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NEW REQUIREMENTS IN THE CREATION OF VARIETIES OF VINE WITH THE ECONOMIC AND ECOLOGICAL EFFECT IN THE CONDITIONS OF CLIMATE CHANGE

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Abstract

Development of culture of the vine during of his evolution has multiple known methods to improve, such as the natural selection or directed (intraspecific, interspecific, clonal, genetic engineering, etc.). A desideratum of the wine world science and practice remains to be the getting of vines varieties for quality, absolutely resistant to attack by phylloxera (root and leaf). The process of obtaining distant hybrids, just like any cross varieties of Vitis vinifera L. with representatives from the donor species of qualities necessary (resistance to diseases and pests winter low temperatures etc.) can bring to the changing spectrum of chemical compounds and biochemical responsible for the aroma, color and taste of berries, juice and wine obtained. According to the European Union requirements in the production of the wine products, chemical composition of the raw material must meet certain strict requirements. Therefore, the current remain the problem of obtaining new varieties of vines agro-biological characters that meet the maximum requirements for table grapes using fresh consumption and for industrial processing to those intended (juices, concentrates, wines, spirits).

Key words**:** *anthocyanin, berry, diglucoside-3,5-malvidin, methyl anthranilate, methanol*

INTRODUCTION

World wine assortment, currently has about 12 000 species and varieties of vine, but so far not created the variety "ideal", that meets the most valuable features: *colour* – golden-pink-red; *flavors* – muscat, citron, rose petals etc.; *taste* – smooth and crunchy varieties for consumption at current, and balanced and juicy on the juice and wine. and balanced and juicy on the juice and wine. It is necessary that these varieties possess and resistance to biotic and abiotic factors of the environment, such as the low winter temperatures and the high arid summer drought, specific diseases of this culture - mildium, odium, gray mold and other as well as various pests. Therefore, the current remains the problem of obtaining new varieties of vines agro-biological characters fully satisfy the requirements of grapes for current consumption and those intended to industrial processing (juices, concentrates, wines, spirits). Vine during its evolution has multiple known breeding methods, such as natural selection or directed (intraspecific, interspecific, clonal, genetic engineering etc.). Today it is very necessary to obtain vine varieties productive and qualitative characters, and absolutely resistant to attack by *phylloxera* (root and leaf). Botanical Garden (Institute) of the Academy of Sciences of Moldova, together with other international scientific centers in this field works were carried out in distant hybridization of grapevine, by applying American vines spontaneous (*Muscadinia rotundifolia* Michx.), who has absolutely resistance to attack by phylloxera, but agro-biological characters of inferior quality in crossbreeding varieties in European species (*Vitis vinifera* L.), who do not possess resistance to attack by phylloxera, but agro-biological characters (productivity and quality) high. The resulting hybrids were created vine that combines these requirements [3, 4, 12, 13].

The process of obtaining hybrids of vines, as well as any cross varieties of *Vitis vinifera* L. with representatives from the donor species of qualities necessary (resistance to diseases and pests winter low temperatures etc.) can bring

to the changing spectrum chemical and biochemical compounds responsible for flavor, color and taste of berries, juice and wine obtained.

According to European Union requirements in the production of wine products, the chemical composition of the raw material must meet some strict requirements, for example diglucozid-3,5-malvidin not exceed the limit of 15 mg/dm^3. Recently the World Organisation of Vine and Wine discussed the issue of reducing the index wines at the limit of 5 mg/dm^3, which requires to be monitored in the selection of vines to try and approve only varieties with low content of this phenolic - diglucozid-3,5-malvidin.

Another important compound juice berries vine hybrids of any order, including the distance is methyl anthranilate (3,4-benzoxazole), which has the main role in the creation of taste and smell (aroma) of frame (naphthalene and / or phenol) [11].

Methyl anthranilate is a chemical compound benzoxazoles group, formed in grapes (especially direct producer hybrids) in amounts of 0,2 to 3,5 mg/dm^3 of must (juice). It is found in the wine with the same concentrations of volatile aromatic another chemical compound - ethyl isoamyl [11]. That is why this important chemical compound in the juice of berries new hybrids intraspecific selection requires determined, studied and taken as a criterion for selection.

International Organisation of Vine and Wine in 2004 established the methanol concentration limit of 250 ml/l for white wines and 400 ml/l for red wines.

Methanol is an alcohol which is synthesized by substituting the methane molecule an atom of hydrogen, with a group - OH (hydroxyl). In the fermentation process of the fruit can be formed by the decomposition of pectin's methanol. Consumption of methanol can cause severe poisoning, it may arise in particular by fractional distillation of alcohol.

MATERIALS AND METHODS

In this study were included grapevine distant hybrids (*Vitis vinifera* L. x *Muscadinia rotundifolia* Michx.), created in the Botanical Garden (Institute) of the Academy of Sciences of Moldova, hybrids of vines with the green-yellow berries: DRX-M4-502; - 512; -571; - 578; -580; - 609; hybrids of vines with the red-violet berries: DRX-M3-3-1; -660. Varieties of vines by *Vitis vinifera* L.: "Feteasca alba" (with the green-yellow berries); "Rara neagra", "Feteasca neagra" and "Negru de Ialoveni" (with the red-violet berries) [2, 3, 4, 9, 21].

These new strains were tested for resistance to attack the root and the leaf *phylloxera*, as well as *mildium*, *oidium*, *botrytis*. Berries juice and the wine were tested on content: methyl anthranilate, diglucozide-3,5-malvidin and methanol [3, 4, 12, 13].

The study included ten varieties of vines created of the VCR (Vivai Cooperativi Rauscedo, Italy), including five varieties of grapes with white berries (Fleurtai (UD-34.111), Soreli (UD-34.113), Early Sauvignon (UD-76.026); Petit Sauvigon (UD-55.098); Sauvignon d´ore (UD-55.100) and five varieties of red berries with juice (Petit Cabernet (UD-58.083); Royal Cabernet (UD-32.078); Royal Merlot (UD-31.125); Petit Merlot (UD-31.122); Julius (UD-36.030) [1].

Varieties of vines created in Germany: Cabernet Jura, Zweigelt, Regent, Monarch and Cabernet Carbon [1].

For the quantitative and qualitative determination of diglucoside-3,5-malvidin, the quantitative and qualitative fluorimetric method was applied.

Qualitative determination (identification of diglucozid-3,5-malvidol). In an Erlenmeyer flask, 10 mL of control wine with 15 mg/L of diglucozid-3,5-malvidin, which have been treated with 1,5 mL of solution of acetic aldehyde, are taken. It is stirred about 20 minutes for combining free SO_2 in wine with acetic aldehyde. In a centrifuge tube with a capacity of 20 mL is introduced 1 mL of wine treated with acetic aldehyde, to which is added a drop of 1 M hydrochloric acid and 1 mL sodium nitrite solution. The tube content is stirred; it is expected for 2-5 minutes the oxidation-reduction reaction of malvin and then 10 mL of ammonia solution are added.

Under the same conditions, in another centrifuge tube, we treat 10 mL of the control wine containing 15 mg or 5 mg malvin/litre. It is stirred and then we wait 10 minutes and then centrifuge it.

We decant the clear liquids from the two centrifuge tubes, two calibrated tubes with ground glass stopper. The fluorescence of the analyzed sample of wine compared with the control sample is examined in UV light at 365 nm.

The wine samples which do not give fluorescence or their fluorescence is far below the control wine, are considered as lacking diglucoside-3,5-malvidin. In case of a slightly lower, equal or higher fluorescence in comparison with the control wine, the quantitative determination of diglucoside-3,5-malvidin is necessary [14, 15, 18, 19, 22, 23].

For determining the methyl anthranilate, the gas chromatographic method was applied. Extraction of methyl anthranilate was performed by absorption on a resin of the type Amberlite XAD-2, followed by elution with azeotropic mixture of pentam-dichloromethane solvent (2:1 ratio by volume). The organic extract is half concentration and injected into a chromatography capillary column of fused silica. When leaving the column the terpineols get into the mass spectrometer to be detected.

In the chromatography column are injected 2 μL of organic extract obtained from must or wine and 2 μL of each internal standard. The scanning area of the mass spectrometer is between 30 m/z and 300 m/z, at an interval of 1 sec./cycle. The spectrum obtained is compared with that of the internal standards of reference and the terpineols content is calculated. [12, 15, 18, 19, 22, 23] .

Distant hybrids of vine (*Vitis vinifera* L. x *M. rotundifolia* Michx.) served as study material.

Botanical description of distant hybrids was performed during all phases of the vegetative stages; the organs of the plants were studied from spring, at bud unfolding, until early autumn, at the fall of the leaves. The bio-morphological characteristics of the organs were studied at the stages of: - bud unfolding – leaf and shoot growth - blossoming – berries growth - grapes ripening, wood maturing and leaf drop. [2, 3, 4, 5]

RESULTS AND DISCUSSIONS

For the cultivation of plants multiannual it is necessary to weigh consider the specifics of at regional level agro-climatic resources. The more so that the climatic conditions are changing.

Territory of the Republic of Moldova is located at the northern limit for the some thermophilic multiannual crops, including for vineyard.

Taking into account that some varieties are very susceptible and vulnerable to determining climate conditions, their knowledge could contribute significantly to enhancing agricultural productivity, especially in the conditions multiannual approval for new plant varieties.

Improving of the vine consists not only in creating the productive of vines variety rights and quality traits, but also resistant to biotic and abiotic factors of the environment.

In contemporary agro biological science are the achievements of the vine selection made, since the second half of the XX century resulted in obtaining new varieties with increased resistance to biotic and abiotic factors of the environment, and acceptable qualities such as those in Germany (Soliaris, Hibernal-GM), Hungary (Bianca), Moldova (Viorica, Legenda, Reton, Luminiţa, Alb de Ialoveni, Negru de Ialoveni and others, for juices and wines; Apiren alb, Apiren roz, Negru de Grozeşti, Kiş-miş moldovenesc, Kiş-miş lucistâi, for fresh consumption and for industrial processing: Moldova, Guzun, Suruceni alb, Leana, Ialoveni rezistent, Codreanca, Tudor etc.).

Has the appreciated achievements National Selection Scientific Center (Institute for Winegrowing and Winemaking "V.Tairov") in Odessa, Ukraine, which varieties or spread throughout in the space of ex-URSS: varieties and forms for fresh consumption (Arkadia, Vostok, Gherkules, Dnestrovschii rozovîi, Zolotistîi ustoicivîi, Kiş-miş tairovschii, Muscat jemciujnîi, Muscat tairovschii şi altele); varieties and forms for industrial processing (Aromatnîi, Golubok, Iliciovschii

rannii, Muscat odesschii, Odesschii Ciornîi, Ovidiopolschii, Rubin tairovschii, Suholimanschii belîi etc.).

Achievements well known in large scale CIS belong Vine and Wine Institute "Magaraci" (Yalta, Ukraine), Federal Institute of Vine and Wine in Russia, established in Novocercask, one in Krasnodar – of the North Caucasus Institute for Horticulture and Viticulture, well as Viticultural Experiment Station in Anapa.

Appreciating the true value of these achievements with concrete agrobiological characters of varieties obtained, it should be noted that the cultivation of these varieties require mandatory grafting vines their North American (resistant to *phylloxera*), which significantly raises the cost of planting material production and the establishment unit - plantation of vines. In addition most of the above mentioned varieties are relatively resistant to the main contaminants during the growing season (*mildium, oidium, botrytis*), and lately is observed sensitivity to *Agrobacterium tumefacium* and *Flovecence d'Or*.

It is encouraging increased resistance to low temperatures this winter varieties, which allow them to grow on trellises and fertile buds diminish losses in years with cold winters. Some of these varieties, however, in some years, are attacked by phylloxera leaf, under which the crop matures weak and immature shoots enters hibernation.

A new research has been carried out successfully since 2006 by the Italian selection of Udine and Milan Universities and Institute of Experimental Genomics in Italy, in collaboration with wine experts from VCR (Cooperative Vivai Rauscedo).

The results were obtained, studied and introduced in the National Catalogue of vine culture in Italy, and in 2015 temporarily included in the register of plant varieties approved in the Republic of Moldova (with limited right of multiplication and planting) ten varieties, of which: five varieties of grapes with white berries (Fleurtai (UD-34.111), Soreli (UD-34.113), Early Sauvignon (UD-76.026); Petit Sauvignon (UD-55.098), Sauvignon d´ore (UD-55.100) and five varieties of red berries with juice (Petit Cabernet (UD-58.083), Cabernet Royal (UD-32.078), Merlot Royal (UD-31.125), Merlot Petit (UD-31.122), Julius (UD-36.030) [1].

Among agrobiologic remarkable characters of these varieties, planted and grown in five wine regions of Italy, Slovenia and Moldova are given early and middle technological maturation of the grapes, high yield stocks, good resistance to *mildium* and *oidium*, and normal sensitivity to *Botrytis cinerea*. Tests for resistance to low temperatures showed that red and white varieties of this new direction vine selection withstand temperatures of minus 22° C - - 24ºC, which allows cultivation with minimum risk of these varieties and in the northern border areas of viticulture in Europe and other growing areas in the world.

Agrobiologic characters analysis of new varieties of vines, obtained by crossbreeding the original of *Vitis vinifera* L. varieties of hybrids intraspecific attests to a similarity with the classic varieties of grapes and yield afternoon (productivity) in a unit area (1 ha). Similarly, the physical and chemical index (oenological criteria) varies between corresponding European red varieties grown in traditional wine-growing regions of Northern Italy (Friuli). And yet, a concentration of anthocyanins 1267 mg/l in berries of the variety of Cabernet Royal and 1133 mg/l in the Petit Merlot and polyphenol content of 4300 mg/l at Royal Cabernet and 4203 mg/l in berries of the variety of Royal Merlot admits, that these new varieties have enormous potential, high reserves of important compounds of organoleptic qualities [11, 16].

An important indicator of the quality wines is the methanol content that accumulate in wine as a result of hydrolytic action of the enzyme pectin-methyl-esterase (PME) on the methoxylated pectin molecules, which contain morphological structures inside the berries.

The methanol concentration is dependent on many factors (the peculiarities of varieties, the content of pectin's, the degree of etherification, PME activity, duration of contact of the liquid with the solid fraction, the temperature of the soaking process, the

concentration of ethyl alcohol and sulphur dioxide, etc.), but the fact is that the closer we get to the origin of the *Vitis vinifera* L, the value of this biotechnology index is reduced in comparison with analogue remote of varieties of the *Vitis amurenzis, Vitis labrusca, Muscadinia rotundifolia* Michx. etc.
The purpose of breeding work of the vine consists in selecting new forms, taking account of the concentration of methanol in wines from these new varieties, along with characters such as precious biological agriculture increased resistance to biotic and abiotic factors of the environment. Once this index has a noxious character he is limited by competent international institutions (World Health Organisation, World Trade Organisation, the World Organisation of Vine and Wine) in this area under special medical investigations. This objective is concerned with ensuring the safety of products uvologice [14, 20].

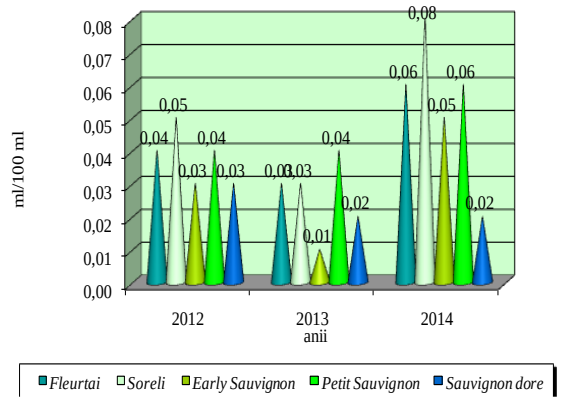

Fig. 1. The concentration of methanol in varieties of vines with white berry.

International Organisation of Vine and Wine in 2004 established the methanol concentration limit of 250 ml/l for white wines and 400 ml/l for red wines (Sheet regulatory OIVV concentration 19/2004).
According to Italian legislation (Law No.82 of 20.02/2006) limit concentration of methanol in white wines is 0.20 ml/100 ml, and for red 0.25 ml/100 ml.
Obtaining varieties of vines of *Vitis vinifera* L. and species donor of the necessary qualities (disease resistance and harmful at low temperatures of winter etc.) can make to changing spectrum of chemical compounds and biochemical responsible for aroma, colour and taste of berries, juice and wine obtained.
European wine-growing practice has started to determine a limit to component-test, a violet-blue anthocyan, chemical name - diglucozid-3,5-malvidin. He is a natural component harmless, clean and do not have harmful properties.

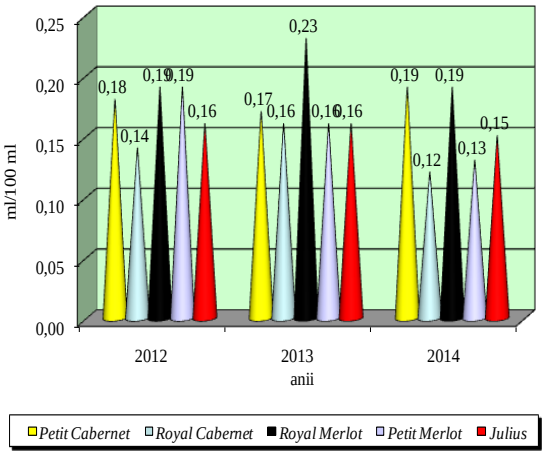

Fig. 2. The concentration methanol in varieties of vines with red berry.

In accordance with legislation of the European Union, concentration of diglucozid-3,5-malvidin in the berries juice should not exceed the limit of 15 mg/l.

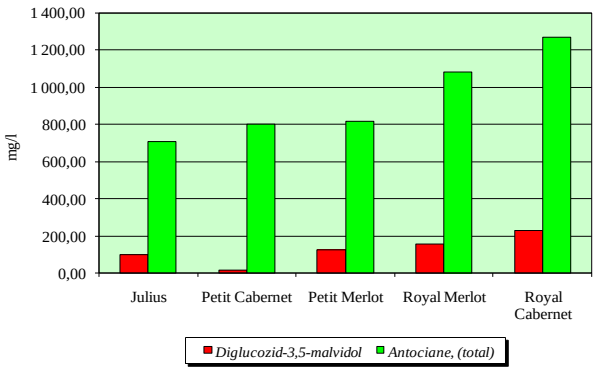

Fig. 3. The concentration of diglucozid-3,5-malvidin and the total anthocyanins.

From the results of present in the Fig. 3. shows that only new variety Petit Cabernet concentration diglucozid-3,5-malvidin not exceed the limits set by 15 mg/l, following so the cluster varieties of the *Vitis vinifera* L.
As varieties of the Julius, Petit Cabernet, Petit Merlot, Royal Merlot, Royal Cabernet showed

an increased concentration of this anthocyanins harmless and curative, but can currently only create obstacles to their approval in some European Union countries. Nutritionists say that the human body in its evolution has created sufficient enzyme systems recovery, anthocyanins metabolism and turning them into other compounds derived necessary and useful [14, 15, 17, 20].

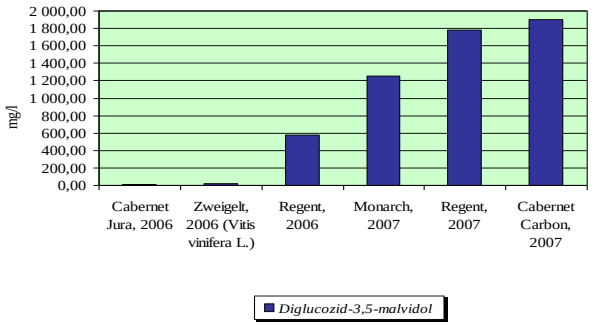

Fig. 4. The concentration of diglucozid-3,5-malvidin juice of red berries vine varieties created in Germany

Interesting results in terms uvologic and oenological were obtained by selecting vine of new varieties in Germany, which demonstrates that the selection of intra-specific can get character agrobiologic important (resistance, productivity and quality), but with a content high in diglucozid-3,5-malvidin (580 mg/l in the wine variety Regent and 1900 mg/l in wine Cabernet Carbon). Of course compared to varieties of *Vitis vinifera* L., Cabernet (Jura, France) and Zweigelt (Rhein, Germany) investigated index do not exceed 15 mg/l, established by specialized international organizations.

In order to create vines with a high resistance to phylloxera root and the leaf, it started to cross *Vitis vinifera* L. x *Muscadinia rotundifolia* Michx., resulting in the creation of new varieties of vine [3, 21].

Studying the physicochemical and biochemical indices of juice berries vine varieties (*Vitis vinifera* L. x *Muscadinia rotundifolia* Michx.), to determine spacers or similarity with the classic varieties of *Vitis vinifera* L.

The test results show that in the fresh juice of berries distant hybrid diglucozidul-3,5-malvidol varies between 7,7 mg/l - 9.3 mg/l (DRX-M4-660; DRX-M3-3-1), but the variety Rară neagră, only 4,9 mg/l of diglucozid-3,5-malvidin.

The concentration of anthocyanins in the varieties of berries colored not show a difference essential difference between hybrids distant and varieties classic Rară neagră and Feteasca albă from 513 mg/l to 640 mg/l for hybrids distant vineyards and content of the 469 mg/l at Rară neagră and 737 mg/l to Fetească neagră - both in group of the *Vitis vinifera* L.

Table 1. The content of methyl anthranilate, diglucoside-3,5-malvidin and anthocyanins in grapes of distant hybrids of grapevine (*Vitis vinifera* L. x *Muscadinia rotundifolia* Michx.)

Form	The content of the compounds, mg/dm^3		
	Methyl anthranilate	Diglucoside-3,5-malvidin	Anthocyanins
Distant hybrids of grapevine (V.vinifera L. x M.rotundifolia Michx.)			
DRX-M4-502	0,08	-	-
DRX-M4-580	0,09	-	-
DRX-M4-512	0,13	-	-
DRX-M4-578	0,15	-	-
DRX-M4-609	0,16	-	-
DRX-M4-571	0,17	-	-
DRX-M4-660	0,21	7,7	640
DRX-M3-3-1	0,24	9,3	513
Varieties of the Vitis vinifera L.			
Feteasca albă	0,11	-	-
Feteasca neagra	0,19	7,4	737
Rară neagră	0,27	4,9	469
Negru de Ialoveni	0,49	74,0	861

The studies found that obtaining distant hybrids are transmitted through hereditary traits - typical direct producer hybrids, whose index methyl anthranilate concentration ranging from 0,30 mg/l of juice to 3,6 mg/l [11, 23].

Hybrids distant with green-yellow berries possess a concentration of methyl anthralinate limits of 0,08 mg/l – 0,17 mg/l. Variety vine classic "Fetesaca alba" with yellow-green berries has a concentration of 0,11 mg/l.

This allows to conclude a resemblance of the distant hybrids of vine DRX-M4-502; -571; -578; -609 in the content of methyl anthranilate with classic grape variety ”Feteasca alba” of *Vitis vinifera* L. (Tab. No. 1.)

Hybrids distant DRX-M4-660 and DRX-M3-3-1 to red-violet berries content of methyl anthranilate is present in the range of 0,21 mg/l – 0,24 mg/l. Vine varieties "Rare neagră" and "Feteasca neagră" of *Vitis vinifera* L., accumulated fresh juice of berries 0,27 mg/l – 0,19 mg/l. methyl anthranilate. (Tab. No.1.)

Based on previous studies we found that the variety of the vine "Negru de Ialoveni" based on gas phase chromatographic method was determined an increased concentration of methyl anthranilate 0,49 mg/l.

CONCLUSIONS

Creating vine varieties with resistance to *phylloxera* root and foliar *mildium* to powdery mildew, botrytis and other biotic and a high resistance to low temperatures in winter and drought, will allow for truly tackling consumer grapes production and for industrial processing biological (ecological). Due to their high agro biological properties, these varieties treated during the season - moderate and limited (strictly in accordance with European legislation "bio") will allow to ensure high hygiene and curativitate berries and grapes.

Uvological and oenological requirements of the new varieties obtained refers to the high quality of grapes fresh for current consumption and those for industrial processing and their use in the production of wines, juices, concentrates (ecological) and distillates.

Uvological and organoleptic requirements berries of new varieties of grape vines consumer include: commercial aspect flawless golden-yellow, pink or red (pomegranate, cherry) colour and blue-violet exceptions, glucose-fructose ratio optimal, which together ensure harmony glucides concentration highly appreciated by consumers, crisp taste and sensations of balance of acidity - the concentration of glucides - astringency.

The new varieties must be early ripening period of the grapes to ensure planting and cultivation their northern border of vine growing on different continents, while ensuring plant and maturing strings for a successful winter, allowing their cultivation trellis medium and high strain to mechanize and automate processes to the maximum possible cutting agro tied, sprayed manually or mechanically with combine harvesting etc.

It is desirable that the varieties of grape vines Consumer possess high transportability to export them from great distances, and the technical - a low capacity to absorb oxygen from the air and protection of berries, juice and wine oxidation (redox low reductive processes). These requirements correspond to the F4 distant hybrids obtained at the Botanical Garden (Institute) of Academy of Sciences of Moldova and new varieties created by scientists coach in Italy (Universities of Udine and Milan, VCR - Rauscedo).

It is necessary to take account of the concentration limits of berries and juice constituents of the wine: diglucozid-3,5-malvidol (= <15 mg/l), methyl anthranilate (= <0.2 mg/l), which they are not harmful, but dislikes.

There is welcome, according to scientists oenologists, increased content in the juice of berries and wines produced cis and trans compounds derived the hexenal and hexanal (less than 0,,2 mg/l) and flavored furaniol hybrids - the direct producer (less than 30 mg/l). They are strictly regulated and limited concentrations of methanol in the juice berries less than 10 mg/l, and the wines produced less than 250 ml/l for white wines and 400 ml/l for red wines (Regulations of the European Union).

Selection of new varieties of grape vines for current consumption puts the onus fructose-glucose ratio that is optimal for fructose value of 1,1 - 1,3 and the ratio of tartaric and malic acid indices within 1,0 – 1,4.

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