Towards a relationship between institutional clonal selection, mass selection and private clonal selection of grapevine cultivars

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Abstract:
Each grape cultivar is composed of a population of individuals that are genetically different. Clonal selection has allowed the purification and improvement of the global quality of the vegetative material for a limited number of grape varieties. But choosing clonal selection as the unique propagation method has decreased considerably genetic diversity. In order to carry out the selection of clones in the future, a diversified background of genetic resources must be available. Institutional collections (conservatory) are not able to preserve sufficient biodiversity. Genetic resources could be conserved by winegrowers through mass selection. 5% of the total acreage planted in vine in Europe done by private mass selection would represent 1000 times the actual capacity of institutional collections. A methodology of private mass or clonal selection is proposed. An economic study shows that the overall extra-charge is 13000 per hectare for mass selection plot and 69000 per hectare for a clonal selection done by a private company. It is urgent to promote private selection in order to preserve vine biodiversity.

Keywords: vine, genetic resources, clonal selection, mass selection, biodiversity

Introduction

Almost all of the cultivated wine grapes belong to the *Vitis vinifera* species. Each grape cultivar is composed of a population of individuals that are genetically different. The existence of some degree of genetic diversity within grape cultivars thus results in variability of characteristics such as fertility, berry size or the ability to accumulate sugars during the ripening period. From a single vine stock, it is possible to obtain a large number of genetically identical individuals through successive cuttings. In biology, a group of individuals sharing the same genome is called clone From the 1960s, the selection of clones aimed to promote the propagation of vine displaying a certain number of predefined characteristics (virus-free, sufficient production, high sugar content, etc). Clonal selection has allowed the purification and improvement of the global quality of the vegetative material planted. Nonetheless, it must be acknowledged that the qualitative potential of the certified clones varies depending on the grape cultivars. Indeed, many high qualitative clones have been selected in Bordeaux area for example for Merlot or Cabernet-Sauvignon, whereas the results regarding Cabernet franc and Semillon are much more elusive. Although there is no reason today to question the validity of clonal selection, it is important to point out that this method of vine stockpropagation contains two important limits. These should bring us to consider other methods of selection to be led in parallel. Firstly, the qualitative impact of clonal selection is very limited in certain grape cultivars like Cabernet franc and Semillon, but also on a great majority of minor cultivars of Bordeaux region like Petit Verdot, Carmenère or Cot (Malbec). Secondly, by choosing clonal selection as the unique propagation method of vine stocks, the genetic diversity that exists within the different grape cultivars is dramatically depleted. The erosion of genetic diversity

prevents any possibility of selecting new clones in the future. This is especially critical as the needs of winegrowers vary in time. In the 70's, the winegrowers needed yield-oriented clones producing grapes with high sugar levels. Today, a high yield is no longer an imperative but the profession wishes to have access to improved disease-resistant clones. Tomorrow, based on global warming predictions, we might need late-ripening clones or ones producing grapes with low sugar content. But to carry out the selection of clones meeting future expectations, a diversified background of genetic resources must be available. There are institutional collections that manage the conservation of genetic resources. These collections are valuable but they cannot alone guarantee the conservation of the grapevine genetic resources. Genetic resources can also be efficiently conserved, and at a lower cost, directly by the winegrowers. To be effective, this system needs strict selection protocols and an evolution of regulations.

Conservation of genetic resources

Institutional germplasm collections have been created to manage the preservation of the genetic diversity of grape cultivars. They certainly are useful but it is unreasonable to rely solely on these collections for the conservation of genetic resources as they have a few weaknesses. Their surface area and their quantity are insufficient to ensure the extent of the grapevine genetic diversity. They rely on public and parapublic funding, which is never guaranteed. Grouping together all the genetic resources within a small number of sites also increases the occurrence of major accidents: climatic catastrophe, disease outbreak, etc. The case of the Vassal site (INRA-France), which regroups the largest number of grape cultivars and clones in France, is a good example of the vulnerability of these collections. It is located on a leased site and INRA has been threatened with eviction several times. Indeed, a real estate promoter wants to build a residential complex on this site, which is close to the Mediterranean Sea. In addition to the institutional collections, genetic resources could be conserved by winegrowers. Without undermining the system of institutional clonal selection, which has proven to be efficient, it is advisable to promote private mass selections on a minor but significant proportion of the planted area, for example 5%. This ratio would allow the preservation of approximately 40 000 ha of genetic diversity in France, 150 000ha in Europe, which represent about 1000 times the actual capacity of institutional collections. Private clonal selections can also contribute, but to a lesser extent, to the preservation of biodiversity. It is important to supervise these private mass and clonal selections, especially to reduce the incidence of viral and phytoplasma transmission. Mass selection is the simplest way to conduct a private selection of vegetative material: the most interesting vines are identified in the vineyard before being mass-propagated by cutting and grafting, without traceability. A better traceability can be acheived by setting up these parcels with repetitions of vine sets originating from a beforehand identified mother vine. Mass selection also provides the possibility to push the selection further to a true private clonal selection. This path evidently requires significant financial means compared to mass selection and only modestly contributes to the preservation of biodiversity.

Which parcels can be used to carry out private selection?

Only parcels planted with non-clonal material are of significant interest for private selection. Most of the time they are old parcels planted before the general commercialisation of clonal material in the 1970's. The majority of the parcels planted before 1970 and the totality of the parcels planted before 1960 meet this criterion. Today, these parcels are coming to the end of

their lifespan, therefore increasing the urgency to save their genetic resources. It is especially worthwhile to undertake selections in the ones having high qualitative potential. Throughout the selection process, every virus-infected vine will be discarded. It is thus not advisable to carry out selections in highly-contaminated parcels as the high rejection rate would increase the cost of selection.

Methodology of mass selection

The selection of vegetative material starts in the field with an acute visual assessment of the candidate parcel. The winegrower must set his own selection criteria. One important selection criterion is the yield, which must be neither too high nor too low and in accordance with the economic and qualitative objectives of the estate. To evaluate the yield, we can look at the level of fertility (number of clusters per pruning bud), cluster size and berry size. The latter criterion is also indicative of the qualitative potential of the vine as it is easier to produce high quality red wines with small berries. Another important selection criterion concerns the morphological aspect of clusters; within cultivars naturally producing tight clusters such as Cabernet franc, the individuals producing looser clusters are less susceptible to *Botrytis cinerea*. The visual observation can be completed with berry tasting. The best time to carry out these observations is during the week preceding harvest.
Once the parcel has been chosen, it must be investigated to mark each vine stock that meets the selection criteria. Those displaying symptoms of virus diseaeses (misshapen leaves, speckles on leaves, high level of *millerandage*, curled discoloured leaves, shortened internodes, fasciation on the shoots) or phytoplasma diseases (*Flavescence dorée*) should be discarded. A large pool of vine stocks (several hundreds per parcel) must be identified the first year of selection. The second year, the marked vine stocks are re-monitored and those still meeting the selection criteria keep their marking. The marking is removed from the vine stocks no longer displaying the selected characteristics. This procedure is repeated a third year. All the vine stocks that have kept their marking for three consecutive years have successfully met the selection criteria. These stocks present a real interest for mass propagation. Each selected vine stockis annotated. A fragment of shoot from each selected vine stock is sampled and sent out to a laboratory to check for the absence of the main viruses (ELISA tests). The existence of many viral diseases makes it impossible to screen for every known virus. The main viruses to test for are those responsible for the *court noué* disease (Arabis Mosaic Virus or ArMV and Grapevine FanLeaf Virus or GFLV) and the leaf roll viruses (Grapevine Leaf Roll Virus or GLRV n°1 and n°3). Once the test results are available, cuttings are sampled from each virus-free selected vine stock. The cuttings are sent to a nursery to be grafted onto the rootstocks requested by the winegrower to make the vine plants. In the case of a traditional mass selection, there is little or no traceability between the mother vine stockand the resulting □grafted vines□ the cuttings are mass-propagated. The main difficulty associated with vine propagation by mass selection is that it requires a certain level of anticipation mostly because of the three-year observation period. As for the means employed, it must be taken into account: the time to carry out the field observations required for the selection (approximately one day of observation per hectare per year during three years), the time to sample cuttings for analyses (2 days of work per hectare), the cost of analyses (approximately 20□plus taxes per tested vine stock), and the extra-cost of made-to-order vine stock (approximately 0.20□plus taxes per vine stock). Around 25 vine stocks can be made from the pruning wood cuttings sampled from a single vine stock(this number can be higher depending on the vigour of the vine). To make 5000 vine stockss, a minimum of 200 virus-free vines must then be selected. Therefore, it is important to mark hundreds of vines during the first year of selection to prevent ending up with a number of vine stocks lower than

required to make the quantity of needed grafts. Once interesting vines are marked, their cuttings can be sampled every year. Two years after planting, it is also possible to use the young grape vine as a cutting nursery. Whether cuttings are taken from the initial pool of selected vines or from the young plantation, it is recommended to test regularly the vegetative material to verify the absence of contamination by viral diseases.
It is possible and advisable that a parcel originating from mass selection be equipped with a system allowing repetitions of the "offspring" of each mother vine stock. Such a system provides full traceability and therefore the possibility, if need be, to undertake a second phase of selection leading to clonal selection.

Methodology of private clonal selection

It is possible to push the mass selection further to the stage of private clonal selection as the first steps of both selection methods are identical: three years of visual monitoring and ELISA screening to confirm the absence of viral diseases. Clonal selection differs from mass selection starting at the stage of grafting. In the case of a clonal selection, full traceability is kept at the time of grafting allowing each vine to be linked to its mother vine stock. Each bundle of cuttings will produce a set of vine plants, which will be annotated with the number of the mother vine stock, and this set constitutes a clone. The extra-cost for the making of the vine stock is higher than for mass selection because of traceability (approximately 1 € plus taxes/vine stock). The vine plants are kept in a collection study and the plantation is divided into blocks. All the numbers (set of clones) are present in each block, for example 5 vine plants from each number in each block. Once the vine starts to produce grapes, it is possible to test each number with replicates in order to perform statistical analysis of the measurement and observation results. Most of the measurements are relatively easy to implement like yield weight per vine, cluster weight, level of fertility, berry weight, sugar content at ripeness, total acidity at ripeness or the level of *Botrytis cinerea* at the time of harvest. After a three-year period of measurements and observations of the collection study, which includes tens of clones, it is possible to identify a few clones perfectly meeting the objectives of the winegrowers. These will be propagated with priority. At this point, it is interesting to perform micro vinifications to evaluate the organoleptic characteristics of the selected clones. If the winery is not equipped to carry out micro vinification, it can be outsourced to a research or development institute.
A clone from the collection study (a few tens of vines) will produce enough cuttings to produce hundreds of vines. It is therefore impossible at the beginning to plant an entire parcel from a single clone derived from a private clonal selection. Each newly planted parcel can serve as a nursery on condition that it is regularly tested to check for the absence of contamination. After a few years, it is then possible to plant the desired one or several clones.
Clonal selection is a fascinating process but it requires a considerable time investment: a dozen of years approximately before the selection of one or a few clones (table 1). The overall cost of clonal selection is obviously much higher than of mass selection but it remains reasonable. The cost mostly comes from observation time, ELISA analyses, grape analyses, cost of micro vinifications and extra-cost of nursery-made vines with full traceability. It is better to maintain the collections pure when dead vines are replaced, which involves having each vine prepared with full traceability. The production from the collection study can be blended into the estate production so there is no yield loss, with the exception of the grapes used for micro vinifications.

Table 1 – Timetable of mass selection and clonal selection

Number of years Mass selection	Step	Number of years Clonal selection
3	Selection in the mother parcel	**3**
1 or 2 depending on the size of the parcel to be planted	Production of vines from the collection study	**1**
2	Time before the collection study or the production parcel starts to produce	**2**
	Minimum number of years of collection study monitoring	**3**
	Tasting of the micro-vinified wines from the collection study	**1**
	Production of vines from the selected clones	**1**
6 to 7 years	**Total**	**12 years minimum**

Cost of mass selection and private clonal selection

A traditional mass selection requires approximately 5 days of work by a highly qualified person (1000€ plus taxes/day). The average cost of serological tests (ELISA analyses) is 8000€ plus taxes. The extra-cost of vine plants, for the planting of a parcel at a density of 5000 vines/ha, is approximately 1000€ plus taxes. Overall, the extra-cost of mass selection is approximately 13 000€ plus taxes for a one-hectare parcel. This investment might seem significant with regard to the first planted hectare. However, afterward, the winegrower can propagated this vegetative material on many other parcels, thus reducing the cost. Mass selection must be considered as a significant initial investment, which is recovered throughout the new plantations.

A private clonal selection requires 23 days of highly qualified work. The cost of the ELISA analyses is 4000€ plus taxes. For 25 clones to be tested, micro vinifications are carried out on the 10 best ones. This represents 30 000€ plus taxes for the three-year study and approximately 2000€ plus taxes/year for chemical analyses. The extra-cost for the making of vine plants is higher than for a mass-selected parcel; an average total of 6000€ plus taxes in part deferred over time. The overall extra-cost for a private clonal selection is approximately 69 000€ plus taxes minimum for a total of 23 days of work. This partly explains why today only the more prestigious estates choose to get involved in the private selection process. As for mass selection, the investment is recovered over newly planted areas.

Table 2 □Extra-cost of mass selection and private clonal selection, with respect to a standard plantation, for a one-hectare parcel planted at a density of 5000 vines/ha

Mass selection	Step	Clonal selection
3 days/ha	Selection in the mother parcel	3 days/ha
2 days, 400 analyses 8000 □plus taxes	ELISA analyses	1 day, 200 analyses 4000 □plus taxes
1000 □plus taxes	Making of vines from the collection study. Extra-cost of 0.20 □plus taxes/vine (Mass selection), 1 □plus taxes/vine (Clonal selection)	6000 □plus taxes
Setting up and maintenance of a standard young vineyard parcel (1ha)	Setting up of the collection study (3 years) or the production parcel	Setting up and maintenance of a standard young vineyard parcel (20a)
	Measurements carried out on the collection study	12 days
	Data analyses 1day/year for 3 years	3 days
	Specific replacement of dead vines	3 days
	Micro-vinifications (1000 □plus taxes x 3 years x 10 clones)	>30 000 □plus taxes
	Tasting of micro-vinified wines from the collection study	1 day
	Analyses of grape musts and wines	6000 □plus taxes
	Making of vines for the production parcel : over-cost of 1 □/plant	5000 □plus taxes
5 days 5000 □plus taxes	Total highly qualified work	23 days 23 000 □plus taxes
>13 000 □plus taxes (5 days)	**Overall extra-charge of selection**	**>69 000 □plus taxes (23 days)**

Conclusion

Institutional clonal selection has played an important role by purifying the vegetative material. It also has a few drawbacks, the major one being the extreme erosion of genetic resources. To maintain the genetic diversity in the vineyard, other methods of selection, such as mass selection and private clonal selection, on a significant surface (around 5%) of the vineyard should be promoted. These alternative selection methods represent an important investment for the winegrowers who decide to use them. Considering that they contribute to the preservation of genetic diversity therefore providing a service to the community, public funding with, among others, a mission of free technical assistance, should be conceivable. Moreover, this kind of framework would prevent that these selection methods become a source of viral transmission. The winegrowers who conduct these alternative methods also need to have a good sense of organisation and anticipation as a serious mass selection requires 3 to 4 years of fieldwork before being able to exploit the vine plants. This period goes beyond 10 years in the case of a private clonal selection.