlike other varieties, which mainly represent *Vitis vinifera* or its hybrids (no more than 10–20 %) with *Vitis riparia*, *Vitis amurensis*, *Vitis berlandieri*), and a strain from the Odeskyi Black variety must, inoculated with a commercial strain of wine yeast at the start of fermentation, were positioned separately from the rest.

In contrast, the strains isolated in 2013, a year characterized by lower temperatures and more precipitation during the first half of the growing season compared to 2012, exhibited greater differentiation. For example, two strains isolated from the Sukholymanskyi White variety formed a distinct cluster, while the remaining strains - regardless of the region (including two strains from vineyards in the Izmail community, as well as strains from the Tairovo and Berezan communities) - clustered together.

In 2018–2021, this genetic diversity was further confirmed by enological testing of five strains of wine yeast isolated on varieties of the NSC “IVW n.a. V. Ye. Tairov” (Boichuk, 2017). It was shown that three of the five studied strains had a positive effect on the polyphenolic complex of dry red wine from the varieties Charivnyi, Odesa Pearl, and Cabernet Sauvignon, enhancing the content of aromatic compounds and improving the wine tasting score.

In general, the study of wine yeasts demonstrated differences between strains isolated in different years, with certain variations depending on the genetic origin of the variety within the plots, even in closely located ones (Vigentini et al, 2015; Gayevskiy et al, 2012; Knight and Goddard, 2015; Gonzales et al, 2023). This highlighted the potential for further research on autochthonous wine yeast strains as a factor not only in improving the quality and authenticity of winemaking products but also in defining the characteristics of the terroir.

Numerous studies using various molecular methods of DNA polymorphism analysis have provided evidence that local populations of *Saccharomyces cerevisiae* exhibit biogeographical patterns and are likely responsible for the regional identity of wine, contributing to the microbial aspect of the terroir concept (Capece et al, 2016).

The influence of regional microbiota on wine characteristics remains a debated issue. Several studies using sequencing have described microbial communities and revealed correlations between grape must, soil microbial communities, and the geography of vineyard locations. These observations have led to the emergence of the concept of “a microbial terroir”, though its validity is still under discussion (Alexandre, 2020).

On one side, evidence shows that yeast biodiversity in vineyards is influenced by macro- and microclimatic conditions, the grape variety, and the geographical location of the vineyard. It has been demonstrated that two neighboring terroirs, which produce wines with different sensory characteristics, may also have distinct microbial profiles. Thus, microbial consortia could partly explain variations in wine types. For example, Gutierrez et al (1999) examined vineyard yeast strains over five consecutive years and found that strain frequencies fluctuated annually, with only one strain consistently isolated across all five years. Similar observations were made by Schütz and Gafner (1994), who found that yeast populations varied from year to year, challenging the idea of a stable yeast terroir.

On the other hand, studies by Schuller et al (2005, 2007) identified the long-term presence of a specific strain in Portuguese vineyards, although this strain was not dominant in every fermentation. Another study conducted by Lopandic et al (2007) in Austria and based on the analysis of amplified fragment length polymorphism isolated different strains of *S. cerevisiae* from various vineyards, concluding that the resulting clusters aligned with the geographical distribution of the yeast strains. Similarly, Gayevskiy and Goddard (2012) reported analogous results in New Zealand. Additionally, Knight et al (2015) provided the first evidence of a positive correlation between microbial profiles and wine aromatic characteristics; supporting the notion that local yeasts, unique to specific vineyard zones, contribute to the terroir and influence the distinctiveness of regional wines.

However, the overall picture appears more complex. Several studies have failed to find strains that are representative of a specific winery or region (Santamaría

et al, 2019; Vigentini et al, 2015; Torija et al, 2001). For example, Torija et al (2001), comparing the biodiversity of *S. cerevisiae* in two Spanish regions (Priorat and Terra Alta) over three consecutive years, reported significant yearly variation in yeast populations. Similarly, Vigentini et al (2015) isolated 270 strains of *S. cerevisiae* from two viticultural areas of northern Italy (six sites in each region) over three years. Their DNA polymorphism analysis revealed that the year of isolation, or vintage, significantly influenced yeast biodiversity, while geographical location, or terroir, had no notable impact.

In another study of *S. cerevisiae* biodiversity, conducted in 11 Spanish wineries in the Rioja region for 3–4 consecutive years, Santamaría et al (2019) found that yeast strains varied annually within each winery, with no single strain being representative of a winery or region. Similarly, Börlin et al (2016), studying three different wineries in the Sauternes region of France, observed significant genetic differentiation between *S. cerevisiae* populations in the three wineries, despite the short distances (less than 10 km) between them. These contradictory results could be due to the different scales of the studies: some of them were conducted on a national level (Gayevskiy et al, 2012; Schuller et al, 2012), while others focused on regional levels (Santamaría et al, 2019; Vigentini et al, 2015; Torija et al, 2001).

In our view, the discrepancies in the study of *S. cerevisiae* populations studies may partially be explained by the multitude of factors influencing wine yeast genetic diversity, many of which are interdependent. For example, the geographical factor influences the year’s specific meteorological conditions, which in turn affect the wine yeast population. This explains why different strains are often isolated depending on the year. Additionally, grape variety can indirectly impact yeast populations by shaping microclimatic conditions, such as variations in vine vigor or the presence or absence of leaf pubescence.

In a study on Malbec (Gonzales et al, 2023), the diversity of *S. cerevisiae* populations was examined over three years (vintages) in two vineyards to evaluate their annual diversity and behavior across different years. A total of 654 *S. cerevisiae* isolates were differentiated by Interdelta-PCR analysis. Although the composition of *S. cerevisiae* strains in the vineyard populations varied significantly across the three vintages, the diversity index values remained stable. In general, *S. cerevisiae* communities were characterized by the emergence of new strains in each harvest season. The *S. cerevisiae* strains

isolated in one vineyard were unique to that particular harvest and were not re-isolated in subsequent years.

Similarly, a three-year study of winery must yeast populations in two Italian wine regions showed that *S. cerevisiae* strains were randomly distributed, with no clear relationship between the Interdelta-PCR profile and factors such as territory, year, or the isolation matrix (Vigentini et al, 2015). Several studies have described this behavior as “unstable” (Schuller et al, 2007; Lopandic et al, 2007; Schuller et al, 2012). Therefore, the majority of studies on *S. cerevisiae* diversity during single or multiple harvests confirm the “unstable” behavior of *S. cerevisiae* strains. Most similar studies did not report widespread distribution of specific *S. cerevisiae* strains across large areas (Vigentini et al, 2015; Schuller et al, 2005; Schuller et al, 2012).

Our findings, though limited in scope, tools, and localization, lend some support to the hypothesis that yeast terroir is associated with the stable diversity of *S. cerevisiae* over time and space, rather than the long-term persistence of specific local strains in a given area or winery.

## CONCLUSIONS

The analysis of environmental conditions across the vineyards where yeast strains were isolated revealed significant differences in temperature regimes, humidity, soil composition, and landscape features (e.g. slope steepness). These factors are important indicators for terroir identification and serve as a kind “background” that supports the genetic diversity of *Saccharomyces cerevisiae* wine yeast strains.

Phylogenetic analysis of 64 strains of wine yeast isolated from 36 technical grape varieties (based on the ITS1-5.8S-ITS2 site) and classified as *Saccharomyces cerevisiae* showed that the clustering of samples was primarily influenced by the year of isolation, highlighting the role of agroclimatic factors as a major component of environmental conditions. The varietal composition of vineyards played a secondary role. Thus, while the wine yeast microbiome may be useful in assessing specificity – particularly with regard to the characteristics of wine product – it does not appear to be a reliable marker for terroir identification.

**Adherence to ethical principles.** No experiments, described in this article, involved the use of animals.

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### Екологічні умови винограду та поліморфізм штамів *Saccharomyces cerevisiae* як передумова ідентифікації теруару

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**Мета.** Оцінка можливості застосування результатів дослідження екологічних умов винограду насаджень та філогенетичного аналізу штамів винних дріжджів *Saccharomyces cerevisiae*, виділених в локаціях Одеської області для ідентифікації теруару. **Методи.** Мікробіологічний, аналітичний, експедиційний, картографічний та порівняльно-розрахунковий методи, використання програми філогенетичного аналізу МЕГА та програмного продукту VIN-CAD-UKR. **Результати.** Показано наявність різниці між екологічними умовами насаджень, розташованих в Одеській та Миколаївській областях і між Таїровською і Ізмаїльською територіальними громадами Одеської області (за характеристиками ґрунтового покриву та мікрокліматичними умовами території). Визначено, що штами *Saccharomyces cerevisiae*, виділені у 2012 та у 2013 роках (різних за погодними умовами стосовно температури повітря та опадів),

переважно кластеризувалися спільно по роках. Окремо позиціонувалися лише штами, виділений із сорту Ізабела (належить до групи гібридів-прямих плідників на відміну від інших сортів, які переважно представляють *Vitis vinifera* або його гібриди з *Vitis riparia*, *Vitis amurensis*, *Vitis berlandieri*) та штаму, виділений із сусли сорту Одеський чорний, проте інокульованого на початку бродіння комерційним штамом винних дріжджів. Показано, що штами, виділені у 2013 р. (більша кількість опадів та більш низька температура повітря у першій половині вегетації порівняно із 2013 р.), показали більш диференційовану картину (окремий кластер склали два штами, виділені на ділянці сорту Сухолиманський білий, проте всі інші виділені штами, незалежно від регіону виділення утворили один спільний кластер. **Висновок.** Філогенетичний аналіз 64-х штамів *Saccharomyces cerevisiae*, виділених на 36 сортах технічного напрямку використання за нуклеотидною послідовністю ділянки ITS1\_5.8S ITS2 продемонстрував, що на кластеризацію зразків переважно впливає рік виділення, тобто агрокліматичні чинники як складова екологічних умов теруару, які є основою для його ідентифікації.

**Ключові слова:** теруар, екологічні умови, штами *Saccharomyces cerevisiae*, секвенування, філогенетичний аналіз.

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