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Targeting the most suitable rootstocks for the cultivation of Gaglioppo

1. A study on the interactive behaviour between grapevine, rootstock and soil

Before the widespread employment of new selections, it is particularly interesting to field-test the suitability of the countless rootstock combinations that can be used with the selected material. As a matter of fact, among the factors which contribute to the final quality of the Gaglioppo grape, all pedoclimatic and cultural conditions being equal, rootstock plays an essential role.

As we know, a vineyard must be implanted grafting the chosen variety on american rootstocks, in order to avoid phylloxera attacks. Every one of the large number of rootstocks commonly used in breeding, though, shows a different tolerance/sensitivity to the physicochemical properties of the soil, and metabolically interacts in a different way with the Vitis vinifera scion. For this reason, different plants with the same scion will show rather diversified vegetative and productive features depending on the rootstock; similarly, their grapes will have different enological potential. It is important to keep in mind that the choice of the rootstock at the moment of implantation is an unchangeable decision, which will affect the productive activity of the vineyard for its whole life. Plant nurseries use a lot of rootstock varieties, therefore it is not easy for winemakers to find their way and make the right choice at the moment of buying his breeding material. European vine variety, soil characteristics, climate, breeding system and wine typology are just a few factors that will interact with the rootstock and affect the plants' response for the whole vineyard's existence. For all these reasons, having the most objective parameters in order to choose the most suitable rootstock at the moment of implantation of a new vineyard is of crucial importance. These parameters, unfortunately, are usually unavailable for Calabrian grapevines, even for those of primary importance for the region such as Gaglioppo. To this day, winemakers rely on tradition, on nurserymen's recommendations or on the available rootstocks at the time, with not so remarkable effects on the vineyard's future quantitative and qualitative response.

With these thoughts in mind, we felt the need to start an experimentation aimed at identifying Gaglioppo's behaviour on different rootstocks, the goal being to locate the most

suitable rootstocks for the culture of the most important Calabrian grapevine in the pedoclimatic environment of inland Cirò. With our study, we did not just evaluate the most common, though essential, agronomic and enological aspects of the different rootstock combinations but, resorting to the most innovative biomolecular techniques, we chose to go deeper, examining the changes in gene expression on different rootstocks with different Gaglioppo scions in water shortage circumstances, and to provide a global vision of the molecular processes combined with the interaction between rootstock and scion.

In 2012, at our Critone estate in the Strongoli area, we created an experimental vineyard grafting the same Gaglioppo clone (I-Librandi CVT 80) on 20 different rootstocks. The selected Gaglioppo clone, the result of years of studies made by our company in partnership with the Virology Institute of the National Research Council in Turin, is characterized by a suitable phytosanitary condition regarding viral diseases, and by an ideal agronomic and enological potential. The clone is therefore capable of best expressing the grapevine's potential. The twenty chosen rootstocks, instead, represent a very wide range of aptitudinal characters regarding the interaction between the scion and the soil. Sixsteen of them are commonly used in the winemaking nusery business, while four of them are experimental rootstocks.

Based on the data we collected in the two-year period of observation (2014-2015), the results, though related to a young vineyard, allowed us to identify a number of grafting combination that can successfully address Gaglioppo's quantitative and qualitative features in Cirò's hot and dry environment. Gaglioppo showed the best productive inclination and high enological potential when grafted on 1103 Paulsen, 775 Paulsen, 3309 Couderc, 17-37 Millardet and de Grasset and 779 Paulsen rootstocks. To this cluster we should add the 110 Richter combination, for its optimal enological potential, unfortunately counterbalanced by a scarce productivity. As a rule, we found that Berlandieri x Rupestris rootstocks (except the not so suitable 140 Ru) and the 3309 C rootstock, belonging to the Riparia x Rupestris cluster, showed the most interesting response in the developing, post-implantation stage and in the vineyard's first productive years, unlike the Berlandieri x Riparia cluster and all the rootstocks with at least one vinifera parent. These roostocks, not surprisingly commonly considered "southern", have also the advantage of being more easily available at the local nurseries, thus simplifying their use for new Gaglioppo implantations.

Of course, Gaglioppo's aptitudinal behaviour on the examined rootstocks will need to be confirmed in the years, following the progressive ageing of the vineyard; however, the established results are a first essential contribution to help the winemakers choose the most suitable rootstock to improve their Gaglioppo grapevines.

2. Rootstocks' effect on Gaglioppo: molecular analysis

While running the previously described agronomic and qualitative tests, we also conducted other sudies aimed at gaining a deeper understanding of the molecular changes caused by different rootstocks on the Gaglioppo clone's scions (I-Librandi CVT 80) in a water shortage situation.

We ran molecular analysis of gene expression on a large scale on the different grafting combinations: in other words, the entire RNA molecular collection inside the leaf (transcriptome) has been analyzed. In particular, among this RNA molecules cluster we evaluated those relating to messenger RNA (mRNA), which specifies the proteins code and possess the greatest informative potential. These analysis showed that the rootstock prompts deep molecular alterations in the scion, which are in turn responsible for phenologic and productive changes. The water shortage that the plants sufferend in July and August may have emphasized those mutations. In those conditions, compared with the other combinations, the GAG-41B grafting responds in a substantially different way to the environmental strains. Diametrically opposed is the GAG-1103P combination, with GAG-KOB, GAG-3309C and GAG-17-37 standing somewhere in the middle, though their response is more akin to the GAG-1103P's one. This partition, revealed by the molecular analysis, conforms to the productivity and strenghtness data obtained for each grafting combination. Regarding the genes that responded the most to the water shortage stress, we gathered that GAG-1103P has the higher ABA levels, as well of VvNCED and VvDH11 genes. Therefore, 1103 P seems to be causing in the I-Librandi CVT 80 Gaglioppo clone a higher sensitivity to water shortage compared to the other rootstocks, thus inducing in the scion a premature protection response to this kind of stress. On the other hand, 41 B seems to be reducing, almost erasing, these protection responses in the scion, showing pretty unchanged ABA and VvNCED e VvDH11 marker genes levels during the long water shortage cycles. We found especially interesting the activation, in some grafting

combination, of a great amount of genes potentially related with protection responses to biotic stresses (glucanases, chitinases, Jasmonate metabolism, disease resistance protein and pathogenesis-related proteins) and to secondary metabolism (phenylpropanoids, among which stiblens). 41 B in particular, compared to 1103 P, used as reference rootstock, has been found responsible for causing in Gaglioppo a greater activation of this kind of genes. Overall, these molecular data partly confirm the previously described results, i.e. that GAG-1103P binomial is the strongest, but at the same time they show that it is more affected by water shortage than the GAG-41B combination. Viceversa, GAG-41B precociously activates its protection responses to several types of stress, including those of a biotic nature, and though less vigorous and with a fluctuating production it could be more suitable to oppose, for example, pathogenic fungal attacks.

This interesting information will have to be confirmed by the next vintages and further in-depth analysis: for example, if and how the different resistance gene modulation in the scion caused by diversified rootstocks may affect the plant's sensitivity towards pathogenic leaf fungi; or if and how the hyper-expression of stilbens in the leaf may affect the qualitative features of the grape.

The results of this technical-scientific work have been published in the scientific essay titled *Studio per l'individuazione dei portinnesti più adatti alla coltivazione del Gaglioppo* (Study to identify the most suitable rootstocks for the cultivation of Gaglioppo), Rubbettino, 2015.