SUMMARY

Keywords: *grapevine, thin layer chromatography (TLC), biochemical descriptor, the fractal dimension, cluster analysis.*

The PhD thesis with the title **Characterization morphogenetic of varieties belonging to Galbenă de Odobeşti sortogroup by determination of free amino acids**, is the first study on the profile of free amino acids in the young leaves from native vine varieties of Galbenă de Odobeşti.

The research focused on five old indigenous vine varieties considered to belong to Galbenă de Odobeşti sortogroup, that are in conservation in ampelographic collection of S.C.D.V.V. Odobeşti. Determinations related to research was perfomed during the period 2009 – 2012, in the research labs of the S.C.D.V.V. Odobeşti. For initiate in the techniques of extraction and separation of amino acids by TLC, I have collaborated with the labs of Center for Research in Biochemistry and Molecular Biology (CCBBM) within Biochemistry and Molecular Biology Departament of Faculty of Biology - University of Bucharest.

Thesis structure. The thesis summarizes a number of 183 pages and is divided into two parts and six chapters:

- Part I – PRESENT STATE OF KNOWLEDGE. Includes chapters I-III that comprising: documentary study on methods for determining the phylogenetic relatedness of vines varieties, an overview of Galbenă de Odobeşti sortogroup and evolution of the researches on Galbenă de Odobeşti sortogroup, a documentary study on the characterization of grapevine genetic material by determining the profile of amino acids.
- Part II - PERSONAL CONTRIBUTIONS. Includes chapters I-VI in that was presented: the research objectives, the biological material and methods used, experimental results on the profile of free amino acids in young leaves and biochemical descriptors for five vines varieties presumed belong to Galbenă de Odobeşti sortogroup, similarity of the leaf architecture using cluster analysis based on measurements ampelometrice, the fractal dimension of leaves, processing and statistical interpretation of experimental results and general conclusions.

Chapter I – Consideration of the phylogenetic relatedness of grape varieties. The traits of cultivars are genetically determined, as characteristic for the species of origin, and modified by anthropogenic conscious action so that some traits were amplified, or conversely reduced. The morphological and production stability of the variety is relative being strongly influenced by a number of biological factors: spontaneous mutations, natural crosses, segregation characters for synthetic varieties obtained by hybridization.

The cultivars that are similar in their morphological characteristics (phenotype), is the so-called ecotypes varieties (sortogroups).

Over time there have been many concerns and attempts to describe the recognition and differentiation of vine varieties based both on ampelometria leaf and on morphological, agro-biological and technological traits of cultivars, materialized in methods that have evolved and have improved in time: ampelometrica method, ampelographic determinators method, descriptors ampelographic method. In 1984 in order to unify the methodology description ampelographic, OIV, UPOV and IPGRI have developed common rules work by having established three categories of descriptors: descriptors for characters ampelographic of varieties (96), descriptions of characteristics agrobiological (25) and descriptions of technological characteristics (5).

Genetic variability of organisms belonging to different species or taxonomic units can be highlighted by the study of genes chemical products, so the enzymes. Isoenzyme analysis also thus found application in researches on the origin of cultivated plants varieties, especially varieties vine.

An upper stage in identify the genetic origin of the varieties was a genome-wide analysis of DNA and RNA, as a result of progress in molecular biology (molecular markers). The most widely used molecular techniques to identify species, Vitis varieties and varieties somatoclonale are: PCR, RFLP, AFLP, RAPD also ISSR analysis of microsatellites.

Chapter II - General considerations on Galbenă de Odobeşti sortogroup. In this chapter are presented the general considerations on the group of vine varieties considered to belong Galbenă de Odobeşti sortogroup.

Starting from the reference also Constantinescu (1971), Galbenă de Odobeşti sortogroup includes native vine varieties with morphological/ampelographic like Galbenă de Odobeşti cv. considered synthetic variety, from which resulted the varieties Zghihară de Huşi and Bătută neagră. For reasons that were based on elements of similarity with Galbenă de Odobeşti cv. For some morphological characters and characteristics were associated with this group and other ancient indigenous varieties of vines as Alb românesc cv., Cruciuliţă cv., and Galbenă verde cv.(Constantinescu *et all*., 1962) and Galbenă uriaşă cv. that was considered a variation of the Galbenă de Odobeşti cv. (Nicoleanu, 1900).

Presence in the area Pontic of the vine culture and other varieties with ampelographic characters more or less similar to those of Galbenă de Odobeşti, was the reason why to this group were associated Cabasma albă cv., Cabasma neagră cv., Negru vârtos cv. and Negru moale cv. Common characteristics with of Galbenă de Odobeşti cv. and Cruciuliţă cv. was signaled for Berbecel cv. whose name and all its synonyms, appears to be connected with yellow berries, as the variety Galbenă de Odobeşti (Bădiţescu, 1974). Except the Galbenă de Odobeşti cv., the origin other varieties considered as part of this sortogroup is still uncertain, in literature there are no precise data on their origin.

Also in this chapter presents the evolution and current state of research on the characterization of vine varieties from Galbenă de Odobeşti sortogroup, by which is justified further approach to research on Galbenă sortogroup.

Chapter III – Bibliographic study on characterization of the vine germoplasm by determining the amino acid profile. The issue of vine germplasm characterization based on free amino acids spectrum in vegetative organs and grapes is exposed in four subchapters, which are presented general considerations on amino acids identified in vine, aspects of amino acid metabolism in vine, methods for determining the free amino acids and the current state of research on characterization the grapevine germplasm based on the free amino acid profile in vegetative organs and grapes.

Bibliographic documentation showed that more than 30 amino acids, of which 20 proteinogenic amino acids specified by the genetic code were identified and Vitis vinifera L. in different stages of growth and development. At grapevine, the total amount of amino acids increases during the maturation process, goes to values between 200 and 6500 mg/l, with variations of each amino acid from one year to another, and from one variety to another (Ţârdea 2007).

Path of forming amino acids in grapes consists of ketoacids amination in the presence of enzymes called transaminases. Ketoacids main underlying the formation of amino acids in grapes are *pyruvic acid*, *á-ketoglutaric acid* and *oxalyl-acetic acid*. Around grape maturation, there is a massive accumulation of free amino acids as a result of the cessation of berries growth and protein synthesis, especially in the years cold, with little precipitation during ripening. During the alcoholic fermentation takes place a rapid metabolism of amino acids from grape must by yeasts, except for proline, arginine, alanine, ornithine, and citrulline (Ough *et al.*, 1991).

The subchapter three contains a brief overview of the main methods of determining the amino acids: chemical methods, chromatographic methods and electrophoretic methods.

Last subchapter present to the evolution and current state of research on amino acid profile in the genus *Vitis*. Bibliographic documentation based on research on free amino acids

especially in the grapes and musts, showed its great variability, suggesting the importance of the genetic involvement in this diversity, and also the need for further research in this area.

Chapter IV – Objectives, biological material and research methods. In this chapter are presented the research objectives, the biological material used and methods of research.

The main research objectives:

- Establishing protocol for extraction, separation and vizualization of free amino acids in young plant tissues of vine varieties studied, assumed to belong at Galbenă de Odobeşti sortogroup by thin-layer chromatography technique (TLC).

- Establishing protocol for the identification and quantitative determination of free amino acids in young leaves of vine varieties analyzed.

- Evaluation of the possibilities of the use of free amino acids profile of the leaves as biochemical descriptor in order to establish lineage and genetic polymorphism that exist between varieties of vines that make up Galbenă de Odobeşti sortogroup.

- Comparing and corroboration of data from the profile of free amino acids in leaves, as descriptor biochemical with the data obtained by other methods and techniques used to characterize morphogenetic of vine varieties (cluster analysis, fractal analysis).

Biological material used: five old native varieties, presumed to belong at Galbenă de Odobeşti sortogroup (Galbenă de Odobeşti, Zghihară de Huşi, Bătută neagră, Negru vârtos and Negru moale). This chapter also presents a brief characterization ampelographic of vine varieties presumed to belong to Galbenă sortogroup.

The last part of this chapter is the largest and presents the research methods: the method of determination of amino acids by thin layer chromatography (TLC), cluster analysis based on measurements ampelometric and fractal analysis - Box - Counting method (program HarFA) to determine the dimension fractal of leaves contour. Are presented in detail steps work, measurements, analyzes and tests carried out. In the case of the method for the determination of free amino acids by thin layer chromatography are given:

- Protocol extraction, separation and visualization of free amino acids in young leaves and roots. The most efficient system has proven to be developing at the base of n - butanol/glacial acetic acid/water (4:1:1 v/v/v), which allowed a good separation of free amino acids in the young leaves of vines.

- Mathematical algorithm identification (retention factor - Rf) ;

- Mathematical algorithm for quantitative determination of free amino acids - image analysis program ImageJ - ver . 1.46r.

Chapter V – Experimental results. This chapter structured in three subchapters present the results of research concerning: profile of free amino acids in young vegetative tissues vine for varieties and the ratios of the predominant amino acids (biochemical

descriptors), determining phenotypic similarity or likeness of the five vine varieties studied on the basis of the architecture of the leaf, the fractal dimension of the leaf contour and the degree of their sects.

To determine the profile of free amino acids in leaves and roots young was tested a total of 17 amino acids, 14 amino acids were identified in samples of biological material (young leaves) in all varieties analyzed. Analysis on the extraction and separation of amino acids in young roots proved to be reproducible for all vine varieties analyzed.

The identification of the 14 amino acids was achieved on the basis of separate retention factor (Rf), which ranged from 0.02 to 0.50. Quantitative assessment of amino acids identified in young leaves was based on profiles or chromatograms obtained by image analysis program ImageJ ver.1.46r, reported to amino acid standard samples containing known amino acid.

The total content of amino acids present in leaves at the vine varieties analyzed ranged from 14.33 µg/mg plant tissues from Negru moale cv. and 18.76 µg/mg plant tissues for Galbenă de Odobeşti cv., with intermediate values for Zghihară de Huşi cv. (14.54 µg/mg), Bătută neagră (15.20 µg/mg) and Negru vârtos (17.74 µg/mg). Predominant amino acids present in all five varieties of vines analyzed were: Aspartic acid (Asp), Glutamic acid (Glu), Serine (Ser), Threonine (Thr), aspartic acid representing betwen 25.3 % (Bătută neagră) and 31.5 % (Zghihară de Huşi).

The reports of the predominant amino acids, considered constant parameters that differ significantly from one variety/cultivar to another (Hernández-Orte, Guitart and Cacho, 1990), can be used as parameters to differentiate vine varieties genetically (biochemical descriptors). In this sense were calculated reports (Asp/Glu, Asp/Ser, Asp/Thr, Ser/Thr, Glu/Ser, Glu/Thr, Glu/Thr), and the ratio Pro/Arg and the ratio γ.

To establish the similarity of phenotypic similarity in terms of architecture leaves of five varieties analyzed was used to multivariate statistical analysis cluster, which has been developed: the dendrogram hierarchical classification of varieties, the levels of linkage hierarchical classification and the histogram of vine varieties. Dendrogram developed based on measurements ampelometrice groups the five varieties of vines into two main groups or branches: Group A consists of chaining varieties: Negru vârtos, Zghihară de Huşi and Galbenă de Odobeşti and Group B resulting from the aggregation varieties Bătută neagră and Negru moale. From the point of view of the similarity the group A has high uniformity as the chaining value or dissimilarity index is lower (17.369) compared to Group B (31.443).

Fractal dimension of leaf contour for the cultivars analyzed, calculated with HarFA program, showed the values between 1.1923 for Negru moale cv. and 1.2730 for Negru vârtos cv., with intermediate values for Bătută neagră cv. (1.2404), Galbenă de Odobeşti cv. (1.2247) and Zghihară de Huşi (1.2067). Average values ampelometrice of d_1/N_2 report

showing level of sectarian leaves the five vine varieties taken into observation ranged between 0.7012 for Negru moale cv. and 0.7516 for Bătută neagră cv. with intermediate values for Zghihară de Huşi (0.7493), Galbenă de Odobeşti cv. (0.7281) and Negru vârtos cv. (0.7262).

Chapter VI - Statistical processing and interpretation of experimental data. Statistically, the total amount of amino acids did not significantly differentiate the five autochthonous vine varieties analyzed, since the calculated value of P (0.93885) is greater than the critical value of P 0.05.

However reports of predominant amino acids (Asp/Glu, Asp/Ser, Asp/Thr, Ser/Thr and Glu/Thr), statistically differentiate the five varieties of vines, considered to belong Galbenă de Odobeşti sortogroup, with a range of of 95%, and can be used as a biochemical descriptors. The ratio proline/arginine (Pro/Arg) shows significant differences between the five indigenous cultivars studied.

Determinations were shown necessary and useful, but not sufficient to entirely resolve the topic addressed. Therefore, we proceeded to corroborate the experimental results and analysis of interdependencies between reports predominant amino acids (biochemical descriptors). For the interpretation of the results were calculated values of correlation coefficients (r). Interpretation of correlation coefficients show a very good association between biochemical following descriptors:

- Ratio Asp/Glu and the reports Asp/Ser (positive correlation) and Glu/Ser (negative correlation);

- Ratio Asp/Ser and ratio Pro/Arg (negative correlation);

- Ratio Asp/Thr and reports Ser/Thr (positive correlation) and γ (negative correlation);

Analysis of variance fractal dimension of leaves shows that among the five vine varieties studied existed statistically significant differences (0.010896). Student test showed significant differences among Negru moale cv. and the cultivars Bătută neagră (0.01847) and Negru vârtos (0.01627). Significant differences exist between Zghihară de Huşi cv. compared to Negru vârtos cv.(0.03825).

Between individual values of fractal dimension of the leaves and the degree of sectarian language (d_1/N_2 ampelometric ratio), there is a moderate negative correlation to good for Zghihară de Huşi cv. (- 0.5469). At the level of the sortogroup on the basis the average value obtained for the fractal dimension of leaves and sectarian degree of leaves there is a low degree of association.

At the end of the thesis are presented **general conclusions** and **bibliography**.