**CLONE SELECTION OF GREEK GRAPEVINE VARIETIES: A SUCCESSFUL VITRO HELLAS-VCR JOINT WORK**

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VIVAI COOPERATIVI RAUSCEDO (VCR) is a cooperative nursery of 250 partners founded in 1933 and settled in Friuli (North Eastern Italy). It is the world leader on the market of the grapevine propagation material. VCR has an yearly average production of over 60 million grafted plants in 4.000 rootstock/scion/clone combinations and has worldwide appreciation for the quality of its plants.

In 1967 VCR established a 30-hectare experimental farm, aimed to selection, evaluation and registration of new clones, equipped with a laboratory for phytosanitary screening (ELISA and PCR) and with a micro-winery for enological clone characterization. At present more than 317 registered proprietary clones are successfully marketed in more than 28 countries across all continents.

Vitro Hellas is a private company established in 1986 in Northern Greece, mainly involved in production and trade of high-quality propagation material. Vitro Hellas produces different cultivars of fruit trees and is the official distributor for the Greek market of VCR grafted vines. Its research activities have been developing through several projects in cooperation with Greek and foreign Universities and research Institutes.

The most significant international and local varieties of the Mediterranean basin have been included into VCR's selection programs. At the end of the eighties, thanks to Vitro Hellas advice, VCR started a forward-looking program for the selection of Greek varieties (Assyrtiko, Liatiko, Limnio, Mandilari, Moschofilero, Moschomavro, Roditis, Xinomavro, etc.), since in those times there were no clone selections for these Greek cultivars. This work led to the registration of 7 clones and to the implementation of a high-level sanitary and oenological genetic platform. The results of these selections and anticipations of the work in progress will be presented.

**MATERIALS AND METHODS**

Pruned wood of the selected genotypes was collected from old vineyards of Assyrtiko, Liatiko, Limnio, Mandilari, Moschofilero and Vidiano located in the most suitable Greek areas for each variety . The sanitary state of the material was assessed by ELISA (ARNADIA protocol), PCR and woody indexing. Additional controls were performed with respect of the latest Italian sanitary selection protocol approved in 2008 (GU n. 195, 21-8-2008) (Table 1).

Healthy genotypes were propagated by grafting on healthy rootstock clone Kober 5BB VCR 102 and planted in adjacent rows of 40 vines each, located in the VCR's Experimental Farm. Standard grafted vines of each variety flanked the selected genotypes. Vines were vertically trained and single-cane pruned. Starting from the third year after plantation and for further 3 years agronomical and oenological assessment were carried out. Phenological phases, in accordance with the OIV scale, cluster and berry features (size, weight, shape) and composition (sugar content, acidity, phenolic profile), fertility and productivity of plants and ripening curve, in comparison with the standard population, were evaluated. The crop yield of each genotype was submitted to small scale winemaking, following standard red and white wine protocols. Chemical analysis on stabilized wines were performed. After a few months of rest in the bottle, wines of two consecutive harvests were submitted to tasting commissions of experts in order to evaluate the sensory profiles of the selected genotypes. Official wine tasting form and ranking sensory test were adopted. Agronomical and oenological data were statistically elaborated by ANOVA.

**Table 1**. Sanitary controls performed at VCR Experimental Center and Viticulture Research Center (CRA-VIT)

| | | | | LABORATORY | | WOODY INDEXING: INDICATOR PLANTS |
|---|---|---|---|---|---|---|
| **FAMILY** | **GENUS** | **SPECIES** | **VECTOR** | **ELISA** | **PCR** | |
| CLOSTEROVIRIDAE | Closterovirus | Grapevine leafroll-associated virus 2 (GLRaV-2) | unknown | X* | X* | *Vitis vinifera* Carmenere (*) |
| CLOSTEROVIRIDAE | Ampelovirus | Grapevine leafroll-associated virus 1 (GLRaV-1) | Mealybugs, soft scale insects | X* | X* | *Vitis vinifera* Carmenere(*) |
| CLOSTEROVIRIDAE | Ampelovirus | Grapevine leafroll-associated virus 3 (GLRaV-3) | Mealybugs, soft scale and scale insects | X* | X* | *Vitis vinifera* Carmenere (*) |
| BETAFLEXIVIRIDAE | Vitivirus | Grapevine virus A (GVA) | Mealybugs | X* | X* | Kober 5BB (Kober stem grooving syndrome) (*) |
| BETAFLEXIVIRIDAE | Vitivirus | Grapevine virus B (GVB) | Mealybugs | X* | X* | LN 33 (Corky bark wood syndrome)(*) |
| BETAFLEXIVIRIDAE | Foveavirus | Grapevine Rupestris stem pitting-associated virus (GRSPaV) | Unknown | | X | Rupestris "Du Lot" (Rupestris stem pitting) |
| SECOVIRIDAE | Nepovirus | Arabis mosaic virus (ArMV) | Nematodes | X* | X* | Rupestris "St. George" |
| SECOVIRIDAE | Nepovirus | Grapevine fanleaf virus (GFLV) | Nematodes | X* | X* | Rupestris "St. George" |
| TYMOVIRIDAE | Maculavirus | Grapevine fleck virus (GFkV) | Unknown | X** | | Rupestris "St. George" |
| Unknown | Unknown | Unknown | Unknown | | X | 110 Richter (Vein necrosis, associated to GRSPaV |

*compulsory; ** compulsory only for rootstock : (GU n.195, 21-08-2008)

## RESULTS AND DISCUSSION

Starting on the 1990, overall 412 samples of the mentioned varieties were collected from the most important Greek viticulture sites and inspected. 30 samples (7,28 %) were negatives at the ELISA test, 12 of those (40 %) were negatives at PCR and woody indexing. Independently from the place of selection and the genetic background of the varieties, the most common single viruses detected by the ELISA test were GLRaV1 (17,96%) and GLRaV3 (25,73%), but multiple infections occurred frequently (24,27%) (Figure 1). Individual variety exhibited a similar pattern. 7 genotypes of 12 were chosen for their high sanitary, agronomical and oenological profile:

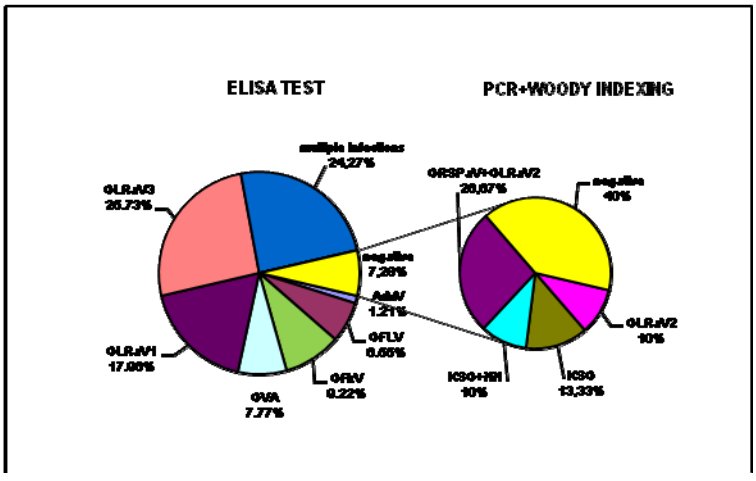


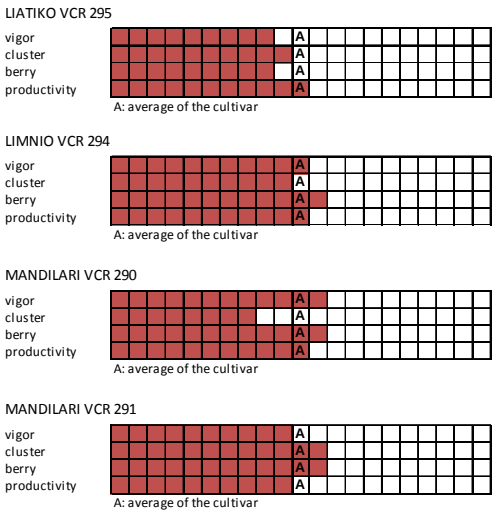

**Figure 1**: Overall sanitary controls on Liatiko, Limnio, Mandilari, Moschofilero and Vidiano varieties from 1990 to 2003. (KSG: "Kober Stem Grooving"; NN: Vein necrosis). ELISA, PCR and woody indexing controls were performed in collaboration with the official National Laboratory of Viticulture Research Center (CRA-VIT) of Conegliano (TV). The sanitary documents issued meet the Italian and European law for clone selection and grapevine propagation.

**LIATIKO**: It is a local variety cultivated mainly in Crete. Despite the similarity with the Italian "Aleatico" there is no evidence to suggest a connection between the two varieties. It is a vigorous variety, medium-precocious, well adapted both in loose and rich soil. It has a high productivity due to the good fertility of the buds but It is sensitive to Botrytis attack during ripening. The Vitro Hellas-VCR clonal research focused on less vigorous biotypes, with good and stable production, a medium size, less compact cluster with a thicker berry skin.

The clone **VCR 295** was isolated from Boutaris' vineyard, in Skalani (Crete Island). The vigor and size of the berry are slightly below the average of the cultivar. The productivity is stable and uniform. The cluster is a bit smaller (Figure 2A) and less compact than the average, cylindrical and elongated, with two noticeable wings. The wine is vivid red and has a very good structure. The palate is full and It is enriched in ripening red fruits flavors and little spicy aromas. The acidity is balanced (Figure 3). It is suitable either for dry or dessert wine since the grapes can be easily withered.

LIMNIO: It is an indigenous variety to the Aegean Island of Limnos now cultivated in the Chalkidiki Peninsula, in central Macedonia and in Thessalia. It's regarded as the most ancient Greek variety since It's believed to be, almost certainly, the same "Lemnia" described by Aristotle. It is a late ripening variety, easily adaptable in gravel and loose soil due to its strong draught resistance. The fertility of the basal buds is good and It fits short pruning. Clonal research focused on small cluster biotype with a good polyphenolic profile-above all what concerns anthocianins- and a favorable acidity supporting the high must weights.

**Figure 2A**: Agronomical profiles of the selected clones in comparison with the average (A) of the standard population of each variety.




The clone **VCR 294** was isolated from Tsantalis'vineyard in Agios Pavlos (Chalkidiki Peninsula). Vigor and productivity are on average. The cluster is a bit smaller than the average, pyramidal, with a short wing. The berry weight is slightly above average (Figure 2A). The skin is thick and equally pigmented. The wine has excellent colour and a robust structure. It is particularly rich in fine red fruits and spicy aromas. The typical fresh herbs quality is agreeably detectable. Tannins are moderate and soft. The acidity is good (Figure 3). It can be used in single-varietal wines (unblended) or blended with other red varieties.

**MANDILARI**: It is an indigenous Cretan variety nowadays cultivated mainly in the Aegean Islands of Paros, Rodos, Evoia and Chios. It is used for the red wine production of high quality appelation OPAP "Rhodes" and "Paros". It is a very vigorous variety, easily adaptable to different pedoclimatic conditions. It has a high but sometimes fickle productivity partly due to floral abortion. In hot-humid areas It suffers from Botrytis, and Downy mildew attacks. Grape ranges from medium to large size and shows a remarkable variability in the feature. Clonal research focused on smaller and/or semi-compact cluster biotype which can limit the mildews susceptibility . Particular care was taken in the selection of stable and regular productive biotype.

The clone **VCR 290** was isolated from Boutaris' vineyard, in Skalani (Crete Island). It is a vigorous clone. The productivity is constant and on the varietal average. The cluster is small, a little elongated, semi-compact. The berry weight is slightly above the average (Figure 2A); pulp is juicy and fleshly. Sometimes a tolerable sweet millerandage (shot berries) is possible. The wine has a good shade and a bright violet colour. It is pleasant, perfumed, with noticeable red ripe fruits aromas and delicate flowers scent (Figure 3). The high tannins content suggests to blend the clone with other varieties, such as Kotsifali, in order to temper its astringency. The clone **VCR 291** was isolated from Boutaris' vineyard, in Skalani (Crete Island). Vigour and fertility are contained. The productivity is slightly under average but stable. The cluster is large, pyramidal, semi-compact. Berry weight is slightly above average (Figure 2A), pulp is juicy and fleshly. The wine has a good colour, with a marked violet hint. It has a broad-aromatic profile of red ripe fruits and violet flower, enriched by a pleasant but soft tobacco and spicy scent. The acidity is notable (Figure 3). It is suitable for fresh rosè wines but It can also be traditionally blended with Kotsifali.

**MOSCHOFILERO**: It is a pink-skinned variety of Peloponnese origin, grown mainly in the Peloponnese plateau, at the 650 m heights of Mantinia. The notable variability of the grapes gives credit to the theory that Moschofilero belongs to a polyclonal family originated from plural mutations of the ancient Fileri variety. It is very vigorous and productive and It adapts to different soils even though It prefers the poor ones where It can reach the best quality results, if water provided. At the contrary, It is sensitive to lasting rainfalls that can cause the flowers fall off and increase Botrytis susceptibility. The aromatic profile of Moschofilero wine resembles the one of Traminer and Muscat. It is used for the production of OPE "Mantinia" . It is usually produced in "blanc de gris" version, but It is also suitable for sparkling and rosè wines. Clonal research focused on less-compact cluster biotypes which are more suitable for hillside and improve botrytis' tolerance .

The clone **VCR 292** was isolated from Boutaris' vineyard in Mantinia (Peloponnese). It is a vigorous biotype. Productivity and berry size are below the average. The cluster is small, cylindric-pyramidal, semi-straggly, visibly winged (Figure 2B). The skin colour ranges from pink to light violet; the pulp is juicy, typically aromatic. The wine is straw yellow with a light body and a pleasant freshness thanks to the good acidity. It is elegant and fragrant with an intense Traminer-like aromas of roses (Figure 3). It has shown good aptitude to withering for the dessert wine production. The clone **VCR 293** was isolated from Boutaris' vineyard in Mantinia (Peloponnese). Vigor is on average. Productivity and berry size are above average. Cluster is a bit bigger than the average (Figure 2B). It is semi-compact, cylindrical, elongated and visibly winged. Fertility of the buds is above the average.

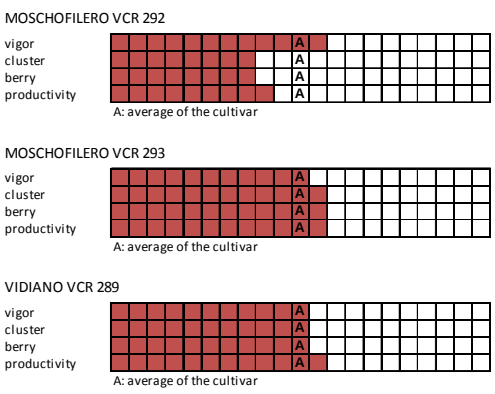



**Figure 2B**: Agronomical profiles of the selected clones in comparison with the average (A) of the standard population of each variety.

The berry skin is pink-purple with red-violet hints. The wine is of an intense straw-yellow colour with a wide aromatic profile of roses, white flowers and citrus fruit. The body is moderate and the acidity is good, giving a fresh and crispy sensation to the palate (Figure 3). The clone turns out to be suitable also for rosè vinification.

**VIDIANO**: It is indigenous to island of Crete, where It is planted in the region of Rethymnon and Heraklion. Very little is known about this variety, however It has proved to have an interesting eoenological aptitude that, in some winemakers opinion , goes near to Viognier.

The clone **VCR 289** was isolated from Boutaris' vineyard, in Skalani (Crete Island). Vigor, berry and cluster size are on the average (Figure 3). Production is abundant and stable . The cluster is cylindrical, elongated, straggly, profusely winged. Berry is oval with a thin, yellow skin and a fleshly pulp. The wine colour resembles that of the berry skin, with light gold-yellow tone. The mouth is rich and fresh; the body is moderate, well balanced by the good acidity. It has a very typical and intense aromas of apple and citrus fruits, with a final note of herbal essences (Figure 4).

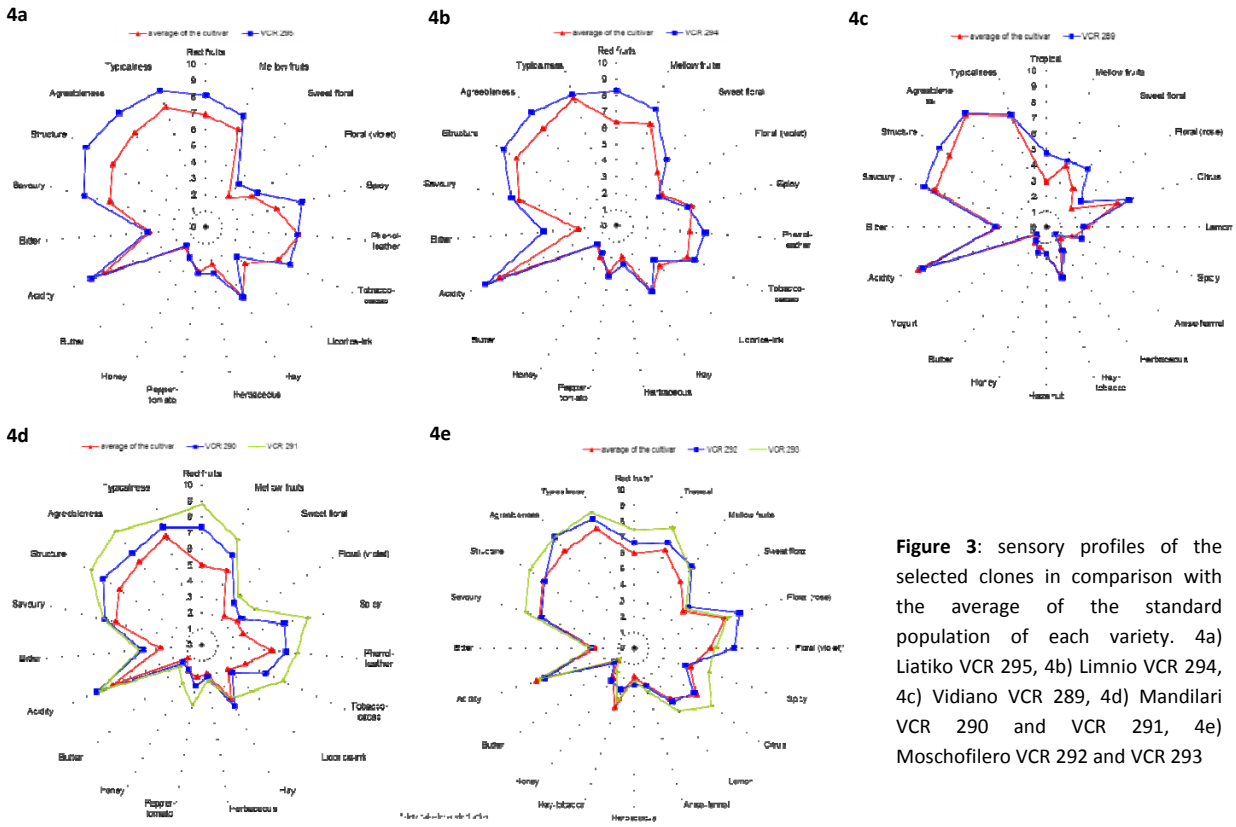



**Figure 3**: sensory profiles of the selected clones in comparison with the average of the standard population of each variety. 4a) Liatiko VCR 295, 4b) Limnio VCR 294, 4c) Vidiano VCR 289, 4d) Mandilari VCR 290 and VCR 291, 4e) Moschofilero VCR 292 and VCR 293

Clone selection has many benefits , since quality is always of significance. The Vitro-Hellas and VCR joint work led to the registration of 7 new clones, implementing the Greek genetic platform with new valuable propagating materials.

**Figure 4**: New clone selection timing of Greek varieties. The number of genotypes are shown in brackets.

| | PHENOTYPE EVALUATION | SANITARY SELECTION | AGRONOMICAL AND OENOLOGICAL EVALUATION | REGISTRATION AND PROPAGATION |
|---|---|---|---|---|
| ASSYRTIKO (1) | | | | |
| ASSYRTIKO (2) | | | | |
| MALAGOUZIA (2) | | | | |
| MOSCHOMAVRO (2) | | | | |
| RODITIS (3) | | | | |
| XINOMAVRO (2) | | | | |

However It is essential that varietal polymorphism not be «eroded» in the process. A weak and wide clone selection is essential to ensure the natural diversity of the variety and to valorize the unique and broad inheritance of Greek viticulture. At present 12 new genotypes are under evaluations. Few of them have already passed the sanitary inspections (Figure 4) and have been planted in the VCR and Vitro Hellas' experimental fields for further agronomical and enological evaluations.

Figure 5: field performance, juice composition and testing scores of the clones selected (All date are expressed as 3 years average values)

| | | LIATIKO | | | LIMNIO | | | MANDILARI | | | | | | MOSCHOFILERO | | | | | | VIDIANO | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | VCR 295 | ST | F | VCR 294 | ST | F | VCR 290 | ST | | VCR 291 | ST | F | VCR 292 | ST | F | VCR 293 | ST | F | VCR 289 | ST | F |
| Agronomical parameters | Fertility of the buds | 1,38 | 1,37 | ns | 1,45 | 1,41 | ns | 1,19 | 1,13 | ns | 0,95 | 1,13 | *** | 1,47 | 1,49 | ns | 1,46 | 1,49 | * | 0,97 | 0,96 | ns |
| | Cluster weight (g) | 249 | 253 | ns | 183 | 189 | ns | 358 | 382 | * | 416 | 382 | *** | 247 | 256 | * | 273 | 256 | * | 384 | 383 | ns |
| | Plant productivity (Kg) | 3,44 | 3,46 | ns | 2,64 | 2,66 | ns | 4,25 | 4,32 | ns | 3,92 | 4,32 | ** | 3,64 | 3,83 | * | 3,97 | 3,83 | ns | 4,38 | 4,26 | ns |
| Must parameters | Sugars (babo) | 18,67 | 17,93 | *** | 18,2 | 17,73 | ** | 17,13 | 16,67 | *** | 17,47 | 16,67 | *** | 18,33 | 17,97 | ns | 18,63 | 17,97 | * | 18,1 | 17,2 | *** |
| | Total acidity (g H2T/l ) | 6,2 | 6,03 | ** | 7,13 | 6,9 | * | 7,07 | 6,6 | ** | 7,06 | 6,6 | *** | 7,37 | 6,7 | *** | 7,07 | 6,7 | *** | 8,17 | 7,3 | *** |
| | Anthocyanins (mg/l) | 457 | 408 | *** | 429 | 355 | ** | 512 | 468 | *** | 507 | 468 | *** | 336 | 315 | ns | 345 | 315 | ns | - | - | - |
| Tasting score | | 79,16 | 74,38 | *** | 73,82 | 72,23 | *** | 77,61 | 74,15 | ** | 78,58 | 74,15 | *** | 78,34 | 75,36 | ** | 80,15 | 75,36 | *** | 80,75 | 77,73 | *** |

Significance level: ns= not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, ***= $p \leq 0.001$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