RESOLUTION OIV-VITI 564B-2019

OIV PROCESS FOR THE RECOVERY AND CONSERVATION OF THE INTRAVARIETAL DIVERSITY AND THE POLYCLONAL SELECTION OF THE VINE IN GRAPE VARIETIES WITH WIDE GENETIC VARIABILITY

THE GENERAL ASSEMBLY,

ON THE PROPOSAL of Commission I “Viticulture”,

IN VIEW of the article 2, paragraph 2 b) i and c) iii of the Agreement of 3rd April 2001, established by the International Organisation of Vine and Wine, and under the point 1.c.iii of the OIV Strategic Plan 2015-2019, which foresees ”Promote knowledge on the functional genomics of the grapevine and micro-organisms”,

CONSIDERING works presented during the meetings of the experts groups and particularly the "Genetic Resources and Vine Selection" Expert group, “Vine Protection" Expert group and following a proposal made by these groups of experts and,

CONSIDERING the Resolutions OIV/VITI 6/1990 concerning clone obtainment, reproduction, conservation and propagation; OIV/VITI 1/1991 on the OIV Standard programme for the clonal selection of grapevine and OIV-VITI 564A-2017 on the OIV procedure for clonal selection,

DECIDES to adopt the definition of "polyclonal selection" and an OIV process for the recovery and conservation of intravarietal diversity and polyclonal selection of grapevines on vines with wide genetic variability

INDEX

A. INTRODUCTION

The preferred targets for the application of this methodology are ancient varieties of grapevine (V. vinifera L.) having high intra-varietal diversity.

The procedure follows quantitative genetic and statistics. It follows the pioneering ideas first proposed by Rives (1) for grapevine selection, which were developed, tested and evaluated to a great extent in Portugal since the 1970’s (2). There are also, in other countries, protocols for clone selection which are referred to and described in the resolution OIV-VITI 564A-2017 that considers the definition of clonal selection and the procedures for obtaining it.

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B. DEFINITION OF POLYCLONAL SELECTION

Polyclonal selection is the selection of a set of genotypes performed according to the following methodology

C. METHODOLOGY

1. Framework

The methodology comprises two cycles:

1. the first cycle is focused on the exploration and identification in the context of a given variety of “starting genetic material” (intra-varietal diversity) for selection;
2. the second cycle is focused on the study of sampled genotypes and on the process of polyclonal selection that will eventually allow for the plantation of new vineyards with stable genetic gains and/or for supplying plants and data for clonal selection;

2. First cycle - prospecting/sampling mother plants in old vineyards of the ancient variety

In order to achieve the highest possible variability, «near-random» sampling of plants visually free of virus and other diseases, is preferred over selection based on phenotypic values of target traits because environmental deviations over individual plants are very high. Sampled plants shall be propagated after being tested for the absence of the main, naturally high-occurring viruses and only then planted into the field for the second cycle.

2.1. Identification of region(s) to be sampled

Prospecting and sampling of the starting material should be performed in one or more wine regions where the variety to be examined is traditionally grown (historical, literary and administrative sources on spatial distribution of the ancient variety should be consulted), as these regions are more likely to contain high genetic diversity of the studied variety. Restricting selection to one single region reduces the amount of diversity submitted to selection and, consequentially, the potential genetic gains to be obtained. Exact identification of regions, in which sampling is to be undertaken, will

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allow for later generalization of results to those regions, discovering the ancient variety's centre of diversity (the most likely location of its domestication) and its historical geographical progression.

2.2. Number of vineyards and plants to mark

The general goal is to safeguard the intravarietal diversity present in each region by identifying a suitable number of plants. Based on real and computer simulation experiments (3), this number oscillates between 50 to 70 plants per region. When a variety is present in just one wine region, the number may substantially exceed 70. Theoretically, the best outcome is obtained by sampling few plants in each of the chosen vineyards. As a rule of thumb, for each region, one should collect 2 to 3 plants in 20 to 30 separated vineyards, ideally belonging to different owners (figures refer to plants free of naturally occurring high-frequency viruses).

2.3. Criteria for choice of vineyards and plants in the vineyard

Plants in vineyards chosen for prospecting should not be grafted with material coming from prior selection processes (i.e. commercial material subject to previous mass and clonal selection work), that is, they must have been planted prior to the introduction of selection programs and distribution of grafted plants, or cuttings for grafting by nurseries.

Geographical distribution of vineyards to be sampled should correspond approximately to the frequency of distribution of those having the target variety in the considered region. Sampling is preferred in vineyards planted with material that is unrelated (for example, not belonging to the same owner at the time of planting).

For each vineyard, chosen plants to mark should be separated from each other at least for 5 rows and for more than 20 meters in the row.

Since the main concern at this stage is to collect a representative sample of intra-varietal diversity of the ancient variety with wide genetic variability, choice of plants for specific traits should be avoided. However, the rejection of markedly undesirable plants is advised (symptomatic of viral or GTD infection, abnormal phenotypic aspects, malformations, etc.) and /or plants that do not correspond morphologically to the selected variety.

A simple reference system, as universally understandable as possible, for locations of chosen plants and their vineyards of origin must be created and maintained, to allow subsequent verification of the representativeness of sampling and for traceability from the final selected genotype back to the original mother plant or its location.

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2.4. Virus diagnosis using ELISA

It is appropriate that harmful viruses be tested at this stage by ELISA, considering compliance with the legal framework for certification of vine plants existing in each country. However, viruses with recognized low-frequency of natural occurrence in the region under prospection may not be tested in this phase, but could await testing in a later phase.

3. Second cycle – large field trial with prospected genotypes for polyclonal selection and conservation of diversity

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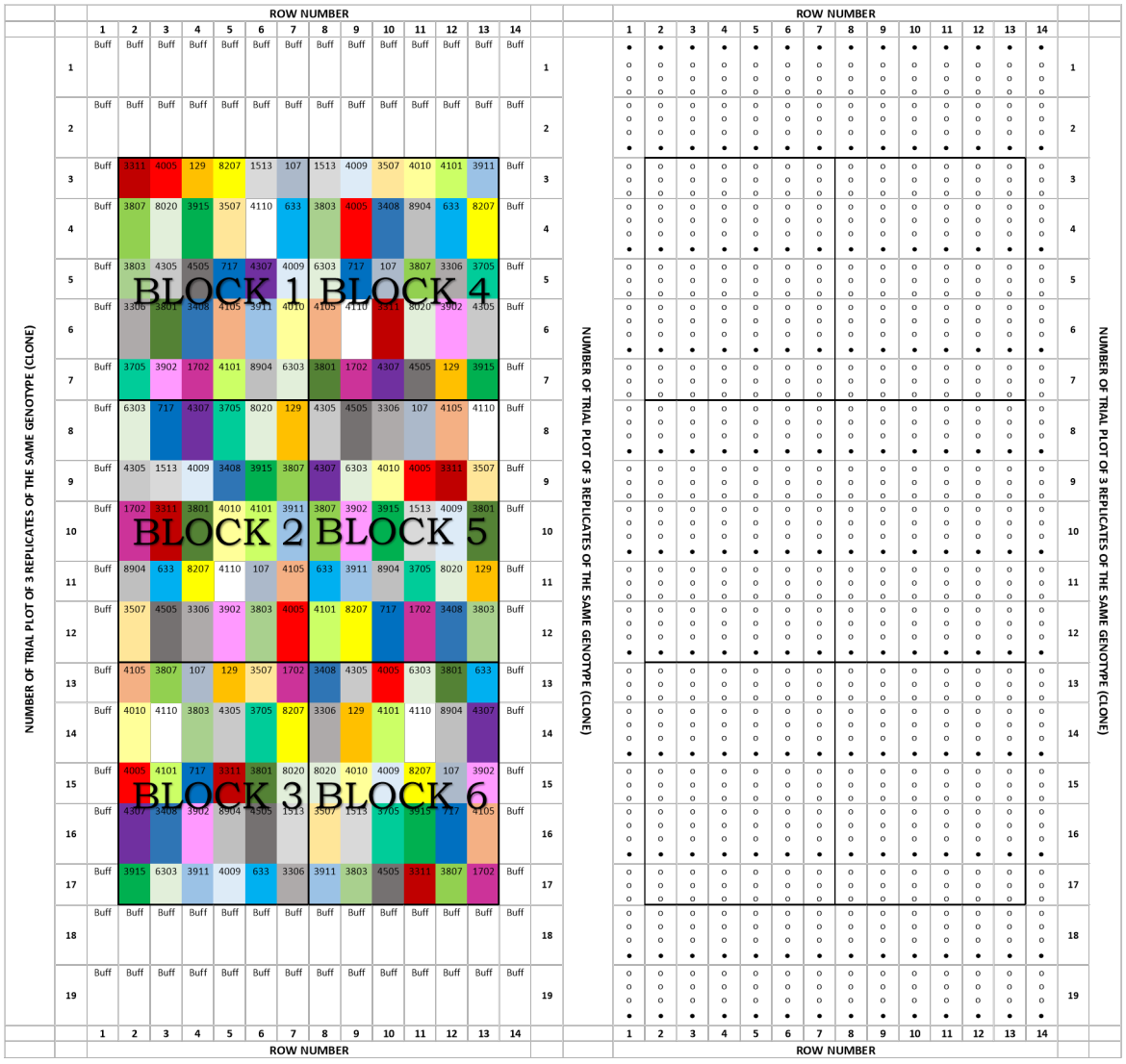

Figure 1 – Diagram example for a RCB (randomized complete blocks) design of an experimental field trial with 30 grapevine genotypes to be compared for a variety with little diversity. Left side details randomisation of the 30 genotypes repeated in each of the 6 blocks. Each cell represents 3 plants, numbers and colours designate genotypes selected Buffer plants encircling the trial area reduce borderline heterogeneities. Right side shows the actual grapevines (o) and row poles (•) inside the plots (lighter lines) and blocks (darker lines). In this small experimental plan there are 18 plants of each genotype, organized in 3-plant plots replicated 6 times, one for each block. For the sake of clarity, however, the design is applicable to higher numbers of genotypes as is the recommended case for ancient varieties having hundreds of plants.

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This cycle consists of a large field trial containing a sample of diversity throughout the studied variety. In such a field trial the methodological tools of quantitative genetics can be applied under optimal conditions. The experimental vineyard combines the two conditions essential to the success of genetic selection (1) it contains all the starting material identified (diversity) and (2) its design is suitable to apply models for data analysis allowing phenotypic evaluations of traits. In addition, it permits analyses to evaluate the distance of obtained values from true genetic values.

3.1. Choosing the experimental design

The experimental design is essential to control the actual environmental variation on a single set of plants consisting of hundreds of genotypes. Solutions may range from randomised complete blocks (RCB, 5-6 blocks x 3 plants, Figure 1) to more efficient solutions: alpha designs, row-column designs and others, depending on methodological and computational resources available in each context. For safer delimitation of experimental units these should correspond to half of the plants between row stakes (usually 3 plants).

3.2. Preparation of the experimental trial vineyard

In general, the set of studied genotypes is managed according to standard agronomic techniques in the region concerned (plant density, training system, pruning methods, etc.), making sure that agronomic operations are applied in a strictly homogeneous way. However, the large number of genotypes and replicates requires unusually strict control during plantation. This control is more efficient using field-grafting (planting the rootstock and grafting genotypes the following year). If using bench-grafted plants, they should preferably be rooted in labelled pots for minimizing error. Rootstocks should be chosen according to soil characteristics that will host them; the same rootstock must be used for all the plants in the trial.

3.3. Data collection

As a principle, the plants in the field trial should be evaluated for all traits of interest for selection purposes. However, the number of analyses in many hundreds of plots (number of genotypes multiplied by the number of replicates) are often limited by the feasibility of evaluation with available resources. In current practice, the most relevant traits are; yield, fertility, bunches and berry size and concentration of total soluble solids, acidity, pH, total phenols and anthocyanins. Must quality components are analysed on samples of, at least, 60 berries per replicate, collected in all the replicates (for speed and feasibility when under scarce resources, using only the 3 more

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homogeneous replicates is a possible alternative). However, given recent advances in automatic analysis (such as FTIR spectrometry) and field sensors, there are realistic prospects of being able to analyse more traits.

Yield is evaluated by direct weighing of grapes (per plot) in the field). Usually, data collection can start from the second or preferably the third year following bench-grafted plantation or field-grafting and continue for at least 3 years. Results from the first analysis of data from these 3 years (or more, if available) provide indications of need for further evaluations. Additional data, (ampelographic, phenological, etc.) may be collected depending on special selection objectives and available resources.

Optionally, before final selection of a set of genotypes (for immediate use) from varieties intended for use in wine production, it would be useful that micro-vinifications of that group and of a representative sample of the entire set in the trial vineyard, using approximately 20 to 30 randomly-chosen genotypes, be conducted following a standardized protocol. Both resulting wines should be blind-tasted by experts, using a duo-trio test to assess whether wine made from selected group carries the sensory characteristics typical of the variety. If differences are statistically-significant, characterization by sensory descriptive analysis should follow to objectively establish sensory differences between the selected group and the whole trial set.

3.4. Data analysis and polyclonal selection

For data analysis, mixed models are fitted. The final objective is to estimate variance components, to find empirical best linear unbiased predictors (EBLUPs) of genotypic effects and calculate genetic gain (R).

For balanced data and models with simpler covariance structures, selection can be based on ranking of phenotypic mean values of genotypes (since in those cases the ranking of phenotypic mean values is equal to the ranking of EBLUPs of genotypic effects). Under these conditions, classical tools of quantitative genetics are applied. That is, to select implies ordering a set of genotypes studied by their phenotypic values, to choose a subset of the most interesting for a given trait and computing the difference to the overall mean (selection differential, S) as well as quantifying the distance between the phenotypic values (observed) and genotypic values (broad sense heritability, h2), to calculate genetic gain (R) according to the following formula:

$$R = S \times h^2$$

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For unbalanced data and more complex models, genotype selection should be based on ranking EBLUPs of genotypic effects and, prediction of genetic gain being computed as the mean of EBLUPs of selected clones.

Selection can be made in favor of one evaluated trait, or several, considered either individually or under the form of a selection index.

The number of genotypes selected which will constitute the polyclonal material is the result of a compromise between desired gain (increases when the number of selected genotypes decreases) and stability of behavior of the selected set of genotypes in different environments, i.e. low G×E interaction (Genotype by Environment interaction). This stability increases with the number of selected genotypes. Experimental results in literature (3) show that the group stability grows sharply from 1 to 7 genotypes and more moderately above that number. Based on these results, obtained polyclonal material should consist of balanced mixtures of 7 to 20 genotypes, depending on the specific conditions of each selection work. However, even though that number may rise above 20, it should never be less than 7. The balance of the mixture implies that each genotype is represented in the group with a frequency of 1/n, being n the total number of genotypes in the mixture. Due to feasibility reasons as dependency on the material available from each genotype and other conjunctural factors, some tolerance for those limits must be accepted. In any case, the frequency of any single genotype must never exceed twice that of the least frequent genotype.

3.5. Second virus diagnosis of the genotypes that form the polyclonal group

Phytosanitary requirements for that selection must follow the regulatory framework of the country where selection is being conducted or of where it will be used for plantation, whichever the strictest.

3.6. Molecular diagnosis of the genotypes in the polyclonal group

The selection of genotypes must be preceded by molecular analysis to confirm their varietal identity, using the best available methods.

3.7. Maintenance of foundation plants of the polyclonal group

Each selected genotype must be submitted to phytosanitary and varietal diagnosis then multiplied in small-scale, to generate foundation plants.

Plants should be kept under conditions of high sanitary security, with capacity for replacement in case of infection and adjustment of their effective to market demands. A possible model for this maintenance is growing foundation plants on an inert fertilized substrate, in pots inside a greenhouse, protected against virus vectors.

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3.8. Conservation of biodiversity

In addition to polyclonal selection, both the vineyard of the extensive field study and the data acquired from it can be used for the preservation of intravarietal diversity, countering the current genetic erosion. Conservation should be guaranteed by the study vineyard during its useful life and, at the end of this period, it must be pursued using other methods.

4. Homologation

If homologation of polyclonal material is envisaged, it must follow the regulatory framework of the country where selection is being conducted. It is desirable that the standards for homologation of selected genotypes are harmonized between the different states that choose this option.

5. Other secondary results and recommendations

In addition to polyclonal selection, both the field trial vineyard described before and the data collected from it can be directed towards other, complementary uses.

Experimental data should be stored under high security measures, such as redundancy of media support and databases. These data will allow for new “on-the-spot” selections responding to new challenges posed to the vine and wine industry, always under constant change.

Additionally, yield and other data can be analysed for the quantification of intra-varietal diversity of the variety by plotting normal curves of probability density of these characteristics or by calculating the corresponding coefficients of genotypic variation. This knowledge may allow an understanding of the origin and geographical expansion of a variety, being an indispensable condition for objective studies of intra-varietal diversity.

The experimental phase for polyclonal selection (see 3.) can also be considered as the first phase of clonal selection itself. In this case, selected genotypes would have to be in higher number to enter a second cycle of regional adaptation trials for the selection of individual clones.

6. Summary of the protocol

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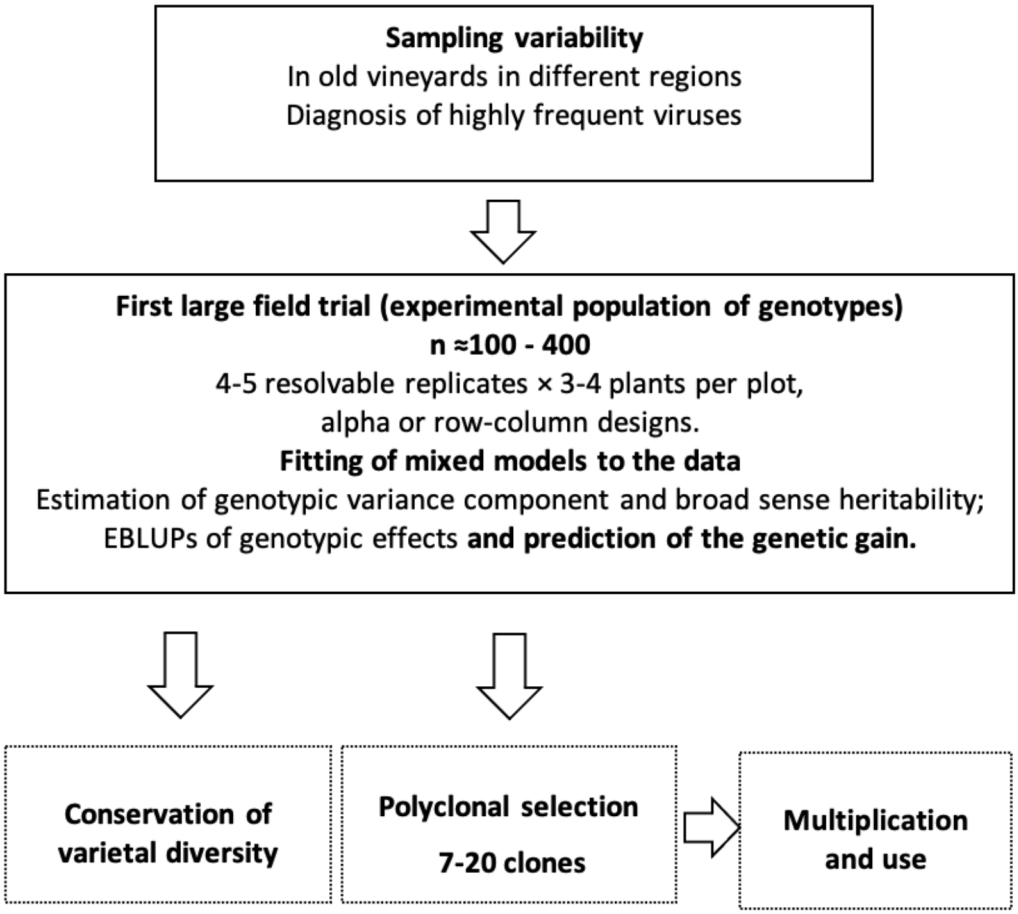

Figure 2 - Methodology of grapevine polyclonal selection and diversity conservation

ANNEX I: GLOSSARY

The terminology and use of this glossary should be only applied within the scope of this resolution' to ensure that the context of the definition applies to the text of the resolution.

Broad sense heritability (h^2)

Genotypic variance of a quantitative trait (referred to the set of genotypes of an ancient variety in a specific field trial) divided by the total phenotypic variance. This genotypic variance can't be obtained directly, but it can be obtained by subtracting

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the environmental variance from the total variance as observed in the trial.

Certification

System of control for multiplication and distribution of plant propagation material to verify compliance with relevant legal requirements. In general, various types of materials, based on sanitary status and other conditions, result from this system. In the European Union, the current existence of the category "certified material" does not exclude other categories (“initial”, “base”, “standard”) from being considered as certified too.

Clone

See definition in (4) resolution OIV-VITI-564A-2017

EBLUP (Empirical Best Linear Unbiased Predictor of the genotypic value)

To learn genotypic value, this is a more accurate predictor than the phenotypic value, because it enables a more efficient removal of environmental variation. Thus, when expressed as deviation from the total mean, it is also a direct quantifier of genetic gain.

Environmental deviation (E in the fundamental equation of the phenotypic value: P = G + E)

The part of phenotypic value determined by the environment, assumed as a distribution with null mean and non-zero variance (5) (6) (7) (8). The environmental deviation is not directly determinable, but its variance in an experimental set of genotypes can be experimentally found.

Foundation plants

Small number of plants representing a selection proposed for official homologation. These plants are managed according to strictest conditions favoring genetic stability and sanitary protection.

Predictable Genetic gain (R in the equation: $R = S \times h^2$)

For any given trait, the difference between its value in vegetative descendants of one or more genotypes, selected from an experimental set of clones, and the average value of vegetative descendants of all clones in that set. It can be obtained by calculating the product of selection differential (S) by broad sense heritability (h2) or as the sum average of EBLUPS of genotypic effects of selected clones (1) (9).

Genetic selection

Selection of a subset of clones within a heterogeneous ancient variety concerning quantitative traits, based on predictors of genotypic values (phenotypic values, EBLUPs or others) for one or more traits, leading to objective genetic gains of those traits.

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Genotype

The genetic makeup of an organism or group of organisms with reference to a single trait, set of traits, or an entire complex of traits.

Genotype by environment interaction (G×E)

A genotype x environment interaction may be defined as a change in the relative performance of a 'character' of two or more genotypes measured in two or more environments. Interactions may therefore involve changes in rank order for genotypes between environments and changes in the absolute and relative magnitude of the genetic, environmental and phenotypic variances between environments (10).

Genotypic value (G in the fundamental equation of phenotypic value: P = G + E)

The genetically determined part of the phenotypic value which is transmitted by vegetative propagation. The genotypic value is not directly determinable but can be approximately known through various predictors based on variance relationships.

The variance of the genotypic values in a field trial equals the difference between phenotypic and environmental variances in the same trial.

Intra-varietal diversity

Differences in quantitative traits of plants of an ancient variety that was initially homogeneous, but that became heterogeneous due to the accumulation of clonal vegetative mutations and to other mechanisms of variation associated to somatic multiplication. This diversity is the prime material for genetic selection and for other uses of ancient varieties.

Mixed models

Models with fixed and random effects.

Phenotypic value of a trait (P in the fundamental equation of phenotypic value: P = G + E)

A trait's value in a plant, or a clone, inside an experimental set, evaluated by direct observation or by any other method. Per a classic model, the phenotypic value is the sum of the genetically determined value (genotypic value, G) and in a given environmental deviation (E) with mean zero and variance different from zero: P = G + E. The phenotypic values are experimentally determinable, so the variance of these values in an experimental set is obtained by simple direct calculation using observed values.

Polyclonal selection

In the context of this resolution, polyclonal selection means the selection of a group of 7 to 20 genotypes from an experimental set of genotypes of an ancient variety, containing most of its intra-varietal diversity. The selection is based on tools from

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quantitative genetics to enable the efficient reduction of environmental deviations and to achieve high, stable and predictable genetic gains. In exceptional circumstances, the number of selected genotypes may be greater than 20 but never less than 7, to prevent interference from G x E interaction. In the final selection to be multiplied for productive plantation, all genotypes should be equal in quantity. However, a tolerance is admissible, provided no genotype exceeds in frequency an effective equal to twice the effective of the less frequent genotype. Target traits to be evaluated and selected for should be, as a minimum: yield, must soluble solids and acidity for white varieties, and all those plus skin anthocyanins, for red varieties.

In this context, a mixture of genotypes resulting from various independent selection processes does not correspond to this definition of polyclonal selection and in order to avoid confusion should be designated as multiclonal mixture.

Qualitative trait

A trait that, in a heterogeneous set of plants, has a discrete distribution, resulting from monogenic or oligogenic determinism and moderate environmental deviations.

Quantitative genetics

Genetics principles allowing for understanding and analysing quantitative traits. Distributions of quantitative traits are sums of genetic distributions and distributions of environmental deviations, so understanding the first implies reduction of random deviations, variance decomposition and related processes. In summary, quantitative genetics is statistics applied to genetics of quantitative traits.

Quantitative trait

A trait that, in a heterogeneous set of plants, has continuous and normal distribution. Normality results from polygenic determinism and/or high environmental deviations.

Sanitary selection

Diagnosis of systemic pathogens, intra- or intercellular, which are transmitted by vegetative propagation (and other mechanisms). In practice, it is mostly applied to viruses.

Selection differential (S in the genetic gain equation: $R = S \times h^2$)

Difference between the mean values of genotypes selected from an experimental set of an ancient variety and the overall mean of genotypes in that set.

Ancient variety (botanical sense) and cultivated variety, or cultivar

In a botanical sense, variety is defined as a group of plants resulting from natural evolution, having similar traits that can be reproduced "true to type" from generation to generation. Per the International Code of Nomenclature of Cultivated Plants (ICNCP), the cultivated variety, or cultivar, is an assemblage of plants (a) selected for a

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character or a combination of characters, (b) distinct, uniform and stable in these characters and, (c) when propagated with appropriate means, retains those characters. Per these definitions, ancient grapevine varieties such as Pinot Noir or Tempranillo, are not botanical varieties because their origin and evolution resulted primarily from human activity. But neither are they exactly cultivars because they were not selected for concrete traits by any identified selector and are far from being uniform concerning important cultural and oenological traits. What corresponds to the definition of cultivar in the ICNCP are the parts of the old variety selected by different selectors which may be markedly distinct from each other, uniform and stable. In the scope of this resolution, and until a better definition has been agreed upon, the term "ancient variety" is used to designate them.

ANNEX II: GENETIC AND PHYTOSANITARY SELECTION OF THE VINE: STATE AND BIBLIOGRAPHIC REVIEW

1. Theoretical bases in quantitative genetics for carrying out genetic selection

By the time of early domestication of the vine, in the geographical space prevalently of Mediterranean Eurasia, the ancient variety of the grapevine would have begun as a naturally homogeneous population. However, the following vegetative multiplication of varietal population entailed countless mitosis and equally numerous DNA replications, these latter entailing genetic mutations and other mechanisms of genetic variation. Those mutations are often visible as berry color, leaf shape and other qualitative traits' mutants.

Almost all the grapevine traits, including those economically important are quantitative (yield, berry sugar, acidity, anthocyanins and many other), thus becoming the natural targets for genetic selection. Quantitative traits are controlled by sets of genes determining small cumulative effects (microgenes or polygenes) but they are also subject to mutations.

Intra-varietal variation amplitude for a trait resulting from centuries- or millennia-old accumulation of mutations may attain extremely high levels: magnitude of variation for yield of genotypes of a variety historically widespread in a given territory may be ten-fold while sugar, acidity and anthocyanin levels in berries may be two-fold. This diversity is the prime-matter for selection opening the field for achieving high genetic gains leading to relevant economic benefits. It paves the way to naturally modify the ancient variety for adaptation to novel biotic and abiotic challenges, including climate

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change. To harness this potential, selection should be conducted upon samples of genotypes large enough to represent, as much as possible, the ancient variety's natural diversity. Estimates from real-life experiments and computer simulations place that sample size between 100 and 400 genotypes. Using samples of this size, the difference between the value of a single genotype (or the average of a group of genotypes) for any given quantitative trait and the whole sample's average will be maximized (selection differential - S). This differential will be the first factor in the evaluation of genetic gain. However, this factor is still over-estimated by environmental deviation and genotype by environment interaction, both non-inheritable, needing correction by quantification of the genetic part of variation as related to total variation within the whole sample's set.

Intra-varietal diversity is determined by many genes, each controlling a certain distribution of diversity. To these genetic factors, random environmental deviations, characterized by null average, non-zero variance and near-normal distribution, add their effect. This means that distribution of total diversity is a random sum of distributions, which, per statistics theoretical foundations, will always be a normal distribution. From these considerations, it follows that, for any given trait, (observed) phenotypic value of a genotype in an experimental set to undergo selection will obey the model

P	=	G	+	E	+	G × E
Phenotype value		Genotype value		Environmental deviation		Genotype by environment interaction

A given genotype's phenotypic value (P) is the only one that can be directly measured. However, the unseen genotypic value (G) is the only characteristic transmissible to the "offspring" and, thus, the only one targeted by polyclonal selection. Therefore, the main goal of the whole selection process should be to reduce, as much as possible, the values of E and G×E to approximate the values of P and G. If this is achieved, the measurable P will become a close predictor of G.

The first step towards obtaining that goal is by conducting selection in field trials, where each genotype is replicated several times. In that way, there will be several mutually-cancelling environmental deviations, whose average will trend towards zero as the number of replicates increases. This property is explained by a basic notion in statistics: the variance of means of samples with n elements of a plant set is the

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variance of the set divided by n.

Computer simulation experiments provide the same conclusion. In practical terms, it means that any selection of individual plants will be highly inefficient because the variance of environmental deviations and the genotype by environment interaction both account for 70 to 100% of total variance. Consequently, genetic variance will vary from 30 to 0% of total variance. Inversely, selection trials using 15 plants per each genotype, following classical experimental design with plots of 3 to 4 plants and 4 to 5 replicates in complete randomized blocks, allow for a reduction of environmental deviation and G×E interaction to values ranging from 10 to 40%, thus increasing genetic variance to values between 60 and 90% (3).

These values of genetic variance, divided by the total observed phenotypic variance, correspond to broad sense heritability (h2) providing the necessary corrective factors of selection differentials (S) to predict genetic gain (R):

R	=	S	.	h^2
Genetic gain		Selection differential		Broad sense heritability

Extensive selection trials founded upon these principles of quantitative genetics and statistics with ancient grapevine varieties from Central and West Europe have frequently resulted in observed gains of yield between 10 and 40% and of must quality traits up to 10%, when compared to the original unselected ancient variety.

Other than using phenotypic values of genotypes as predictors of respective genotypic values as described above, in optimised contexts it will be possible to use methods leading to more exact predictors: Empirical Best Linear Unbiased Predictors (EBLUP) of the genotypic value. These are methods based in trials using incomplete blocks experimental design and data adaptation to mixed models and estimation of variance components through Restricted Maximum Likelihood (REML).

The above-mentioned methodologies are applied to a large initial trial including all the diversity of the target ancient variety and meant to provide a selection of a group of genotypes displaying significant gain towards the desired trait(s). Like it happens with environmental deviation (E), when this group is planted fully mixed, G×E interactions of different clones will be mutually cancelled: the group's G×E interaction and environmental deviation both tend towards zero. That group will be the result of polyclonal selection and will provide stable behavior across different environments.

In this methodology, environmental deviation (E) and G×E interaction are dealt with

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together not being necessary to separate and estimate their respective values.

2. Historical evolution of selection methodologies applied to agricultural crops and to grapevine varieties

The roots of plant improvement date back to the first half of the 19th century. At that time, several plant hybridisations (for vegetables, cereals, fruit trees, etc.) were made with the goal of obtaining offspring presenting more efficient outcomes for farmers. It was a very productive moment, at a time when there was no genetic knowledge base, something that was first mentioned when Mendel published his work with the pea plant (11) but only accepted by the wider scientific community when his work was rediscovered in the early 1900's.

In the last quarter of the 19th century, genetic improvement techniques by hybridization started to be applied also to the grapevine (crossing Vitis vinifera with American Vitis species) achieving success and great impact by overcoming the severe crisis of downy mildew, powdery mildew and Phylloxera that pushed European viticulture to the brink of termination. The first works on mass and clonal selection of ancient varieties, mainly in Germany, date from the same period.

Despite these historical milestones, Plant Improvement, as a science and practice, only became continuous and notable from the 1920's. This late development can be attributed to the fact that almost all relevant traits of plants (same as with animals) are mostly quantitative, making its heritability not totally explainable by Mendelian genetics. However, also in the 1920's the appropriate scientific field allowing for their better understanding started to develop: biostatistics also known as quantitative genetics.

A famous pioneer of this development was Ronald Fisher, a researcher in Plant Improvement and Selection, among other interests, who was struggling with methodological insufficiencies to explore large datasets he had available at the Rothamsted Experimental Station in England. Fisher's fundamental theorem of natural selection states that «the rate of increase in fitness of any organism at any time is equal to its genetic variance in fitness at that time » (12). The birth of quantitative genetics and other important statistic science branches (analysis of variance, regression and others) derived from needs first identified in Plant Improvement. Naturally, once it became formalised and structured, Plant Improvement received a major boost from the application of several methodological innovations.

From that (and other) boost, Plant Improvement knew fast development since the 1930's. Major visible cases were the great success of corn improvement in the USA and

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other countries, by exploration of hybrid vigor and improvement of plants conducted by CIMMYT (International Maize and Wheat Improvement Center[1]) allowing to curb hunger for millions of people around the world.

Recently, plant improvement has been considered by several authors (3) as the technology responsible for half of the great yield and quality increments of main cultures in the USA and the world in the last 100 years, that is as much as all those resulting from the sum of all other farming techniques. Several powerful methodologies are used today to obtain genetically improved varieties, of which the following are to retain: commercial hybrids (based on exploiting hybrid vigor of crossbred plants, such as maize); pure lines (homozygous, self-fertilizing plants such as wheat); free pollinated varieties (heterogeneous, obtained by mass selection, such as crossbred forest species), clones (the most homogeneous varietal type, used for vegetative propagated plants).

The grapevine (Vitis vinifera) is a special case in the context of plant improvement, mainly because the concept of wine quality incorporates many historical and psychological factors leading to the preference of ancient varieties of natural origin. Therefore, improvement techniques involving the creation of new variability (hybridization and others) struggle to be accepted in the real world. Yet, mass and clonal selection are based on natural intra-varietal variability, have zero counter indications and should, therefore, be resolutely applied.

The first condition for mass and clonal selection in the grapevine is the existence of intra-varietal diversity and the understanding of its amplitude, geographical distribution, affected traits and so on. The first approach to this subject was conducted in the 1930's in Germany and the USA but with results, apparently, not consistent with the existence of intra-varietal variability: that variability was found in traditional German varieties but not in those cultivated in the USA. For further information see: (13) (14) (15). In the 1950's, in France, Levadoux reinterpreted those incoherent observations and proposed that intra-varietal diversity in Germany resulted from its ancestry, whereas lesser diversity in the USA suggested plants in there were imported from Europe (after selecting a few varietal accessions) and contained a fraction of, not the whole, total diversity (16). These studies, however, were not continued towards clarifying the origin of diversity, its objective quantification, geographical distribution and role as a factor for genetic gain as expected from selection.

The second factor of genetic gain – broad sense heritability – resulting from separating total variance in its genetic (heritable) and environmental (non-heritable) components, was not fully studied for application in the context of grapevine

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Secretary of the General Assembly
Pau Roca

selection. Rives, in France, conducted a rigorous synthesis of genetic foundations for modern and efficient selection, but his proposals were not met by widespread effective application (1).

Still, a few exceptions (3) to this picture, in terms of aligning grapevine selection methodologies with Plant Improvement standards for species with agricultural interest in general, have happened.

In summary, long-standing and well-proven theories in statistics and quantitative genetics, as several reference works in Plant Improvement from several places in the world and solid, documented, experience of application to the grapevine are the basis for the methodology proposed in this document.

3. Present situation and risks of Vitis vinifera genetic resources

Ancient grapevine varieties would have been originally a homogeneous genotype who have started to generate and accumulate diversity resulting mainly from somatic mutations of quantitative traits. In the past, most plants were propagated asexually, therefore, their genes were not lost upon their death; they were instead transferred to new plants who would add more mutations, the process going on and on along generations.

This process, at work since millennia gave origin to the extraordinary diversity that we can today observe within many ancient varieties. It was, however, limited when new vineyards started to be planted with commercially-prepared bench-grafted propagules originating from a much-reduced number of specialized vineyards (used for multiplication). This means that all plants of that ancient variety are still cultivated and accumulating mutations, but these are not transmitted anymore to plants in new vineyards: the process by which created variability in each year was accumulated and transmitted has been reduced.

This phenomenon is further worsened by the wide generalisation of varietal selection, most especially when this selection is narrow-band (cultivation of a few selected genotypes).

This situation can only lead to homogenisation of all vineyards and to a «genetic freezing» of the variety ultimately resulting by ending selection possibility in medium or even short terms, unless in classical clonal selection of a given variety, a weak selective pressure was not adopted which led to the selection of many clones. Deadline for intra-varietal diversity extinction in each country depends essentially on the work of public and private clonal selection started in the 1970s and when it started using bench-grafted plants and the renovation rhythm in older vineyards. In Eurasia,

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Secretary of the General Assembly
Pau Roca

where the Vitis vinifera species has more diversity, some countries may have already lost most of the diversity they originally had, while others may still be on time to organise conservation strategies.

Alternatives for conservation are quite scarce: wide-band conservation under cultivation (on farm) is not widely viable, as it would entail cultivating extremely heterogeneous plants, something difficult with modern and competitive viticulture. Only conservation in dedicated vineyards using representative samples of each variety's diversity offers viability against erosion by classical clonal selection.

Some countries already developed a theory to guide this type of conservation. To be representative, the sample should have, at least, 70 or more genotypes for each region where the ancient variety is grown, numbering in several hundred when a given ancient variety is, for long, cultivated in several regions of several countries. Conservation should be redundant, based preferably on one collection exclusive for conservation (this may be done in flowerpots) and another in a productive vineyard under usual farming practice, for conservation and evaluation of all genotypes in the sample.

Conservation is an experimental action with many interdependencies towards selection. Firstly, genotypes to be prospected for both goals can be largely the same. Secondly, evaluation of conserved clones can be put to good use as a pool for selection and, conversely, selection trials can be used as conservation structures.

In summary, conservation of intra-varietal diversity is today a high-priority action to ensure continuity of selection efforts together with the high genetic and economic gains they may generate.

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[1] http://www.cimmyt.org/

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