SUMMARY

OF THE DOCTORAL THESIS

THE STUDY OF THE MORPHOLOGICAL CHARACTERISTICS AND OF THE BIOTECHNOLOGICAL PROPERTIES SPECIFIC TO SOME TYPES OF VINES ON THE BASIS OF AMPELOMETRIC SYSTEMS

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THE IMPORTANCE OF THE TOPIC

In order to identify and describe various types of vines it is necessary to use a common language all over the world. That would lead to a better management and preservation of the existing vine genofund. Such an aim became a reality when O.I.V, U.P.O.V. and Bioversity elaborated a *List of the descriptors specific to the varieties and types of the Vitis genus*, the second edition (2002); this represented an almost complete adjustment of all the descriptors used in this respect.

The adding on the O.I.V. list of 18 ampelometric descriptors completes the methodology of vine type description, at the same time reconsidering the ampelometric method.

The usage of this method in describing and characterising vine types has come into fashion since computer science and data processing have been developing. Thus, there have been obtained many precious information on vine types division into sort-groups, on their kinship degree and differentiation.

Nowadays, when in viticulture there are used more than 5,000 vine sorts without considering interspecific hybrids and their synonymies (at least 40,000), the necessity of choosing a complex identification and description methodology becomes fully justified. The International Office of Vine and Wine elaborated a resolution regarding the granting of the necessary support in order to continue the efforts made to maintain the germplasm of the Vitis genus. It was pointed out the necessity of

identifying and differentiating all grapevine sorts and genotypes through various methods. At the same time, a systematic collecting and storage of genetic resources requires international cooperation and information standardisation.

RESEARCH OBJECTIVES

Our research made in the domain of the topic under discussion focused mainly on old Romanian vine sorts which exist in the ampelographic collections of SCDVV Drăgăşani and SD Tâmbureşti pertaining to the University of Craiova: the traditional vine sort of the Drăgăşani Vineyard (Crîmpoşie, Gordan, Braghina and Romanian Tămîioasă), other autochthonous sorts or sorts considered autochthonous because they have been grown in Romania (Roşioară, Gordin, Negru moale, Negru vârtos, Babească neagră, Fetească neagră, Fetească albă, Fetească regală, Coarnă albă, Coarnă neagră). We also analysed Haiduc and Pandur vinesorts, created by professors Aurel Zăvoi and Marin Gheorghiță (University of Craiova) at S. D. Tâmbureşti.

To reach the stated objectives, we established and followed the next directions of reseach:

- The evaluation of the elementary ampelographic descriptors specific to some old Romanian vine sorts in order to synchronize Romanian literature on the topic with the latest international description methods.
- The evaluation of Haiduc şi Pandur vine sorts in point of their purity and typicality in order to preserve authentic genetic material and to identify new biotypes within these vine sorts;
- The identification of the descriptors with discriminating features which can prove useful in vine sorts identifying and describing;
- The evaluation of some autochthonous vine sorts in point of their fenotypical features;
- Making up the ampelographic charts for some autochthonous vine sorts according to the methodology elaborated by OIV, UPOV and Bioversity.

RESEARCH METHODS

- *The Ampelometric method* constitutes the necessary primary data basis in order to observe the ampelometric descriptors and to make the statistic calculus.
- *The Ampelographic Descriptors Method* (according to OIV descriptor list);
- *Variance Analysis Method* which uses ampelometric measurements as variables;
- *Main Components Analysis;*
- *Direct Observations* made within specific phenophases and by making a comparison with referential vine sorts in order to adequately record the level of expression of each feature;
- *Observations* made by using *a video camera stereomicroscope* in order to establish the presence and density of erect and prostrated rows of hairs.

RESULTS OBTAINED

In order to attain the first objective ***- the evaluation of the elementary ampelographic descriptors specific to some old Romanian vine sorts –*** we made a series of observations and measurements, each feature being recorded according to ampelometric descriptors. Our conclusions are the following:

- ✓ Elementary descriptors contribute to obtaining a quick vine sort description, representing an important data base for their identification.
- ✓ The description of autochthonous vine sorts according to the latest norms adopted by international organisations will lead to a better management of Romanian genetic vine patrimony.
- ✓ Ampelometric descriptors complete vine sorts description through specific, stable, genetically-determined parametres.

The second objective - ***The evaluation of Haiduc şi Pandur vine sorts in point of their purity and typicality in order to preserve authentic genetic material and to identify new biotypes within these vine sorts –*** was attained by applying the simple variance analysis, using as variables the ampelometric character of an adult leaf. For this purpose, 10 equally vigorous vines were marked and from each vine there were prelevated 10 leaves located on the middle segment of the offshoot, during the ripening phase. The offshoots had the same development and insertion level.

We made a series of measurements according to the ampelometric characteristics listed among OIVdescriptors:

- *Median main nervure length N1;*
- *Lateral upper main nervure length - N2;*
- *Lateral lower main nervure length - N3;*
- *Lower secundary nervure length – N4;*
- *The size of the angle between N1 and N2- α;*
- *The size of the angle between N2 and N3 – β;*
- *The size of the angle between N3 and N4 – γ;*
- *The size of the angle between N3 and the tangent: petiole point - N5 extremity;*
- *The distance between the petiole point and the base of the upper lateral sine-d1;*
- *The distance between the petiole point and the base of the lower lateral sine - d2*;
- *The number of teeth between N2 and the first secundary nervure;*
- *The distance between N2 and its first main nervure.*

The following statistic indices were calculated: *the mean, the variance, the standard deviation, the variation index.* After analysing the significance of differences we drew the following conclusions:

- ✓ H4, H8, P6 and P9 elites may constitute new biotypes and may be analysed in order to further evaluate their agrobiological characteristics.
- ✓ H1,H3, H7, P2, P4 and P7 elites don’t show any difference in relation to the general average, representing an essential material for vine sorts multiplication and authenticity maintainance.
- ✓ The variation index was higher than 30%, only in case of P5, considering the feature *The number of teeth between N2 and the*

first secundary nervure, the samples maintaining within homogeneity limits and being representative for the elements under study.

The third objective consisted in ***Identifying morphological characteristics which can be codified and used in identifying and describing vine sorts.*** These characters concern ***the tendril***, ***the petiole*** and ***the flower***.

1. **The tendril** represents a vine sort identifying element, its relevant characteristics being the ramification degree, fluffiness and color. The observations are to be made during the flowering period.
   Considering their ***ramification,*** tendrils can be bifurcated, trifurcated or they can have other forms, the notations being 1, 2 and 3 corresponding to the three variants.

With some vine sorts, ***tendril fluffiness*** represents a strong recognition characteristic and can be noted as follows: 1- glabrous; 2 - hairy; 3 - fluffy.

As with other organs, ***tendril color*** can be simple, uniform, made up of two basic colors, or having spots or other nuances overlapping the basic color. To simplify the notations, there can be made three classes, depending on the predominant color: 1 – for the uniform green color; 2- for the reddish green; 3- for the copper color and 4 – for the violet color.

2. The leaf petiole can also offer indications for vine sort identification. The main characteristic is represented by the length measured in relation to the length of the main nervure, according to the OIVdescriptors list. Apart from this feature, we can also notice the color, the striation, the hairs and the average petiole length.

The color can vary from light green to purple, depending on the presence and intensity of the anthocyanic coloring. The codification could be made either by noting with 1- the feature *absent* or *green* and with 9 – the feature *present* or *red*, or by noting color intensity with 1- green, 2-winy red, 3- purple.

The petiole surface can be either smooth or striated, the numbers 1 and 9 representing the presence of the striations and their absence respectively.

The petiole hairiness can be classified into three types noted as follows: 1- glabrous, 2- hairy and 3 – fluffy.

The petiole length. To synchronize with the presentday notation system the contiguous classes can be cumulated and noted as follows: 1,3,5,7 and 9.

3. The flower. The flowering phenophase arouses a special interest for vine sorts identification and description. Though limited in time for a certain sort (10-14 days), it was given a lot of attention by ampelographs such as P. Bolgarev (1928); D.I. Sosnovski and L.S. Mirimanova (1928) made vine sorts classifications, using flower elements as a basic criterion.

Most specific features are encountered when analysing the next flower characteristics: *the morphological type*; *petal opening way; stamen length and stamen position in relation to the flower axis.*

The morphological type. With most vine sorts, the flower is type 5. There are also sorts which displays a flower isomorphism, with stamens and petals type 4, 5 and 6, but also sorts having a certain predominant type, this being a potential distinctive characteristic.

Petal Opening Way. During flowering, the petals detach starting from the base, like a hood. Usually, the corolla is falling, leaving the stamens free, but there are also cases with cleistogamous flo*wering, the corolla remaining on the flower till after flower producing (Tămâioasă* românească). This phenomenon is easily noticeable even from the distance, the inflorescence becoming brownish after the flowering.

Another way of petal opening, considered to be an anomaly (Gh. Constantinescu 1935, 1940), is the star – flower. Its petals open from the top not typically, like a hood. The corolla has pulpy, longitudinally hollowed petals on its upper side. This type of flower was encountered at a biotype of the Braghină vine sort and could represent an important characteristic in discovering new forms.

Stamen Length – It varies from 1,5 to 5 mm ; stamens are considered short if they are less than 2,5 mm, medium between 2,6 and 3,5 mm and long if they are more than 3,5 mm length. After analysing the stamen length for 170 vine sorts cultivated in our country, Gh.

Constantinescu and his collaborators (1952) mention that for 80% of the studied vine sorts the stamen length ranges between 3 and 4 mm.

The stamen position in relation to the flower axis displays an important ampelographic characteristic. After the petals fall, the filaments lean outwardly at a 45 degree angle and the female function flowers re-curve downwards, bringing the anthers to the lower part of the receptacle, a characteristic typical to this type of flowers.

There are vine sorts with female function flowers, whose stamens incline at a 90 degree angle, without re-curving. It implies that the morphological type is not always connected to the functional type. This characteristic, together with the others referring to flower morphology, can help differentiating among new biotypes or among vine sorts which are very similar from the point of view of the other characteristics in the phase under discussion.

- ✓ The characteristics to be codified have a low variation degree within the same vine sort and are not influenced by environmental conditions.
- ✓ The vine sorts presented as having these characteristics can be considered as reference sorts from the perspective of those characteristics.

The evaluation of certain phenotypical characteristics with some autochthonous vine sorts was made by using the methods specific to the field, taking into consideration OIV (International Office of Vine and Wine), U.P.O.V. (International Union for the Protection of New Varieties of Plants), I.B.P.G.R. (International Board for Plant Genetic Resources) and I.C.V.V. Valea Călugărească regulations. The ampelometric measurements were made during the period between fruit-bearing and ripening. The samples were represented by 10 adult leaves per vine sort, leaves placed in the middle segment of the offshoot, above the grape. 21 ampelometric variables were determined: the length of the main nervures (N.m.; N.l.s.; N.l.i.;N.l.t.); the angles made by the main nervures (α,β,γ); $\Sigma_{\alpha+}\beta$, $\Sigma_{\alpha+\beta+\gamma}$); the length and width of the leaf lamina; the distances between the base of the lateral sines and the petiole point (d_1 , d_2); the ratio between the length and the width of the leaf lamina (r L/l); the ratio between (superior, inferior and tertiary) lateral nervures and the length of the median nervure (A= L n.l.s./L n.m.; B = L n.l.i./L n.m.;

C = L n.l.t./L n.m.); the ratio between the distances at the base of the lateral sines and the length of the nervure which supports the sines (d_1 / L n.l.s; d_2 / L n.l.i.); the petiole length.

The statistic ampelometric data processing was made by using the Microsoft Excel 2003 software and appealing to the method of the analysis into main components.

The purpose of the analysis into main components is to present the information contained in the ampelometric matrices in a graphic form (the circle of correlations, the plane determined by the main components), to be able to express the fact that the closer two elements or two variables are in these graphic representations, the more similar they are. To demonstrate that, we first calculated the correlation matrix between the variables based on Pearson correlation coefficient.

Pearson correlation coefficient correesponds to the classical linear correlation coefficient. Its value ranges between -1 and 1 and expresses the degree of linear correlation between two variables. The square value of Pearson correlation coefficient gives a hint about the influence of one characteristic on the variability of another. P values calculated for each coefficient allow us to test the null hypothesis- the coefficients are not significantly different from 0.

Analysing the correlation matrix for the vine sorts under study, the conclusion was that the majority of significant correlations (58.8 %) had been registered for the following variables: the length of the lower lateral nervures, the length of the tertiary lateral nervures and the leaf lamina length. The fewest significant correlations had been registered for the variables: the size of the beta angle (the angle between lower and upper lateral nervures), the ratio between the length and the width of the lamina and the ratios between the distances at the base of the lateral sines and the length of the nervure which supports the sines (d_1 / L n.l.s; d_2 / L n.l.i.).

As far as the determination of the values and vectors specific to variables are concerned, within the space created by the main components, we notice that the inertia percentage of the first two main components is 67, 34 %, of which 48,11 % on axis 1 and 19,23 % on axis 2. The analysis of the correlation circle leads to the conclusion that the highest values of the correlation coefficient on axis 1 (the

correlations are significantly positive for α = 0,05) are registered for the variables which define the leaf lamina size - length (0,916), NLI(0,887) ; NLT (0,876)- but also the depth of the sines.

The analysis of the sorts (individuals) coordinates on the main axes is of interest from the point of view of the contribution that these vine sorts had in defining the main components. After analysing the data we have gathered, we may conclude that in defining the main component number 1, the prominent contribution was that of Crâmpoşie (49,808%) and Călina (15, 348%) vine sorts. The main component number 2 was predominantly defined by Novac (26,989 %) and Băbească neagră (23,801) vine sorts.

- ✓ The applying of the analysis into main components in ampelometry opens new perspectives in defining and specifying the ampelometric descriptors to be used in order to describe and identify vine sorts.
- ✓ The inertia percentage of the first two components is 67, 34 %, of which 48,11 % corresponds to the first component and 19,23 % to the second component.
- ✓ The bidimensional representation of the plane determined by the main components evinces the linear connections among the 21 variables analysed for 16 vine sorts.

The making of ampelometric charts for the autochthonous vine sorts under study was based on giving each characteristic the adequate notation, reflecting the registered observations and determinations. For this purpose, the choice of the biological material, the manner of discussing the expression of the characteristics and also the period in time when observations were made depended on the instructions found in OIV *Descriptor List*, the second edition. The observations on the offshoot top, on tendrils, on young leaves and offshoots were made during the flowering period. To notice the characteristics of the mature leaf, the observations and measurements were made in July - August (the ripening phase). The grape was studied when fully mature. For each characteristic there were studied a number of 10 healthy organs, with the same development and insertion level.

To give the adequate notation, for quantitative characteristics we used the mean values specific to three successive years (2006, 2007 şi 2008) to diminish the variations due to weather conditions.

- ✓ Ampelographic charts constitute an important data base in the process of computer procesing vine sort identification.
- ✓ The synchronisation with international methodology in describing vine sorts is an element which helps evaluating the real biodiversity specific to *Vitis vinifera* species, by eliminating the synonymies and the confusions generated by the existence of a multitude of biotypes within the same vine sort.
- ✓ The vine sorts for which ampelographic charts were made can be used as reference sorts in vine sort identification within the collections and plantations where they exist, taking into account that the vine sorts mentioned in OIV are seldom encountered in our country.