

GRAPE BREEDING FOR THE RESISTANCE TO DISEASES AND THE QUALITY OF MUSTS

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Two hundred years after the birth of Charles Darwin (1809-1882), it is worth remembering what the father of the evolution theory said about the living organisms, that is they accumulate over time mutations on which the natural selection operates by favouring the survival of the 'best adapted' individuals. Grapevine does not make exception and, like other species in nature, is subject to natural selection pressure, related to changing climate and biological environment that surrounds it.

The man tends to select genotypes with superior features and keep them growing for as long as possible, especially when the vegetative propagation - it is the case of the grapevine - helps overcoming the heterozygosity problem. Growing the same genotypes for long periods creates traditions of great historical and cultural interest, but from the biological point of view it will prevent those genotypes from reproducing, evolving and adapting to the changing conditions of life.

In the past century, the need to protect crops from pests and pathogens has prompted the development of synthetic chemistry so that nowadays about 10 metric tons of phytosanitary products are used in agriculture each year.

The situation has long been regarded as unsustainable and since the second half of the last century, breeding programs for introducing resistances to different pathogens and parasites have been initiated in many species, with the intent to reduce the pressure on the environment and human health caused by pesticides. Grapevine is one of the crops that has received less attention by plant breeders. As a result, grape protection within the European Union requires 65% of all fungicides used in agriculture, compared with an area occupied by grape that reach only 3.3% of total arable land (Eurostat report 2007).

Indeed, several breeding programs aimed at introducing resistance to the three diseases introduced from the Americas in the '80s (downy mildew, powdery mildew and *Phylloxera*) have been active over 100 years in some European countries, USA and Canada. Results achieved so far are interesting with regard to resistance (Fisher et al 2004), but less interesting as far as the quality of must is concerned, due to high heterozygosity of the grape genome and the consequent unpredictability of the results of cross breeding (Bavaresco 1990).

The information gained from grape genome sequencing projects just completed (Jaillon et al 2007, Velasco et al 2007) seem to open also for grape the new frontiers of the marker assisted selection, which promises to get in few cross generations more interesting results than those obtained by traditional breeding techniques used throughout the whole last century.

The most interesting results achieved right now deal with the resistance to pests and diseases. Three genes/QTL of resistance to downy mildew, two genes and four QTLs of resistance to powdery mildew, one gene of resistance to *Xylella fastidiosa*, one QTL of resistance to *Phylloxera*, and one gene of resistance to nematodes of the genus *Xiphinema* have been identified, mapped, and in some cases cloned so far (Testolin et al 2008). There are already programs aiming to pyramidize some of these resistances, that will make them more durable over time and the molecular markers associated with those resistances are already routinely used for the early selection of seedlings (Testolin et al 2008).

Regarding the quality of musts, the story is more complex as the quality of must is the product of hundreds of compounds that, in connection with soil and climate, contribute to create

those cultivar-*terroir* combinations, which have made great several viticultures and from which we are not willing to break away unless with a justified concern.

Even in this case, selection methods are making rapid steps forward. Consider, for example, some classes of compounds such as anthocyanins and volatile compounds.

Unlike other species, in which anthocyanins are synthesized by relatively simple biochemical pathways, the grape in the course of its evolution has experienced an expansion of the family of flavonoid hydroxylases, particularly F3'5'H, those ones that convey the synthesis towards the anthocyanins that give the darkest colours to the wine (delphinidins, malvidins, and petunidins). As many as 16 different copies of this gene have been identified in grape, thanks to the recent sequencing of the genome. Many of them have specialized functions in relation to the evolution of berry ripening and climatic characteristics (light, temperature, and water stress) (Falginella et al 2010). The knowledge of these isoforms allows for a targeted selection of seedlings, depending on the type of anthocyanins desired in wine.

Another fascinating topic is represented by the volatile compounds that contribute to wine aroma. Such compounds are found in berry, must and wine at very low concentrations and this makes it difficult to detect them with traditional instruments; moreover, these compounds are often bound to or interacting with other compounds (sugars, amino acids) and this make more tricky their extraction from the matrix where they are. The development of new extraction techniques such as 'Solid Phase Micro Extraction' (SPME) and 'Stir Bar Sorptive Extraction' (SBSE), as well as the introduction of more sensitive instruments like gas chromatographs coupled with new generation mass spectrometers allowed to develop suitable methods to analyze those complex matrices, extracting information on many compounds, some of which not yet known.

With current technologies, a few hundreds of compounds can be simultaneously extracted and analysed, but we could foresee that thousands of compounds can be analyzed in a single run within a few years, following a further refinement of instruments and the underlying chemistry. Once the compounds have been identified with such rapid analytical methods, two roads open: the first one deals with the selection of seedlings on the base of their aromatic profiles; the second one, even more ambitious, regards the study of the genetic control of those compounds and the molecular marker assisted breeding. In other words, knowing the genetic determinants of a number of key compounds of the flavour profile, an early selection of seedlings can be carried out by means of molecular markers associated to genes that control the synthesis of those compounds, without waiting for sexual maturity of plants and the production of grape clusters (Di Gaspero e Cattonaro 2009) .

We cited two examples of assisted selection for two important components of wine quality, the anthocyanins and the aroma compounds. Another few cases are more focused on classes of flavour compounds that are already well characterised at the chemical level, such as terpenes or methoxypyrazines, for which the identification of genetic determinants is well advanced (Mattivi et al 2008).

To conclude we would like to point out that the vine, which did not enjoy great attention in the past by geneticists, is experiencing a period of great scientific excitement, thanks to the grape genome sequencing projects. We are paving the way for a new approach to the selection, based not only on the phenotype (agronomic evaluation, chemical analyses, microvinification), but first of all on the genotype, that is all information on genes and genetic factors that control their expression, which come out from the project of sequencing and annotation of the grape genome.

It is not an easy task and the work done so far is only a very small part of what is possible to achieve with the resources and the knowledge currently available and those that the scientific community will make available in future years.