SUMMARY

The doctoral thesis on **"PHYLOGENETIC RELATIONSHIP RESEARCH ON NATIVE VARIETIES OF VINE BY ANALYSIS OF DNA (DEOXYRIBONUCLEIC ACID)"** is mainly aimed at determining genetic parentage of indigenous varieties of vines.

Structure. The thesis is divided into seven chapters: the general part, which includes Chapters I-III and the experimental part that assemblies Chapters IV-VII. In the general section the genetic heritage of the vine varieties (Chapter I) is briefly described, in Chapter II is presented an overview of indigenous varieties and the methods used to determine phylogenetic relatedness of vine varieties are described; the analysis in Chapter III is presented to deoxyribonucleic acid / DNA description.

The experimental research has objectives (Chapter IV), methods for investigating the genome native vine varieties by determining deoxyribonucleic acid, the biological material is described and presented as well as the working method (chapter V). The experimental results are presented (Chapter VI) and finally one finds the interpretation of experimental data (Chapter VII).

The research objectives were:

- genetic profiling of indigenous vine varieties under study;
- establishing genetic parentage of the studied varieties;
- choosing the most appropriate methods and molecular markers, by which we can investigate the genome of vine varieties.

1. The genetic patrimony of the vine varieties (chapter I). Ecological plasticity of *Vitis vinifera* L. has led to thousands of varieties/cultivation spread to all continents of the world today. It has a striking phenotypic variability, which often leads to uncertainty and confusion about the identity of vine varieties.

Worldwide, we are witnessing a reduction in crop genetic diversity, a phenomenon called "genetic erosion". No vine has not been bypassed, a phenomenon seen both in the vine varieties cultivated and wild forms of vines. The major wine growing countries such as France, Italy Spain and Portugal have the richest vine gene pool. For example: France has about 633

indigenous grape varieties, MJ Boursiquot and collaborators present variety Gouais as European ancestral variety. Italy prides itself with the ancestral variety "Greeks" / "Grechetti" from which many varieties grown today originated, Spain is registered with more than one hundred native species in conservation (Gazette of Agriculture, Arad, "A Brief History of the vine", 2007).

The twenty-first century brought a new challenge with the economic globalization and the globalization of trade, including wine trade. The effects of globalization of trade which Romanian viticulture should face are the alignment of Romanian wines to international standards for a globalized trade and revaluing local grape varieties as well. Romania as a wine country, has a narrow genetic heritage, about 40-50 fruitful vine indigenous varieties, which were formed by natural selection, aided by anonymous winegrowers (Ţârdea, 2008).

2. Methods for determining the phylogenetic relatedness of varieties (chapter II). They are briefly described in the paper. These are:

The ampelometric/botanical method, is to examine the morphological characteristics of the leaf, the ampelography of the main body, their expression measurements and numerical values. The idea belongs to Professor Herman Goethe, Higher School of Agriculture in Vienna, the international ampelographic Commission session in 1876, from Marburg, draws attention to the relationship between leaf shape and angles that form the main veins between them (Ţârdea, 1992).

The ampelography descriptors method. The O.I.V. together with the International Plant Protection Organizations (UPOV) created it in 1984. The aim was to characterize the encoding of morphological and technological traits of agrobiological varieties for computer processing.

The enzymatic method. Enzymes are specialized proteins that catalyze biological reactions, ranking among the most remarkable biomolecules known because of their specificity and catalytic extraordinary power, far greater than that of chemical catalysts (Lehninger A.L., 1970). The possibilities of separation of proteins are based on differences in size, properties, solubility, electric charge, affinity adsorption and biological behavior of a protein for a specific liason. The best separation methods are: disc electrophoresis, isoelectric focusing, ion exchange chromatography. One can determine enzymes in grape and wine for determining the genetic relatedness between varieties.

Amino acid fingerprint. Amino acids are monomeric forms of proteins. Their molecule is characterized by two opposing functions: a carboxylic (acid) one and another amino (basic) one, both related to the same carbon atom called C α. Share amino acids in the grapes is high and represents 20-30% of the total nitrogen compounds (Poux, Ournac , 1970).

It was found that the amino acids contained in grapes varies from year to year, while not significantly changing the amino profile, but the report proline/arginine remained basically the same in each variety (Târdea, 2009).

DNA analysis. According to the hypothesis formulated by G.W. BEAD and E.L. Tatum (1941) conception of the gene was complemented by the formula "one gene = one enzyme." The gene controls the synthesis, function and specificity of the enzyme. The fact that genes are complex molecules consisting of nucleic acids, DNA or RNA (material that contains hereditary information) changing therefore the old school theory on genes.

Current state of research. Internationally there are already genetic mapping studies of vine varieties, which led to organizing databases with information about the mapping and sequence genes, which include protein databases. Such investigations take place nationally too, in Cluj, Iaşi, Bucharest, Craiova. Inquiries are directed mainly towards cosmopolitan vine varieties and indigenous varieties towards which specialists are growing increasingly concerned.

3. In the last chapter (III) of the general part, the description of deoxyribonucleic acid, its functions and the types known and described by geneticists are presented.

4. In chapter IV are presented **research objectives** and methods of investigation of deoxyribonucleic acid are described. The most widely used methods are:

- Analysis of randomly amplified DNA polymorphisms - RAPD) based on polymerase chain reaction (polymerase chain reaction - PCR). This method uses one or two primers with arbitrary sequence, rich in G and C to obtain PCR products from genomic DNA. Since 1990 several laboratories have introduced a new strategy for PCR amplification of genomic DNA sequences.
- Length polymorphism analysis of amplified fragment (Amplified fragment length polymorphism - AFLP). This is a combination of RFLP and PCR method (Vos et al., 1995). After DNA digestion with restriction enzyme fragments attached to the ends of double-stranded specific sequences (adapters) using DNA ligases.
- These sequences, together with adjacent sequences represent restriction site of the enzyme and will be placed as attachment of primers for PCR amplification.
- Restriction fragment length polymorphism analysis - RFLP. The best known method for DNA fingerprinting is the restriction fragment length polymorphism RFLP. RFLP is a set of specific DNA fragments generated by digestion of genomic DNA suitable endonuclease, which varies in length from one individual to another, or two alleles of genes within an individual.
- The microsatellite analysis, which are short sequences of DNA, found in many DNA fragments which are themselves the result of repetition times up to 20-30 units of 2-3

bases. Example: cytosine-adenine (CA) or guanine-adenine-thymine (GAT). The method involves PCR amplification of a microsatellite specific for a given fragment of the genome.

5. Observations and measurements made (chapter V). A study on quantitative and qualitative determination of deoxyribonucleic acid was conducted. For the quantitative determination made to quantify the analyzed samples, occurring with a certain amount of DNA purity. The data has been accumulated and represented the starting point of our research. Qualitative determination was made after a series of steps of isolation and purification of deoxyribonucleic acid.

The following describes the biological material and working methods addressed. The biological material was represented by 12 indigenous varieties: Bătută neagră, Busuioacă de Bohotin, Coarnă albă, Coarnă neagră, Fetească albă, Fetească neagră, Fetească regală, Furmint, Galbenă de Odobeşti, Grasă de Cotnari, Tămâioasă românească and Zghihară de Huşi. Of these varieties, only ten still cultivated, while Coarnă albă, Coarnă neagră are in conservation ampelographic collections in the country. Biological material was taken from two ampelographic collections: ampelographic Collection of Faculty of Horticulture and ampelographic collection of Research and Development Station for viticulture and wine, Iasi.

6. Experimental data (chapter VI). For the cluster analysis were extracted mean a total of 30 variables, all parameters are centralized and processed in a specific software. Data was obtained on the chaining phenotype/morphologic varieties, with the coefficient of kinship or affinity (similarity). The final results of the hierarchical variety histogram show the relatedness of sorts. It was found:

- Nearest architecture leaf varieties were Tamaioasa romaneasca and Busuioaca de Bohotin whose similarity index value was equal to 0, 1148;
- The following varieties are aggregated and are thus similar in terms of architectural - Furmint and Grasa de Cotnari with a similarity index value of 0, 1337;
- similar phenotype were shown to be varieties of Zghihara de Husi and Batuta neagra, with a similarity index of 0,3721.

Based on all these data, one was able to achieve a classification dendrogram of first indigenous varieties.

Development of varieties hierarchical dendogram classification was made according to the principle of minimum loss of inertia (generalized Ward criterion). By analyzing the dendogram, three optimal groups (branches) divided into two sub-branches branch of A1 and A2 were found: Fetească regala, Fetească alba, Fetească neagra, Coarna neagra and Grasa de Cotnari and Furmint, branch B - the result of aggregation variety: Tamaioasa romaneasca, Busuioaca de

Bohotin and Coarna alba, mainline C, consisting of varieties Galbena de Odobeşti, Zghihara de Husi and Batuta neagra.

The measurement of DNA extraction consisted of young leaves, where the protocol of Lodhi et al. (1994), modified by R. Pop et al. in Cluj (2004) was used. Extraction of DNA by this method yielded colorless solutions in almost all DNA samples. Doyle & Doyle (1990) tried a different extraction protocol, which recorded the results were lower and of poorer quality of the bands visualized after amplification, leading us to rebuild a part of the working stages.

Deoxyribonucleic acid analysis could be performed in several stages, such as DNA extraction, amplification with specific primers or a series of randomly chosen and electrophoresis products resulting from amplifications. View of product amplification was performed under UV light, the bands were automatically detected using a program that sets the size of DNA fragments by comparing them with a DNA standard (DNA Ladder). The used DNA standard consists of 40 fragments ranging from 100-4000 bp. In the statistical analysis were included only bands with a high luminous intensity. Polymorphic bands were scored 1, and the monomorphic 0. The presence of bands (marked with 1) and absence of bands (marked with 0) were entered in a matrix form of binary table.

A total of 244 amplified bands, 163 obtained from 24 primers and 81 of the 12 primers which have amplified DNA samples, was obtained. Interpretation of results obtained by RAPD in the two processes of extraction and amplification with different primers with similar working principle is basically the same. The difference was the working method, which was somewhat different so that the centralizing and laboratory processing was specific to each part, and calculating coefficients were also different.

Class grouping genetically related variants (cluster Analyses) was performed using the program NTSYS - pc 2.1., using as variables the coefficient of genetic similarity and UPGMA (Unweight Pair-Group Method Arithmetic Average). UPGMA method was chosen among other similar methods that best highlights the groups (clusters) existing (Miligan, 1980). The result of these groups was reflected in the achieved dendrogrames.

7. Establishing the phylogenetic relatedness of indigenous vine varieties.

Following research on the genetic analysis of grape varieties cultivated in Romania, one was able to confirm or disapprove the ideas sustained by some data in specific literature.

- There is evidence of the degree of phylogenetic relatedness of varieties Grasa de Cotnari and Furmint, showing the common origin of these varieties, all three being found in the same dendrogram node.

- Feteasca regala also confirms that its genitor Grasa de Cotnari is very close to it by registered similarity coefficients.
- Variety Feteasca alba of the parents Feteasca regala variety appears in two out of three at the same dendrogram node and similarity coefficients higher, and the third Feteasca alba aggregate dendrogram something beyond.
- Tamaioasa romaneasca and Busuioaca de Bohotin, cited in the literature as related support that view only a single biological dendrogram. The other two occur at different nodes, together with other varieties of oriental origin.
- The group composed of varieties Batuta neagra, Galbena de Odobeşti and Zghihara do not confirm relatedness between varieties. In no dendogram do they appear at the same nodes. They seem to be much closer to red wine varieties Batuta neagra and Feteasca neagra, appearing in two of three nodes in the same dendrogram, with close similarity coefficients. Under no circumstances varieties that resemble phenotypically Galbena de Odobesti and Zghihara de Husi known in literature as bud variation of the Galbena vine variety, do not seem to have any connection, genetically, they are more spaced in the three dendrogram architecture.
- The varieties Coarna alba and Coarna neagra, of oriental origin and with a phenotypic similarities only seen in the grapes, but not in any of the other morphological organs, do not seem to be related , being found in three different nodes in the dendrogram structure.
- It is left to discussion the relatedness of aromatic varieties (Tamaioasa romaneasca, Busuioaca de Bohotin) with that of table grapes (Coarna alba and Coarna neagra), whose aggregation appears often in architectural dendrogrames.

The research that has been started in this doctoral study, based on determination of DNA in local vine varieties, leads the way for further studies on the same topic.