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Clonal selection of vitis vinifera cv. malbec: Confluence of science and nature

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Abstract. It is not overstated that Argentinean viticulture identifies with Malbec, the vine which long ago was introduced in the country from France and which has marvelously naturalized here. However, the variety Malbec has many different expressions, depending very much on environmental and cultivating conditions and on natural mutations occurred over time. A modern viticulture cannot do without the capability of exactly identifying and differentiating clones of the same variety and from the ability to do that over contingency. This work on clonal selection, conceived and developed by a very polyvalent team, focuses exactly on defining instruments to unequivocally distinguish and select different clones and using these instruments to analyze, classify and select all different clones representing the highest variability of Malbec in Argentina ever sampled. The work bases on traditional instruments – phenotypic and enological analysis – and on a molecular marker selection program. Through the synergy of all these methods the team has come to the selection of 16 superior clones of Malbec and will proceed by sharing and mapping three of those clones on the country different micro-environments for grapevine growing regions, giving Argentinean viticulture a key instrument to identify its most valuable grape wine variety.

Introduction

It is in the semi-desertic climate of the province of Mendoza, in the foothills of the Argentine Andes, where Malbec expresses his utmost. The preference for arid areas with large temperature variations between day and night makes it particularly suitable for these regions and this is why it has become the symbol of Argentine viticulture, getting a strong international recognition. The Argentine Malbec, however, is characterized by a huge variety of genotypes, which can be attributed to the different environmental conditions in which it is grown, that has caused a series of spontaneous mutations, accumulated over the years, a specific feature of the genus Vitis.

This fact, though it has been multiplied over the centuries by vegetative propagation has a great genetic variability that can be attributed to different types of mutations and transpositions. The result is the presence of different phenotypes even at the clonal level, which differ for phenotypic, agronomic and oenologic traits.

Modern cultivation techniques applied to grapevine growing and the production of high quality wine can not ignore the possibility of distinguishing in a univocal way the different clones of the same cultivars in order to allow their identification, certification and monitoring for traceability. To date, the identification of Malbec clones is made only on the basis of phenotypic or oenologic characterization, but this method has major limitations and does not allow to unambiguously distinguishing the different clones of the said variety. It also requires many years of observations with results that are often masked by environmental influences, pathogen agents, soil characteristics, nutritional status of the plants and last but not least, climate that play a vital role in determining the phenotype and the oenological quality of the grapes.

Previous studies of clonal diversity of different grape varieties were based on the use of molecular markers such as microsatellites, some of which are able to detect even low levels of polymorphism in a group of clones (Imazio et al. 2002, Pelsy et al. 2010). Therefore, these molecular markers have been very effective in distinguishing the differences between clones.

The use of molecular markers S-SAP (Sequence-Specific Amplified Polymorphism) – which are based on

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the presence of retro-transposons can be an alternative to the technology of AFLP (Amplified Fragment Length Polymorphism) – has allowed the identification of reliable and reproducible clones from different grape varieties (Stayner et al. 2009), among other species. The retro-transposons are a class of mobile elements in the eukaryotic genome and it is quite common also in grapevines. Their presence often causes the activation or deactivation of some genes with effects even at the phenotypic level (i.e. Pinot). They are widely dispersed throughout the genome, from a few to thousands of copies per haploid genome. They are used as a source of information because of their ability to integrate in different loci of the genome, generates an insertional polymorphism among the different clones of the same variety. These markers were used to identify clones of apple (Venturi et al. 2006), several species of Vitis (Moisy et al. 2008), and differentiate grape varieties and clones (Labra et al. 2004, Stajner et al. 2008).

The results of these analyzes at the genomic level, and based on molecular markers, together with the ampelographyc analysis and the quality assessment of the wine produced by an individual clone are the key to a clear and unambiguous identification of a given clone, in order to permanently select the best from its quality point of view. With this understanding the family type winery Tempus Alba, of Italian origin, started an ambitious and pioneering research on clonal selection of Malbec, which began 13 years ago in the belief that this variety is able to express itself in a much more differentiated manner than what we see today.

Description of the work and its stages

First stage (start: 2000)

The first phase of the work was meant to create a gene bank on which to conduct the future selections. This had to be as wide as possible, genetically speaking, and possibly represents all the micro-zones climate conditions of Mendoza where Malbec has proven over time to be able to give quality wine. To this end, a total of 8000 Malbec vines were collected from those microclimatic zones, and were planted on a parcel of 2.5 hectares of land in Coquimbito (Maipú, Mendoza). These genotypes allowed the interdisciplinary working team to do the clonal selection research work. The authors are convinced that this proceeding has permitted them to collect a variety of Malbec individuals, never done until now, and that it truly represents o very good approximation to the real variety of Malbec in Mendoza.

Second stage (early: 2000)

At the same time the grapevines were planted, Tec. Marisol Cortez – responsible for the biotech lab of the winery- and Ing. Agr. Miguel Cirrincione, proceeded with the development and fine tuning of the in vitro plant micro-propagation process adjusting this universal method for Malbec vines. This work included rustication or acclimatization of the new plants for further growth under ex vitro conditions, first in an isolated chamber and later on, in a shaded greenhouse.

The following parameters were defined:

- Ideal conditions for transplanting the in vitro produced seedling to ex vitro: this includes the stage of development of the plant, type of substrate to be used, type of container, conditions of temperature, humidity, light, ferti-irrigation treatments if any;
- Ideal conditions for transplanting the new vines to a greenhouse and then to the open field.

Photos of the different steps of the micro propagation process are shown below:

Mother plants for initiation of tissue culture.

Clean material to start the initiation phase Medium preparation to induce roots.

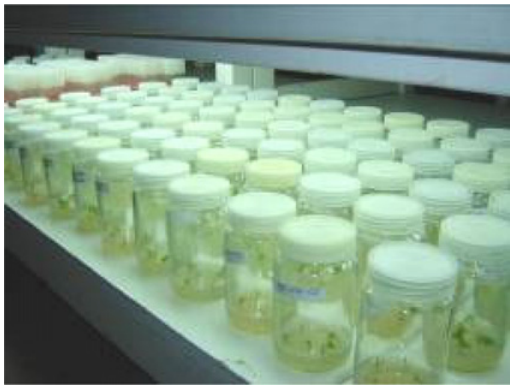
Seeding a tube with green material.

Tissue culture under controlled conditions.

Ex vitro rustication or acclimatization of new plants.

End of the initiation phase.

End of rustication phase in recyclable pots.



Beginning of propagation phase in isolated chamber.

Transplantation to a vineyard in an open field.

In vitro micro propagation phase.

Third stage (start: 2004)

After the vineyard has reached an adequate level of production, the first phenotypic analysis, was started. They have led to the selection of 589 genotypes then implanted at the site of Anchoris, Lujan de Cuyo. In the new vineyard each genotype was planted with repetitions in order to have enough grapes to make micro vinifications and physico-chemical analyzes and blind tasting of the wine produced.

The criteria for selection of the 589 plants were qualitative, based on an index specifically point to it, namely:

$$Iq = (1 - c)[50 - (v - 3)^2 - (t - 3)^2 - (k - 3)^2] \quad (1)$$

where Iq is the potential quality of grapes for winemaking.

The other parameters are: v = vigor of plants.

t = size of the berries.

k = yield of each plant measured in kg/plant.

c = propensity to millerandage problem (expressed in %).

v, t, and k vary from 1 to 5 with an optimum value of 3.

Fourth stage (start: 2007)

This phase lasted three seasons (2007/8, 2008/9 and 2009/10) in which a much more accurate assessment of the quality of the grapes was conducted, based on various types of analyses, namely:

- Sensory analysis of the quality of the grapes.
- Physical-chemical analysis of the grapes at the optimal harvest time.
- Physical-chemical analysis of wines obtained from microvinification.
- Blind test of wines obtained from microvinification of clones.

Sensory analysis of the quality of grapes

This analysis was conducted by Carlos Domingo Catania, following the methodology used by the Co-operative Institute of Wine in France (2003). With this method, he analyzed the general physical conditions of the grape, and evaluates the structure of the berries (color and size), the skin (ease to separate from the pulp, acidity and aroma), pulp (consistency, tannin intensity, acidity, astringency, flavors) and seeds (color). Based on this assessment the clones under study were divided into four groups: low, medium, high and outstanding quality. The final results indicate that clones TA 16, TA 24 and TA 28 were of an outstanding quality scoring 90% of the maximum potential value. Clones TA 14, TA 17, TA 18, TA 19 and TA 25 were of high quality standard with a score around 80% and the remaining clones were categorized as of an average standard of quality.

Physico-chemical analyzes of the grapes at harvest time

These analyzes were conducted by Norberto B. Richardi and led to:

- determination of anthocyanins in grapes and wine (Method of discoloration of SO_2 – Ribéreau Gayon et Stonestreet, (1965), Glories (1984);
- determination of free anthocyanins by the fractionation of phenolic compounds with PVPP (Glories 1976), quantification of tannins in grapes and wine (Method for absorbance at 280 mu – Somers et Ziemelis (1985), N. Vivas, N. Vivas de Gaulejac, M.F. Nonier, and adapted by N.B. Richardi;
- determination of total polyphenols F.T. Poux (1958), Ribereau Gayon (1970);
- determination of the average molecular weight (AMW) and the average degree of polymerization of tannins calculated from the index MDMACH;
- analysis of color in wine (intensity and deepness) – method de Saudreau;
- analysis of tannic power by the method of V. De Freitas – PhDThesis (1995);
- determination of the color of the seeds;
- organoleptic assessment – Moscowitz and Chandler, (1977).

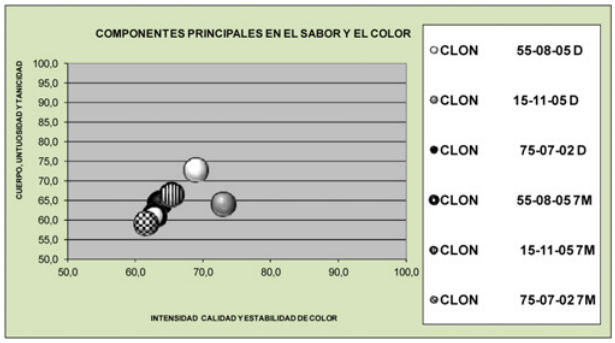
With the results of the physico-chemical analyzes on the grapes, Richardi has designed a quality indicator called IMCE as its acronym in Spanish (Indice de Madurez y Calidad Enológica) which was used in the oenological quality assessment of the different clones. Based on the values calculated for each clone during three consecutive vintages (2007/10), as compared to the average value for Malbec in Mendoza, a quality rank was made and permitted to classify the clones in three quality categories: Outstanding (TA 15, TA 16, TA 18, TA 22, TA 25 and TA 28); Excellent (TA 14, TA 17, TA 19, TA 24, TA 26, TA 27, TA 30 and TA 31) and Very Good (TA 20 and TA 23).

Physico-chemical analysis of wine coming from microvinification of clones at two different points in time

The analyzes were conducted at two different times: at bottling time (April 5th, 2010) and 7 months after bottling (December 15th, 2010).

The main results are summarized in the following Table.

Blind test of the wines coming from the microvinifications of the different clones performed by a team of Oenologists leaded by Norberto Bartolomé Richardi



Principal components in wine: color intensity and stability, body, tannins and greasiness.

At the end of the year 2010 16 clones were selected capable of producing a wine of superior quality when compared with a control sample: a Malbec clone identified by the Argentine National Institute of Agricultural Technology (INTA) and a COT grapevine taken from INTA's collection.

Fifth stage (2012/2013 campaign)

This phase was characterized by two types of work, namely the characterization ampelografica of the 16

IDENTIFICACIÓN CLON Nº	CLON 55-08-05	CLON 15-11-05	CLON 75-07-02	CLON 55-08-05	CLON 15-11-05	CLON 75-07-02
FECHA	05-abr-10			15-dic-10		
Alcohol % v/	14,82	14,35	14,10	15,11	14,30	14,24
Extracto Seco Total g/l	32,07	30,15	32,01	28,15	28,77	29,74
Azúcar Reductor g/l	2,74	2,09	2,23	2,19	2,50	2,31
Acidez Total g/l	6,90	7,59	7,31	5,52	5,73	5,80
Acidez Volátil g/l	0,13	0,08	0,12	0,54	0,37	0,40
Ácido Málico g/l	2,79	2,34	2,17	0,80	2,56	2,20
pH	3,60	3,26	3,39	3,68	3,79	3,78
SO2 Total Real mg/l	48	42	38	65	71	69
SO2 Libre Real mg/l	3	6	5	25	21	22
SO2 Molecular mg/l	0,08	0,33	0,18	0,52	0,33	0,36
INT. COLOR	1917	2256	1863	1185	1366	1259
MATIZ	0,45	0,41	0,44	0,67	0,62	0,63
ANT. TOTALES g/l	1,06	1,07	0,99	0,63	0,65	0,59
ANTOC. LIB. g/l (POR PVPP)	0,81	0,15	0,14	0,23	0,22	0,23
ANT-TANINOS g/l	0,24	0,92	0,84	0,40	0,42	0,35
IPT	44,6	41,3	39,3	44,9	44,2	41,8
IND. DMACH	55,9	55,5	50,4	33,3	28,1	27,5
PODER TÁNICO	4,5	8,4	6,1	6,6	7,9	3,7
PESO MOL. MEDIO TAN.	1638	1666	1816	2677	3137	3204
TANINOS g/l (por IPT)FACT.12	1,75	1,47	1,45	2,49	2,40	2,31
REL MOLAR TAN/ANT	0,51	0,41	0,41	0,74	0,60	0,62
ETANAL TOTAL mg/l	68,3	43,4	36,8	27,4	34,2	32,3
ETANAL COMB. AL SO2 mg/l	12,9	10,2	9,4	9,5	13,2	12,5
ETANAL LIBRE mg/l	55,5	33,2	27,4	18,0	20,9	19,8
ÍNDICE DE ETANOL	9,9	6,2	6,6	4,6	7,5	4,5

selected clones led by Ing. Susana Matus, and the genetic characterization with DNA analysis conducted by Ing. Celeste Arancibia and Dr. Liliana Martínez.

Ampelographic characterization

It was done considering the parameters suggested by OIV for different varieties of Vitis vinifera. The observations were carried out on the plant, the clusters and the stems.

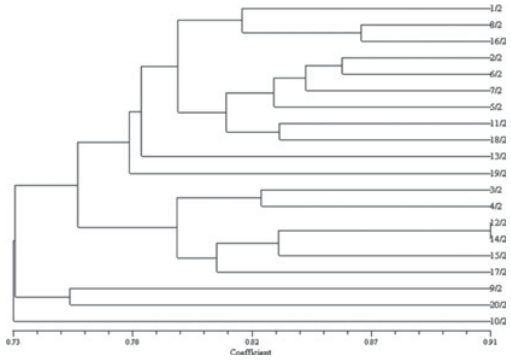
Phenlogy: as for the time of sprouting, all clones behave similarly (early date). As respect to veraison, three types of behavior have been registered: early-term (only one clone), middle-term (9 clones) and late-term (6 clones). Adult leaves: the leaves are medium in size, orbicular shaped, flat, entire and lobed. Except for clone TA 15, that shows weak blisters, the rest of them have medium to strong blisters. The teeth are straights and medium in length. The petiole sinus is semi-open without any particular characteristic i.e. the presence of teeth. Petiole sinus flush is absent. The upper lateral sinuses are open and have an average depth in most of the clones, except for clones TA 14, TA 15 and TA 16 in which are superficially marked. The anthocyanin pigments of the major veins in the upper face of the leaf is below average in most of the clones, except again for clones TA 14, TA 15 and TA 16 in which are very weak. Spider hairs presence is average in the majority of the clones, and low in clones TA 14 and TA 15. Pubescence is absent or very little, with the exception of clones TA 14, TA 15 and TA 16 which present a greater quantity. Except for Clone TA 14, in which the length of the petiole is shorter than the length of the main vein, in the remaining 15 clones the length of the petiole is slightly shorter or equal to the length of the main vein. Inflorescence: as it is very typical of Malbec, all the 16 clones present perfect hermaphrodites flowers.

Clusters are medium in size, loose and with short peduncle.

Berries: they are small, round, blue-black color, c skin, invisible navel; soft, juicy and colorless pulp; difficult separation of the pedicel, and the presence of seeds. Branches are grooved surface and reddish-brown color. Shoot, young leaves and tendrils: the apical portion (apex) is open, with medium intensity anthocyanin pigmentation, woolly, with erect branches, with the dorsal side of the internodes green with red stripes and the ventral side totally green. The pubescence in the branches is null o very weak. In most of the clones, tendrils are discontinuous, short to medium length. Only clone TA 20 has medium length tendrils. Leaflet are coppery, with medium density spider hairs and little pubescence.

Genetic characterization with DNA analysis:

It was conducted with the use of molecular markers S-SAP. These have allowed us to clearly distinguish all the clones (the Simple Matching coefficient varies between 0.73 and 0.91), but the genetic clusters thus obtained do not reflect the groupings of the clones made based on the quality of the wine produced. This technique has however proved to be useful to distinguish unambiguously all clones and can be used also in the future as a method of clonal selection.



Dendrogram and genetic grouping of Malbec clones.

Conclusions

The project described here is a work of great scope, which aims not only to the interest of a company and a time horizon, but look to the future of Argentine wine. It is an important contribution for the better knowledge of Argentine Malbec and maybe considered a pioneer work on clonal selection of this variety in the sense that there have been no many attempts to tackle this area of work in such a wide and comprehensive manner.

The selection work presented here has many implications. It aims to develop selection tools appropriate for the unique recognition of Malbec clones and this will make it possible in the future to respond more effectively to the need to distinguish clones, clearly and quickly, on the basis of their unique characteristics.

In particular, here we are thinking about:

- the ability to distinguish clones that have specific capability to adapt to different weather situations, a theme that is becoming increasingly urgent for viticulture and for agriculture in general as a result of climate change evidence in course;
- the development of tools for the unique identification of superior clones of Malbec – the **"VERO MALBEC CIRCLE"** – that represent the best that can give Malbec in Argentina dates in environmental conditions, then put at the disposal of long-term representatives of 'Argentine wine;
- the opportunity to give Argentinian winemaking tools to identify and distinguish uniquely their own clones, related to its terroir;
- the possibility to develop in the near future a scientifically based sensory map of Malbec, for the main wine producing regions in Argentina.

On a closer inspection, this work could have many effects on viticulture in Argentina, by providing for the first time an instrument of identity, as well as identification. In this context, therefore, we believe that this effort has not only a scientific importance for the improvement of winemaking, but also a social and socio-economic impact for viticulture in Argentina.