THE APPEARANCE INCIDENCE OF SOME OXIDATIVE YEAST SPECIES IN THE ECOTOPE BANU MARACINE

INCIDENȚA APARIȚIEI UNOR SPECII DE LEVURI OXIDATIVE ÎN ECOTOPUL BANU MĂRĂCINE

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Abstract. *The research has been made at the vineyard Dealurile Craiovei, S.D. Banu Maracine during 2006-2008, and focused on the appearance incidence, under the influence of the climatic factors (temperature and humidity), of the Candida spp. yeast. The temperature and the humidity of the air, also the rainfall constitute essential features of the climate in a certain viticultural area, with decisive impact on the microorganisms biological, physiological and biochemical processes, with implication on their distribution on the viticultural area. In order to obtain corect correlation between the depending factors (the number of yeasts strain isolated) and the independent factors (temperature and air humidity), all the data has been percentage expressed. For complex ecosystems, including the spontaneous flora there has been developed new methods for inventorying the yeast from a sample – without avoiding the cultural and isolation stages – and measures regarding the global evaluation of the development dynamics and of all the physiological index which reflect the metabolic and celular activity.*

Key words: oxidative yeast, climatic factors, ecotope Banu Maracine

Rezumat. *Cercetarile au fost efectuate în plantația viticolă de la S.D. Banu Maracine 2006-2008, şi s-au axat pe incidența de apariție a unor specii de levuri oxidative, sub influența factorilor climatici (temperatura şi umiditatea). Temperatura şi umiditatea aerului, inclusiv precipitațiile constituie caracteristici importante ale climatului în anumite zone viticole, cu un impact decisiv asupra proceselor biologice, fiziologice şi biochimice ale microorganismelor, cu implicații asupra distribuției acestora în arealul viticol (Popa, A. Teodorescu Şt. 1990). Pentru a obține corelații corecte între factorii dependenți (numărul de specii izolate) şi factorilor independenți (temperatura şi umiditatea), toate datele au fost exprimate procentual. Pentru ecosisteme complexe, inclusiv flora spontană, sunt dezvoltate noi metode pentru a realiza inventarul tulpinilor aflate într-un eşantion – fără a ocoli etapele de cultură şi izolare - şi măsuri privind evaluarea globală a dinamicii de dezvoltare şi a tuturor indicatorilor fiziologici care reflectă activitatea metabolică şi celulară.*

Cuvinte cheie: levuri oxidative, factori climatici, ecotopul Banu Maracinie

INTRODUCTION

The yeasts are eukariotic microorganisms made from a single cell. Like anyother microorganism, the yeasts are opened thermodynamic systems, changing matter and energy with the environment. These functioning conditions become clearly defined only if the environmental parameters are equaly well defined. In

order to understand and forecast the microorganisms behaviour, it is necessary the characterization of the metabolic and cellular state of the microorganisms placed in their micro-climate (Bouix M., Leveau J.Y., 2001).

For complex ecosystem, including the spontaneous flora, there has been developed new methods for inventorying the strains from a sample – without avoiding the cultivation and isolation stages – and measures regarding the overall evaluation of the development dynamics and all the physiological index that reflect the meatbolic and cellular activity.

During the past years there has been realized many ecological studies for knowing the dynamics, the quantification and the microflora composition responsable for the spontaneous fermentation of the must.

The largest part of the yeasts population are distributed in the soil, water and air. They form comunities of species, well defined in their habitat. The yeast being chemo-organotrophic, they are encountered only in that areas where they found the sources of organic carbon needed for the growth and development. Because different species need different sources of organic carbon (selective nutrition), in the biosphere their dispersion it is very well determined on species categories (Belin J.M., 1981).

MATERIAL AND METHOD

The research has been made in the vineyard of the Didactical Station Banu Maracine, during 2006 – 2008 and focused on the apearance incidence of some oxidative yeasts, under the influence of the climatic factors (temperature and humidity).

The temperature and air humidity, including the rainfall constitute important features of the climate in certain viticultural areas, with a decisive impact on the biological, physiological and biochemical of the microorganisms, with implication on their distribution in the viticultural area.

For the data base used in the statisticall analyze, we have selected only the climatic data with maximum values (table 1, 2), the use of the average and of the temperature degree summ can introduce yearly errors to the calculus and results.

In order to obtain corect correlation between the depending factors (number of yeast strain isolated) and the independents factors (the temperature and air humidity) all the data has been expressed percentage (table 5).

For the collecting of the soil samples used for isolate the yeast strains from the viticultural are Banu Maracine, there has been established 25 collecting stations.

The spreading degree of the data has been verified through the variance analyze, and the estimation of the differences between the isolated species has been verified through the calculation of the transgression probabilities, obtaining p=0,05516% for all the research years; we conclude that the results of the statisticall analyze are significant for t=3,5984 and 21 liberty degrees.

RESULTS AND DISCUSSIONS

For the mathematical modelling of the entire experiment, there has been tested many type of curves: liniar, exponential, logarithmical, sygmoide, square and cubical. The type of curve that has represented most accurate the experiment

has been second degree function, with the interaction of the independents factors, the selection of the function being made through calculus as well the selection of the regression coefficient with the highest value (closer to the value 1).

There has been calculated the coefficient of simple correlation between the distribution frequency of the isolated yeasts strains from the vineyard soil during March – October and from the grapes during June – October (depending factors), as well the raw values monthly temperature and humidity, during the 3 years of research 2006 – 2008, these data being presented in the table 3.

In the table 4 there are presented the regression function characteristic for each isolated species, where „x" represent absolutely all the values of the air temperature during 2006 – 2008, and „y" represent absolutely all the values of the air humidity during the same period. The fifth therm of the equation represent the interraction of the two independents factors and mathematically reproduce the cumullated value of the their influence on the distribution frequency of the isolated yeast species from the viticultural ecotope Banu Maracine. We mention that all the results of the statisticall analyze and the mathematical modelling reflect the researched period 2006 – 2008.

The results obtained after the isolation does not respect the reality in the nature (soil, grapes, must and wine), that it is why the yeast species disperssion has been presented only after a previous statistical analyze through their disperssion frequency, percentage expressed (table 4).

Thus from the data presented in table 4 it come out that the appearance incidence of the strains species *Brettanomyces claussenii* has been of 0,35% from the total of the isolated yeast population. Although the variation of the air relative humidity had a lesser influence, the percentage development of the species population in 2008 has been attributed to the higher levell of the rainfall from the spring, being known the rapid development of this species in liquid media.

From the species of the *Candida* genre, *Candida stellata* had an appearance incidence of 0,4%, higher than the one of the species *Candida vini* which had presented an appearance incidence of 0,38% from the total of the isolated yeast populations. The two species have presented similar curves of the frequency during the research years: from average values in 2006, they suffer a decline in 2007 due to the lack of rainfall from spring; the rainfall from 2008 have positively acted on the increasing of the strains frequency. From the climatic factors taket in to study, the temperature had a significant positive action only on the appearance frequency of the species *Candida stellata* and the air relative humidity had a significant positive action only on the appearance frequency of the species *Candida vini*.

Table 1

The maximum value of the temperature recorded at the Meterological Station Banu Mărăcine(2006-2008)

Year	Month												Yearly average
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2006	0,3	3,2	10,1	12,3	21,3	23,2	31,4	29,5	23,7	18,2	11,4	4,0	10,9
2007	8,4	7,3	12,3	15,4	21,5	25,4	33,8	31,0	23,4	13,9	8,6	3,4	12,4
2008	0,1	5,7	13,8	18,5	22,2	27,0	30,3	32,4	28,5	21,2	15,2	7,6	11,9
Average 2006-2008	2,9	5,4	12,0	15,4	21,6	25,2	31,8	30,9	25,2	26,3	17,7	5	11,7

Table 2

The air relative humidity recorded at the Meterological Station Banu Mărăcine(2006-2008)

Year	The air relative humidity (%)												Average U.R./year (%)
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
2006	85	83	73	70	65	72	61	70	69	75	73	90	73
2007	69	72	64	46	60	57	36	66	71	77	85	91	66
2008	91	73	57	69	65	62	55	50	66	76	71	85	68
Average 2006-2008	81,6	76	64,6	61,6	63,3	63,6	50,6	62	68,6	76	76,3	88,6	69,4

Table 3

The coefficient of the simple correlation between the strains frequency of the isolated yeasts from soil and grapes and the climatic factors

Nr. crt	Non-sporogenous yeast	Correlations with the temperature	Correlations with the humidity
1	*Brettanomyces claussenii*	0,284	0,372*
2	*Candida vini*	-0,114	0,495**
3	*Candida stellata*	0,520**	0,422*
4	*Torulopsis bacillaris*	0,783**	0,189
5	*Kloeckera apiculata*	0,254	0,441*
6	*Rhodotorula mucilaginosa*	-0,071	-0,270

*Significant correlation for p=0,05%; ** Significant correlation for p=0,001

Table 4

The equation of the regression curves with the factor interaction

Species	Equation
Brettanomyces claussenii	$1,3817-0,1807x-0,5878y+0,044x^2-0,032xy+0,0981y^2$
Candida vini	$-1,545 +0,7809x+0,0042y-0,0576x^2-0,0738xy+0,0414y^2$
Candida stellata	$-1,032 +0,2408x+0,231y+0,0003x^2-0,0475xy+0,0026y^2$
Torulopsis bacillaris	$-0,8829-0,0277x+0,3894y+0,022x^2-0,0134xy-0,0327y^2$
Kloeckera apiculata	$6,0797-0,9085x-2,1282y+0,0218x^2+0,1969xy+0,1756y^2$
Rhodotorula mucilaginosa	$3,6399-0,9043x-0,7608y+0,0428x^2+0,1314xy+0,0229y^2$

x=air temperature şi y=air relative humidity

Table 5

The strains appearance incidence of the yeasts isolated from the soil, grapes, must and wine during the three years of research (2006-2008 Banu Mărăcine)

Nr.	Species	Total strains 2006-2008	The appearance incidence %
1	*Brettanomyces claussenii*	40	3,54
2	*Candida vini*	42	3,85
3	*Candida stellata*	43	4,02
4	*Torulopsis bacillaris*	53	4,95
5	*Kloeckera apiculata*	84	5,57
6	*Rhodotorula mucilaginosa*	35	3,12
Total non-sporogenous yeasts		294	25,05

The species *Torulopsis bacillaris* had constant levell of the appearance incidence (aproximatively 0,5%), the evolution tendency being significantly positively influenced in all the research years, by the variation of the temperature, 64,0%.

The strains distribution high levell of this species in the viticultural area has encountered also in the microflora from the must,. The air relative humidity had no influence on the appearance incidence of this species strains.

Known as the most spreaded species in the yeast populations that control the fermentation process, *Kloeckera apiculata*, with 0,55% from the total of the yeast strains during 2006, has presented a drastic decrease of the apearance incidence levell during the summer of 2008, 0,24%. The factor that acted significantly positive to the increase of the appearance incidence of the species (19,4%) has been the air relative humidity.

From all the yeast species isolated from the viticultural ecotope Banu Maracine, *Rhodotorula mucilaginosa* has the most affected species with an appearance incidence of 0,31% from the total of the yeast strains.

The way that the yeast responde to the climatic factors action has been different, emphasizing the different character of the species. During 2007 the amount of the rainfall was small, almost droughtness, affecting the number of the yeast species. Thus, in 2008, has increase the number of the oxidative species, especially for the species *Candida*, *Brettanomyces.* Through the positive evolution of the species *Kloeckera and Torulopsis*, we can forecast that in the following year these species will be majoritary during the first days of the fermentation.

CONCLUSIONS

Analyzing the appearance frequency of the non-sporogenous yeast during the three years of research, it came out that within the viticultural biocoenosys Banu Maracine, the non-sporogenous yeast population has passed through a decline period, the major reason being the climatic changes that occured at the beggining of 2007. In generally, the appearance frequency of the non-sporogenous yeast has decrease.

In any vineyard, the spontaneous fermentation it is not conducted by the same strains year after year; there is no strain specificity and thats why it does not participate to the vineyard features.

The study of the yeast ecology can be a perpetual research theme, tack into consideration the permanent changes of the climatic factors.

REFERENCES

1. Belin J.M., 1981 - *Biologie des levures liees a la vigne et au vin*. These Docteur en Sciences Naturelles, Universite de Dijon.

2. Bouix M., Leveau J.Y., Cuinier C.,1981 - *Conn. Vigne Vin*. 15, p.41–52.

3. Castelli T., 1967 - *Ecologie et systematique des levures du vin*. Il´eme Symposium International d'Enologie, Bordeaux-Cognac, INRA Ed. Paris.

4. Delfini C., 1995 - *Scienza e technica di microbioloia enologica*. Il Lievito, Asti.

5. Heard G.M., Fleet G.H., 1987 - *Appl. Environ. Microbiol.* 51, 539–45.

6. Popa I.A. şi Teodorescu C. Şt., 1990 - *Microbiologia vinului*. Ed. Ceres, Bucureşti.