The yeast on the grape berry surface influenced by climatic factors

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Abstract. Republic Moldova is a country with long history of winemaking. Understanding the microorganism on the grape surface is very important to the winemaking process, and it's also a national strategy of development of the wine industry. In this study, twenty seven samples from three regions and three vintages in Republic of Moldova were studied. The conventional microbiological methods combine with molecular methods (PCR-DGGE) have been used for study the quantity and the quality of microbes. The result show that the yeast population on the berries are variable in different vintages, and in the climatic factors, the Cool nigt index (CI) affect the yeast most. From the identification result, *A.Pullulans* and *R.glutins* are two culture which are easy to be found on the Moldova grapes. The autochthonous *S.cerevisiae* also been identified, but it shows a different results from different vintages.

1 Introduction

The wine quality and the characteristic are influenced by the microorganisms present in the fermentation process. [1] The natural yeast not only plays an important role in the winemaking process, but also in the "terrior". The population and the diversity of natural yeast on the berry surface are shaped by many factors such as vintages, the location of the vineyard and the climate.[2] Although, researchers have studied the relationship between microorganism on the grape and the climatic factors and the regional factors. [3]But it's still not clear how these factors influence the diversity and the population of microorganism on the grapes.

In this study, the grape berries collected from three protected geographical indications (PGI) regions in Republic of Moldova, conventional microbiological methods combine with molecular methods (PCR-DGGE) have been used for study the quantity of microbes and identification.

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2 Material and method

2.1 Samples

Grape samples collected from several vineyards in three PGI in Republic of Moldova: PGI Codru (PGI C), PGI Valul lui Traian (hereafter referred as PGI VLT) and PGI Stefan Voda (hereafter referred as PGI SV). The sampling time is when the grapes reach the technological maturity in every year. For each sample, 1 kg berries are took, the whole sampling process took place in sterile condition. Eight commercial varieties which can represent the regional characteristic were studied: Feteasca neagră, Fetesca Albă and Sauvignon Blanc et al. (detail in Table 1)

Table1. Characteristics of samples.

PGI name	Coordinates Sampling Place	Variety	Vintage	Sample Code
PGI "Codru"	(E47.41,N27.98)	Feteasca Neagra	2018	FN_C_18
			2019	FN_C_19
			2020	FN_C_20
	(E47.06,N28.51)	Feteasca Alba	2018	BG_C_18
			2019	BG_C_19
			2020	BG_C_20
	(E47.22,N28.52)	Sauvignon Blanc	2018	SA_C_18
			2019	SA_C_19
			2020	SA_C_20
PGI "Valul lui Traian"	(E46.39,N28.73)	Feteasca Neagra	2018	FN_VLT_18
			2019	FN_VLT_19
			2020	FN_VLT_20
	(E46.19,N28.63)	Feteasca Alba	2018	BG_VLT_18
			2019	BG_VLT_19
			2020	BG_VLT_20
	(E45.65,N28.47)	Merlot	2018	ME_VLT_18
			2019	ME_VLT_19
			2020	ME_VLT_20
PGI "Stefan Voda"	(E46.53,N29.87)	Feteasca Neagra	2018	FN_SV_18
			2019	FN_SV_19

			2020	FN_SV_20
	(E46.48,N29.94)	Carbernet Franc	2018	CF_SV_18
	(E46.48,N29.94)	Pinot Noir	2019	PN_SV_19
			2020	PN_SV_20
	(E46.53,N29.87)	Rara Neagra	2018	RN_SV_18
	(E46.53,N29.87)	Viorica	2019	VI_SV_19
			2020	VI_SV_20

2.2 The acquisition of data on climatic factors

In this study three synthetic and complementary viticultural climatic indices are used: 1) Heliothermal Index (HI) which corresponds to Huglin's heliothermal index. 2)Cool night index (CI), an index works as thee indicator of night temperature conditions during maturation. 3) Dryness index (DI), which is an indicator of the level of presence-absence of dryness.

The CI is the minimum temperature in September, and the other two index values obtained from formula:

$$HI = \sum_{01.04}^{30.09} \frac{[(T-10)+(T_x-10)]}{2} d \tag{1}$$

where the T is the average air temperature (℃), Tx is the maximum air temperature (℃), d is the length of day coefficient.

$$DI = W_0 + P - T_v - E_s \quad (01.04\text{-}01.10) \tag{2}$$

W_0 is the estimated regional mean, usually the adopting value 200 mm is used. P is the precipitation, T_v and E_s are calculated by potential evapotrasnpiration[4].

The acquisition of primary climatic data: T, T_x, P, ETP were performed using i-metheos resorts.

2.3 The method of yeast quantity analysis

Grapes were randomly collected, using ethanol sterilised shears (100 berries). 300g of berries were placed in a sterile 500 mL flask containing an isotonic peptone solution (10g/L Bacto Soytone, 2mL/L of Tween 80) to wash for 3 hours at 30 ℃.The samples and dilution series was plated out on yeast peptone glucose plate (yeast extrac 10g/L, Bactotryptone 10g/L, glucose 20g/L, agar 25g/L, pH adjusted to 5.0 using orthophosphroc acid), at 25 incubation 10 days.The numeration was made on plates. The population of yeast was obtained from the formula:

$$CFU/berry = \frac{C \times V \times m}{M} \tag{3}$$

where, C is the number of colony forming units (CFU) per mL,V is The volume of the dilution, m ishe average weight of the berries, M is total weight of the berries.

2.4 The method of yeast quality analysis

Amplification and sequencing were performed as described previously for analysis of yeast.[5] Briefly, the D1 region of 26 rRNA gene was amplified by PCR using the primer NL1-GC (5'-GCG GGC GCG ACC GCC GGG ACG CGC GAG CCG GCG GCG GGC CAT ATC AAT AAG CGG AGG AAA AG-3') and primer LS2 (5' -ATT CCC AAA CAA CTC GAC TC-3'). PCR was run in a final volume of 50μL containing 0.5μM of each primer, 4μL of commercial mix (Invitrogen) and 2μL of DNA. The amplification was carried out as condition : 95℃ for 5 min, 40 cycles of 95℃ for 1 min, 52℃ for 45s and 72℃ for 1 min, and final 72℃for 7 min. 5μL aliquot of the amplified DNA was analysed by 1.5% agarose gel electrophoresis to verify that the PCR worked prior to DGGE. The DGGE separation was performed on a BIO-RAD DGGE system.

2.5 Statistical analysis

PCA analysis are used for studying the climatic factors. Graphics were made by R 4.04. Analysis of variance (ANOVA) was applied to the experimental data for the population analysis in different vintages and regions. The means were analysed using the R 4.04. The significant differences were determined by the mean of one-way ANOVA, and the results were considered significant if the associated P values were ≤ 0.05.

3 Results and discussion

3.1 Quantity result

In order to evaluate the influence of the regions and vintages, variety factors are ignored, the population of yeast (CFU/berry) is the average of three varieties in the same region and same vintage. Graphics were made by Origin 2018 Lab.

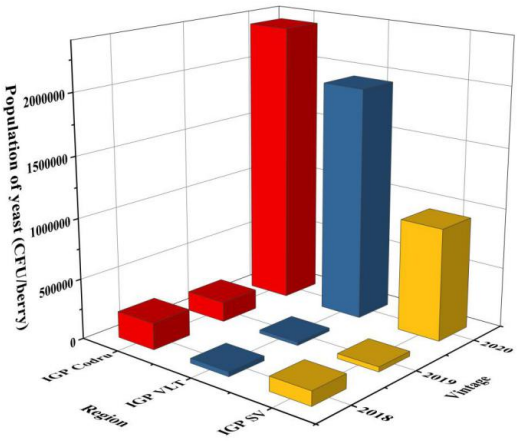


Fig. 1. The quantity of yeast in three regions in vintage2018, 2019 and 2020. ANOVA test for Vintages (P = 0.00466), Regions (P = 0.807)

The Figure1. shows a comparison of population of yeast on the grape surface in different vintages and regions. It's clear that the geographical factors is not significant, but when it comes to vintage, the populations show a significant variation(P<0.05), especially

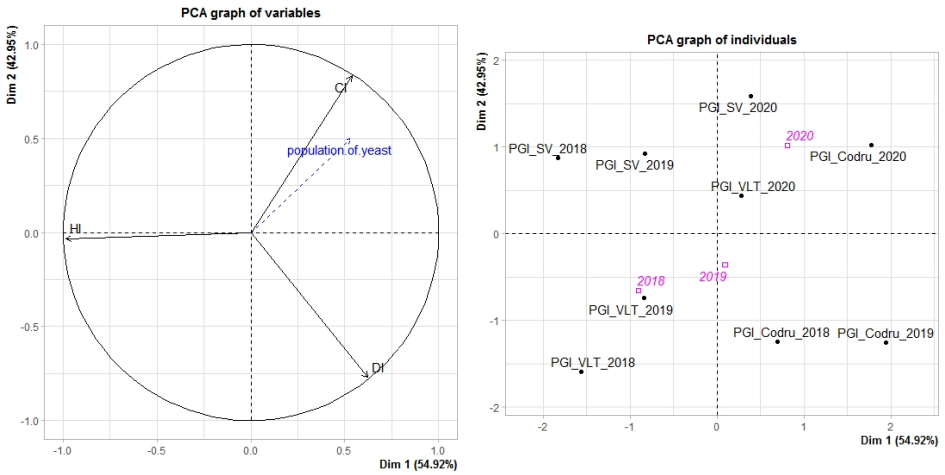
the vintage 2020 the population of yeast reach a high level.

Table 2. The value of climatic index (data from ng.fieldclimate.com) and the result of yeast quantity.

Sample Code	The population of yeast (CFU/berry)	The average population of yeast (CFU/berry)	Climatic Index*		
			HI (℃ • day)	CI (℃)	DI (mm)
FN_C_18	6.00E+04	2.20E+05	2311.73	2.85	141.49
BG_C_18	3.00E+05				
SA_C_18	3.00E+05				
FN_C_19	3.30E+05	1.64E+05	2100.12	4.13	176.01
BG_C_19	1.60E+05				
SA_C_19	2.80E+03				
FN_C_20	1.00E+06	2.33E+06	2174.00	10.55	75.19
BG_C_20	5.00E+06				
SA_C_20	1.00E+06				
FN_VLT_18	1.00E+05	3.90E+04	2659.16	-0.92	84.81
BG_VLT_18	9.70E+03				
ME_VLT_18	7.20E+03				
FN_VLT_19	1.70E+03	2.72E+04	2479.39	1.14	43.42
BG_VLT_19	3.70E+04				
ME_VLT_19	4.30E+04				
FN_VLT_20	8.00E+05	1.93E+06	2398.00	7.04	53.45
BG_VLT_20	1.00E+06				
ME_VLT_20	4.00E+06				
FN_SV_18	2.00E+05	1.20E+05	2662.69	4.57	-56.57
CF_SV_18	6.00E+04				
RN_SV_18	1.00E+05				
FN_SV_19	1.90E+03	4.08E+04	2503.83	5.90	-28.00

PN_SV_19	1.20E+05				
VI_SV_19	4.10E+02				
FN_SV_20	6.00E+05				
PN_SV_20	2.00E+05	9.33E+05	2292.41	8.80	-28.90
VI_SV_20	2.00E+06				

** Data collected from the network of weather stations installed on the experimental plots (ng.fieldclimate.com)*



(a) (b)

Fig. 2. The PCA result (a) the population of the yeast in three vintages and three region (b) the influence of climatic factors on grape yeast.

In Fig.2. (a) it can be observed: CI is the most influential factor on population of yeast, and the influence is positive. The HI has a negative influence on the population of yeast. When it comes to DI, it seems very little influence is.

Fig.2. (b) shows that vintage 2020 the population of yeast with a high level and the vintage 2018 and 2019 are close. In vintage 2020 the CI is bigger than other two vintages. However the vintage 2019 is drier, 2018 is warmer. In PGIs, the PGI SV is wetter, to the contrary, the PGI Codru is drier. And in the vintage 2018 and 2019 the PGI VLT with a low CI value.

Considering that CI is an indicator of night temperature conditions during maturation,. it can be speculated that the climatic factors in the maturation period or veraison are important to the microbes on grape berry, it should be studied in the future.

3.2 Quality result

Every year we chose to isolate 4 types of yeasts whose colonies presented interesting morphotypes. The result shows in Table 3.

Table 3. The result of yeast quality on grapes in three vintages.

2018 Sequencing result	*R.glutinis, M.pulcherrima, A.pullulans, H.uvarum*
2019 Sequencing result	*R.glutinis, R.Gramins, A.pullulans, S.cerevisiae*
2020 Sequencing result	*M.pulcherrima, S.cerevisiae, A.pullulans, R.glutinis*

Among the identified microbial species, *A.pullulans* and *R.glutins* were observed in three years of continuous.

H.uvarum and *M.pulcherrima* which is often the main species found on grapes. Both of them frequently appears in spontaneous fermentation. Killer strains of *H.uvarum* species may inhibit the fermentation of *S.cerevisiae*.[6] The *M.pulcherrima* is also supported by the expression of many extracellular activities, such as enhance the release of varietal aromatic compounds. *M.pulcherrima* has a respiratory metabolism that can help to lower ethanol content when used under aerobic conditions.[7] In addition it shows good compatibility with *S.cerevisiae* in producing a low to medium acidity and, function of reducing level of H_2S. *R.glutins* is a specie with high adaptive capacity against environmental changes.[8] It can explained that why the *R.gulutins* can be observed in all vintages studied.

In every vintage, *S.cerevisiae* straines are isolated to study, the FIG.3. is the comparison of genetic profile in three vintages.

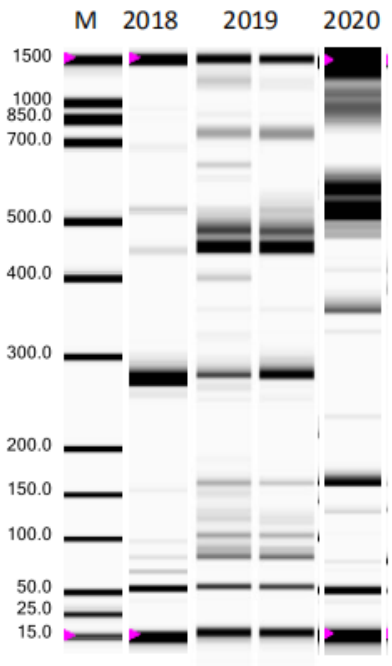


Fig. 3. Genetic profile in vintage 2018, 2019, 2020.

It's clear that the *S.cerevisiae* strains in different vintage are totally different. All these strains are put in collection (freezer at -80℃) to proceed to further analysis.

4 Conclusion

In the past three years, the population yeast on Moldova grapes with a annual variation, in which 2020 the population is highest. In the climatic factors, the influence of CI is most obviously, and the DI and HI have negative influence. *A.Pullulans* and *R.glutins* are two culture which are easy to be found on the Moldova grapes. The autochthonous *S.cerevisiae* found from grapes are totally different among different vintages.

Acknowledgments

This research under the support of the National Office for Vine and Wine of Republic Moldova (ONVV).

References

1. R.M. Callejona, A. Clavijob, P. Ortigueirac, A.M. Troncosoa, P. Panequeb, M.L. Moralesc, Anal. Chim. Acta, **660**, 68-75 (2010)
2. A. Barata, M. Malfeito-Ferreira, V. Loureiro, Int. J. Food Microbiol. **153**, 243-259 (2012)
3. N. Bokulich, T. Collins, C. Masarweh, G. Allen, H. Heymann, S. Ebeler, D. Mills, Mbio, **7(3)**,e00631-16 (2016) doi: 10.1128/mBio.00631-16
4. J. Tonietto, A. Carbonneau, Agric For Meteorol. **124**,81-97 (2004)
5. V. Renouf, O. Claisse, et al. Aust. J. Grappe Wine Res., **11,** 316-327 (2005)
6. F. Radler, M.J. Schimit, B. Meyer, Arch. Microbiol., **154**, 175–178 (1990)
7. A. Morata, I. Loira, C.Escott, J. Fresco, M. Banuelos, J. Suarez-Lepe, Fermentation, **5(3)**, 63 (2019)
8. E. Longo, J. Cansado, D. Agrelo, T.G. Villa, Am. J. Enol. Vitic., **42**, 141-144 (1991)