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ORAL PRESENTATION

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GRAPE BREEDING OF THE FORMER SOVIET UNION

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Dear Colleagues! On behalf of our Institute, I want to express my sincere thanks to the International Vine and Wine Organization and the Steering Committee of the VIth International Symposium on Grape Breeding for the honor to host this most prestigious meeting. We are delighted that such a great number of distinguished scientists, from home and abroad, have come to participate.

Grapes are rich gifts of sunshine and skilful hands of man. This rewarding crop has always lended our life charm ad beauty.

It is well known that the amount of grape varieties is enormous; 36000 of them are contained only in the Inrternational data bank of Prof. G. Alleweldt. Many of these varieties are unique and possess excellent qualities. However, none of them meets all requirements rendered necessary under present conditions when prices of energy, chemicals and all kinds of materials increase, environment has to be protected from anthropogenic influences and man himself is subject to harmful effects of modern farming technique of grapevine. In zones with extreme climatic conditions, low frost resistance of the existing grape assortment also causes a problem.

That is why search for improved cultivars will always remain actual. Grape breeding is conducted both in regions where grape cultivation is of commercial importance and in more northerly areas. For instance, in the Podmoskovie region this work is important both for amateur growers and for the farther successful introduction of these cultivars In the assortment of southern regions. For the last 50 years, originators of the former Soviet Union have made considerable progress in the breeding of grape cultivars with desired characters. These activities are greatly promoted by international cooperation. However, "ideal" cultivars still cannot be developed. In this connection, one cannot overestimate results of the breeding activities of Prof. P.Ya. Golodryga, Chief of the Breeding Department of the Institute "Magarach", and. his colleagues who developed "models" of ideal cultivars. Of great importance are also results achieved in the line of classification and identification of grape cultivars by Prof. P.Olmo, D.Boubals, P.Galet, N.Nemeth and G.Alleweldt.

Nevertheless, considering the doctrine of models of ideal cultivars, one hes to admit that the present level of grape breeding and. the world's economical situation lay certain restrictions on these models. They have to be not universal but regional and should make efficient use of the ecological factors of their region of cultivation. Besides, they have also to meet requirements caused by long-tenn economical trends of different countries and their alliances. In addition to that, all. models are to contain characters of universal importance, such as resistance to pests, viruses included, and other unfavorable factors. In definite regions, breeding for resistance to frosts, drought, carbonates and. other factors may be conducted.

All this, in my opinion, renders it unnecessary to pursue phylloxera resistance of newly bred cultivars as the obtention of this character in a hybrid is very problematic and is combined, as a rule, with lower quality of fruit. This problem can be solved very successfully through an efficient and ecologically safe method of grafting to phylloxera-resistant rootstocks, as the costs linked with this business can be neglected compared to longterm profits of yields.

Meanwhile, the possibility to cultivate own-rooted. vines is very important in regions where death of old wood is highly probable.

In the former Soviet Union whose disintegration gave way to new independent states grape breeding has been very successful and, as a result of this, different trends have arisen.

The breeding school of the Institute "Magarach", for instance, whose first research was attempted in 1828, practised gene combination within *V. vinifera* over a certain period and now the breeders of me Institute try to combine *V. vinifera* polygenes responsible for high yielding capacity and good quality of fruit with those of resistance to unfavorable environmental factors typical of French-American hybrids.

The first breeding stage of our Institute which lasted 100 years has led to the development of excellent cultivars such as Bastardo magarachski., Rubinovy Magaracha and Ranni. Magaracha. These cultivars have gained worldwide recognition and are now in commercial cultivation with a total area of 10 000 ha. The originators of these cultivars are N.V. Paponov, V.V. Zotov and the world-known grape breeder P.Ya. Golodryga.

The second stage of breeding conducted by our Institute has given rise to new cultivars with complex resistance. These are such as Pervenets Magaracha, Antei Magarachski, Aurora Magaracha, Yubileiny Magaracha, Podarok Magaracha included in the State registers of Ukraine and Russia. These cultivars are gaining wide commercial distribution aid the area of their cultivation amounts now to) 3000 ha. Currently, they are in constantly increasing demand. In addition to these, 16 resistant cultivate such as Kentavr magarachski, Tavkveri Magaracha, Citronny Magaracha, Granatovy Magaracha, Dance, etch are now under the State Trials.

The breeding school of the Tairov Institute for Viticulture and Enology in Odessa P.K. Aivazian, E.N. Dokuchaeva, L.F. Meleshko) has raised 16 recognized cultivars. Of these, to be mentioned are Sukholimanski.

bely, Odesski cherny, Odesski suvenir, Muscat tairovski, Lesia, etc. developed based on *V. vinifera* as well as Golubok, Dnestrovski rosovy, Zolotisty ustoichivy, Ovidiopolski, Lanka, Muscat odesski, Rubin tairovski developed with the use of interspecific hybrids. The total area of their cultivation is 8 500 ha.

Also in Odessa, Prof. N.A. Dudnik from the Odessa Agricultural Institute has developed table grape cultivars Yuzhanka OSHI and Yantar OSHI as well as seedless cultivars such as Mechta and Kishmish OSHI.

The Russian school of breeding headed by Prof. Ya.I. Potapenko and Prof. E.I. Zakharova and located on the northern border of commercial grape cultivation pursued the development of high-yielding and frost-resistant cultivars based on the hybridization of the best representatives of *V. vinifera* and *V. amurensis*. Breeders of Russia have released such popular frost-resistant cultivars as Saperavi severny, Fioletovy ranni, Vydvizhenets, Cabernet severny, Stepniak, Tsvetochny and Chaaselas severnaia. At present, disciples of Prof. Potapenko practise insertion of genes responsible for disease resistance into genomes of valuable newly bred cultivars. As a result of this approach, cultivars Agat donskoi., Vostorg and Grushevski bely have been released. These cultivars have found recognition both with commercial and amateur growers.

The breeding school of Russia includes a trend started by K.P. Skuin. This researchist developed a theory of breeding frost-resistant cultivars and developed such ones as Burmunk, Moskovski and Yubilei TSHA. The breeder L.M. Filipenko who continues to continue the work of his teacher A.A. Kuzmin developed cultivars Muromets, Izo gorianki, etc. based on Euro-Asian sources and Amur frost-resistant forms. In the post-war time, the All-Russia Potapenko Institute released cultivars of Euro-Asian origin such as Belorosovy, Narodny, Desertny, Zorevoi, Osoby, etc. These cultivars - developed by Prof. M.A. Lazarevski., A.M. Aliev, F.M. Gramotenko and other originators.

The Armenian school of breeding (S.A. Pogolian, S.S. Khachatryan, M.V. Melkonian) conducts both fundamental research and practical breeding. For about 50 years, Armenian breeders concern themselves with theoretical problems such as grape breeding for immunity to pests and diseases, frost resistance, precocity and heterosis for a number of qualities. They have also released 33 table, wine and universal cultivars, frost-resistant and 4- phylloxera-resistant ones among them, based mainly on inter- (*V. vinifera* and *V. amurensis*) and interspecific (*V. vinifera* hybridization). 28 of these cultivars have been allowed for commercial cultivation, some of them (Megrabuir, Nerkarat and Burmunk) being disease-resistant. Besides, these cultivars withstand frosts of up to -30°C . A number of Armenian cultivars are in efficient commercial cultivation in Russia, Ukraine, Kazakhstan and Kyrgyzstan.

The Armenian Institute for Agriculture conducts efficient breeding under the guidance of Prof. P.K. Aivazian. As a result of this work, cultivars Akhtanak, Anushik, Kangun, Podarok Rossii, Ereuni, etc. have been developed.

Research for immunity of grapevine to pests and diseases (D.D. Verderevski, K.A. Voitovich, P.N. Nedov) has been conducted in Moldova since 1960s. As a result, theoretical principles and practical methods of multistage selection have been developed and cultivars Doina, Onitskanski bely, Liana, Suruchenski bely, Kriulianski, etc. have been released. The breeding school established by M.S. Zhuravi (now deceased) and his colleagues N.I. Guzun and P.N. Nedov have been studying for the recent years theoretical and practical ways of breeding cultivars with complex resistance to diseases, pests and phylloxera. Using gene sources of *V. vinifera* and French-American hybrids, these originators obtained wine cultivars such as Viorica, Negru de Yaloven, Plamenny, table cultivars such as Dekabrski, Moldova, Strashenski, Zvezdny, Godrianca, Frumoasa alba and others included in the State register. Russian and Ukrainian ampelographers consider these cultivars and those which are now under the State trials to be worthy of confidence, and now they are being propagated intensely.

The Middle-Asian breeding school headed by Prof. A.M. Negroul and his successor Prof. K.V. Smirnov has also achieved considerable success. As a result of this work, excellent table grapes with high yielding capacity were obtained in Uzbekistan, such as Gouzal kara, Muskat uzbekistanski, Oktiabrski, Pozdny VIR'a, Ranni VIR'a and Rizamat. This breeding school released also such seedless cultivars as Kishmish VIR'a, Kishmish Khimrau, Tarnau, Kishmish Zarafshan, Kishmish samarkandski., etc. P.V. Mikhailova a breeder from griddle Asia, developed wine cultivars Magarachski., Tashkentski, Rubinovy and Record which enriched the scarce wine grape assortment of Uzbekistan.

In the neighbouring republics of Middle Asia, breeding research has been conducted for 30 or 40 years. The breeders V.P. Ponomarchuk and R.T. Teknoriadnova developed cultivars Alma-Atinski ranni, Kara-koz and others in Kazakhstan, E.I. Sosina et al. released cultivars Vartan, Kirghizski ranni, Madeleine muscatny, Mairam, Olesia, Olga, etc., and A.U. Savchenko obtained cultivars Anzob, Ghissarski ranni. and Zarif in Tajikistan.

Georgia possesses a richest genofond. of indigenous grapes (more than 500 varieties), and to create superior genotypes was uneasy work. However, as a result of 60 years of research, five cultivars which are now recognized and allowed for commercial cultivation have been developed: Kartuli saadreo, Kolkhuri, Saamo, Rkatsiteli muskatvili., Tbilisi (originators R.M. Ramishvili, V.I. Kantaria, R.M. Kikagheishvili et al.). The Telavi. experiment station released the famous rootstock Berlandieri x Riparia Teleki 8B vazisusbanski (originator A.G. Gavaketashvili) which is widely distributed in Georgia and outside this republic. Clonal selection is practised on a large scale by this experiment station, the Georgian Institute for Horticulture, Viticulture and Enology and the Georgian Agricultural Institute. As a result of this work, many valuable clones have been selected which are now being tested industrially.

On the Kirovabad experiment farm, M.A. Mirzoian developed wine cultivar Karaghez which entered the recognized grape assortment of Azerbaijan.

Thus, breeders of the former Soviet Union have released about 90 grape cultivars which are recognized now and allowed for commercial cultivation. Of these, about 50% are resistant to one or several unfavorable environmental factors (mildew, oidium, gray rot, phylloxera, low temperatures). Of 40 resistant cultivars, 11 are table grapes, six are universal and 23 are wine cultivars. Just for comparison grape assortment of the EEC countries contains only 24 resistant cultivars!

On the contrary, clonal selection has never been an intense trend of research in the former Soviet Union. Only eight clones have been submitted to the State trials and none of them has been included in the recognized grape assortment. This is due to inefficient methods of testing. Nevertheless, phytosanitary selection seems to be rather developed in Moldova, Ukraine and Russia: the grape and wine industries of these countries are supplied with considerable amounts of sanitized propagating materials.

Among new approaches in grape breeding to be mentioned is gene engineering. First steps in this direction are being made by the Institute for Plant Physiology and Genetics (Academy of Sciences of Ukraine) in collaboration with the Institute for Vine and Wine "Magarach" where plants were developed from explants treated with agrobacteria containing plasmids carrying genes which code for resistance to crown gall and the herbicide preparation "Glifosat". Of such plants, those resistant to canamicin were selected and they are being propagated now in order to conduct molecular-biological analysis to see whether their genomes contain the corresponding genes, and to study expressivity of the genes introduced.

Summing up the results of grape breeding throughout the former Soviet Union, to be mentioned is the tremendous effort that has been made to collect and preserve the grape genofond in ampelographical collections as no breeding is possible otherwise. Ampelographical collections can be found in the Crimea (those of the Institute "Magarach" containing 3 220 grape varieties, forms and species), Moldova (about 2 000), Russian (more than 600), Armenia (580), Kyrgyzstan (1 060), Turkmenistan (620) and Georgia (1 200). This is a unique treasure, which is to be used on an international scale, and we are always ready for cooperation and joint research.

Ladies and gentlemen! Being the local organizer of this Symposium, I would like to conclude by wishing you fruitful discussions and a pleasant stay in the Crimea.

GENETIC VARIANCE AND STABILITY ABOUT SOME QUANTITATIVE TRAITS IN A POPULATION OF VINE (*VITIS VINIFERA*)

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Abstract

Selection for improvement of quality is common in grape breeding programs. Efficient utilization of different germ plasm source depend on knowledge of quantitative traits variation within and among ecotype. The purpose of this experiment was to quantify variations for ten characters among 17 ecotypes of cultivar *Sangiovese* and *Prugnolo gentile*, typical cultivars of Tuscan region (centre of Italy). Broad-sense heritability was calculated for all the variables and the stability analysis was made to value the Genotype + Environment interaction. By the multivariate analysis (Principal Component Analysis) it was identified the ecotypes that could be used to (1) sustain selection in a longer-term program, (2) infuse new variability into existing populations undergoing selection, or (3) maintain variability in short-term population improvement program.

Key words : *Vitis vinifera*, genetic variability, quantitative traits, stability, selection.

Introduction

For a successful breeding program, the knowledge of genetic variability of desirable characters is very important (Firoozabady and Olmo, 1987). The cultivar *Sangiovese* (*Vitis vinifera*) is cultivated a lot in Italy, especially in Tusca region where it is wellknow like as Brunello in Montalcino area and where it integrates itself and it is often confused with another important cultivar, the *Prugnolo gentile*, in Montalcino area. Recently these cultivars have attracted constant attention from clonal selection researchers.

Most characters, that form the object of the genetic improvement study, show a variability so that individuals of the same populations can't be grouped in distinctly classes (Ottaviano, 1968). The differences between various individuals depend on the expression grade of the character and they can't be reverred to the segregation of one or few mendelian character (Poni, 1989). This variability is said continuous and its character are called quantitative.

In 1909 a Johansen study appeared; it was destined to give a considerable contribution to understand the factors which control the continuous variability. This auctor studied about the weight of bean seeds and demonstrated that the expression of a quantitative character can be influenced both by the genotype and by the environment; so, the phenotypic variability includes a not hereditary part.

Variability in p population is influenced by heredity and environment. Together they play their respective role on the concept of hereditabilty. The partitioning of the variance into its components allows us to estimate the relative importance of the various determinants of the phenotype, in particulat the role of heredity versus environment, or nature and nurture. The relative importance of a source of variation is the variance dued to the source, as a proportion of the total phenotypic variance. The relative importance of heredity in determining phenotypic values is called the heritability of the character (Falconer, 1981).

The inability of plant genotypes to maintain consistent performance over a given range of environments constitutes the genotype \times environment (G \times E) interaction (Onokpise and Mortensen, 1988).

The mean yield of a crop genotype measured over several years or locations has often been used as a measure of performance or adaptability. While this characteristic provide one indicator or performance, it does not provide information on the underlying basis of the performance. For example, a moderate mean value for a particular response would occur if several test locations reported moderate values, or if a few sites reported high values while a similar number reported low values (Pritts, 1990). An assessment of adaptability or yield stability would be quite different in these two cases. In one sense, stability can be thought of as a homeostatic state, defined as low variation in yield when measured over various locations or years (Pritts, 1.c.). Consistent performances across different sites and/or years are referred to as stability (Fernandez, 1991).

Moreover, quantitative traits are useful in taxonomic contest also because they allow the affinity among groups of individuals to be easily related to environmental factors. For these reasons, a classification of a germoplasm collection of a given species based upon quantitative traits, allows either the choice of the most interesting types to be included in a breeding program or/and the study of the evaluative history of the material .

Most important yield characters had to be examined in populations of different origins for their genetic variability with the aim of exploiting these for genetic improvement of *Sangiovese* (and/or "Brunello") and *Prugnolo gentile*.

The aim of the present study is : at first to value the stability of the characters and at second to choose the ecotype where to select for the highest improvement.

Materials and methods

Sampled population

Research was conducted in the three years period, from 1988 to 1990, and the plants sampled were 370 coming from 17 populations, corresponding to 17 old vineyards made with old genotypes (tab.I). The sampled populations was three of *Brunello*, then of *Sangiovese* and four of *Prugnolo gentile*. The chosen locations were representative for the morphology and life history variation present in these cultivars in Tuscan region (centre of Italy). A short description of the populations is given in table 1.

Morphological measurements

From each plant was measured the following quantitative traits:

- number of clusters per plant at vintage
- yield per plant at vintage
- mean weight of clusters at vintage
- mean weight of 100 berries at vintage
- number of bud per plant
- sugar content in must at vintage ($^{\circ}$ Brix)
- titratable acidity content in must at vintage (g/l)
- pH in must at vintage
- malic acid content in must at vintage (g/l)
- tartaric acid content in must at vintage (g/l)
- potassium in must at vintage (g/l)

Statistical analysis

Analysis of variance were computed for all data collected on ecotype assuming all effects to be random. Genotypic variability, interaction variance G x E, and heritability in broad sense H^2 according to Falconer (1981, I.c.) were estimate from the control populations measured for three years (Vogel, 1992)

Genotype x Environment (G x E) interaction was evaluated with two stability parameters, ecovalence stability index (W_1) (Wricke, 1962) and stability variance (σ^2) (Shukla, 1972). The estimation of ecovalence is an intermediate step in calculating stability variance and therefore stability variance is a coded value of ecovalence (Fernandez, 1991). Shukla's method allowed for partitioning of G x E into relative component value, stability variances, for each genotype (Deren, 1992).

The ecovalence stability index (W_1) and the stability variance (σ^2) allow to do a dissimilarity matrix based on the statistics of a joint regression. The residuals estimated from regression model were used for plotting the ecotype for each variable and the matrix is used for a clustering process (Lin, 1992)

For the 17 ecotype, mean values and variances were used to generate two 17 x 6 matrices - one containing family means for each significative variable and the other containing within-family variance for each significative variable. Both matrices were subjected to Principal Component Analysis (Wolff, 1991) using the correlation matrices among the six variables (Caster, 1991).

Multivariate statistical procedures are used when several quantitative characteristics are considered. These method makes it possible to obtain a synthetic description of the overall variability, to assess the relative contribution of each trait to the differences among populations and to group similar population together on the basis of information that can be used in a breeding program.

The scatterplot of the principal component for family means versus the principal component for within-family variances were graphed.

The Analysis of Variance and the Principal Component Analysis were calculated using the statistic package SAS/STAT version 6.0, while the ecovalence stability index (W_1) and the stability variance (σ^2) were calculated using the S116- Genotype X Environment Interaction Program, coming from the Department of Agriculture Canada.

For graphics was used the SYSTAT ver. 5.0 Package.

Results

Differences in morphological characters

Variation among families (ecotypes) was detected statistically significant ($p \geq 0.01$) for all the variables measured (tab. 2). The AP family had the highest weight of 100 berries, while the LV family was clearly different from the others populations with the less value.

The bud number and the cluster number per plant was highest in SV ecotype.

The same ecotype had also the less mean weight of cluster, titratable acidity and malic acid. The less bud number and number of cluster had the MF ecotype. The yield per plant was highest in LV ecotype and less in ZN family. The ecotype NN had the highest value of sugar content in must, while the AP ecotype had the less. The malic acid and pH was the highest in YS ecotype; the less pH was noticed in ZN family. The tartaric acid was highest in UC family and on the other extreme in AP ecotype.

For all the characteristics measured, the interaction genotype x environment (Ecotype X Year) was a significant component of variance, while the year was resulted significant on weight of 100 berries, pH, sugar content, titratable acidity, malic acid, tartaric acid.

The heritability was very different for several traits. In fact, the highest heritability was 0.89 for bud number. The cluster number per plant (0.85), yield per plant (0.65), tartaric acid (0.69), mean weight of cluster (0.60) were very high.

Heritability of pH was moderately high, but enough to consider high the degree of genetic determination of this character. The others characters had a low heritability.

Analysis of stability

The ecotype GT (tab. 3) had the less G x E interaction about the weight of 100 berries, while the highest interaction was into family LV. The clustering process created three groups with different G x E interaction (fig. 1a-b).

The highest value of ecovalence and stability variance of the bud number showed itself into ecotype SV, while the family XC had the less value. Four groups of G x E interaction had been created by clustering process (fig. 2a-b).

The number of cluster per plant was very influenced by G x E interaction into ecotype SV. The interaction into family VC was very low. The clustering process showed three groups (fig. 3a-b).

About the yield per plant the cluster showed three groups. The highest value of interaction with environment underlined the family YS, while the less one the XC ecotype (fig. 4a-b).

The ecotypes YS and MF had the highest value of G x E interaction in relation to mean weight of cluster; the ecotype PC had less G x E interaction. The clustering process had made four groups (fig. 5a-b).

The pH was very interactive with environment into ecotype YS, on the contrary into ecotype CN.

The dissimilarity matrix had produced a cluster with three groups (fig. 6a-b).

The sugar content was very genotype x environment interactive in family MF and very low in ecotype UC. The cluster showed three groups (fig. 7a-b).

Both the suggest content and the titratable acidity were very interactive with environment into genotype MF, while the families NN and XC were less interactive (fig. 8a-b).

The ecotype PC had the highest value of G x E interaction in relation to malic acid; the less value was seen into SV family and the clustering process had made three groups (fig. 9a-b).

The ecotype AP had the greater interaction about tartaric acid, on the contrary in the family VC the interaction is very low (fig. 10a-b).

Multivariate analysis

Only the characters with high h^2 were used in multivariate analysis. Principal component analysis of mean values resulted in the formation of three interesting components which described 89,5% of the variation among ecotype means (tab. 4). The first component (PC1mean) had high value that was strictly correlated with the bud number per plant, the cluster number per plant and the yield per plant. The PC1mean can be considered a component of production. The second component (PC2mean) had high value that reflected above all the pH and the tartaric acid content. The third component (PC3mean) had high value strictly correlated with the cluster mean weight.

For within-ecotype variance three components described 84,7 % of the variation among ecotype within-variance (tab...). High values of the first component of variance (PC1var) were associated with the bud number per plant, the cluster number per plant and the yield per plant. High values of the second component (PC2var) for variance were correlated with the mean cluster weight. The third component (PC3var) for variance were associated with the pH of must.

The figures It, 12, IS show the results in scatterplot of the principal components for within ecotype variance versus the principal components for ecotype means.

Discussion

The ecotype SV was expected to provide the highest probability of success for yield improvement. This family had high variance for yield and high G x E interaction.

The RS ecotype was similar in performance for PC1 mean values, but had a near-average PC1var score. ZN and MF germ plasm source were expected to provide the lowest probability of success for yield.

The MF germ plasm should be the most useful in a breeding program designed to improve the mean weight of cluster, with a secondary goal of maintaining a high level of genetic variability.

The germ plasm XC behaved like MF, too, but the less PC3mean value couldn't obtain the same improvement.

The ecotype LV with highest PC3mean value did not adequate mean weight of cluster variation to meet this objective.

The OS, FO, CN, UC germ plasm would be entirely inadequate for this objective.

The YS germ plasm appeared to be extremely valuable, in term of mean

Discussion

performance about the pH and the tartaric acid content, but did not possess adequate variation for improvement programs. UC and RS germ plasm were the only families useful for a selection program to improve pH of must and tartaric acid content.

In conclusion:

1. Only the following quantitative traits had enough Hereditability to provide high probability of success for selection:

- number of cluster per plant
- yield per plant
- mean weight per cluster
- pH
- tartaric acid content

2. The several ecotype had different G x E interaction for the quantitative traits to select.

3. The following ecotypes could be used for obtain a high improvement:

3.1. The ecotype SV was expected to provide the highest probability of success for yield improvement. This family had high variance for yield and high G x E interaction.

3.2. The MF germoplasm should be the most useful in a breeding program designed to improve the mean weight of cluster, with a secondary goal of maintaining a high level of genetic variability. Your high value of PC3mean and PC2var suggested high level of mean weight of cluster variation that could be used to (1) sustain selection in a longer-term program, (2) infuse new variability into existing populations undergoing selection, or (3) maintain variability in short-term population improvement program.

3.3. UC and RS germoplasm were the only families useful for a selection program to improve pH of must and tartaric acid content; their PC2mean hadn't very high mean value, but should be useful with a secondary goal to maintaining a high level of genetic variability.

Acknowledgment. Thanks a lot to Consorzio Vino Nobile of Montepulciano and to Consorzio Vino Chianti of Firenze that have financed this research and also to the vinegrowers that have given their vineyards.

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References

Tab. I. Ecotype sampled in three years period (1988-1990)

ECOTYPE	CULTIVAR	LOCATION
AP	Sangiovese	Fatt. Pagnana
BB	Sangiovese	Fatt. Bacchereto
CN	Sangiovese	Fatt. Castellare
FO	Prugnolo gentile	Az. Fogngano
GT	Sangiovese	Fatt. Poppiano
LV	Sangiovese (Brunello)	Az. Banfi
MF	Sangiovese	Fatt. Montecastello
NN	Sangiovese	Fatt. Montellori
OS	Sangiovese(Brunello)	Az. Case Base
PC	Sangiovese	Az. Pomino
R3	Prugnolo gentile	Az. Tre Rose
SV	Sangiovese	Az. I. Selvatici
UC	Prugnolo gentile	Az. Contucci
VC	Sangiovese(Brunello)	Az. Banfi
XC	Sangiovese(Brunello)	Az. Banfi
YC	Prugnolo gentile	Fatt. Del Cerro
ZN	Sangiovese	Az. Nipozzano

Tab. 3 Wricke's ecovalence and Shukla's stability variance:

ecotype	weight of 100 berries		buds number		number of cluster		yield per plant		mean weight per cluster		pH		sugar content		titratable acidity		malic acid		tartanic acid	
	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2	W_i	σ_i^2
AP	364.4	324.2	0.11	-0.19	1.35	0.22	0.84	0.84	.00	.00	.01	.01	4.18	4.49	.31	.24	.02	.00	1.77	1.97
BB	7.82	-79.9	0.04	-0.27	3.21	2.33	0.88	0.89	.00	.00	.00	.00	.06	-.18	2.32	2.51	.01	-.01	.16	.14
CN	351.2	309.3	0.39	0.13	0.29	-0.98	0.56	0.53	.00	.00	.00	.00	10.9	12.11	.95	.97	.41	.45	.21	.19
FO	191.6	128.3	1.21	1.06	30.5	33.2	4.23	4.68	.00	.00	.00	.00	10.2	11.33	2.37	2.58	.00	-.02	.02	-.02
GT	0.03	-88.7	9.74	10.7	14.2	14.7	0.60	0.57	.00	.00	.01	.01	2.33	2.40	.00	-.10	.14	.14	1.48	1.64
LV	12216	13756	0.47	0.22	11.4	11.6	0.69	0.68	.00	.00	.00	.00	2.40	2.47	.80	.80	.64	.70	1.74	1.93
MF	96.25	20.3	1.06	0.88	17.5	18.5	3.68	4.06	.01	.01	.00	.00	11.6	12.93	12.8	14.4	.02	.00	1.00	1.10
NN	2091.9	2282.1	9.02	9.90	43.1	47.5	2.03	2.20	.00	.00	.01	.01	.13	-.10	.01	-.09	.44	.48	.60	.64
OS	5.70	-82.3	0.80	0.59	1.70	0.61	0.18	0.10	.00	.00	.00	.00	.03	-.20	.30	.23	.73	.81	.13	.10
PC	409.6	375.5	2.64	2.67	4.10	3.34	0.69	0.68	.00	.00	.00	.00	.37	.18	.12	.02	1.05	1.17	.08	.05
R3	1.16	-87.4	9.36	10.3	47.1	52.1	0.53	0.49	.00	.00	.01	.01	1.71	1.70	.31	.24	.06	.05	.11	.09
SV	1186.9	1256.4	25.8	29.0	83.1	92.9	1.57	1.68	.00	.00	.01	.01	1.46	1.42	.90	.91	.00	-.02	.63	1.80
UC	39.52	-43.9	4.48	4.76	24.4	26.3	1.07	1.11	.00	.00	.01	.01	.00	-.24	.06	-.04	.00	-.02	.31	.31
VC	2132.1	2327.6	0.20	-0.09	0.00	-1.31	0.23	0.15	.00	.00	.00	.00	1.46	1.41	2.31	2.51	.98	1.09	.01	-.04
XC	428.7	397.1	0.00	-0.31	2.82	1.88	0.14	0.06	.00	.00	.01	.01	.57	.41	.01	-.10	.10	.09	.40	.41
YS	212.5	152.1	2.36	2.36	29.7	32.3	7.19	8.05	.01	.01	.05	.05	3.62	3.86	1.43	1.51	.30	.32	.12	.10
ZN	1564.3	1684.2	8.43	9.24	1.16	0.00	0.34	0.28	.00	.00	.00	.00	7.07	7.77	1.14	1.19	.24	.25	.20	.19
TOTAL	21300		76.2		80.95		315		335.3		25.5		27.05		.03		.03		.11	
L																				

Table 2 : Analysis of variance and heritability broad sense (h^2)

DF	weight of 100 berries		budd number per plant		number of cluster per plant		yield per plant		mean weight per cluster		pH		sugar content		titratable acidity		malic acid		tartanic acid	
	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F	M/S	Pr>F
Ecotype	18369.9	***	1156.3	***	2111.0	***	68.09	***	0.079	***	0.152	***	80.05	***	18.99	***	5.08	***	36.17	***
Year	151464.1	***	39.6	n.s	68.22	n.s	13.6	n.s	0.017	n.s	0.149	***	385.5	***	148.1	***	88.8	***	7.6	***
Ecotype X Year	16295.2	***	74	***	251.07	***	18.5	***	0.026	***	0.049	***	35.05	***	15.2	***	2.35	***	7.1	***
Heritability h^2	0.10		0.89		0.85		0.65		0.60		0.42		0.16		0.10		0.05		0.69	

Statistic and variable	Eigenvector		
	1	2	3
Within-ecotype variance			
bud number	0.551362	0.050334	0.105211
number of cluster	0.523124	0.0411021	0.311256
yield per plant	0.458441	0.387536	-0.235347
pH	-0.181812	0.413818	0.847119
tartaric acid	0.364684	0.066219	-0.054941
weight of cluster	-0.214762	0.818516	-0.325051
explained variance	50.2	18.2	16.3
Ecotype means			
bud number	0.585234	-0.196150	-0.064615
number of cluster	0.591969	-0.117330	-0.140768
yield per plant	0.495327	0.083372	0.456505
pH	0.224619	0.642730	-0.055021
tartaric acid	0.026005	0.700919	-0.271774
tartaric acid	-0.102933	0.190838	0.831098
explained variance	43.9	23.8	21.0

Fig. 1a. Dendrogram of several ecotype based upon the dissimilarity index in relation to mean weight of 100 berries

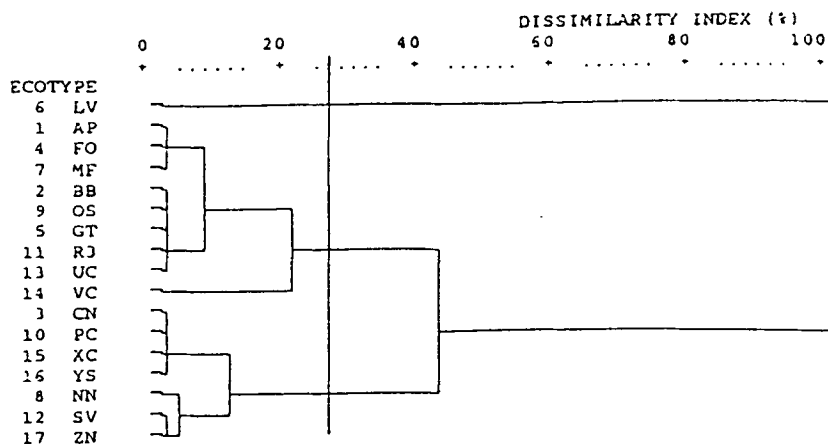


Fig. 1b: Plot of residuals estimated from Anova model in relation to weight of 100 berries

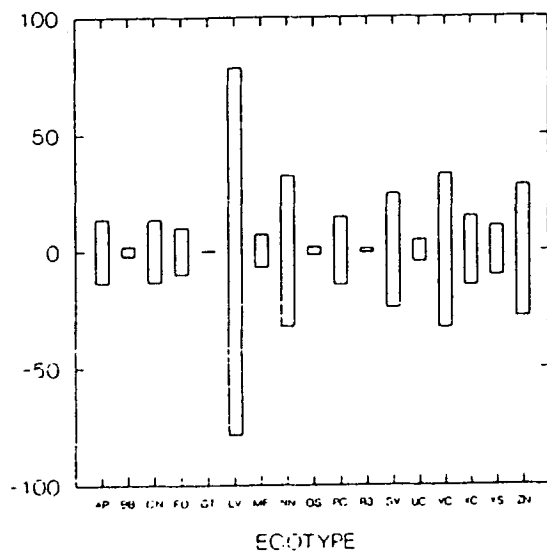


Fig. 2a: Dendrogram of several ecotype based upon the dissimilarity index in relation to bud number per plant

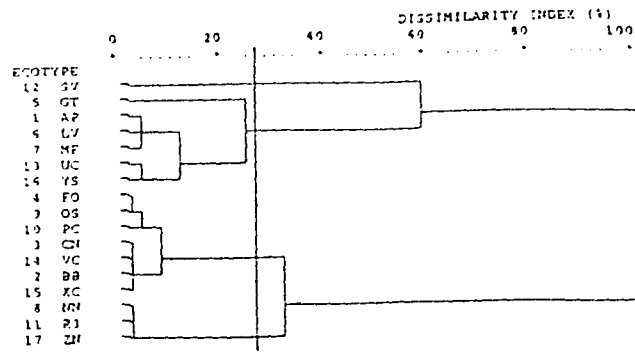


Fig. 2b: Plot of residuals estimated from Anova model in relation to bud number

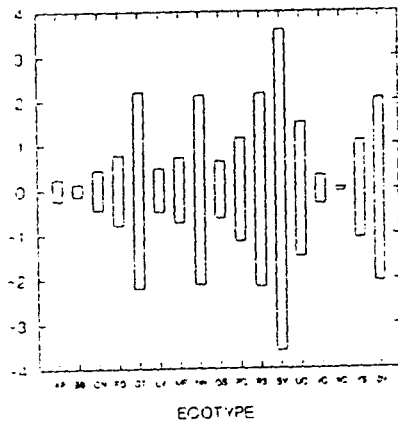


Fig. 3a: Dendrogram of several ecotypes based upon the dissimilarity index in relation to number of cluster per plant

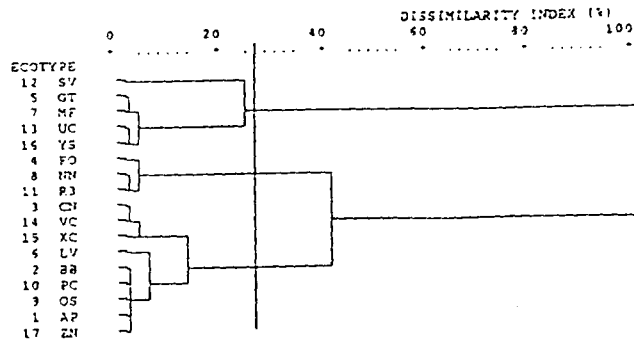


Fig. 3b: Plot of residuals estimated from Anova model in relation to number of cluster per plant

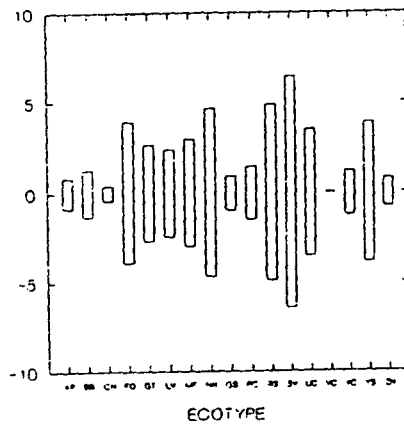


Fig. 1a. Dendrogram of several ecotype based upon the dissimilarity index in relation to yield per plant

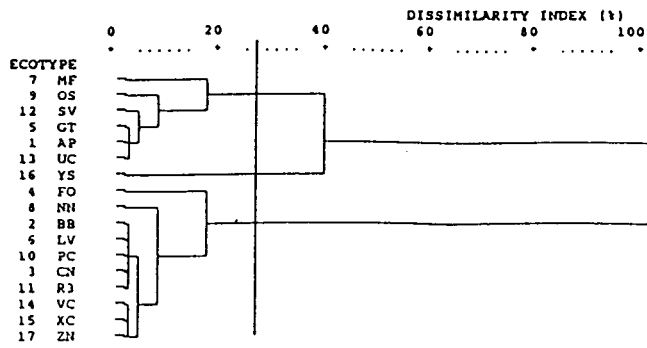


Fig. 1b: Plot of residuals estimated from Anova model in relation to yield per plant

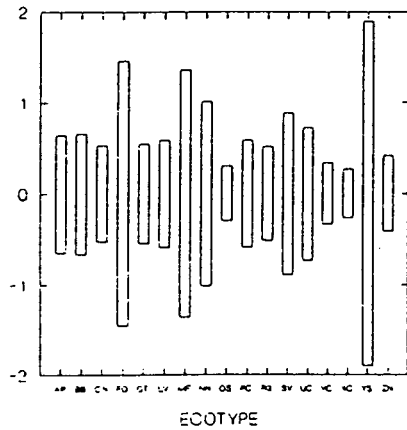


Fig. 5a. Dendrogram of several ecotype based upon the dissimilarity index in relation to mean weight of cluster

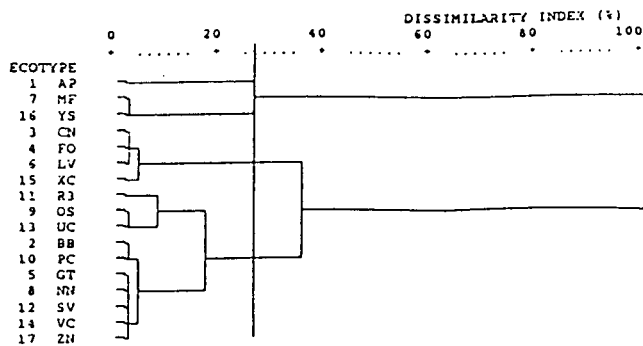


Fig. 5b: Plot of residuals estimated from Anova model in relation to mean weight of cluster

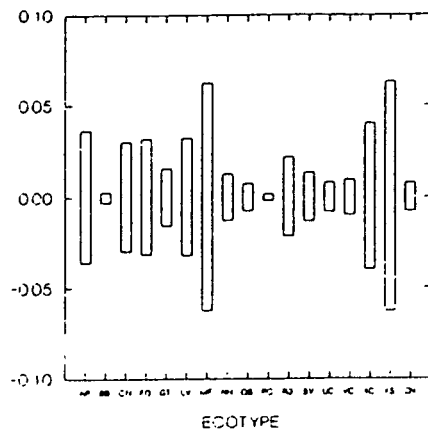


Fig. 6a. Dendrogram of several ecotype based upon the dissimilarity index in relation to pH

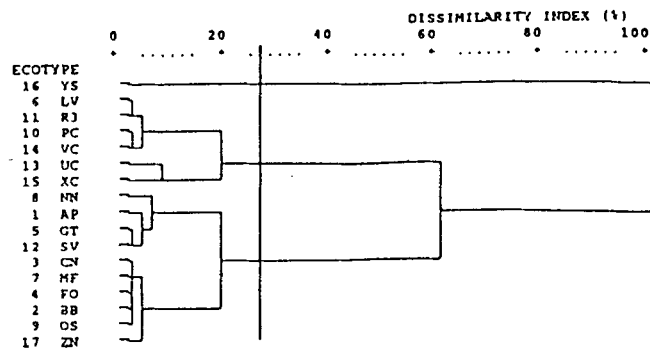


Fig. 6b: Plot of residuals estimated from Anova model in relation to pH

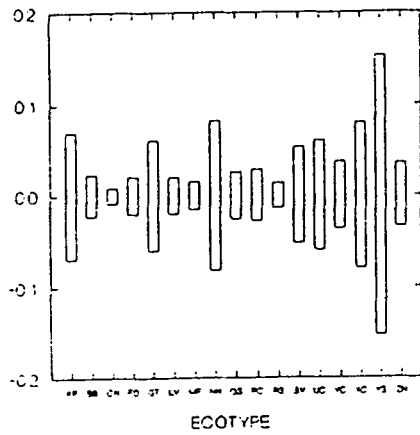


Fig. 7a. Dendrogram of several ecotype based upon the dissimilarity index in relation to sugar content

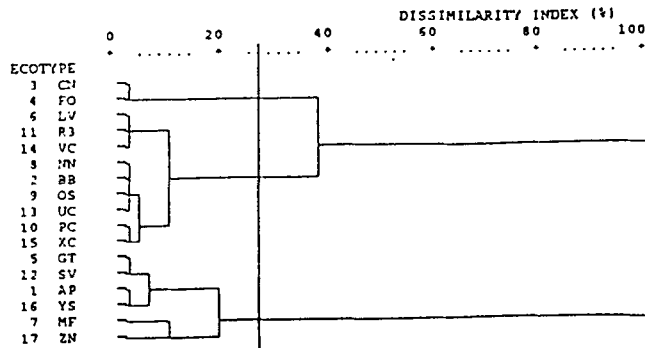


Fig. 7b: Plot of residuals estimated from Anova model in relation to sugar content

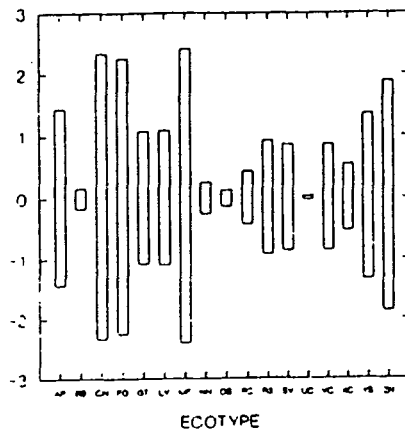


Fig. 8a. Dendrogram of several ecotype based upon the dissimilarity index in relation to titratable acidity

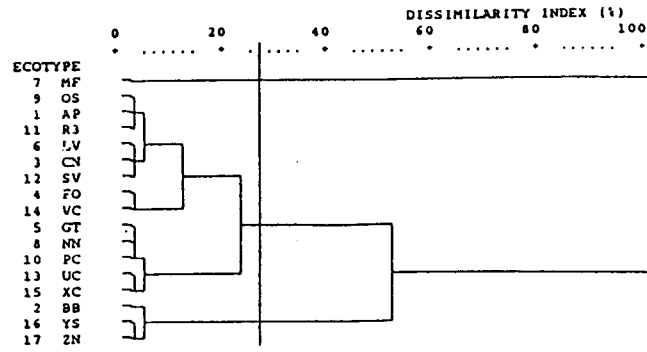


Fig. 8b: Plot of residuals estimated from Anova model in relation to titratable acid

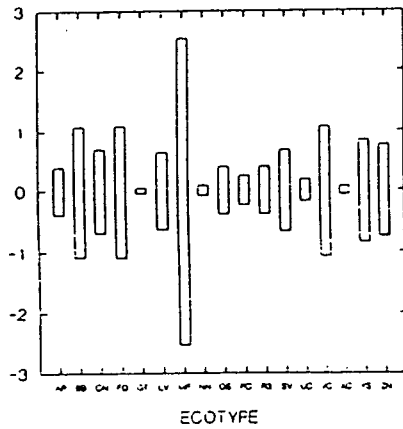


Fig. 9a. Dendrogram of several ecotypes based upon the dissimilarity index in relation to malic acid

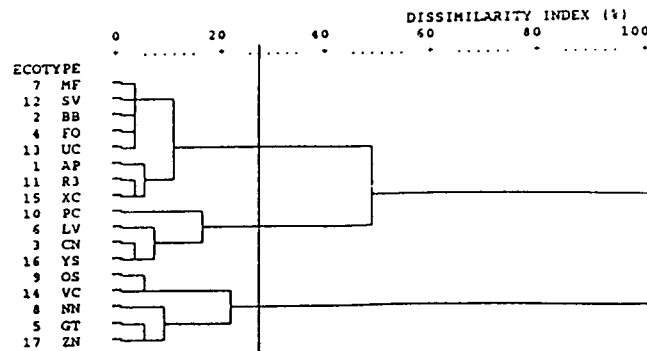


Fig. 9b: Plot of residuals estimated from Anova model in relation to malic acid

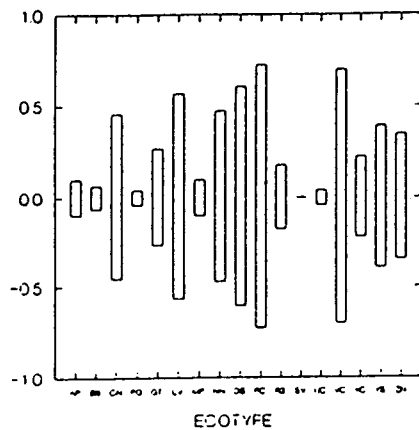


Fig. 10a. Dendrogram of several ecotype based upon the dissimilarity index in relation to tartaric acid

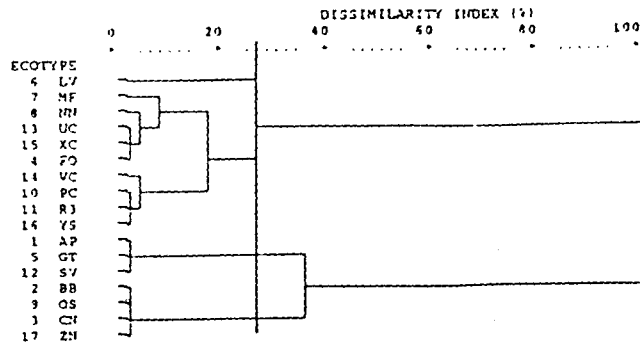


Fig. 10b: Plot of residuals estimated from Anova model in relation to tartaric acid

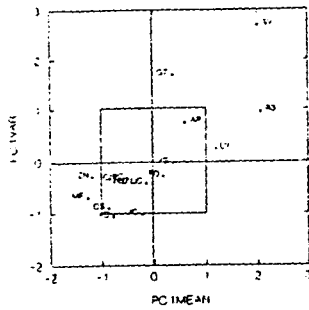
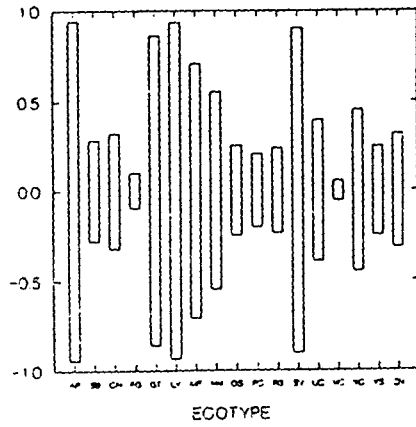


Fig. 11. Scatterplot of the first principal component for within-ecotype variance (PC1var) versus the first principal component for ecotype means (PC1mean).

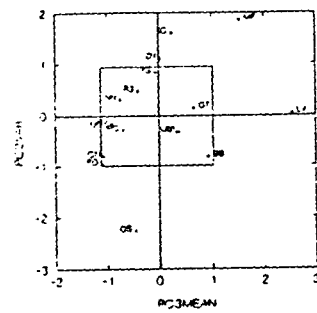


Fig. 12. Scatterplot of the second principal component for within-ecotype variances (PC2var) versus the third principal component for ecotype means (PC3mean).

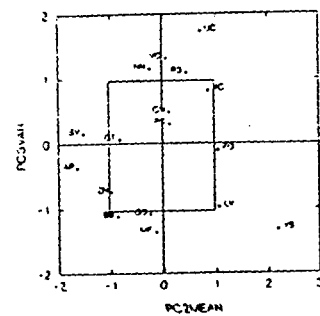


Fig. 13. Scatterplot of the third principal component for within-ecotype variances (PC3var) versus the second principal component for ecotype means (PC2mean).

SESSION 1 : GERMPLASM

DATABASE FOR GRAPEVINE VARIETIES AND -SPECIES.

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Introduction

The worldwide awareness of the loss of grapevine genetic resources is leading to responsible actions. The motives differ: international organizations, like FAG, OIV and governments, have economical, ethical and historical reasons, the breeder's success however depends on germplasm resources.

At the request of IBPGR and FAG and with their financial help the Expert Group "Selection of the Vine" of the Office International de la Vigne et du Vin (OIV) started a survey of all the viticultural research centres in the world which are in the possession of a collection of *Vitis*, aiming to establish a worldwide inventory on the genetic resources of *Vitis*. The results were published in the "Repertoire mondial des collections de *Vitis*" in 1987.

In 1992/93, the Institute for Grapevine Breeding Geilweilerhof has carried out a second survey of all the viticultural research centres in the world with collections of *Vitis*. The new facts were published in the "World List of Grapevine Collections", 2nd edition, in 1994.

Passport data (specifications to the geographical site and the grapevine collection) are published in:

	Total number of countries	Number of collections	Number of accessions
Repertoire mondial des collections de <i>Vitis</i> (OIV 1987)	30	131	41.648
World List of Grapevine Collections (Institute for Grapevine Breeding Geilweilerhof 2nd edition 1994)	40	121	30.311

In the World List of Grapevine Collections (Geilweilerhof) only those collections with more than 50 genotypes were considered, therefore the number of collections is inferior than that of the OIV list.

1. Inventory

a) OIV carried out an inquiry asking governments to list the recommended and authorized grapevine cultivars and their synonyms in their country. The resulting compilation lists 1383 cultivars which are commercially grown in 27 countries. This listing was not yet published officially.

b) In 1984, the Institute for Grapevine Breeding Geilweilerhof started the inventory of the worldwide existing *Vitis* species and -cultivars, in the beginning with the financial help of IBPGR and the help of the OIV. For each cultivar FAG passport data are registered in a database. These are:

- prime name, synonyms, berry color, origin, parentage, *Vitis* species, occurrence in collections, use, bibliographical references.

Information's on cultivars were obtained from the 121 institutes listed in the World List of Grapevine Collections and from 360 ampelographic publications and 558 single cultivar descriptions. In total 15.916 different prime names and 14.208 synonyms were collected together.

c) The inventory is available as:

- The Genetic Resources of *Vitis*, Parts I and 2
3rd edition 1994

A disk version of "The Genetic Resources of *Vitis*" was elaborated too. Besides the FAD passport data bibliographic references like author, title etc. can be displayed on the screen for each cultivar.

2. Maintenance of *Vitis* germplasm

a) in situ maintenance

The in situ maintenance is problematic because biotopes are more and more constricted and difficult to supervise so that the loss of germplasm is risked.

b) ex situ in vivo grapevine collections

In 121 collections of *Vitis* a total of 30.311 I cultivars are existing. From those 30.311 cultivars 10.659 are different. Presuming that a double conservation of each cultivar is required to avoid loss of germplasm, still about 10.000 unnecessarily maintained cultivars would remain. Therefore, international activities should be initiated to improve the efficiency in the maintenance of grapevine germplasm.

c) in vitro long term conservation

At the Institute for Grapevine Breeding the in vitro collection comprises about 70 different genotypes, particularly *Vitis* species and old autochthonous German cultivars. Obviously there seems to be no further institute in the winegrowing world maintaining *Vitis* germplasm under in vitro long term conservation conditions.

d) Long term conservation of seeds

Maintenance of the genetic diversity of *Vitis* species in situ is difficult to control. On the other hand the ex situ in vivo conservation is very expensive. Investigations on the long term conservation of seeds were started at the Institute for Grapevine Breeding. Seeds are dried slowly to a seed moisture content of 3-5% and stored at minus 20°C. At the moment, *V. silvestris* is preserved under those conditions. Seeds of further *Vitis* species will be stored this autumn. Here, too, no other institute is known practicing the long term conservation of seeds.

3. Identification

a) by morphological descriptors

- OIV Descriptor List for Grapevine Varieties and *Vitis* Species

In the beginning of the 1980's the OIV, UPOV (International Union for the Protection of New Varieties of Plants) and the FAO (Food and Agriculture Organization) decided to harmonize the descriptive characteristics of grapevines, which resulted in the OIV Descriptor List for Grapevine Varieties and *Vitis* Species. This list contains descriptors for 128 characteristics. Morphologic descriptors comprise shoot, inflorescence, leaf, bunch and berry characteristics. Through accurate definition recording was made more objective.

- Descriptions according to the OIV Descriptor List

After the establishment of the OIV Descriptor List the Expert Group "Selection of the Vine" decided to publish the descriptions of the most important cultivars of each winegrowing country according to that list. 58 cultivars were described by 11 countries. The results were published by OIV in the "Description of world vine varieties". But cultivars were described only at one site, and therefore environmental influences are not considered. This is comparable with classical ampelographies.

- Preliminary Minimal List

During the 5th International Symposium on Grape Breeding, which took place in 1989 in Germany, in an ampelographic workshop the "Preliminary Minimal Descriptor List for Grapevine Varieties" was elaborated. This list includes 41 characteristics, from which 12 descriptors were taken over from "OIV Descriptor List for Grapevine Varieties and *Vitis* Species" without any adjustments, further 19 descriptors of that list were modified and 10 new descriptors were added. The application of the minimal descriptor list was recommended by OIV and an international ampelography project was initiated. 35 collection holders from 20 countries have participated in the description of cultivars. They recorded 17 visual- and 3 berry descriptors and sent leaf and seed samples for verification and measurement to our Institute. 18 leaf- and 5 seed characteristics were measured. With the results obtained so far a group of statisticians at the Federal Center of Breeding Research on Cultivated Plants in Ouedlinburg are working on an identification procedure using discriminant analysis. The results may reveal that the Preliminary Minimal List needs to be modified through the addition of new and the elimination of non-suited characteristics. This will be discussed in detail in the Ampelography Workshop, which will be held in the scope of this symposium. As reference material specimen of 1.000 cultivars are maintained in the herbarium. Leaf comparison of cultivars with the same name but from different sites and the comparison with illustrations revealed that 98% of the cultivars are kept under the correct name or synonym in grapevine collections. Participating collections receive the results of our leaf checking.

b) through molecular genetic methods

Fingerprint techniques are considered to be a very helpful tool and may be applied if identification is not possible by morphologic descriptors. But until now there is no standardized method leading to uniform results. In addition, at the moment the analysis is too expensive.

4. Evaluation

The OIV Descriptor List for Grapevine Varieties and *Vitis* Species comprises besides morphological descriptors those of breeding importance, like the begin of berry ripening, degree of resistance to Oidium, resistance to drought etc. On the basis of these descriptor sheets we started to review literature on resistance against fungus diseases. Descriptors for *Plasmopara viticola*, *Uncinula necator*, *Botrytis* were adopted from the OIV Descriptor List. Thirteen new descriptors were added: Degree of resistance to *Elsinoe ampelina* (Anthracnose), *Glomerella cingulata* (Ripe Rot), *Guignardia bidwellii* sp. *euvitis* and sp. *muscadinii* (Black Rot) *Isariopsis clavispora* (*Isariopsis* leaf blight), *Phaeoramularia dissilens* (*Cercospora* leaf spot) *Phomopsis viticola*, *Pseudopezicula tracheiphila* (Rotbrenner) and the degree of resistance to the bacterium *Xylella fastidiosa* causing Pierce's Disease. As an example the descriptor sheet on the degree of resistance to *Guignardia bidwellii* is to be seen on table I .

We would recommend to add the new descriptors to the OIV Descriptor List. After the establishment of the descriptor sheets cultivars' resistance was evaluated according to indications found in literature. Various screening procedures, different evaluation criteria and inconsistency in terminology made a final classification difficult. In spite of these difficulties, those data were compiled in the "Preliminary List on Resistance Genes in *Vitis*" (1994). Table 2 informs on the diseases evaluated and the number of cultivars recorded as resistant with notations 7-9 and the number of cultivars described as non resistant with notations 1-6.

Final remarks

Activities on the *in situ* conservation of *Vitis* are missing as well as activities in the field of cryoconservation or the long term conservation of seeds. More emphasis should be laid on the collection of germplasm for example in the Asian centers of origin.

The 4-th International Technical Conference for the Conservation and Utilization of Plant Genetic Resources, organized by FAO, will be held in Germany in 1996. Country reports may help to assess how far the conservation of the grapevine genetic resources has progressed in other countries which could lead to better coordination among OIV, European Community, FAD and further nations.

Table 2: Number of cultivars found as resistant or

DISEASE	EVALUATION	
	resistant (not. 7-9)	susceptible (not.1-6)
Anthracnose	253	494
Black Rot	57	106
Botrytis	39	256
Brenner	12	170
Cercospora	61	203
Isariopsis	23	95
Oidium	740	637
Peronospora	285	628
Phomopsis	0	30
Pierce's Disease	70	31
Ripe Rot	14	32
White Rot	0	5

SOME CHARACTERISTICS OF TROPICAL GRAPEVINES

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THE TROPICAL VITIS

The grapevine genus *Vitis* is one of the few plants whose natural distribution spans both temperate and tropical zones in both hemispheres. Of tropical origin, these vines are the oldest living repository of *Vitis* genes available. Most of the *Vitis* species that are indigenous in tropical regions have yet to be catalogued and described. Botanical studies have often relied on very few herbarium specimens that lack complete representation of essential organs, such as primary shoot tips, mature leaves, inflorescences, fruit and seeds. Field studies of the natural habitat and the physiology and geographic distribution of these taxa are fragmentary and have increased speculation and confusion amongst both botanists and viticulturists.

Difficulties have impeded the collection and study of the tropical *Vitis* flora in situ:

inaccessibility of the terrain, lack of winter hardiness in temperate climates, difficulty in propagation from cuttings, language barriers in communication and the traditional a priori belief that these unknowns are "sans valeur vinifere," essentially not fit for winemaking.

Tropical forms can be classified into two large geographical groups, American and Asian. The American complex extends from southern Florida, spans the Caribbean Islands, reaching Venezuela.

Everbearing Vines of Tropical Species:

Many vinifera cultivars in use today, especially table grapes, are characterized by a high output of vegetative growth in relation to yield of harvested fruit. There is a large loss of photosynthate removed in vegetative pruning that has not been diverted to fruit production. This is caused by the poor inherent distribution of the inflorescences in the shoot structure that develop in competition with vegetative growth and are confined to a basal portion of the shoot. It is of considerable interest to investigate a solution that would bring vegetative growth and fruit production into balance by a parallel development of flowers and fruits. The concept of "everbearing" vines is of interest in this regard, especially in tropical climatic zones.

Within the populations of the American complex, some vines have a cyclic production of flowers and fruit when conditions remain favorable for growth. This has been observed in seedlings derived from *Vitis gigas* Fennell of southern Florida and specimens from the island of Puerto Rico and Venezuela. The existence of self-fertility in some of these examples indicates they are derived from male vines and flower and fruit set is influenced by cytokinins (6). After pruning to a single dormant eye, the primary bud develops a shoot bearing two large inflorescences, in the same manner as many vinifera cultivars. If this shoot is weak, its clusters set well and ripen their fruit. However, if the shoot growth from the primary bud is quite vigorous, the lateral buds also produce side shoots that repeat the cycle. In each succeeding cycle the inflorescences become smaller in size with fewer flowers. At the time of normal maturity of the primary crop, one finds flowers and fruit in all stages of development.

Such an abundance of potential crop can be manipulated by green pruning to prolong the harvest season or regulate crop load and composition of the fruit. This may be of economic benefit if conditions warrant separate harvests and temperature summations are sufficient to ripen the fruit. Such intermittent harvests could prove most profitable with table grapes.

In California, the so-called "second crop" derived from lateral shoots of the Ruby Seedless provide a late season harvest, an added source of income. The everbearing characteristic is inherited and has appeared in crosses with vinifera cultivars. Although rare in cultivated vinifera, some varieties are known that show this characteristic.

The Black Morocco is apparently of Turkish origin. Perold () imported the 'Trifere du Japon' from Montpellier in 1910, which proved to be the same as the Henab Turkey then grown in the Cape of South Africa. It is cultivated to a limited extent in Morocco and Algeria in warm, dry growing areas, as the crop ripens over an extended season. The clusters and berries are very large and were considered to be one of the best for export to England.

The variety is most easily recognized by its everbearing characteristics. Perold notes "when its shoots are lopped, it develops laterals with numerous second-crop bunches. When the main bunches are ripe, one finds on the vine bunches (or laterals) which turn color, are half-grown, are small, are flowering and do not yet flower.

The second crop clusters grow to a fair size and ripen up well under suitable conditions." This variety is of historical interest because it was the continuous blossoming that enabled some of the first *vinifera-rotundifolia* hybrids to be produced. Floral Biology of Some Tropical Vines:

The floral biology of *Vitis* is the basis of the widespread genetic variation in many native populations. Since most wild *Vitis* are dioecious, separate vines function as male or female, the offspring then results from cross-pollination. If different wild species are sympatric, the opportunity for natural cross pollination is increased and the hybrid plants often show increased vigor and are fertile, as shown by numerous experimental interspecific hybrids (1). Deforestation and conversion of land to cultivation offers new niches for the establishment of hybrid progeny and eventual selection of better adapted cultivars by the resident villagers.

The number, size and fertility of inflorescences are the principle selective factors determining vine yield. The number of flowers in the female vine of the *jacquemontii* type is 50 to 100, with only one inflorescence per shoot. On the other hand, a related species in northern India produces inflorescences of up to a meter in length with as many as 20 thousand flowers. There, hybrid descendants have given rise to new local cultivars such as the Rangspay in which the cluster branches arise from a central base and are of equal length, resembling a horse's tail, hence the name. This recalls the famous grapes of the Palestinian Valley of Eschol where two men are shown transporting a huge cluster of grapes suspended from a horizontal staff resting on top of their shoulders. Both in the American and Asiatic complexes, the tropical vines are sometimes more correctly classified as polygamo-dioecious, the male inflorescences are in part converted to the hermaphroditic state and are partially fruitful.

Progeny Vines:

Seedling 58-3 (Fig. 1) appears to represent the parental type of the wild species that has contributed most to the evolution of a new class of cultivars in northwest Pakistan. This vine is exceptionally vigorous, producing very long shoot growth, up to 8 meters or more. The vine begins budding out two weeks after most *vinifera*. The canes have very long internodes. The tendrils are bifid, very strong and long, usually show coiling of the minor branch, which dries back completely and remains attached at maturity.

The young shoot tip is covered with a rusty colored tomentum. As the leaves reach full size and begin aging, the rusty tomentum of the underside of the leaf surface becomes lighter and the color changes to a silvery cast. The leaf has a very long, thick petiole. The leaf blade loses most of its hairiness and on aging the surface becomes blistered, the veins appearing depressed. Only a single inflorescence of 50 to 200 flowers is borne on each shoot, being the best developed tendril at the fourth or fifth leaf axil above the base of the shoot. The inflorescence has short right angled side branches and with good fruit set is 5 to 10 cm in length.

There is some parthenocarpic fruit set and also some green nubbins of ovaries that fail to color. The berries are bluish-black in color and have a heavy waxy bloom. They are spherical in shape, 2 to 3 mm in diameter. The berry pulp is green and juicy and the skin is very resistant to cracking. The berries harvested at Davis on August 28 tested 18° to 19° Brix, were palatable, but slightly acidic in taste. The flesh clings to the seed on extraction, freeing the skin from the pulp.

Scion and seed collections of *Vitis* were collected in northern Pakistan by Thompson and associates. Two wild species were identified:

1. *Vitis jacquemontii* Parker and a possible glabrous form;

2. *Vitis parvifolia* Roxb. A weak trailing vine with very small glabrous leaves, small clusters of black inedible fruit, at higher elevations of 1,200 to 1,400 meters.

Seed collections of fruit obtained in village markets thought to be cultivated clones of *jacquemontii* were shipped to collaborators and national grape repositories in Geneva, NY and Davis, CA. I received 30 seeds each of the following accessions - of the Davis National grape Repository, 2349 - 2355. Plants grown from this material showed a very wide range in variation. Typical leaves from each seedling plant are shown in Figure 2. Many fruit characteristics are inherited from a *jacquemontii* type species. The long cluster stems and leaf petioles of this parent are also evident.

The most plausible theory is that most of the suspected *vinifera* clones are actually complex hybrids involving *vinifera* and the wild type *jacquemontii*, which would explain the rusty shoot tomentum not present in known *vinifera* cultivars. The example of introgression is similar to that observed in *californica* x *vinifera* hybrids (7).

The morphological characteristics in the seedlings and clones that are most typical are: shoot tips and young leaves are covered with a heavy rusty-colored tomentum, but less than the wild species.

The leaves of most of the cultivated clones of *vinifera* that were propagated and in fruit at the repository are very deeply lobed, and the lobes tend to curl inward. The margins are serrate with large acute teeth. The petioles are very long, often exceeding the length of the midrib. The mature canes have long straight internodes and are light brown when mature.

The inflorescences are medium to small in size, usually short conical or globular in shape. The peduncle is very thin but is highly tensile, hut not woody, often as long as the fruiting body itself (Fig.)

Fruit clusters are borne singly on the cane, usually at the fourth or fifth node from the base. Most clones observed have white, pink or red fruit with medium sized berries, hut irregular in set and final berry size. The spherical or oblate berry shape is more common than in *vinifera* cultivars.

It is evident that most of these are not *vinifera*, hut hybrid cultivars involving

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SESSION 1 : GERMPLASM

A METHODOLOGY FOR DESCRIPTION AND EVALUATION OF GRAPEVINE GERMPLASM

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1. INTRODUCTION

In Italy, in order to limit the loss of genetic variability due to the reduction of varieties grown in vineyards, the National Research Council and the Experimental Institute for Viticulture of Conegliano promoted in 1981 a program, in collaboration with other institutes, a research program, having among its main objectives:

1. the ampelographic description of local varieties;
- 2 the establishing of germplasm collections for the conservation and evaluation of these varieties.

First results were reported in a Symposium which was held in 1992 in Alghero on FruitGermplasm

Our Institute takes part to this project.

The aim of this study was to develop a suitable and practicable methodology for (1)description, (2) identification and (3) evaluation (yield and fertility characteristics, disease resistance, ecological and sensory properties) of grapevine varieties cultivated in the past which are now uncommon.

The methodology was tested in a viticultural district (more than 20,000 hectares of vineyards) in northern Italy (Oltrepo Pavese).

Major grapevine varieties nowadays grown in Oltrepo Pavese district.

Table1

	ha	%
Barbera	7212	39.98
Croatina	4942	27.39
Uva Rara	720	3.99
Riesling Italico	1619	8.98
Pinot nero	1534	8.50
Pinot grigio	452	2.51
Moscato	825	4.57
Malvasia	206	1.14
Cortese	185	1.03
Altri	345	1.91
Totale	18.041	100

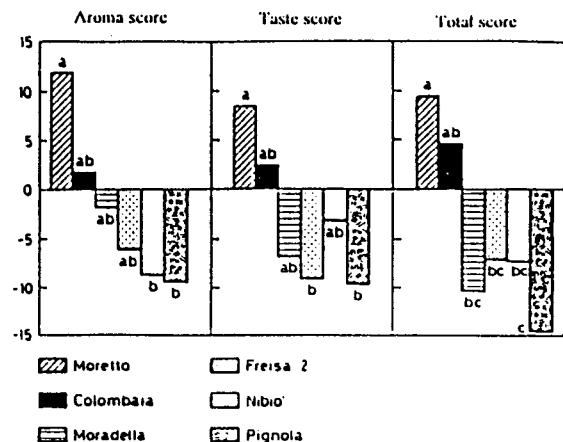


Table2

Sensory properties of some old varieties and of a tester wine (Barbera) obtained by a pair test. Sensory scores were standardized to the tester wine. Only Moretto and Colombaia wines got higher scores than Barbera.

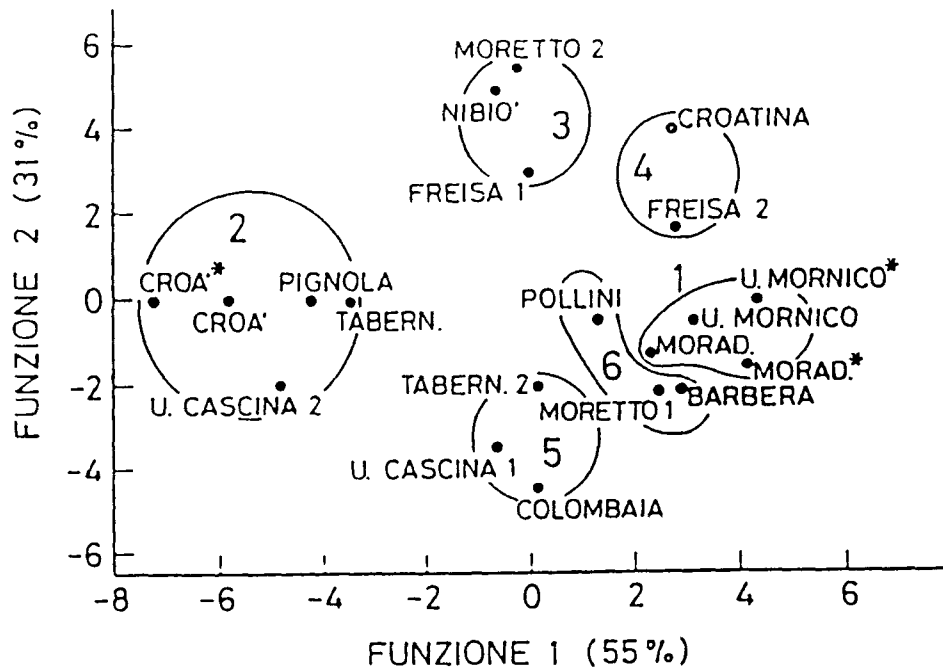


Table3

Diagram of the discriminant analysis relative to sensory characteristics of local varieties and tester wines (Barbera and Croatina) Standardized coefficients of the first two canonical functions were related to wine colour and acidity and to wine alcohol, body and tannin content, respectively.

*:replications of the same wine.

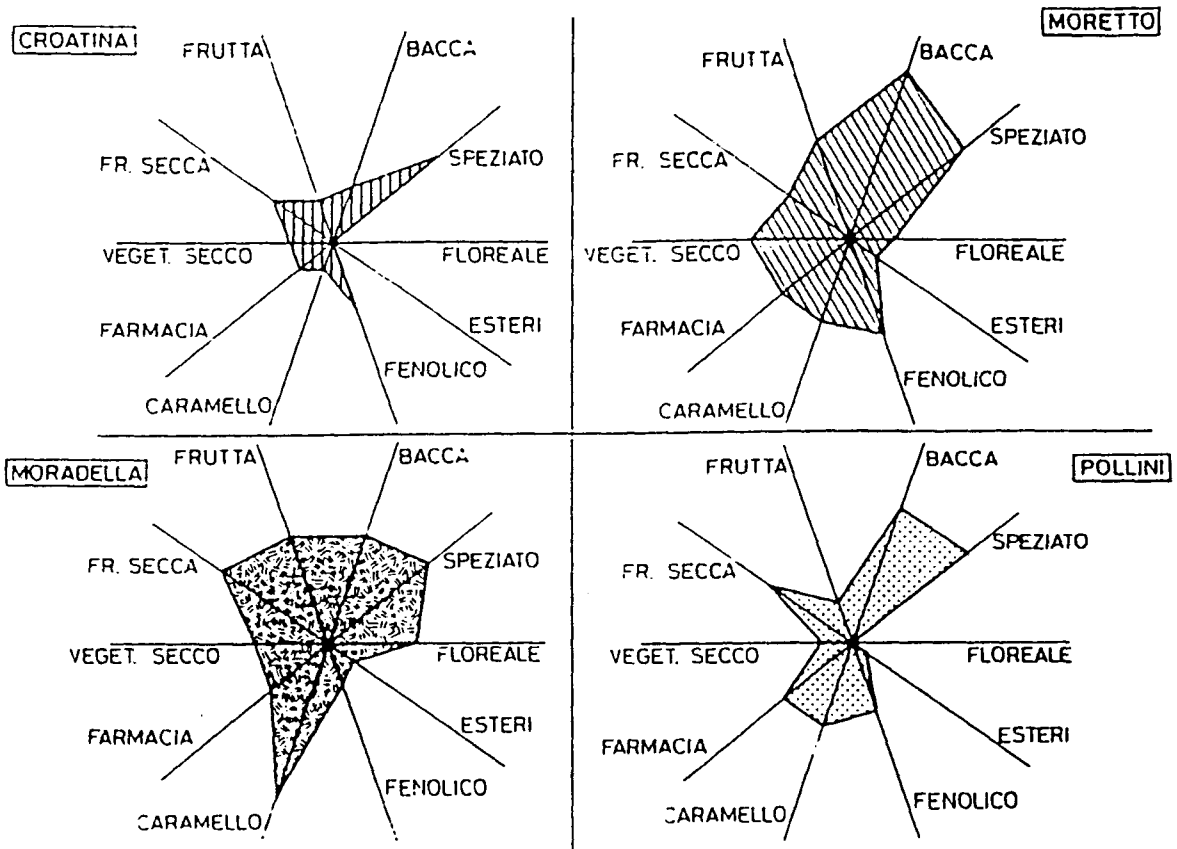


table4

Aroma characteristics of some local old varieties and of a tester wine (Croatina). Quantitative Descriptive Analysis (QDA) was applied to sensory data recorded by a trained panel of 15 evaluators in 1990 and 1991 .

Minor grapevine varieties grown nowadays in Oltrepo Pavese district.

List of varieties cultivated in the past in Oltrepo Pavese area which have been collected in C.I.VI.FRU.CE. Germplasm Collection in Torrazza Coste (PAVIA).

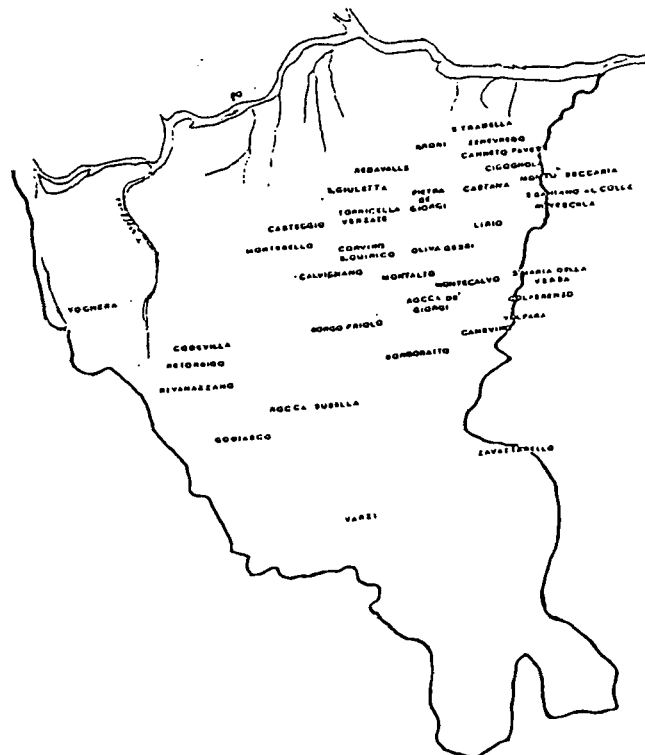
10-100 hectares
Bonarda piemontese Biancame Bosco Clinton Dolcetto Freisa Isabella Lambrusco di Alessandria Merlot Muller Thurgau Nebbiolo Pinot bianco Riesling renano Trebbiano toscano Verdea Vespolina

1-10 hectares
Albana Albarola Aleatico Alicante Ancelotta Arneis Baco Bombino b. Bonamico Cannonau Carignano Colombana n. Corvina veronese Favorita Fortana Garganega Grechetto Greco b. Greco di Tufo Grignolino Lambruschi Molinara Montepulciano Monti Ortrugo Prunista Prosecco Raboso Ribolla gialla Sangiovese grosso Sangiovese piccolo Schiava lombarda Timorasso Tocai Trabbiano Vermentino

less than 1 hectare
Abbuoto Aglianico Albana n. Ansonica Avana Avarengo Bellone Bianchetta Biancoletta Bovara Bracciola Brachetto Cabernet Francese Cabernet sauvignon Cagnolari Catarratto Casanese Cilegiolo Cococciola Coda di Volpe Colorino Corvone Durella Empibotte Erbaluce Falanghina Fiano Fogarina Forostera Francavidda Gamay Girò Greco n. Groppello Incrocio Terzi Mauica Mammico Marzemino Mazese Mocasina Molinara Monica Montonico Negroamaro Nerello m. Neretta cuneese Pavana Petite rouge Pignola Primitivo Rondinella Rossara Sauvignon Schiava grigia Seyve Villard Sylvaner Verde Syrah Tintora Turca Verdea Verdello Verdoschia Vermentino Vernaccia Vespolia altri vitigni

- Colombaia b.*
- Colombaia b. (V)*
- Colombaia nera*
- Croà rosso*
- Croà rosso 2*
- Croatina b.*
- Croatina nodo C*
- Freisa*
- Freisa 1*
- Freisa 2*
- Moradella (V)*
- Moradella 1*
- Moradella 2*
- Moretto (V)*
- Moretto 1*
- Moretto 2*
- Nibiò*
- Nibiò 2*
- Pignola*
- Pollini Barbera*
- Pollini bianca*
- Pollini ignota*
- Pulitana*
- Tabernello 1*
- Tabernello 2*
- Timoraccio 2*
- Timorasso 1*
- Uva Cascina*
- Uva Cascina 1*
- Uva Cascina 2*
- Uva di Mornico*
- Vermei*
- Vespolina (V)*

Locations in Oltrepò Pavese district which were surveyed in the Germplasm programme.



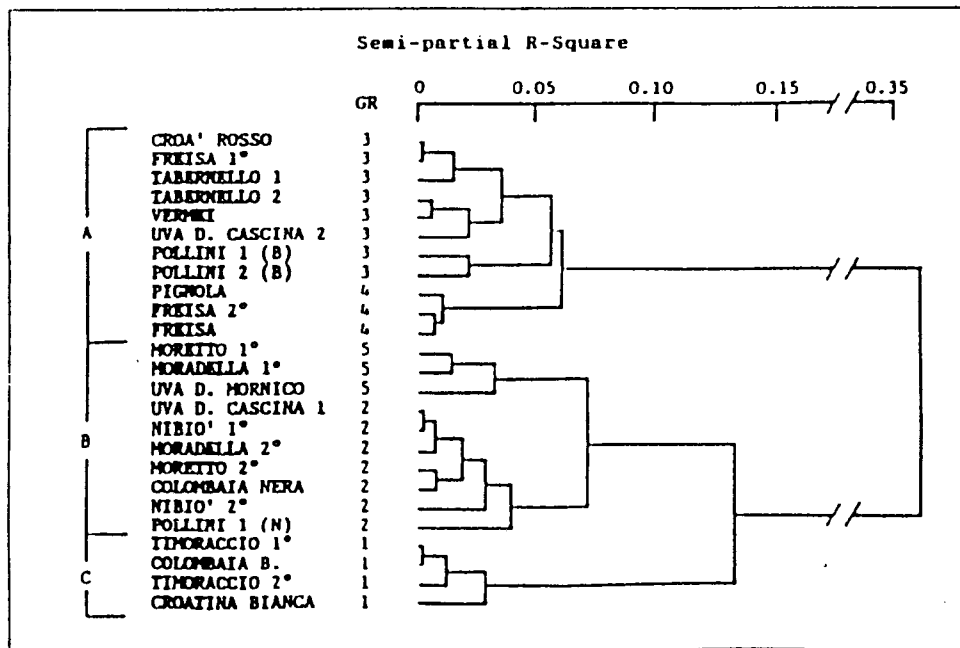
Mean of yield and must composition characters recorded in 1990-1993 period

Variety	yield kg/vine	cluster weight (g)	bud fruitfulness	soluble solids (°Brix)	titratable acidity (g/l)	pH
Colombaia b.	6.91	378	1.09	19.3	7.68	3.17
Colombaia b.(V)	4.64	293	1.05	18.1	7.20	3.12
Colombaia nera	8.89	372	2.09	20.9	5.42	3.36
Croà rosso	13.04	384	1.74	21.0	7.39	3.10
Croà rosso 2	9.17	347	1.66	17.1	8.15	3.01
Croatina bianca	8.66	349	1.48	18.5	6.27	3.09
Croatina nodo C	5.30	369	0.92	22.6	5.85	3.26
Freisa	4.55	238	1.53	21.0	6.14	3.25
Freisa 1	7.02	314	1.57	21.7	9.31	3.31
Freisa 2	4.52	219	1.52	21.0	6.83	3.21
Freisa (V)	2.94	130	1.70	19.8	5.59	3.57
Moradella 1	5.57	183	1.69	19.1	7.19	3.30
Moradella 2	6.59	273	1.64	19.8	7.19	3.35
Moretto (V)	1.22	179	0.77	20.7	5.32	3.47
Moretto 1	2.35	135	0.98	20.2	6.07	3.40
Moretto 2	4.78	232	1.08	20.2	6.92	3.18
Nibio	4.45	95	2.50	20.8	5.55	3.26
Nibio 2	2.40	127	1.44	20.5	4.94	3.30
Pignola	1.99	161	1.20	21.6	7.60	3.16
Pollini Barbera	3.32	155	1.56	23.9	7.73	3.23
Pollini bianca	4.64	178	1.51	20.9	4.16	3.54
Pollini Ignota	3.01	180	1.13	18.0	5.42	3.39
Pulitana	7.26	558	0.83	15.4	6.30	3.20
Tabernello 1	13.08	356	1.77	20.3	6.51	3.36
Tabernello 2	6.67	272	1.54	19.1	6.86	3.11
Timoraccio 2	5.49	346	1.07	25.4	5.51	3.24
Timorasso 1	6.65	249	2.00	24.0	5.60	3.35
Uva Cascina	10.00	227	2.32	20.0	6.00	3.40
Uva Cascina 1	19.43	362	2.19	18.9	5.80	3.45
Uva Cascina 2	7.71	326	1.48	22.1	6.84	3.26
Uva di Mornico	6.36	302	1.38	19.6	5.28	3.32
Vermei	7.33	295	1.34	22.9	6.48	3.21
Vespolina (V)	8.71	259	1.45	20.7	8.50	3.00
MEAN	6.14	277	1.86	20.4	6.37	3.26

Soluble solids (°Brix) and titratable acidity of some grapevine varieties grown in Oltrepo Pavese recorded in 1883 vintage.

Località	Azienda	Vitigno	Zuccheri %	Acidità ‰
Casteggio	Truffi Giovanni	Moradella	18	8
		Uva rara	20	7
		Barbera	21	14
Corvino	Cornaro Paolo	Croà	19	10
		Croatina	20	7
		Ughetta	22	10
		Dora	16	11
Casteggio	Giulietti Carlo	Dora	18	11
		Ughetta	18	8
		Vermiglio	17	10
		Croatina	19	18
Oliva Gessi	Debenedetti Abramo	Moretto	18	10
		Dora	17	14
		Barbera	20	11
		Moradella	18	14
		Ughetta	23	7
Roveskala	Guffanti Angelo	Dora	18	9
		Pignolo	19	6
		Barbera	22	9
		Croà	19	8
		Croatina	22	6
		Vermiglio	23	6

Groups obtained by cluster analysis (Ward's method) applied on the 18 ampelographic characters used to describe grapevine varieties in Italian National Research Council Project on Grapevine Germplasm Preservation.



Anthocyanins in the skin.

Classification of Oltrepo Pavese local grapevine varieties for the five monoglucoside concentrations (expressed as percentage chromatographic area at 520 nm), the summation of acetic esters and the summation of p-cumaric esters.

Varieties are divided in five groups according to results of cluster analysis.

GROUP 1.

Croa' acino piccolo (10/90), Croa' Rosso (3/90-91), Croa' Rosso (14/91), Croa' Rosso (16/91), Incognita 4 (8/90-91), Incognita 5 (8/91), Moradella 1 (13/91), Moretto 2 (3/90), Rossarone acino grande (10/90), Rossarone chiuso (14/90-91), Rossarone gentile (10/90-91), Tabernello 2 (3/90), Tabernello (3/90-91), Uva Rara (1/90), Doux d'Henry (9/91), Ignota 1 (16/91), Sangiovese (16/91), Uva dura (14/91), Vermei (3/91), Ignota 4 (16/91), Rossarone maschio (14/91).

GROUP 2.

Bonardina (6/90), Brachetto (9/90), Freisa 1 (3/90-91), Freisa 2 (3/90-91), Freisa (6/90-91), Incognita 5 (8/90), Incognita 6 (19/90-91), Moscato nero (9/91), Pignola (3/91), tipo Pulitana (6/90-91), Uva Rara (4/90), Uva Rara (5/90), Uva Rara (6/90), Uvetta (16/91), Bonarda (3/91), Ignota 2 (16/91), Ignota 4 (15/91), Uva nera particolare 2 (9/91), Ignota 1 (15/91), Ignota 1 (16/91), Ignota 2 (15/91), Ignota 3 (16/91), Rossarone (15/91)

GROUP 3.

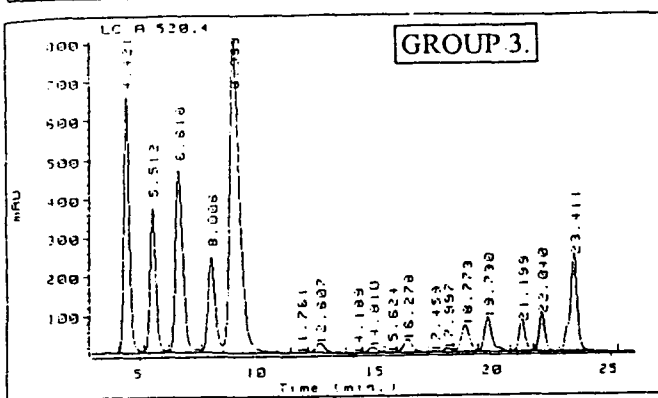
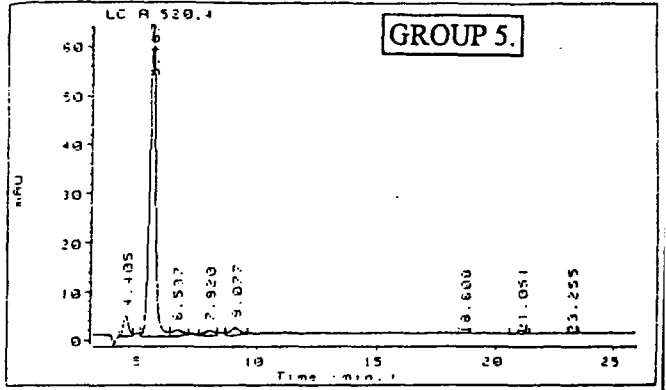
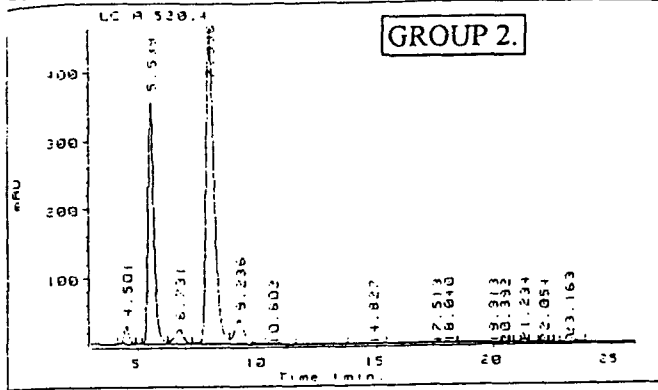
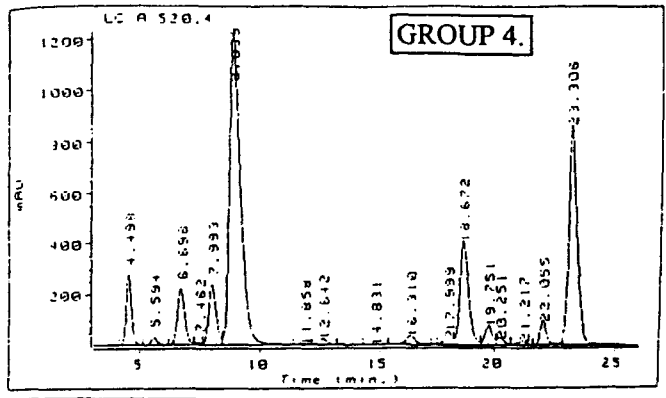
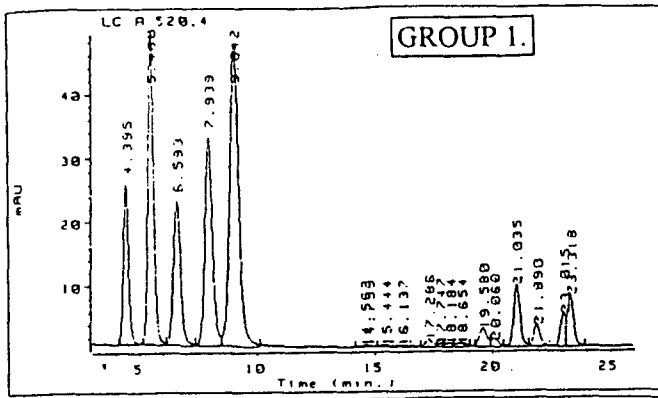
Barbera (1/90), Barbera (2/90), Barbera (3/90), Barbera (4/90), Barbera (5/90), Barbera (6/90), Barbera (19/90), Barberone (6/90-91), Basgano (7/90), Basgano (8/91), Basgano (14/91), Colombaia nera (3/90-91), Croa' grappolo grande (11/90), Croa' grappolo grande (14/91), Croatina (2/90), Croatina (6/90), Croatina (12/90), Freisa (3/91), Incognita 1 (6/90-91), Incognita 3 (8/90-91), Moradella 1 (13/90), Moretto (3/90-91), Moretto (6/90), Moscato rosa (9/90-91), Moscato nero (9/90), Moscato nero (14/91), Moscato nero (16/91), Pignola (3/90), Pollini Barbera (3/90-91), Uva crova di Montaito (13/90), Uva della cascina 2 (3/90), Uva della cascina (9/90-91), Uva della cascina (3/91), Uva di Mornico (7/91), Uva Rara (3/90), Uva Rara (19/90), Bermestia (9/91), Bonarda (15/91), Ignota 3 (13/91), Pissadella (8/91), grappolo lungo (17/91), Moradella 2 (3/91), Moscato rosa (16/91), Ignota 2 (13/91), Ignota 2 (16/91), Uva rossa 1 (17/91), Uva rossa 2 (17/91).

GROUP 4.

Basgano (8/90), Ciliegiolo (6/90-91), Croatina (3/90), Croatina (4/90), Croatina (5/90), Croatina (13/90), Croatina (19/90), Moradella 1 (3/91), Nibio' (3/90), Uva della cascina 2 (3/91), Uva di Mornico (3/90), Uva di Mornico (7/90), Uva di Mornico (13/90-91), Uva di Nigazzo (13/90-91), Uva dora (13/90-91), Uva nera particolare (9/90-91), Uvetta (13/90), Moradella grossa (18/91), Nibio' 2 (3/91), tipo Tintoria (13/91), Vespolina (3/91), Ignota 1 (13/91).

GROUP 5.

Tipo Pulitana (3/90), Pulitana (3/91).



Chromatographic patterns of grape skin anthocyanins of some local varieties. Time as min.

SESSION 2 : IN VITRO CULTURE

Micro propagation of the grapevine : results often years of experiments in the Champagne vineyard and results of the first vinifications.

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ABSTRACT

The traditional technique of woody grafting grapevine multiplication could be usefully complemented by the *in vitro* micro-propagation process and by the green grafting method. In this work, the possibilities and limits of the *in vitro* propagation process are studied. About two hectares of experimental *in vitro* grapevines are planted in the Champagne vineyard. The viticultural and enological results after ten years of experimentation have been gathered and are presented

Compared to control vines, no morphological difference and no phylloxera damage have ever been observed on the 41B (*Vitis vinifera cv chasselas x Vitis Berlandieri*) and 333EM (*Vitis vinifera cv Cabernet Sauvignon x Vitis Berlandieri*) from *in vitro* propagation, planted in the field in 1985 (8 years old)

Leaves down side and one year old stem of Chardonnay and Pinot noir from *in vitro* vines present some vegetative differences compared to the control grapevines. As a consequence of a poor flower differentiation, the *in vitro* grapevine production is about 50 % lower than the production from control vines but the developed berries are normal (shape, size, maturity,..). Nevertheless, all these abnormal differences progressively disappear. The results of vinifications from 1989 to 1993 are similar to the control results.

No significant preference in Chardonnay and Pinot noir base wines from micro propagation and control grapevines has been detected by a panel.

INTRODUCTION

While trying to verify the hypothesis according to which apical meristems were virus free, G. Morel and C. Martin (13) gave the *in vitro* culture method its rank of sanitary selection tool.

The *in vitro* multiplication process has been used since the sixties in order to solve sanitary problems (elimination of certain dangerous viruses). So Gauzy (7) obtained sound plants out of severely fan leaf virus infested *Vitis rupestris* cuttings, after heating at 35 °C for 90 days. Afterwards, other serious virus diseases have been eradicated through *in vitro* thermotherapy : leaf roll, stem pitting, bacterial necrosis (10).

Some of these results rapidly found a viticultural application for clones of the root-stock and vine material improved by this method has been legally approved and distributed to the nursery industry.

In return, the results obtained on the *Vitis vinifera* varieties have not permitted any practical use today, even though they are satisfying from a sanitary point of view. The *in vitro* multiplication seems to induce juvenile morphological changes : jagged leaves, pigmentation of young stems and change of the production capacity (9).

In order to test in the vineyard the results of these first preliminary works on the Champagne grapevines begun 1984, MOET & CHANDON has undertaken viticultural and enological studies on the *Vitis vinifera* Chardonnay and Pinot noir produced through vegetative *in vitro* multiplication and nursery green grafting.

This work had three aims :

- * Study technical conditions of *in vitro* micro cutting multiplication, greenhouse acclimatization and green grafting in order to evaluate the industrial use of that vegetative multiplication process for grapevine
- * Plant an important area of *in vitro* grapevine to determine the uniformity and conformity of performance relative to standard material
- * Vinify the grapes of these vines to evaluate the organoleptic quality of the musts and the wines obtained from these *in vitro* plants.

MATERIALS AND METHODS

Vegetative Material

Two varieties of *Vitis vinifera* are studied in this article : Chardonnay clone 96 France and Pinot noir clone 388 France. This clone of Chardonnay, selected in Burgundy is grafted on the rootstock 333 Ecole de Montpellier standard (*Vitis vinifera* cv Cabernet Sauvignon x *Vitis Berlandieri*). The planting took place in 1986 on the Chouilly terroir (0,2 ha). The density is 7500 plants per hectare (1,10 m each row and 1,20 m each plant) The pruning method is the traditional Chablis champenois (120000 buds per hectare) and the trellis system is the vertical shoot positioning 1,30 m high.

The clone 388 of Pinot noir selected in Champagne grafted on 41 B Millardet de Grasset clone 86 France (*Vitis vinifera* cv Chasselas x *Vitis Berlandieri*) in 1986. The density is 8300 plants per hectare (1m x 1,2m). The pruning method is cordon from Champagne : this is a cordon de Royal with a rejuvenation of one stock per five. The trellis system is the same as for Chardonnay.

The control parcels are located on the same terroirs. The soils resulting from chalk alteration are typical from Champagne Formation pruning lasts seven years at least for Chablis champenois and five for the cordon pruning system

In vitro micro propagation :

The vine cuttings came from the Comite Interprofessionnel des Vins de Champagne (CIVC) collection. The rootstock cuttings came from the Etablissement National Technique d'Amelioration de la Vigne (ENTAV). The ligneous cuttings were taken in March-April and were grown under a plastic tunnel in the following substrate : a mixture of turf/perlite (70/30) fertilized at the beginning with 0,5 g/L of a solution composed of Nitrogen (N)/Phosphorous(P)/Potassium(K) (28/14/14) and micro elements.

All through their development, ligneous stems are treated with a fungicide (type anti Botrytis and Downy mildew). After one to two month growth, herbaceous nodes are set apart on the growing herbaceous stems to be introduced in vitro culture after an appropriate disinfection (20 % diluted commercial sodium hypochlorite for 15 min, 70 % alcohol for 5 min and rinsing with sterile water).

The in vitro introduction was done on a Murashige and Skoog (14) culture medium modified with addition of thiamine-HCl (5 mg/L), myo-inositol (100 mg/L), pyridoxine-HCl (1 mg/L), calcium pantothenate (1 mg/L), nicotinic acid (1 mg/L), saccharose (25 g/L) and activated carbon (500 mg/l) It was solidified with 7 g/l of agar (Touzaud et Matignon) and adjusted to pH 6 with a 0,1 N NaOH solution That medium with no added growth regulator, was used both for budding and rooting The medium was dipped (140 ml/jar) in glass jars (750 ml) from the "Verrerie mecanique champenoise", screw-capped with plastic Meli type caps and sterilized during 20 min at 120 °C (under a 1,2 kg/cm² steam pressure).

Ten explants were vertically set down in each jar They were grown under "photo periods" of 16 hours (30 W Osram neon tubes "lumiere du jour", placed at 50 cm from the jars, giving a 60 mE/m² light intensity to the explants) at a temperature of 25 °C ± 1°C.

Every 45 to 60 days subcultures were done by node cuttings, that is to say taking a stem fragment and its bud (± 1,5 cm).

Greenhouse acclimatization :

Greenhouse acclimatization was done on 20 days old *in vitro* plants, which mean small plants with 3 to 4 leaves (5 cm high) and with 1 to 3 emergent I to 3 cm long roots.

The in vitro plants are drawn out of the agar and pricked out in horticultural substrate from Grodan Society : mixture of yellow turf, brown turf and perlite (20 %), fertilization 0,5 g/L with N/P/K and micro elements solution. They were set under a plastic tunnel for 8 to 10 days in a relative saturating moisture at 20-25 °C. Then the plastic tunnel was progressively opened to gradually bring relative moisture equal to atmosphere humidity Next, plants are grown for 6 to 8 months to get 2,5 mm stem diameter grapevines and rootstocks. Then the simple slide herbaceous grafting technique (3) was used to graft these plants.

Grafted plants are fertilized to facilitate their lignification and they are stored bare-rooted during winter in a cold room (4 °C) with relative saturating humidity Usually, planting takes place in spring. The bare-rooted grafted grapevines are planted manually or with a pick.

Measurement methods in the vineyard

Boubals method (1) is used to test the phylloxera sensitivity of the plants

During the harvest several parameters are assessed on both control and in-vitro plants.

- weight of grape per stock repeated 10 times
- weight of 10 clusters per stock repeated 10 times.
- potential alcohol (refracting method 16,83 g of sugar corresponding to 1% alcohol).

Repetitions allow statistically significant differences to be tested by the analysis of variance (ANOVA) method (5%)

Vinification process ;

Sampling : Grapes are harvested in September or October, depending of the state of ripeness of the grapes. The lots from the same soil are harvested separately the same day. The harvest is set in 90 L bottom punched boxes Four boxes are collected for each lot (approximately 200 kg).

Pressing : the grapes are immediately brought to the CIVC experimental pressing centre. the boxes dumped in small capacity (180 kg) vertical press "COQUARD", and go through the same pressing cycle. While pressing, the out lawing juice undergoes a manual sulphiting (40 mg/L). The must is collected in 90-L stainless steel vats A sample is taken for laboratory analysis.

Settling of the must: in stainless steel vats (static settling) at room temperature during 12 to 15 hours

Racking : the batches are racked in two 35-L carboys containing 30 L of juice or in a 70-L stainless tank containing 60 L of juice. Musts of the same origin are in identical containers

Chaptalisation and yeast addition : sugar is added to the different must in order to reach a potential alcohol degree of 11,2 % Then the musts are inoculated with a selected yeast preparation (.Saccharomyces cerevisiae strain Moet et Chandon) in order to bring a concentration of 3.10^6 yeast/nil.

Alcoholic fermentation : all through the alcoholic fermentation, temperature and density are recorded daily, up to the total consumption of sugar.

Malolactic fermentation: lactic bacteria (*Leuconostoc oenos*) are added as soon as the alcoholic fermentation is over. Total acidity and pH are recorded weekly and this helps to follow the fermentation evolution. The end of the fermentation is confirmed by a measure of malic acid.

Racking and sulphiting: the wines are racked in 25-L carboys and undergo a 20 mg/L sulphiting. A set of samples is set aside in order to taste the wines.

Wine stabilization is reached through storage of the carboys in a cold room (2°C), while waiting for bottling

Bottling and bottle fermentation: the wines are racked after natural fining. Sugar (24 g/L), yeast (10⁶ /ml) and bentonite as fining agent are added to the wines. Second fermentation and ageing take place in the cellar at a temperature of 12°C

RESULTS AND DISCUSSION

Result in the vineyard

The planting method: Insect damage on roots or Botrytis attacks on rootstock were detected in some cases before planting. These problems were solved by removing the soil from the roots. Damaged plants were eliminated to reduce the failure risk although such problems could also be solved by suitable chemical treatments A 90 % success rate is now possible (at first, only 10 %) by controlling the main quality factors of grafted plants : normally wood maturation and winter preservation.

Planting on very heavy soil that does not allow proper rooting, particularly in soils with a high rate of clay or using very weak plants (diameter of the shoot under two millimeters) were proven to be the main other reasons for plantation failures.

The phylloxera resistance: The rootstock obtained in-vitro are as resistant as the control 41 B (6), when assessed with the Boubals test In the vineyard, all the plants we have put out in the soil are uninjured by phylloxera .

Nodosities or tuberosities of phylloxera have never been found

Plant Development: In vitro plants are small (3 to 6 mm depending on under glass cultivation conditions) but have an intense development : three years after planting, no differences can be observed between *in vitro* plants and traditional ones.

According to Fouret (4) and Gallet (5) an important development of hairy roots, uncommon on vine has been observed as a feature of youth plants During the first years, young in vitro plants are quite different Continuous blush is observed all along the shoot. The leaf lateral sinus are much deeper (Fig 1) and unusual villosity, is observed on veins (inferior side) on both young and adult leaves.

About the seventh year, those differences fade on Chardonnay (Fig. 2) and Pinot noir.

According to Grenan (10), blush disappears on stems and the lateral sinus become normal.

Harvest : In Champagne, a two year old vine can produce several clusters (unfit for the appellation wine production). The production becomes effective when the vine is three years old.

In-vitro young plants produce as many inflorescences as control ones, but a large part of their flowers fall during their differentiation and never blossom. This generalized abscission reduces significantly the production (50 % by comparison to the control harvest).

Huglin (11) suggested the hypotheses of an excessive vigour induced by *in vitro* cultivation and causing the abscission Experiments were undertaken by modifying the main factors of vine vigour (2) : no fertilisation , the number of buds after pruning, the date of the pruning period

Chardonnay : After three years the weight of pruned wood did not show a significative influence of these factors on vigour As yet, the reason for premature abscission of flowers on Chardonnay *in vitro* plants has not been determinated

Harvest yield of *in-vitro* plants progresses year by year whatever is the pruning method (normal Chablis or long pruning method). But these level stay significantly different and less than the control harvest yield (analyse of variances), eight years after plantation .

Nevertheless, mainly inflorescences are normal (Fig. 3)

Pinot noir : Whatever the studied factors, harvest yield of the *in-vitro* plants located on Verzenay terroir has increased regularly and can be now compared to control.

The pruning method Cordon champenois, that leaves an important arm of old wood is perhaps one of the reasons why these *in vitro* plants recover a normal harvest level.

Results of musts analyses

Analytical differences are usually correlated to the weight of grapes on the vine. Young plants often have that kind of problem for they show an obvious difference in productivity. It mostly influences the maturity rating (sugar g/l / total acidity). Musts analyses done for three years on grapes produced from 5, 6 and 7 years old Pinot noir and Chardonnay vines gave no significantly different results (similar maturity).

Whichever the year, there was no difference in ability to ferment, both for alcoholic and malolactic fermentation's There was no sugar left in any wine Bottle fermentation rates have always been comparable

Tasting results

Pinot noir : there was no significant preference on tasting 1989 to 1993 base wines.

Chardonnay: the control wine was significantly preferred in 1990. There was no significant preference on tasting 1992 and 1993 base wines.

CONCLUSION

Today grapevine vegetative multiplication using the *in vitro* process is hardly to be considered for *Vitis vinifera* vines (8) (15), as long as the problem of delay to get a normal productivity is resolved Present studies are trying to understand the real *in vitro* process influence on flowering disfnctioning. It is also noted that some authors do not report such grapevine juvenility problems following *in vitro* process (12)

A solution which permits plants (raised from sowing and so juvenile) to rapidly get adult characteristics in greenhouse (10) is already known It consist in taking buds from the 40th node onwards however it demands a long growth period in green-house which is not compatible with a commercial plant production

Nevertheless, this solution would allow a rapid multiplication of the selected material in a perfectly safe way (basic clone A or B in the French sanitary improvement project □ plan♦, genetically improved vines,...)

Concerning the rootstocks, the *in vitro* multiplication technique could be used as described, because, up to this day, no variation has ever been observed, at least on the 333EM and the 41B rootstocks

ACKNOWLEDGMENT

The autors thank the CIVC (Comite Interprofessionnel des Vins de Champagne) and the ENTAV (Etablissement National Technique d'Amelioration de la Vigne) for providing the variety and rootstock clones and Peter HAYES from Grape and Wine R&D corporation/South Australia for reviewing this paper

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Figure 1 : Left : Control leaf
Right : *In vitro* Chardonnay's leaf of three years old grapevine



Figure 2 : *In vitro* Chardonnay's leaf of 6 to 8 years old grapevine.
The morphology of leaf is similar to the control one.

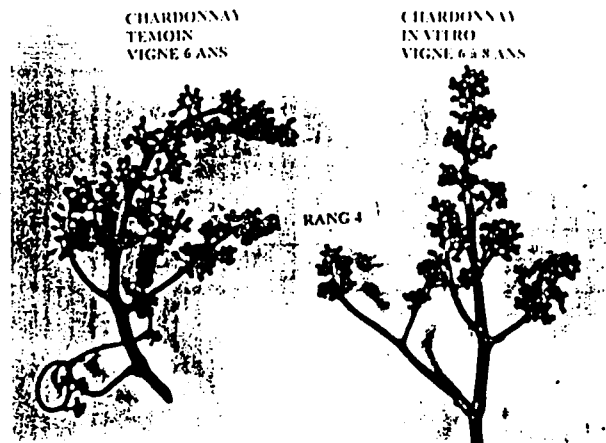


Figure 3 : Left : Control inflorescence
Right : *In vitro* Chardonnay inflorescence of 6 to 8 years old in vitro grapevine, which is similar to the control one.

SESSION 2 : IN VITRO CULTURE

Micro Regeneration system on explants of the grapevine

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Summary: The development of a high-yielding regeneration system on leaf discs of grapevine cv. Seyval is described. After 4 years of examinations which were focused on the effect of the origin of the starting material, basal media, phytohormone combination and concentration, influence of amino acids and culture conditions, a regeneration protocol could be outlined. Best embryo induction (80% embryogenic explants) has been obtained, when leaf discs were excised from *in vitro*-plantlets and cultivated on a modified NN69-medium supplemented with 20 μ M NOA and 4 μ M TDZ. The application of 2,5 μ M phenylalanine to the induction medium promote embryo production significantly. By a subsequent cultivation of the initial explants on NN69 medium without any additions resulted in further embryo production. The embryogenic capacity could be maintained for at least more than 18 months.

Key words: somatic embryogenesis, explant source, amino acid, phytohormone Abbreviations: BAP, 6-benzylaminopurine, LS, LINSMAIER and Skoog (1965), MS, Murashige and Skoog (1962), NN69, NITSCH and NITSCH (1969, NOA, β -naphthoxyacetic acid, PHE, L-phenylalanine, TDZ, Thidiazuron (N-phenyl-N'-1,2,3-thiadiazol-S-yl-urea)

Introduction

The application of tissue culture techniques as additional tools within existing reeding programs is hindered by the unsatisfying regeneration of grapevine tissues. But the use of the interesting perspectives of tissue culture for breeding purposes, resp. the culture of protoplasts, like somaclonal selection, genetransfer, somatic hybridization (Alleweldt 1989), elucidates the necessity of an effective regeneration protocol .

This report gives an overview about the efforts to establish a regeneration protocol with a high standardization. Therefore the examinations were concentrated on fulfilling special requirements like an unlimited availability of the starting material and a high productivity of regenerates over long periods intending to transfer the obtained experiences to protoplast culture.

Because the interspecific cultivar Seyval has successfully regenerated from leafderived callus (Krul and Worley 1977), and was used for isolation and cultivation of protoplasts (Reustle and Alleweldt 1996), this promising variety has been examined in our study.

Material and methods

A detailed description about the cultivation of the starting material from greenhouse or *in-vitro*-culture and the excision of explants is given by Harst and Alleweldt (1994, in press). Media preparation and culture of induced embryos and recovered plantlets is carried out according to Harst-Langenbucher and Alleweldt (199S).

Results and discussion

Because even all explants taken from field grown grapevines were highly contaminated (data not presented) only leaves from greenhouse cultivated grapevines were used as explant source. For induction of somatic embryos on leaf discs of greenhouse deriving starting material only a modified NN69 medium has proved to be suited. Compared with BS- and MS-medium where no regeneration could be observed, on NN69 medium about 6 % of the plated leaf discs showed embryo induction in the first year of our examinations.

So far, a special phytohormone combination which inforced embryo induction could not be outlined. Therefore in subsequent investigations different phytohormone combinations and concentrations were tested. As auxin compounds 2,4-D was examined versus NOA, and as cytokinin components SAP versus TDZ. When 2,4-D has been applied, no embryos were induced, but the application of NOA combined with both cytokinin components caused an embryo induction (Table I). With increased BOA concentration and a simultaneous increase of both used cytokinins a better regeneration rate could be achieved. Quite obviously was the maximum concentration of 20 μ M NOA combined either with 40 μ M BAP or with 4 μ M TDZ. The cytokinin effect was much stronger when BAP was applied. Because higher NOA concentrations as well as increased cytokinin concentration resulted in a decrease of the regeneration rate, in further studies these both promising NOA/BAP and NOA/TDZ-combinations were used. TDZ has first been applied for the induction of somatic embryogenesis on grapevine by Matsuta and Hirabayashi (1989). They tested TDZ on leaf discs of greenhouse grown starting material of grapevine cv. Koshusanjaku. Their results were vice versa because they obtained a higher induction

rate when TDZ (5-10 μ M) was supplemented in combination with 2,4-D. About 4 % of their explants showed embryo induction when TDZ was applied, and only about 2 % embryogenic explants have been observed when BAP was used.

By selecting the suited phytohormone application the leaf position on the shoot of the mother plant has been neglected. A possible improvement of the still insufficient regeneration rate was expected by screening a probably different regeneration capacity in dependence of the leaf position. From the apical to the basal part of greenhouse grown grapevines, younger (position 3) and older (position 7) leaves were taken. Best results have been obtained by using older leaves for the excision of explants (Fig. 1). Leaf discs from more basally inserted leaves showed a higher regeneration capacity than those being excised from younger, more apically inserted, leaves. The different reaction of the leaf discs to the applied cytokinin compound in dependence of the leaf position is evidently demonstrated.

By using the above outlined phytohormone combinations and the use of greenhouse starting material as explant source as well as considering the phyllotactic position of those leaves being assigned for the excision of explants, the regeneration rate could be improved to more than 8 % (see Fig. 1)

For a further improvement of the regeneration, in-vitro-plantlets were tested in their suitability as explant source to obtain a seasonal-independent availability of explants which should be a prerequisite for an effective use of a regeneration system. When the origin of the mother plant for excision of explants from greenhouse deriving material was substituted by in-vitro-plantlets and using the previously mentioned phytohormone combinations NOA/BAP and NOA/TDZ in the induction medium, an interesting result was achieved (Fig. 2). The most obvious result is the much higher regeneration capacity of leaf discs deriving from in-vitro-plantlets according to the results of Stamp- and Meredith (1988) with Cabernet Sauvignon. Also quite impressive is the opposite effect of the phytohormone combination. Whereas the BAP-application caused a considerably higher embryo induction than TDZ on greenhouse derived explants, however, the TDZ-effect on leaf discs from in-vitro-plantlets was twice as high (59 % embryogenic explants) than the reaction of the explants to BAP (27 %).

Because TDZ has proved to be best suited for embryo induction on explants of in-vitro-plants, this cytokinin compound was used in the subsequent examination testing the effect of amino acids in the induction medium.

Various amino acids were applied alone or in combination to the induction medium. In improving effect was only found when PHE was used in combination with other amino acids, but best results (78 %) were obtained when applied alone in a concentration of 2,5 mM (Fig. 3). On PHE-treated explants embryo induction occurred 4 weeks earlier compared to untreated leaf discs. Embryos induced on PHE-treated leaf discs showed in the subsequent cultivation on hormone- and phenylalanine-free medium a higher germination rate, and plant recovery was much better than of those embryos which were induced on untreated explants (Fig. 4).

The effect of amino acids on induction of somatic embryos on anthers of Cabernet Sauvignon was tested by Mauro et al. (1986) They obtained best results when L-glutamine and adenine was used, the application of PHE had no influence on somatic embryogenesis.

Summarizing the results of each examination the regeneration system was established for grapevine cv. Seyval, as demonstrated in Fig. 5.

Leaf discs are excised from in-vitro-plantlets and incubated on NN69-medium which is supplemented with 20 μ M NOA/4 μ M TDZ and 2,5 mM PHE for the first 4 weeks of cultivation. The cultivation of the explants and the induced embryos is carried out under permanent darkness. After about 2 weeks on this induction medium, callus is induced on the explants. In further cultivation the initial explants are transferred in 4-week-intervals on NN69 medium without any other compounds. Only some days after the first subcultivation on basal medium the first embryos can be observed. After about two further weeks some of the embryos start to germinate. These germinating embryos are excised from the leaf discs and transferred on LS-medium without any supplementation in culture tubes and cultivated in the light (16 hours light, 40-50 μ M $m^{-2} s^{-1}$). This first excision of germinating embryos is called generation A to distinguish this first charge of regenerating embryos from subsequent secondary regenerations induced on the initial leaf discs which are subcultured routinely every 4 weeks. That means that in every subculture phase a new generation of embryos germinate and have to be excised and cultivated in the described manner. These new continuously resulting generations of each subculture are called B, C, and so on.

Several of our more than 12 tests produce secondary embryos since more than 1 1/2 year by a regular transfer of the explants, and the explants are still producing embryos. From all experiments more than 500 plantlets were recovered from somatic embryos, but the total output would have been higher unless a lot of embryos have been used for other studies.

This regeneration protocol could successfully be adapted to leaf discs explants of *Vitis thunbergii* the interspecific hybrid Chancellor, and with some restrictions to *Vitis vinifera* cv. Cabernet Sauvignon, and finally to protoplasts deriving from in-vitro-plantlets of Seyval (Reustle et al. 1994, in press).

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Table 1: Effect of different concentrations of NOA in combination with different concentrations of BAP and TDZ on leaf discs of grapevine cv. Seyval after 16 weeks of culture. Data are given as means \pm S.D. [% of embryogenic explants, N=36 leaf discs]

NOA (μ M)	BAP (μ M)				TDZ (μ M)			
	2	20	40	50	1	2	4	10
5	0	0	0	0	0	0	0	0
10	0	1,2	0	0	0	1,6	0	0
20	0	0	6,5	0,4	0	0	2,9	0
40	0	0	0	0	0	0	0	0,3

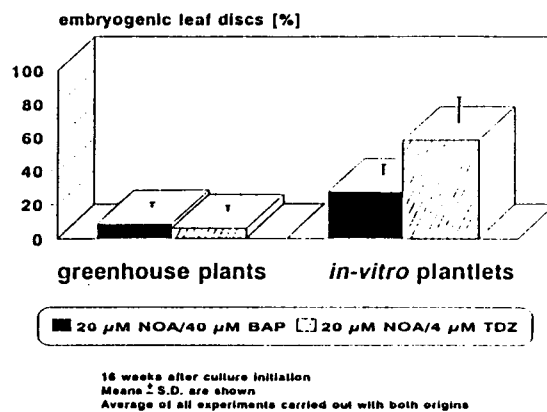
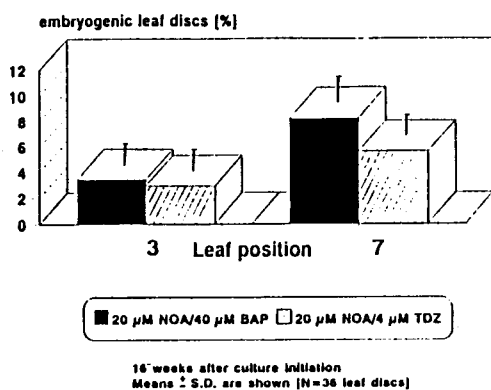


Fig. 1: Influence of the leaf position at the mother plant on induction of somatic embryogenesis on leaf discs of grapevine cv. "Seyval"

Fig. 2: Effect of the origin of the starting material on induction of somatic embryogenesis on leaf discs of grapevine cv. "Seyval"

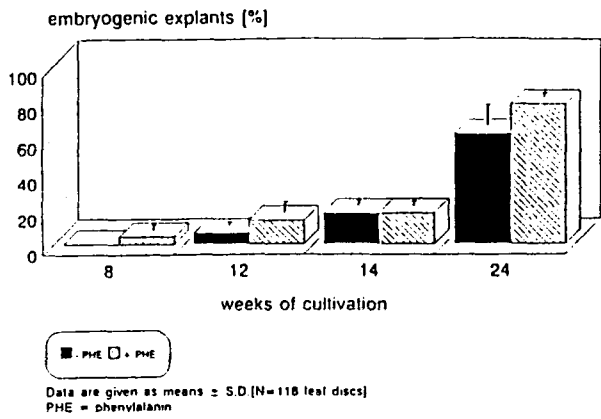


Fig. 3: Effect of PHE-treatment (2.5 mM) on somatic embryogenesis on leaf discs of grapevine cv. "Seyval"

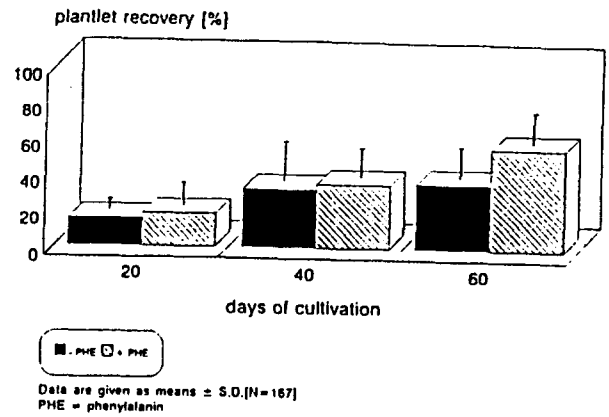


Fig. 4: Effect of PHE-treatment (2.5 mM) on plant recovery of somatic embryos of grapevine cv. "Seyval"

Fig. 4: Effect of PHE-treatment (2,5 mM) on plant recovery of somatic embryos of grapevine cv. "Seyval"

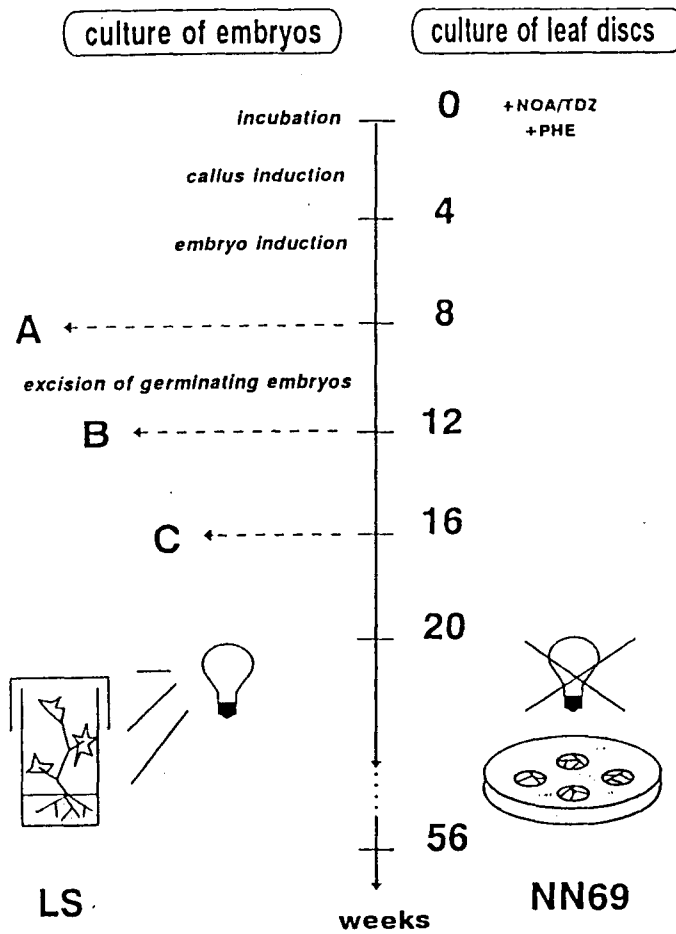


Fig. 5: Regeneration protocol for leaf discs of *in-vitro* grown grapevine cv. Seyval

NON-TRADITIONAL TECHNOLOGIES IN GRAPE SELECTION, BASED ON "IN VITRO" CULTURE METHODS

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"In vitro" culture has been firmly established in the research work, carried out at the laboratory of cyto-embryology and tissue culture of the Selection Department of the National Institute of grape and wine of the Moldova Republic.

The first investigations were initiated in 1978, the aims remained the same: the elaboration of non-traditional technology, contributing to the acceleration and the improvement of selection process in the viticulture.

During this period the following results were obtained:

1) the original biotechnology of clonal micro-propagation of grape was elaborated and used in industrial conditions (Inventor's Certificate N 1764206 "A method of vegetative propagation of grape"), followed by producing of thousands of ownrooted seedlings for mother material nurseries for planting of new varieties and clones, phytosanitary clones as well as planting material for intermediate selection;

2) the technology of hybrid embryoculture, including embryos from seedless and precocious genotype crossings, was elaborated and introduced;

3) the investigations on practical employment of a method of long-term conservation of test-tube plantlets at the low positive temperature were completed (earlier the method of long-term conservation of grape pollen at the extra-low temperature was elaborated and is being used at our laboratory);

4) an original method of green grafting of plants, obtaining "in vitro", after their adaptation to the "in vivo" conditions is now at development stage;

5) and finally, nowadays we endeavour to elaborate with the use of the available achievements of other investigators, a method of mass production of callus tissue regenerants, to promote attaining of good results in selections process.

Bearing in mind, that scientists of several other viticultural organizations pursue the same objects in their investigations with the use of "in vitro" culture, the exchange of scientific information on some principal questions seems to be expedient. So, in particular, we have to state, that the clonal propagation, while being already a routine laboratory work, in most cases has not overpassed the laboratory and nursery scale. We see here two main reasons: technological unfeasibility of many stages of such propagation and the scepticism of viticulturers, hardly accepting new non-traditional methods.

The lack of technological feasibility is conditioned, firstly, by the absence of clear elaboration of every main stage of the method: characteristics of the material, used in the acceptic culture, and its sterilization regime, depending on its preparation place (a field, nursery, laboratory), the test-tube culture, in particular, all parameters of culture place, peculiarities of transplantation of plantlets from test-tubes to adaptation substrate, cumbersome pot culture. Namely the technological feasibility of every process was the object of our special attention. Some

of these processes will be discussed further on, without presenting the table data on the investigation results, as these results, together with the nutrient medium composition, one can find in the above mentioned Inventor's certificate SU 764206 AI.

Secondly, the technological feasibility can be ensured by the appropriate technical means, upon observing the corresponding parameters.

In the course of mass clonal micro-propagation performance our special attention is paid to donor plants. Field material is used for culture only on emergency, the forcing of green shoots from the mature cuttings at laboratory conditions being preferred.

In the case of prolonged propagation of definite varieties or their clones after phytosanitary the cultivation of donor plants in greenhouse lysimeter is the most expedient. On using definite pruning system one can obtain sufficient number of buds for introduction in the culture at any time of the year and with any frequency.

Handling micro-cutting by means of immersion into the boiling water with the use of nutrient media of two or more compositions should be considered as very unreasonable and expensive. We use universal nutrient medium. It was tested on more than 250 grape genotypes, including rootstocks. One exception is to note: for introducing of the material into the culture and to obtain the inicial sterile test-tube plantlets, the concentration of nutrient medium should be reduced by 25-30% and the benzyladenine should be substituted for the pentyl-adenine.

The results of our numerous investigations on a great number of test-tube plantlets showed, that observing of physical parameters at the culture place, especially soon after the preparations of micro-cuttings (25,5-26,5

50 °C, 75-85% air humidity, 1500-2500 Lux, 16 hrs-lighting a day) ensures higher adaptability to the constant composition of nutrient medium. In the course of culture of the initial sterile test-tube plantlets, first a bud proliferates and then roots appear, while after the micro-propagation by cuttings first roots appear and only 10-15 days after a bud proliferates.

In the case of the micro-propagation by cuttings the number of the plantlet node should not exceed 12, if possible. Otherwise, its leaflets undergo fast fading with subsequent perishing of the whole plantlet. When the test-tube plantlet attains 3-4 nodes, it should be placed under mixed lighting (natural and artificial) with lower relative air humidity, that contributes to further micro-propagation and to more successful adaptation in the future.

Bearing in mind the technological feasibility and economic aspects, we have refused the pot culture or any other greenhouse culture with the aim of adaptation. We have constructed of polyethylene film (A. Litvak, N. Guzun) so called packet-vessel, that can be substituted for any pot. As a substrate, the perlite sand (1-5 mm fraction) is used in suspension with artificial soil of bionine-type, elaborated at the Institute of physico-organic chemistry, Science Academy of Belorussia. pH of this soil is 6,4-7,2.

Significant advantage of the substrate, containing the bionine, is its suitability for the long distance transportation of plants in the polyethylene packet-vessels. Survivability on this substrate attains 90 and more percent. The bionine is easily dosed, mineral

substances are not washed out in the course of irrigation, the substrate maintains very well the humidity, ensuring the plant nutrition during long transportation and adaptation. Moreover, a reinfection of plants being free of bacteria or viruses, does not occur.

As the practice has shown, the test-tubes with the plantlets, obtained "in vitro", can be sold to specialized farms only. Unfortunately, there are very few such viticultural farms. The plantlets should be sold only after their adaptation. We have solved the problem of selling by other way: the plantlets are transplanted from the polyethylene packet-vessel into larger polyethylene packets 10-11 cm in diameter and 20-25 cm high, filled with a substrate of a soil and sand. Preliminarily, some gravel is placed on the bottom of packet. Cost saving of this method is expressed in the fact, that up to 72 packets can be placed on 1 m² of shelf area of a greenhouse. Under good care conditions on own-rooted seedling with mature internodes can be obtained within 3-4 months. The farms willingly purchase such plants, that quickly become adapted to the field conditions. Moreover, the roots are clearly seen in the packets and the plants can easily be sorted, non-standard ones being left for completion of growth. Plantations of seedlings, obtained in Moldova according to our technology, do not differ from these, produced by traditional means. Along with the significant acceleration of propagation process, the production cost of own-rooted seedling, obtained by means of the culture, does not exceed more than twice the cost of an ordinary seedling.

Having in our possession rootstock plants 50-60 cm high, well rooted in the polyethylene packets, as well as "in vitro" cultivated grafts, we could perform the grafting manually. Having ensured growing together of herbaceous 2,5-3,0 mm shoots, we obtain a grafted seedling with own roots.

Due to the propagation by soft cuttings of the packet plants, the yield of own-rooted seedlings increases significantly. In this case the plants easily grow up, and the cuttings, planted into the packet-vessels, quickly become rooted and further on, due to the above mentioned polyethylene packet, easily adapt themselves. All packet-vessels are placed in small portable trays on lighted shelves of specially-equipped packet-holding boxes.

We have brought into the laboratory-greenhouse production line one more non-traditional technology, based on an embryo-culture. It is known, that a great number of hybrid seeds are eliminated from the selection process due to their structural or physiological deficiency. Any portion of "saved" hybrid embryos, and obtaining of normal plants-regenerants is of high importance, because sometimes, as we know, such plants are carriers of properties, required for the selection practice.

The successful development of the research work on embryoculture was supported by the suitability of our basic artificial nutrient medium and appropriate technical equipment. According to the development anomalies, hybrid seeds can be subdivided into 3 categories: with developed endosperm but without embryo or with very poorly developed one; with degenerating endosperm but with an embryo of various deficiency grade; empty seeds. We have shown (A.Korkodel, 1994) a possibility of X-ray determination of the deficiency grade of intact grape seeds by Mukhortova method. Explantation of several thousands germs from hybrid seeds has shown their significant structural differences. We could not obtain regenerants of germs at "globule" or "heartlet" stages. A positive result with normal plant producing was obtained, when the germ has reached to the moment of extraction the stage of "torpedo".

It should be noted, that the optimal explantation stage is specific for every crossing combination and depends on the year conditions. So, for crossing combinations "Kodrianka x Dekabrsky", "Kodrianka x Frumosa albe" and some other ones, the optimal time for the germ extraction is 35-45 days after pollination; for combinations "Koroleva vinogradnikov x Dekabrsky" and "Muscat yantarny x Dekabrsky" - 45-60 days, and for germs, obtained by reciprocal crossings, the optimal time is the berry ripeness stage. Defective germs should be extracted together with a small portion of endosperm, and during the first 5-7 days they should be cultivated at 26 °C in obscurity. The beginning of cotyledon development will show the necessity of their further development in the light (16-hrs day lighting, 25,5-26,5 °C, 2000 Lux, 75-85% air humidity). The lighting intensity should be gradually increased. Further on, the cultivation regime for clonal micro-propagation is to be considered as suitable for embryo-culture as well as well.

The main deficiencies of the growth and development of the plantlets, obtained by embryo-culture, comprise: lack of development (of the growing point, of the root), necrosis of the stem and of the root, shortened internodes, morphological anomalies of the primary leaflets (development asynchronism, grown together, leaf plates, different pigmentation etc.).

We have accumulated much methodical experience, that contributes to the survival of perishing plants. The further development conditions of the plantlets of "saved" germs is identical to those of clonal micro-propagation, but with more care and with observance of all required physical parameters. A great number of plants-regenerants from embryo-culture in 1994 will be brought into fruiting stage in the open ground. Yet more care is required in the case of embryo-culture of defective germs, extracted from hybrid seeds, obtained by crossing with seedless varieties. Nevertheless, tens of such plants-regenerants have attained a normal development stage.

Thus, basing on the experience of two technologies-clonal micro-propagation and embryo-culture of hybrid germs - one can draw a conclusion, that "in vitro" culture serves already practical tasks of viticulture, in particular, of grape selection. Due to the use of test-tube and packet plants, a number of experiments on chlorosis phytopathology, bacterial defense and nutrition etc., could be done, that will be reported separately in the future. The "green light" should be given to the "in vitro" research work, connected both with new developments and with improvement of existing ones.

REGENERATION OF GRAPEVINE

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Summary: The adventitious regeneration of grapevines was developed as the basic tool for transformation approaches. Leaf disc method was performed using in vitro propagated grapevines. The leaves were incubated under different growth conditions and more than 100 different media were tested for shoot induction. The culture conditions of the donor plants and of the regeneration process influenced formation of shoots. We ascertained increasing shoot formation by modifying the regeneration temperature, agar concentration of the media, light spectrum and obviously media composition. The addition of amino acids derived from hydrolysed proteins could improve regeneration rate. Yield of shoots on rootstock leaves was higher than of V.v. cultivars. They proliferated shoots commonly to the seventh leaf. Cultivars only responded to the second leaf. Plants were regenerated from the rootstocks Kober 5BB, SO4, Teleki 5C, Teleki 125AA, Cosmo, the I.C. FS4 and from the V.v. cultivars Gruener Veltliner (main cultivar of Austria) Blauburger (a crossing of our department) Blaufraenkisch, Pinot blanc, White Riesling and Traminer.

Key words: regeneration, shoot induction, rootstock, cultivar, kanamycin, light spectrum

Breeding of grapevines by the use of gene transfer needs a well established regeneration protocol. With the help of this technique it should be possible to regenerate plants from single cells transformed by *Agrobacterium*. The essence of almost all transformation procedures is to improve the existing genotypes by the addition of a trait coded by a single gene. That is the reason why it is a necessity to elaborate a regeneration protocol of our valuable cultivars and clones. Nowadays gene transfer is useful in genotypes cultivated by private growers but no longer to model vines. The transgenic variety will be easier accepted for grape production if the genotype remains stable except the transferred gene. The method of regeneration and the plant tissue used fort, effect the stability of a genotype. The idea of gene transfer is lost if any mutation changes the phenotypical characteristics of a vine. The choice of the plant tissue and the extent of growth regulators are responsible for the genetic stability. It is a fact that the totipotence of plant cells decreases with age and extent of differentiation. Consequently juvenile and plasma dense cells are more appropriated for regeneration than older and vacuolized cells. Zygotic tissue or germinating seedlings contain juvenile and very reproductive cells. Regeneration of plants from these tissues was documented by Vilaplana and Mullins (1989). They regenerated for the first time transgenic grapevines using this procedure (Mullins et al. 1990). They transferred the model en β -glucuronidase into the model plant *Vitis rupestris*.

Few scientists intended to use embryogenesis as a transformation system. However, tissue competent to undergo formation of embryogenic structures is sparse. With the use of anther culture a protocol could be established to produce heterozygotic plants via embryogenesis (Rajasekaran and Mullins 1979). This method allows additionally to eliminate viruses (Goussard et al. 1991) and was adapted for valuable cultivars (Harst-Langenbucher and Alleweldt 1993). The critical intermediate callus stages and a high auxin level could favor mutations. Despite using 2,4-D or NOA in embryogenesis no genetic aberrations were observed in grapevines already acclimatized to greenhouse (Stamp and Meredith 1988, Reustle 1989). The first report concerning transformation of an agronomically valuable rootstock with a resistance gene published last year was based on this procedure (Kastranova et al. 1993,

Walter et al. 1994). Nevertheless, stability of the involved genotypes can be attested after some years in the field. Regeneration by leaf disk method is ensured to be the most stable system concerning somaclonal variation. Using this method leaves were cut with a razor blade and incubated on the regeneration medium for shoot formation. The two phases of shoot proliferation and elongation demand special treatments. Successful regeneration by leaf discs was reported from different laboratories (Cheng and Reisch 1989, Clog et al. 1990, Stamp et al. 1990).

They agreed with the announced tendency that other *Vitis* species responded in higher shoot production than *Vitis vinifera* cultivars.

Once the fragmented shoot apex was used for the transformation process (Baribault et al. 1990). They failed in regenerating homogen transgenic shoots. The growth of chimeric plants consequently resulted if tissue already differentiated to form shoots was induced for organogenesis.

Formation of chimeras seems to be the main problem using leaf disc method for transformation (Colby et al. 1991). However, this phenomenon is not the result of a false regeneration protocol, rather the lack of an

appropriated selection system. Grapevines are very sensitive to kanamycin (Colby and Meredith 1990) and that inhibited an easy selection as performed with many other plants. The low level of selection agent favors the formation of chimeric or untransformed shoots (Meredith et al. 1990). Chimeric shoots stopped developing and deteriorated later on (Berres et al. 1992).

However, if regeneration of grapevine cells coincides with the transformed cells transgenic shoots will arise.

Material and methods

plant material:

Leaves were taken from in vitro cultured grapevines. They were grown on MS medium ($\text{Ca}(\text{NO}_3)_2$ instead of CaCl_2) containing 2,9 μM IAA and propagated by shoot tip and nodal cultures. Plants were kept at a temperature of 25°C and a photoperiod of 14h with intensity of - 40 $\mu\text{Mol}/\text{m}^2$. The following light sources were used :

- Gro Lux - (Silvania) special spectrum for plant growth
- Day light de Luxe - (Phillips) TLD F 30 W/186
- White warm light - (Osram) L 38 W/31 Lumilux
- Cool white light - (Osram) L 30 W/ 20

Plant material should be juvenile and was commonly grown for onemonth in glasses (d=6 cat) covered with the half of a petri dish and closed with parafilm. The leaves were removed one by one from the intact plant, cut from the lateral side perpendicularly to the veins beginning at the distal end. The dissected leaves were placed on the medium upside down.

The temperature of the regeneration process was varied (20°C, 25°C, 28°C, 30°C), the intensity of illumination was kept constant and the 4 different spectra were compared in regeneration: Media used in these experiments consisted of the basal medium MS (Murashige & Skoog) LS (Linsmaier & Skoog) and NN (Nitsch & Nitsch) . We combined macro and micro elements of LS and HS with vitamins of NN and defined them LSN or MSN. For shoot induction they were supplemented with growth regulators. We tested the addition of 4,4 - 6,6 - 8,8 - 11,0 - 13,2 μM BA (Benzyladenin), further we used the combination of BA (8,8 and 11 μM) with gibberelins GA₃ 0,28 - 0,56 - 1,4 μM , cytokinin 2 - IP (Isopentyl adenosin 0,5 - 2,5 μM , Zeatin 2,8 - 4,2 - 5,6 μM , TDZ (thidiazuron) 2,25 - 4,5 - 6,7 - 9 - 13,5 μM and IAA (indolyl acetic acid) 0,58 - 1,2 and 2,9 μM . In further experiments the addition of charcoal (0,5 - 1 g/l), Chinosol 0,01%, carrot extract 0,5 - 2 ml/l and skinned milk powder (0,25 - 0,5g/l) was tested. As a source of amino acids we used hydrolysed Casein 0,5 - 1g/l and hydrolysed Lactalbumin (LA) (0,25 - 0,5 - 1 g/l). Sucrose content was kept at 2 % and agar concentration was varied from 1%, 0,8%, 0,5% to liquid medium.

Shoot elongation was performed on LS / NS medium with 8,8 μM BA and 0 - 2,9 μM IAA. Shoots were rooted on NS medium containing 2,9 μM IAA.

Results and discussion

The adventitious regeneration is a complex development influenced by several parameters. The main factors effecting the formation of shoots are the donor plant material, the regeneration procedure and the medium. All main factors consist of several sub factors which determine the quality of regeneration. The effect of some of these parameters was tested and will be shown here:

As already mentioned by other scientists the genotype influences the regeneration rate massively. The different rate of shoot formation and elongation of some genotypes is shown in Table 1. Rootstock varieties like sober 5 BB, SO4, Teleki 5 C, Teleki 125 AA and Cosmo responded to shoot induction with a high rate of proliferation. Cultivars such as Gr. Veltliner, Blauburger, White Riesling etc. are still recalcitrant to regeneration. Furthermore the I.C. FS4 showed a similar response as observed with Vitis v. cultivars. However, this difference in regeneration could be an indication that organogenesis of cultivars still needs modification of media and growth conditions. The fact that regeneration capacity decreases with age of the leaves was reported (Stamp et al. 1990) and was confirmed by our results. One month old shoots grown in vitro are most favorable for the regeneration process. The trial to produce shoots using leaves from older or under other conditions cultivated plants showed a decrease in regeneration. The successful regeneration of shoots from plants grown in the greenhouse was diminished as the youngest leaves were damaged by desinfection and older ones were contaminated .

(table 1) influence of genotype on regeneration rate

Donor plants of some genotypes differ in vitality and growth as a consequence of gradients in temperature and light intensity. We incubated our donor plants at 25°C and put them under daylight regime. In a special experiment different light spectra were used to incubate donor plants, and the same light sources were involved in regeneration process.

genotype	number of shoot-forming leaves	transferred shoots (%)	regeneration rate (%)
5 BB	- 7	70	50
504	- 6	60	50
5 C	- 6	60	50
125 AA	- 3	50	40
Cosmo	- 3	50	40
FS 4	- 2	30	30
Blauburger	- 3	- 50	40
Gr.Veltliner	- 2	- 50	40
Traminer	- 2	- 50	40
Blaufränkisch	- 2	- 50	30
Pinot blanc	- 2	40	30
White Riesling	- 2	40	20
1355/1/116	- 2	50	30
Neuburger	----		
Cabernet franc	----		
Baco noir	----		

(table 2) effect of light spectrum on regeneration of Kober 5BB

regeneration light	DL	WWL	CWL	GrL
donor plant incubation				
DL	++	+	+	+++
WWL	+	+	-(+)	++
CWL	-(+)	-	-	-(+)
GrL	+	+	-	+(+)

- no regeneration
- + weak regeneration (one or two leaves responded)
- ++ moderate regeneration (two to five leaves responded)
- +++ very productive regeneration (shoots at each cut surface)

effect of light spectrum on regeneration of Gr. Veltliner

regeneration light	DL	WWL	CWL	GrL
donor plant incubation				
DL	+	+	-	+
WWL	+	+	-	+
CWL	-	-	-	-
GrL	-	-	-	-

Effect of growth conditions on regeneration

The data of table 2 illustrate the influence of different light sources on regeneration of Kober 5BB. Daylight spectrum seems to be the most favorable illumination to keep donor vines before regeneration. Plants incubated in cool white spectrum responded worst in subsequent regeneration. Further parameters of the donor plants were kept constant. Nevertheless, the improvement of these parameters could be a further potential to augment shoot production. The second factor involved in the illumination experiment is the light spectrum used in regeneration process. Yield of shoots reached a maximum if regeneration was performed with plants grown at daylight regime and leaf discs exposed to Gro lux lamps. This combination of different light sources resulted in shoots at each cut side in the experiment with Kober 5BB. The regeneration rate was stabilized at a high level when this procedure was applied to Gr. Veltliner. Temperature is known to be a critical parameter in shoot induction. We intended to find the most favorable incubation temperature for regeneration. Host genotypes did not respond to a 20°C climate. An increase in shoot formation was ascertained by incubating at temperatures of 28°C or 30°C.

(table 3) influence of temperature on regeneration

temperature	5 BB	5 C	SO 4
- 20° C	+	-	-
- 25° C	++	++	+
- 28° C	+++	+++	++
- 30° C	++	++	++

The yield of shoots from rootstocks showed a maximum at 28°C. Cultivars proliferated most shoots at 30°C and showed no regeneration at 20°C and 25°C except the cultivar Blauburger.

temp.	Gr.Veltliner	Blauburger	W.Riesling	Pinot blanc
20° C	-	-	-	-
25° C	-	+	-	-
28° C	+	+	-	+
30° C	+	++	+	+

Effects of medium on regeneration

We tested as one of our first steps the influence of agar concentration on regeneration. All involved genotypes produced more shoots in lower agar concentration. This phenomenon could be the consequence that cells are better supplied with nutrients caused by intenser contact to the medium and a higher availability of water.

All other used regeneration media contained 0,5% agar or no agar.

An additionally advantage of liquid medium is the facilitated elimination of agrobacteria subsequent to transformation.

(table 4) effect of agar concentration on regeneration

agar conc.	5 BB	SO 4	GV
1 ‰	- 3 leaves	3 leaves	-
0,8 ‰	- 4 leaves	3 leaves	-
0,5 ‰	- 7 leaves	6 leaves	2 leaves
0 ‰	- 7 leaves	7 leaves	2 leaves

The basal media MS, LS and NN were used in preliminary experiments. We find out that in our laboratory regeneration reached a maximum using media based on LS or MS macro and micro elements combined with NN-vitamins.

To regenerate rootstocks cytokinin was added to medium at a level of 4,4 - 13 µM BA. The most favorable amount was found at 11 µM. Less shoots or reinforced growth of callus was achieved when BA was supported by other growth regulators. In numerous experiments the effect of additives was tested. Improvement of regeneration could be noticed by addition of hydrolysed Lactalbumin (0,5 g/l). A ION increase in regeneration was observed whereby the elongation and growth of shoots were improved. Other additives increased growth of callus (milk powder, hydrolysed casein and carrot extract) or caused a browning of the leaves (charcoal, chinosol). we supposed that regeneration protocol of some rootstocks was elaborated sufficiently to perform transformation. The improved media consist of:

5 BB: LSN BA 11 µM LA 0,5g/l
 SO₄: LSN BA 11 µM LA 0,5g/l
 FS 4: LSN BA 4,4 µM TDZ 13µM

Regeneration of cultivars remains limited to a few juvenile leaves and should be further improved for transformation approaches. The basal media LSN and MSN were supplemented with many different combinations (see material and methods) of growth regulators. The additional media were designed as a consequence of failed regeneration using 11µM BA alone. The combination of 13µM TDZ and 4,4µM BA stimulated formation of shoots. Further improvement especially in elongating shoots was achieved by the addition of 0,5 g Lactalbumin.

All other additives could not positively affect regeneration.

Shoot proliferation of cultivars was performed on the following media :

Gr. Veltliner	LSN	BA 4,4 µM	TDZ 13µM	LA 0,5 g/l
Pinot blanc	LSN	BA 4,4µM	TDZ 13µM	LA 0,5 g/l
Blauburger	LSN	BA 13µM		LA 0,5 g/l
Traminer	LS/MSN	BA 4,4µM	TDZ 13 µM IAA 0,5µM	LA 0,5 g/l
W.Riesling	LS/MSN	BA 4,4µM	TDZ 13µM IAA 0,5µM	LA 0,5 g/l

The intensive callus growth in cultivar regeneration is the consequence of high levels of growth regulators in the medium. Shoots of cultivars could be transferred and regenerated at a minor rate (table I) than from rootstocks. Regeneration to plants requires the double time and medium was changed once more.

However, these regeneration protocols are a step forward to breeding by gene transfer.

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PROGRESS IN GRAPEVINE (VITIS SPP.) PROTOPLAST CULTURE

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S u m m a r y: Intensive investigations were carried out in order to improve the system of grapevine protoplast culture. Using stems of to vitro grown grapevines as protoplast source, a very effective system for the production of micro- and macrocalli could be established. However, no regeneration to plants was achieved with this material. Using leaf disk derived embryos and embryoids of the cultivar Seyval, protoplasts with high embryogenic competence could be isolated. After a 4 week induction treatment in NN-69 medium supplemented with NOA (4.0 ppm) and TDZ (0.9 ppm) and subsequent subcultivation in hormonefree medium, high rates of somatic embryos arose. After spontaneous germination of the embryos, typical in vitro grapevines could be regenerated.

K e y w o r d s: protoplast, embryogenic tissue, somatic embryogenesis, regeneration.

Introduction

The use of biotechnological methods in breeding programs presume effective regeneration systems.

The development of a protoplast-to-plant system would open new perspectives for breeding of disease resistant grapevines by the use of somaclonal variation, somatic hybridisation and genetic engineering.

Although intensive efforts has been made during the last decade (LEE and WETZSTEIN, 1991; REUSTLE and ALLEWELDT, 1990; SHIMIZU 1985; WRIGHT, 1985; Mll et al., 1991; UI e@ at., 1990), no successful regeneration protocol for grapevine protoplasts could be developed so far. In this paper a brief summary about experiments with grapevine protoplasts is given. Furthermore, to our knowledge, the first successful protocol for regeneration of grapevine protoplasts is described.

Materials and methods

Protoplasts from stems.

Protoplasts were isolated from stems of in-vitro-grown grapevines of several cultivars (Seyval, Vidal, Riesling, Kober 5BB) as described by REUSTLE and NATTER (1994). After over night digestion and subsequent purification, the derived protoplasts were resuspended in i) modified V/KM-medium (BINDING and NEHLS, 1978) when cultured in a agarose-bilayer-system or in ii) 0.65 M mannitol (pH 5.8) for alginate culture. Protoplast density in both cases was adjusted to 1×10^6 cells per ml to reach a final density of 0.5×10^6 cells.

The method of protoplast cultivation in the agarose-bilayer-system was previously described by REUSTLE and NATTER, (1994). For alginate culture, the method described by KARESCH et. al. (1991) was adapted to the requirements of grapevine protoplasts as followed: the protoplast-mannitol-suspension was mixed with an equal volume of 2 % Na-alginate (Sigma) in 0.55 M mannitol (pH 5.8). Aliquots of 0.75 ml of these mixture were placed in 5 cm petri dishes onto 5 ml of solidified medium (0.3 % gelrite) containing 20 mM CaCl_2 , and 0.6 M mannitol. After 2 hours of incubation, 2 ml of a solution containing 10 mM CaCl_2 and 0.62 M mannitol (pH 5.8) were added to each petri dish to complete the gelification process. Within two to three hours, using a spatula, alginate-films could be transferred into new petri dishes containing 2 ml of culture medium. As culture media for both cultivation systems, modified V/KM-medium (BINDING and NEHLS 1978), modified CPW-13 medium (FREARSON et al., 1972) and MS- based medium (MURASHIGE and SKOOG, 1965) were tested. As standard hormone treatment 1.0 ppm 2,4-D and 0.5 ppm BAP was applied. Osmolality of the media was adjusted to 600-630 mosmol/kg by the addition of glucose. Protoplasts were cultivated at 24 to 26 °C in the dark.

Protoplasts from embryogenic tissue.

Somatic embryogenesis was induced on leaf disks of Vitis sp. cv. Seyval blanc (HARST and ALLEWELDT, 1994). Somatic embryos and embryoids were harvested from the leaf disks and used for protoplast isolation.

The isolation protocol was corresponding to the method used for the isolation of stem material. After purification, protoplast density was adjusted to 5×10^6 cells per ml to reach a final concentration of 2.5×10^6 cells. For cultivation, protoplasts were embedded in Na-alginate as described before. As culture media a modified CPW-13 medium (FREARSON et al., 1973) with 0.54 M glucose and NN-69 medium (NITSCH and

NITSCH, 1969) with 0.6 M glucose, were used for the initial cultivation step. Each medium was supplemented with 1.0 % PVP- 40. During the initialising period, two hormone treatments i) 1 ppm 2,4-D and 0.5 ppm BAP or ii) 4.0 ppm NOA and 0.9 ppm TDZ were tested. As control treatment, a hormone-free variant was used.

After 4 weeks, alginate layers were transferred to hormone-free NN-69 medium (for 4 weeks) containing 0.4 M glucose and 1% PVP-40, followed by a further subcultivation (for 4 weeks) in the same medium but with reduced glucose concentration (0.2 M).

Finally, fragments of the alginate layers (1.5 to 2.0 mm in size) containing microcalli of different types and sizes were transferred in petri dishes (10 cm) onto solidified (0.3 % gelrite) NN-69 medium with 0.2 M glucose.

Results and discussion

Protoplasts from stems.

To obtain more reproducible results, the effect of several factors on protoplast development was studied.

Donor plants / pretreatment:

Reduction of the daylight (8 h), applied to the donor plants, 5 days before protoplast isolation, yielded in a significant increase of rate of cell division compared to protoplasts derived from control plants (14 h photoperiod). Application of reduced daylight 5, 10, 20 and 30 days prior to protoplast isolation showed no clear difference compared to the control treatment (RULER, 1995).

A reduced nitrogen level (NH_4NO_3) in the culture media of the donor plants could improve rate of cell division in some experiments. However, in most cases the positive effect was superposed by other unknown factors (BECKER, 1992).

A cold treatment of freshly isolated protoplasts with 4 °C for 1 hour up to 4 weeks could neither improve cell division nor induce somatic embryogenesis (FIDDLER, 1995).

Rates of cell division and microcallus formation were mainly affected by the genotypes used for protoplast culture. In our experiments, Vidal and Seyval provided the best results.

Cultivation system

The method of cultivation is of high importance for protoplast growth due to the sensitivity of protoplasts to mechanical stress. As the most suitable and most careful cultivation method for grapevine protoplasts, the immobilisation in agarose-medium or in thin alginate-layers was found. Both systems led to similar high rates of microcallus formation. Finally, the alginate-system was preferred because of its easier handling concerning media variations and media changes. Furthermore, gelification of alginate is temperature independent, that means a reduced stress for protoplasts during the process of immobilisation.

Culture media

Concerning suitable culture media for protoplasts, V/KM medium, CPW-IS medium and a MS-based medium were found to be suitable for grapevine protoplast culture. Although the compositions of these media are quite different, they provided similar rates of cell division and microcallus formation. However, the reproducibility of the results was best with the CPW-13 medium (NATTER, 1993).

Media additives:

A main step towards reliable results was achieved by the addition of polyvinylpyrrolidone (PVP) to the medium. PVP is able to absorb phenolic compounds, released from active protoplasts into the surrounding medium. In this way PVP prevent the accumulation of these substances to toxic concentrations. The addition of 0.5 % PVP to the initial culture medium led to high rates of microcallus formation. In contrast, activated charcoal was found to be unsuitable for the used in protoplast cultures (REUSTLE and NATTER, 1994).

The addition of Thryptophan, Phenylalanine and Histidine in several concentrations did not improve plating efficiency. Addition of Glutamine to the culture medium in concentrations of 100 and 200 ppm improved formation of microcalli. However, non of the applied aminoacids could induce embryogenesis or organogenesis (HARTMANN, 1992).

Hormone treatments:

In accordance to the successful experiments with leaf disks of Seyval (HARSH and ALLEWELDT, 1994), induction of embryogenesis by a 4 week treatment with identical hormone concentrations and subsequent subcultivation on hormone-free medium was tested. The induction treatment of the protoplasts was applied at three different developmental stages: either at day 0 (freshly isolated), or day 14 (stage of first cell division) or day 30 (microcallus formation) of cultivation. The experiments were carried out with Vidal and Seyval. Although high rates of microcallus formation with both genotypes and in all three application variants were obtained, neither the protoplasts of Vidal nor those of Seyval showed any indications towards embryogenesis (SCHAARSCHMIDT, 1994).

Altogether, based on the results of these investigations, a very effective system for the production of microcalli and calli from protoplasts of Vidal and Seyval was established, but non of the tested factors effected differentiation towards plant regeneration. All efforts to induce embryogenesis or organogenesis in the derived micro- and macrocalli by means of several hormone combinations and concentrations were unsuccessful.

Regarding this, it seems obvious, that stem material as source for protoplasts was not suitable for protoplast regeneration due to their missing embryogenic competence.

Protoplasts from embryogenic tissue.

With the applied isolation method, yields of 0.8 to 4.7×10^6 protoplasts per gram material were achieved. The size of protoplasts varied from 10 to 50 μm and most of them were rich in cytoplasm.

Beginning and rate of cell division depended on the media used and on the applied hormones (Tab.1). When CPW-IS medium was used, protoplasts started to divide during the second week of cultivation independent on hormone treatment. The rate of dividing protoplasts was up to 20 % using the standard hormone treatment (2,4-D/BAP) and up to 50 % with NOA/TDZ application. Using the NN-69 medium, cell division occurred only after NOA/TDZ application. In contrast to CPW-IS medium, first cell division could be observed at the earliest 4 to 5 weeks after culture initiation. Without hormones, protoplasts formed new cell walls within 2 weeks, however cell division could not be observed.

Best development of divided protoplasts to microcallus arose in CPW-IS medium with NOA/TDZ as hormone treatment. 6 to 8 weeks after culture initiation, friable microcalli, consisting of large cells, developed and the microcalli reached sizes of 0.5 to 1.5 mm. In case of 2,4-D/BAP application, growth of the divided protoplasts was low. Using NN-69 medium with NOA/TDZ as initialising variant, the developed microcalli consisted of smaller cells and the texture remained compact. The positive effect of CPW-IS medium on protoplast division and microcallus formation was already found in earlier studies with grapevine protoplasts (REUSTLE and NATTER, 1994).

Small embryogenic cell aggregates (100 to 200 μm) could be observed in the alginate layers at the earliest after 8 weeks of cultivation when NN-69 medium with NOA/TDZ was used in the initial cultivation step. Ten weeks after culture initiation, somatic embryos and embryoids were visible on the alginate layers with the naked eyes. After transfer of microcalli containing fragments of the alginate layers on solidified NN-69 medium, on up to 40 % of these fragments somatic embryos arose within 4 to 6 weeks. Only very few of the transferred calli, obtained after 2,4-D/BAP treatment in CPW-13 medium, formed somatic embryos and however, no embryogenesis could be induced with CPW-13 in combination with the NOA/TDZ treatment during this time.

Regarding these results, it is evident that the CPW-13 medium supports undifferentiated growth of protoplasts or / and promotes the development of cells without embryogenic competence. In consequence, the development of embryogenic cells is suppressed. Using NN-69 medium in combination with NOA/TDZ, growth of protoplast with embryogenic competence is promoted. The negative results with NN-69 medium in combination with 2,4-D/BAP and the reduced growth of divided protoplasts in the CPW-IS medium with this hormone treatment, could be due to a high sensitivity of these protoplasts to 2,4-D.

During subcultivation of the embryos on solidified hormone-free NN-69 medium, few of them elongated spontaneously. When the germinated embryos reached a size of 0.5 to 1.0 cm , they were transferred to solidified (0.3% gelrite) LS-medium (LINSMAIER and SKOOG, 1965) in culture tubes and cultivated under 16 h photoperiod and 100 $\text{Em}^{-2} \text{s}^{-1}$ light intensity. Within 4 to 6 weeks most of them turned green and typical *in vitro* plantlets developed.

Conclusion

Using embryos and embryoids as source for protoplasts, high rates of cells with embryogenic competence could be isolated. After a 4 week induction treatment with NN-69 medium in combination with NOA/TDZ hormone treatment, somatic embryos arose in the subsequent subcultivation in hormone-free NN-69 medium. Somatic embryos germinate spontaneously and regenerate into plants on hormone-free LS-medium. With the here described protocol for grapevine protoplast regeneration, a first step towards the use of protoplast techniques in grapevine breeding is done.

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Table 1

Induction of somatic embryogenesis in grapevine protoplasts (*Vitis* sp. cv. Seyval). Influence of different induction treatments.

induction variants	cell division	microcallus formation	somatic embryos	plating efficiency
CPW-13				
2,4-D/BAP	+++	++	+	0,5%
CPW-13				
NOA/TDZ	++++	++++	-	-
NN-69				
2,4-D/BAP	-	-	-	-
NN-69				
NOA/TDZ	++	++	++++	40%

*: rate of microcalli on which somatic embryos arose within 6 weeks of subcultivation on solidified hormone-free NN-69 medium;

-: no reaction;

+: intensity of the reaction.

INVESTIGATIONS ABOUT THE INHERITANCE OF POWDERY MILDEW RESISTANCE FOR GRAPEVINE

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Summary: In a field test, the powdery mildew infection of a seedling population of interspecific crossings was determined, including 20 offspring populations from two female and 10 male parents. No plant protection measures were carried out in this year with the 6-year-old seedlings. In accordance with the scheme of OIV (Office International de la Vigne et du Vin) the ratings were done middle of August, separately for leaf and berry.

The results show strong differences in the infection level within and between the populations as well. The mean population values differ between 3,2 - 5,2 (leaf) and 2,4 - 5,1 berry. Although a significant correlation between oidium infection of leaf and berry could be established, the coefficient of determination of $r^2=0,43$ exhibits only a weak relation between the resistance performance to oidium of leaf and berry. The determined coefficients of heritability range markedly higher for the berry ($h_e^2=0,78$) than for the leaves ($h_e^2=0,31$). Furthermore, the frequency distributions for infection show a minor standard deviation for berries.

These results point at a partial differing hereditary process of oidium resistance for leaf and berry. Important for breeding purposes is the fact that results obtained from early screening methods for leaves cannot be transferred with sufficient reliability to berries.

Key words: genetics, heritability, crossing, selection, powdery mildew, resistance

Introduction

It is known from a long line of investigations and observations that the grapevine's resistance to powdery mildew is of a polygenic nature. This means that there is generally a very wide splitting of this characteristic in the offspring of crossings. For the purpose of making resistance breeding more efficient, it would thus be very helpful to know more about the inheritance process. The inheritance of polygenic characteristics can be based on additive and/or dominant gene effects. In addition, the degree of the characteristic can also be influenced by epistatic effects, i.e. by interaction of the loci. In the case of primarily additive gene effects, it can be expected that the population mean of progeny of a certain characteristic will correspond to the mean of the parent varieties. Then the general combining ability of a variety is of great significance. In the case of primarily dominant gene effects a strong difference in the characteristic mean in the offspring from that of the parents can be expected, whereby the population mean may shift up or down compared to the parent mean. In traditional crossbreeding this type of shift can only be assessed empirically in individual cases. In this case we are talking about specific combining ability in contrast to general combining ability.

One important figure for inheritance is the coefficient of heritability. The coefficient of heritability in a broader sense accounts for the entire genetic part of the phenotype, whereas the coefficient of heritability in the narrow sense is a measure for the additive gene effect in the genetic material investigated as a whole. Ideally, a diallel cross scheme with many crossing combinations is desirable for determining such figures. In many cases this is not feasible in grapevine breeding due to technical problems, especially in making the desired crossing combinations. One other limiting factor is the enormous amount of space required for planting the seedlings. Therefore, such investigations into the inheritance of certain characteristics generally have to be carried out on a smaller investigation scale. When doing so, it must be considered that the results are only an estimated value due to the limited genetic material.

Materials and methods

The investigations were done in 1992 on a test plot in which no plant protection measures were carried out. In August the oidium infection was determined on both berry and leaf separately. The ratings were done in accordance with the scheme of the OIV according to EIBACH, 1994. This year's extremely high oidium infection pressure was very favorable for the investigations. The offspring of two mother varieties (Sinus, Staufer) and ten father varieties (Baco blanc, Bellandais noir, Castel 19637, Marechal Joffre, Meynieu 6, Planter, Seibel 15051, Seyve Villard 1-72, Vidal blanc, Villard blanc), i.e. 20 crossing populations, were investigated in a factorial design. The mother varieties called Sirius and Staufer are new varieties established at the Institute for Grapevine Breeding Geilweilerhof. They show mediocre to good resistance to oidium. The father varieties used in these investigations are all part of the group of so-called French hybrids. Except for four populations there were

about 150 to 200 seedlings per population For the crossings with Meynieu 6 and Castel 19637 there was only a small number of seedlings between about 50 and 70 genotypes each

Results and discussion

Table I shows the population mean of oidium infection on the leaf of all 20 crossing combinations. The results show the quite considerable infection differences between the populations. The levels range from 3.2 for the crossing of Sirius and Seibel 15051 to 5.2 for the crossing of Staufer and Bellandais noir The father varieties are shown in increasing order according to the medium infection over both mother varieties According to this the least amount of infection is found in the Seibel 15051 crossings and the most infection in the Bellandais noir crossings. It is remarkable that the order of the mother varieties is nearly identical, i.e. there is hardly any interaction. Only slight differences between the means of the mother varieties were observed. At 4.1 the Sirius offspring show only slightly less infection compared to Staufer at 4.3 However, on the basis of the large number of genotypes tested (N=2951) the difference is statistically significant. The majority of the infection differences between the populations can be statistically supported, as the Fig. 1 reveals The mean berry infection levels of the individual populations in Table 2 are analogous with the leaf levels. Similar to the case with the leaf levels, interaction between the mother and father varieties is hardly noticeable here. However, compared to the leaf levels the extremes of the mean berry infection levels are farther apart They range from 2.4 for the crossing between Staufer and Meynieu 6 to 5.1 for the crossing between Staufer and Bellandais noir A further difference shows up in the order of the father varieties The best example of this is the Seibel 15051 variety, which ranks seventh here, but shows the least amount of infection in the offspring in the leaf levels This is a hint of a partially differing inheritance of oidium resistance on leaves and berries.

The statistical calculation gives evidence of a higher percentage of statistically significant differences between populations in comparison to leaves (Fig 2). For example Meynieu 6 differs from all the other father varieties tested in its statistically significantly lower oidium infection of the berries, Both mother varieties have a mean infection level of 3.8 over all father varieties.

The coefficients of heritability in the narrow sense (Table 3) can be calculated both with the help of the estimation of the components of variance and by the regression method (ALLARD, 1960, FALCONER, 1981, MATHER and JINKS, 1982; WRICKE and WEBER, 1986) The difference in the levels of the two methods is partly due to the test model, which does not correspond to the ideal of a diallel cross scheme With the exception of the quantitative differences, both procedures nevertheless tend to lead to the same results, i.e. to considerably higher coefficients of heritability for berries The percentage of additive genetic variance is thus significantly higher for berries. Again this implies that the inheritance of oidium resistance for leaves and berries varies.

In Fig. 3 the population means of the father varieties over both mother varieties have been diagrammed in a four-field table The point of intersection of the axes is the mean of the infection on leaves and berries for all the parent varieties used in the investigations The offspring of the father varieties Meynieu 6, Vidal blanc and Serve Villard 1-72, which are found in the bottom-left square, had a lesser amount of infection on berries and leaves than the total mean These results offer the breeder important information, because it shows which crossing combinations can be expected to have the highest degree of resistance However, no conclusions can be derived from this as the degree of resistance of the parent varieties remain unaccounted for In Fig. 4 this situation is taken into account For each crossing population the axes cross at the mean of the degree of oidium infection of the parent varieties against which the relative deviation of the individual offspring populations is placed. It can be seen that nearly all the populations are in the top-right square. This means that the mean degree of infection of the parent varieties was lower than mean degree of infection of the corresponding offspring populations in all cases. Serve Villard 1-72, which is closest to the point of intersection, can be judged most favorably with regard to leaf and berry. Meynieu 6's offspring show the same mean degree of berry infection as the parent varieties, but a higher mean degree of leaf infection. The opposite is true for Seibel 15051, whose offspring indicate the same degree of leaf infection, but a higher degree of berry infection The worst oidium resistance showed up for Baco blanc, Villard blanc and Bellandais noir, whose population means were in some cases as much as two rating levels higher than that of the corresponding parent mean In Fig. 5 the individual levels of the genotypes from the crossing with Meynieu 6 as the father variety are remarkable The wide deviation within the family is obvious. The largest number of observations are in the upper-right square, i.e. the oidium infection of this genotype was heavier than the parent mean. Nevertheless a total of 15 genotypes were found in this population, for which lesser infection was determined with regard to both leaves and berries compared to the parent mean, and thus show a transgression in the desired direction. The findings concerning the transgression need to be confirmed in the next few years As the results show, there are apparently differences in the leaf resistance and the berry resistance to oidium Three years of observations of interspecific varieties in the Institute's variety collection confirm this. In the Table 4 each maximum infection level from berries and leaves from the years 1991 to 1993 are indicated in summary It is apparent that the variation of the leaf infection within the berry infection level differs considerably in some cases and may range up to six rating

levels, This is similarly true for the variation of the berry infection within a leaf infection level. In the crossing materials investigated statistically significant correlations between $r=0,54$ and $r=0,59$ can be calculated. However, the coefficient of determination shows that only a maximum of 35% of the variation of the berry infection is based on the variation of the leaf infection and vice versa. A further difference between the leaf and the berry with regard to oidium becomes apparent when studying the frequency distribution. The variance of the leaf infection levels are 2.9 in the mean of all 20 populations tested. The same calculations for the berries indicated a statistically significantly lower mean variance of 2.1. This means that the frequency distributions of the leaf oidium infection run flatter compared to berry oidium infection and cover a higher percentage of rating levels which are farther from the mean level. This is shown in Fig 6

Conclusions

1. The clearly varying coefficient of heritability of leaf and berry on the one hand, the loose correlation between leaf infection and berry infection on the other hand and, third of all, the varying frequency distributions indicated point to an inheritance of oidium resistance in both organs which varies at least in some cases. Several different resistance mechanisms of grapevines to oidium have been described in publications (Blaich, 1987). The reason for the differing reactions of leaves and berries might be due to the varying importance of these individual defense reactions in each organ

2. The heritability levels which are considerably higher compared to the leaf reveal that additive gene effects play a greater role in the inheritance of oidium resistance for berries. It can be concluded that the general combining ability can be derived from the phenotype of a variety with greater probability for berries than for leaves

3. Concerning early diagnosis procedures for selecting oidium resistant genotypes it is important for breeding purposes that it is not adequately safe to conclude the degree of infection of the berry from the degree of infection of the leaf. In vitro resistance tests on leaf discs or intact plants (DIEHL, 1987) can be a valuable screening process for determining the degree of leaf resistance. However, further investigations are required to determine berry resistance exactly.

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♂ \ ♀	SIRIUS	STAUFER	both females
SEIBEL 15051	3,2	3,6	3,4
S.V.1-72	3,8	3,7	3,7
MEYNIEU 6	4,3	3,4	3,8
VIDAL BLANC	3,7	4,1	3,9
MARECHAL JOFFRE	4,1	4,0	4,3
PLANTET	4,3	4,4	4,3
VILLARD BLANC	4,3	4,4	4,4
CASTEL 19637	4,1	4,7	4,4
BACO BLANC	4,7	4,9	4,8
BELLANDAIS NOIR	4,8	5,2	5,0
\bar{x}	4,1	4,3	4,2

Table 1: Mean powdery mildew infection rates on leaves of not sprayed seedling populations

♂ \ ♀	SIRIUS	STAUFER	both females
MEYNIEU 6	2,7	2,4	2,5
MARECHAL JOFFRE	3,1	2,9	3,0
VIDAL BLANC	2,9	3,2	3,1
CASTEL 19637	3,0	3,1	3,2
S.V.1-72	3,6	3,0	3,3
BACO BLANC	3,6	3,6	3,6
SEIBEL 15051	3,6	3,7	3,7
VILLARD BLANC	4,1	4,0	4,0
PLANTET	4,6	4,2	4,4
BELLANDAIS NOIR	5,1	5,1	5,1
\bar{x}	3,8	3,8	3,8

Table 2: Mean powdery mildew infection rates on berries of not sprayed seedling populations

	h_c^2	
	components of variance	regression
leaf	31	17
berry	78	66

Table 3: Coefficients of heritability of powdery mildew

Oidium-leaves	Oidium-berries									Total
	1	2	3	4	5	6	7	8	9	
1	-	1	1	-	-	-	-	-	-	2
2	13	13	17	3	-	-	-	-	-	46
3	20	67	94	14	12	-	1	-	-	208
4	3	15	27	12	6	1	-	-	-	64
5	3	8	26	8	10	3	5	2	1	66
6	2	4	12	5	8	5	5	-	-	44
7	-	-	4	4	7	3	3	-	-	24
8	-	1	2	2	6	3	2	2	-	18
9	-	-	-	-	1	-	3	1	4	9
Total	44	109	183	48	50	15	19	5	5	475

Table 4: Total survey of grouping into oidium infection levels (n=475 varieties) based on the maximum infection ratings during the years from 1991 to 1993

male	x	SEIBEL 15051	S.V.1-72	MEYNIU 6	VIDAL BLANC	MARECHAL JOFFRE	PLANTET	VILLARD BLANC	CASTEL 19637	BACO BLANC	BELLANDAIS NOIR
SEIBEL 15051	3,4				*	*	*	*	*	*	*
S.V.1-72	3,7					*	*	*	*	*	*
MEYNIU 6	3,8						*	*	*	*	*
VIDAL BLANC	3,9	*					*	*	*	*	*
MARECHAL JOFFRE	4,3	*	*				*	*	*	*	*
PLANTET	4,3	*	*		*		*	*	*	*	*
VILLARD BLANC	4,4	*	*	*	*		*	*	*	*	*
CASTEL 19637	4,4	*	*	*	*		*	*	*	*	*
BACO BLANC	4,8	*	*	*	*	*	*	*	*	*	*
BELLANDAIS NOIR	5,0	*	*	*	*	*	*	*	*	*	*

Fig. 1: Powdery mildew infection rates on leaves of not sprayed seedlings. Mean values of populations with Sirius and Staufer as females. (Differences of mean population values of females (Staufer=4,3; Sirius=4,1) are significant)

male	x	MEYNIU 6	MARECHAL JOFFRE	VIDAL BLANC	CASTEL 19637	S.V.1-72	BACO BLANC	SEIBEL 15051	VILLARD BLANC	PLANTET	BELLANDAIS NOIR
MEYNIU 6	2,5		*	*	*	*	*	*	*	*	*
MARECHAL JOFFRE	3,0	*		*	*	*	*	*	*	*	*
VIDAL BLANC	3,1	*	*		*	*	*	*	*	*	*
CASTEL 19637	3,2	*	*	*		*	*	*	*	*	*
S.V.1-72	3,3	*	*	*	*		*	*	*	*	*
BACO BLANC	3,6	*	*	*	*	*		*	*	*	*
SEIBEL 15051	3,7	*	*	*	*	*	*		*	*	*
VILLARD BLANC	4,0	*	*	*	*	*	*	*		*	*
PLANTET	4,4	*	*	*	*	*	*	*	*		*
BELLANDAIS NOIR	5,1	*	*	*	*	*	*	*	*	*	

Fig. 2: Powdery mildew infection rates on berries of not sprayed seedlings. Mean values of populations with Sirius and Staufer as female. (No differences of mean population values of females: Staufer=3,8; Sirius=3,8)

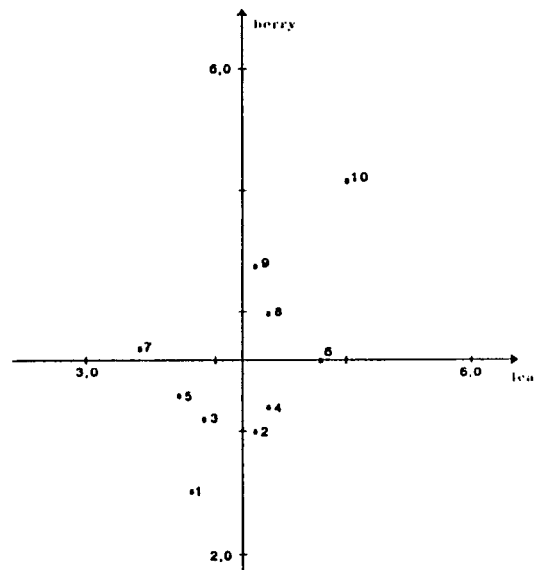


Fig. 3: Mean population values of powdery mildew infection of leaf and berry (1=Meynieu 6; 2=Marechal Joffre; 3=Vidal blanc; 4= Castel 19637; 5=Seyve Villard 1-72; 6= Baco blanc; 7=Seibel 15051; 8=Villard blanc; 9=Plantet; 10=Bellandais noir)

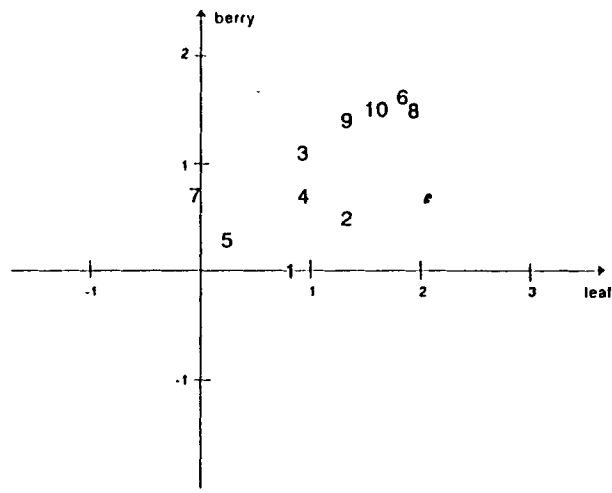


Fig. 4: Mean population values of powdery mildew infection on leaf and berry related to the parents mean (1=Meynieu 6; 2=Marechal Joffre; 3=Vidal blanc; 4= Castel 19637; 5=Seyve Villard 1-72; 6= Daco blanc; 7=Seibel 15051; 8=Villard blanc; 9=Plantet; 10=Bellandais noir)

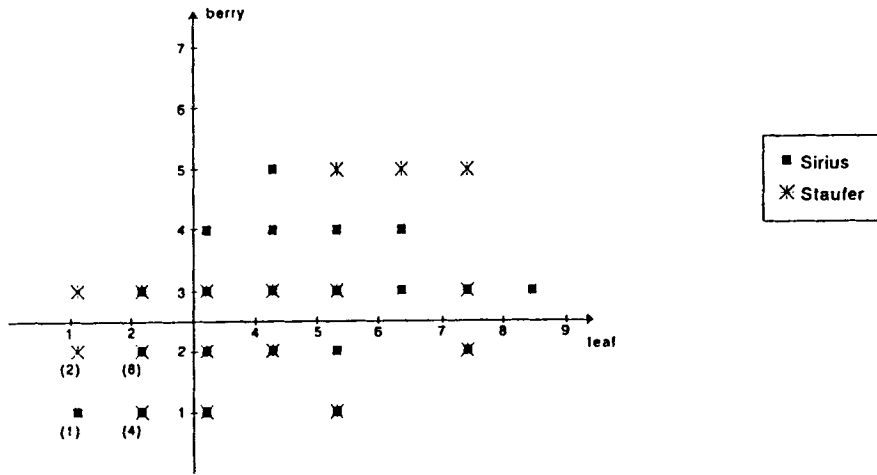


Fig. 5: Powdery mildew infection of leaf and berry in populations of Meynieu 6 (male) with Sirius and Staufer (female) related to the parents mean.

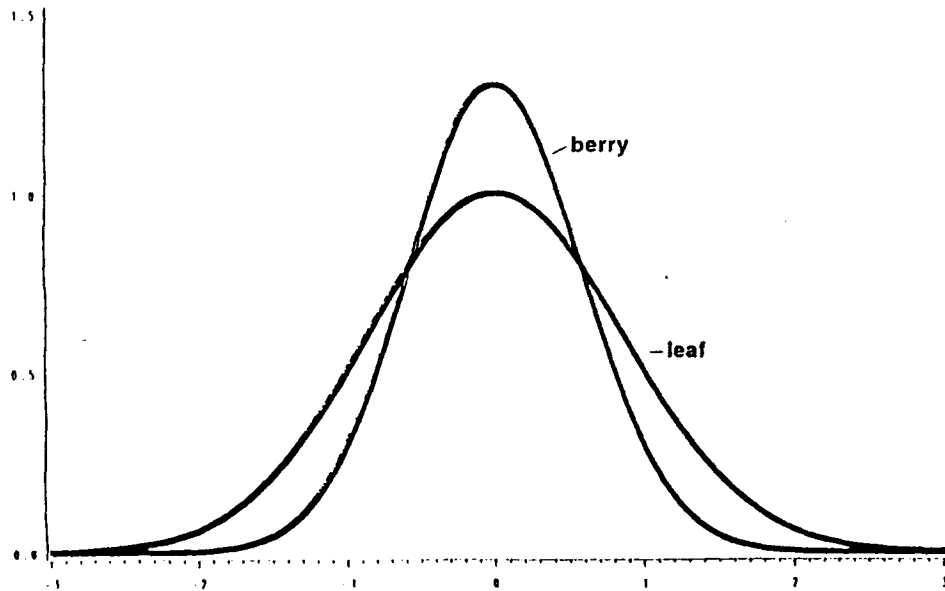


Fig. 6: Schematic frequency distributions of powdery mildew infections on leaves and berries derived from seedling populations

THE PHENYLPROPANOID PATHWAY GENES IN GENUS VITIS AND THEIR ROLE IN PLANT DEFENSE AGAINST BIOTIC STRESSES

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The strategies that plants adopt in response to stress conditions represented by a pathogen attack rely on both constitutive barriers and inducible activation of numerous defensive genes (Dixon and Harrison, 1990). In such situations genes are activated that are responsible for the biosynthesis of pathogen-related proteins as well as of substances known generally under the name of phytoalexins. This is a broad range of compounds produced by plants in a significant amount only as a consequence of stimulation by phytopathogens or by the presence of physico-chemical factors, the elicitors, related to a phytopathogenic condition.

Studies on model systems like maize, parsley, bean or *Petunia* have widely assessed the crucial role that the genes belonging to the phenylpropanoid pathway play in the early steps of this overall gene activation mechanism. For instance it has been demonstrated a positive correlation between either elicitor treatment, pathogen attack or physical agents like UV radiation and the induction of enzymes like phenylalanine ammonia-lyase (PAL), chalcone synthase (CHS) or stilbene synthase (StSy).

These are key enzymes in the pathway, being phenylalanine ammonia-lyase the first one, while chalcone synthase and stilbene synthase catalyze the reactions that lead respectively to the synthesis of flavonoids and stilbenes (the latter in grapevine represent the precursors of viniferins, compounds to which have been ascribed properties of phytoalexins).

In this work we investigated the effect of *in vitro* treatment of grapevines (*Vitis riparia* and *Vitis vinifera* L. var. Lambrusco) with two compounds, salicylic acid and oligogalacturonides, known in literature to act as elicitors of defensive mechanisms in conditions of biotic stress such as a pathogen attack (Malamy and Klessig, 1992 and Ryan, 1987): in particular we considered the effect on the activation of the phenylpropanoid pathway genes.

Data obtained were compared with those from true infections carried on using a natural pathogen of grapevine.

Experimental evidences were based both on molecular biology techniques to assess the rate and degree of activation of the genes under study and on HPLC (high pressure liquid chromatography) quantification of the phytoalexins produced by the activity of the stilbene synthase enzyme: resveratrol and viniferins.

Salicylic acid

Preliminary northern blot experiments probing total RNA extracted from leaves sprayed with solutions at different concentrations of salicylic acid with the stilbene synthase cDNA indicated that induction may be achieved with concentrations ranging between 5 and 50 mM.

Following this procedure changes were determined in mRNA levels in order to test how rapidly gene expression is switched on. The analysis of mRNAs present at different stages after elicitation demonstrates a marked increase during the first 6 hours after treatment. The time course of appearance of the phenylalanine-ammonia-lyase (PAL) and stilbene synthase mRNA appear to be coordinated. From this estimation of gene expression and by densitometric scanning of autoradiograms it was deduced that 22 hours after treatment on intact leaves the degree of transcription for the stilbene synthase gene is about 400 fold that at time zero. A noteworthy difference appears when the profiles of activation of stilbene synthase and UFGT (UDP glucose: flavonoid 3-O-glycosyl transferase) are compared. In fact UFGT, which is a terminal enzyme of the phenylpropanoid pathway branch that leads to the biosynthesis of the colored compounds known as anthocyanins, is not induced at all over the time period considered. Such a behaviour may be explained with the assumption that this branch of the pathway is non critical for the plant in the situation of facing a biotic stress (real or simulated by means of artificial elicitors). A weak increase of stilbene synthase mRNA was evidenced also in parts of the plant not directly interested by the salicylic acid treatment. This fact may account for the involvement of a signalling mechanism which makes the plant sense the effect of the stimulus far from the application site.

In order to demonstrate the real accumulation of compounds which, directly and indirectly, derive from the activity of stilbene synthase (namely resveratrol and its spontaneous products of condensation: viniferins) an HPLC analysis was performed, after treatment, on leaf extracts that were enriched and purified for the stilbenic fraction.

Total methanolic extracts were loaded on chromatographic columns packed with an Amberlite-type resin highly specific for phenolic compounds. Multiple washing steps with different solvents of varying polarity preceded the elution in ethyl acetate, where most of the stilbenic fraction was represented in a purified form.

Comparison of the HPLC data from the control and treated tissues revealed, as expected, different qualitative and quantitative profiles, reflecting in the latter case the increase of enzyme activity.

Oligogalacturonides

The same experimental scheme was followed for the tests using oligogalacturonides. Research on the biochemistry of plant defense has revealed that fragments from fungal and plant cell walls can act as powerful signalling agents to activate plant defensive genes (Toubart et al., 1992).

In the present work oligomeric fragments of a partial digest of polygalacturonic acid were provided by prof. Cervone of the University of Rome, Italy. They were obtained by combined action of a fungal polygalacturonase and a bean polygalacturonase-inhibiting protein under (PGIP) controlled conditions in vitro.

Tests on grapevine were conducted by injecting small volumes (usually 5 microliters) of concentrated solutions into the leaves and following the changes in mRNA accumulation by northern blots of total RNAs extracted from treated samples.

Results paralleled those obtained with salicylic acid treatments since induction of the gene for stilbene synthase was evident as well as the one for phenylalanine-ammonia-lyase (PAL), while no appreciable induction was observed in the case of the UFGT gene.

Furthermore, as for salicylic acid induction chemical data from HPLC analysis confirmed the differences in chromatographic profiles between controls and treated samples. Methodological controls, also made repeating northern blot and HPLC determinations on tissues infected with a true pathogen (*Botrytis fuckelina*), reflected completely the pattern of induction observed for the two previous simulated stress conditions.

Concluding, these data demonstrate the effectiveness of salicylic acid and oligogalacturonides as elicitors of the phenylpropanoid pathway genes and represent a clue to their possible role as signalling agents to activate plant defensive genes. Moreover the activation of the pathway, either under natural conditions (a pathogenic attack) or artificially induced with elicitors like salicylic acid and oligogalacturonides, seems to regard only the biosynthetic branch that is interested by the enzymes responsible for the production of defensive compounds.

Acknowledgments

We thank prof. Felice Cervone (University of Rome) who provided the synthetic oligogalacturonides, prof. Franco Faretra (University of Sari) for the grapevines infected with *Botrytis fuckelina* and Dr. Fulvio Mattivi (Istituto Agrario di San Michele all'Adige) for the protocol and helpful suggestions on purification and HPLC analysis of stilbenic compounds.

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SESSION 3 : GENE ANALYSIS

THE RAPD ANALYSIS OF VITIS

GERMPLASM

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INTRODUCTION

Traditionally *Vitis* germplasm has been described using morphological traits by means of ampelography. In recent years, other methods that rely again on characteristics of the plant which are expressed have been applied for the identification of grapevines. DNA molecular markers are considered to be superior to examine the genetic relationships between accessions because of the availability of a large number of potential polymorphic sequences. Among techniques for detection of DNA polymorphism the arbitrarily primed polymerase chain reaction (AP-PCR) which does not require prior sequence information, large amount of DNA substrate and the use of radioisotopes, provides a faster and easier approach. The random amplified polymorphic DNA is a subset of AP-PCR that uses single or combined short primers of arbitrary nucleotide sequence to amplify unspecified regions of genome.

AIMS

We report here a survey of RAPD markers in *Vitis* species and cultivars and their possible application to grape germplasm characterization.

MATERIALS AND METHODS

Grape material

All plant material was obtained from the germplasm collection maintained at the Istituto Agrario of San Michele all'Adige, Trento. Young fully expanded leaves were separately harvested from two plants per genotype, frozen immediately in liquid nitrogen and stored at -80 °C until DNA extraction. The accessions listed in table I were used in this study.

DNA isolation

DNA was extracted and purified according to the hexadecyltrimethylammonium bromide (CTAB) method of Doyle and Doyle (1987) for fresh tissue.

Data analysis

Amplified band was named by the primer used and its size in bp, RAPD profiles were scored visually. Data were recorded according to the presence or absence of an amplification fragment of a given length. Only those among major fragments whose presence was unambiguous were retained. Genetic similarity between each pair of accessions (n. 171) and the phylogram tree were calculated using NTSYS-pc package (Numerical Taxonomy and Multivariate Analysis System, version 1.8) developed by Rohlf.

RESULTS

The amplification reactions with the 40 decamer primers of arbitrary sequence performed using the DNA of grapes belonging to different species of *Vitis* genus, have shown the existence of a high polymorphism of RAPD markers. In many cases, a single primer has produced band profiles able to differentiate all species which were being examined. This result confirms the possibility of using the RAPD approach for the analysis of genetic diversity also in *Vitis*. The attempt to interpret the phylogram tree originated by the analysis of similarity between RAPD profiles is limited by the low number of individuals analysed for each species. However it is interesting to note that all accessions belonging to Eurasian species:

V. vinifera, *V. caucasica*, *V. betulifolia*, *V. coignetiae* and *V. amurensis* have been put in the same cluster.

The North American species: *V. rubra*,

V. rotundifolia, *V. cinerea* and *V. baileyana*, on the contrary, have been separated from the species

V. riparia, *V. champini* and *V. doaniana*. the last two species have also been considered hybrid species of *V. rupestris* x *V. candicans* Engelman and of *V. riparia* *V. candicans*, respectively. A somewhat intraspecific polymorphism of the amplification products can be observed in the case of *V. riparia* and *V. rupestris* whereas that observed among *V. vinifera* cultivars turns out to be too complicated and not very practical as to be used as fingerprint. The attempts to use RAPD markers for variety identification are still limited to the comparing of few cultivars and, according to our experimental findings, the multi-banding patterns which can be obtained still require some interpretation.

A more accurate way of identifying cultivars can come from the amplification via PCR of microsatellite repeat sequences, but it will require the availability of specific primers. The RAPD approach, for its high capacity of generating ANA markers, allows a genome scanning limited only by the number of primers involved. Therefore it seems to be helpful in elucidating the genetic diversity which is present in *Vitis* germplasm collections.

RESISTANCE TO PSEUDOPEZIZA TRACHEIPHILA (M-Th.) OF SOME DOWNY MILDEW RESISTANT HYBRIDS AND VARIETIES

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The plant protection against downy mildew usually could save the plantations from refer brenner infection, and distracted the attention from it.

The chemical plant protection against downy mildew has been left off entirely in the plantation of new, resistant varieties (e.g. Bianca), and after a few years there could be noticed roterbrenner infection. According to LEHOCZKY (1967) the occurrence of this disease is not common in: Hungary, it breaks out regularly only in certain grapevine districts. It was unknown in plain grapevine region, as in Kecskemet grapevine district. In the northern grapevine regions roter brenner regularly breaks out seriously. According to BRANAS (1974) every Vitis species and their derivatives are susceptible, and the varieties, the rootstock varieties and the direct producer grape varieties are also susceptible, but in different degree BOUBALS (1984) published about a serious refer brenner infection in a plantation not been protected against fungus diseases. He evaluated the susceptibility of rootstock varieties and mentioned, that there could be seen great patches on the leaves of So 12375 (Villard blanc), they were grown usually without any protection against downy mildew. JURKU (1978) founded in Moldova, that varieties originated from V. labrusca were very susceptible, the Riparia X Rupestris 101-14 was little susceptible, he could differentiate susceptible and medium susceptible hybrids among European varieties. In the Research Institute for Viticulture and Enology of Hungary there has been on progress resistance breeding since more decades in order to create resistant varieties, and there were obtained numerous valuable new varieties. In order to establish the further breeding work it has been founded a new collection of varieties, a new gene bank in Kecskemet. There was imported more hundred varieties from abroad, both from Eastern and Western Europe.

A few years ago (1988-89) in our experimental non-protected plantation in Kecskemet (Katonatelep) broke out refer brenner disease. It was a great surprise for specialists. The varieties has been evaluated there, and the same varieties were evaluated in Keszthely (close to Balaton lake) in Western Hungary, in more humid, wet conditions

Material and methods

There were examined in our experiment wild species, rootstock varieties, new clones and hybrids of rootstock varieties, old and new direct producer hybrids, and new resistant varieties.

In Kecskemet in the collection there were evaluated 5-20 pieces vine-stocks of varieties. In Keszthely in the resistance testing garden there were planted 10-10 pieces vine-stocks of varieties, for 3,0 x 0,2 m distance between the rows and vines.

The evaluation took place with a visual review. We have created a scale, it was similar to the OIV code. The results are published according to the Code No. OIV476., published by Erika DETTWEILER (1993):

1. Very large attacked patches, limited by veins on >50 % of the leaf blade. Leaves necrotic, involute. Leaf drop.
3. Large attacked patches, limited by veins on >25% of the leaf blades. Necroses between main veins.
5. Attacked patches 1-2 cm diameter limited by veins on >10% of the leaf blade, partly necrotic.
7. Small attacked patches, limited by veins on >10 % of the leaf blade.
- 9 Punctuated necroses or no symptoms.

Evaluation

Symptoms of roter brenner disease in Kecskemet there could be noticed seldom or hardly in spring. But in the second half of August the infection widened out in such measure, that during a week the great part of foliage of susceptible varieties has been destroyed and has fallen down.

In Keszthely the symptoms there could be noticed earlier, but the disease became serious also in August.

Results

Among the Northern-American wild species V. labrusca proved to be very susceptible (Table I.)

The distribution of susceptibility of rootstock varieties is the next:

- there was not any very susceptible variety
- there could be found as medium susceptible Berlandieri x Riparia T5C and Berlandieri x Riparia SO4
- there was found as little susceptible Fercal, Berlandieri x Riparia 5BB, Georgicon 28, and Solonis x Riparia 1616C

The other rootstock varieties or the sub clones of Berl x Rip SBB, '18B, 125AA showed good resistance.

Among Franco-American hybrids the progenies of Serbel 6468 k Serbel 6905 cross were susceptible, especially SV 12375, which was the most successful resistance source in the last breeding period (Table 2.).

According to our examinations SV 18315 was less susceptible, it had good resistance against refer brenner SV 20365 and SV 23657 (Varousset) were susceptible. Among Seibel hybrids S. 5455 and S. 5450 were resistant and the others were susceptible.

The Hungarian downy mildew-resistant hybrids being the progenies of SV 12375 were very susceptible (Table 3.).

Bianca has a high resistance against downy mildew and powdery mildew and it is of high quality but against roter brenner it showed susceptibility. Progenies of Zalagyongye (backcross with varieties of *V. vinifera*) were susceptible e.g. R79 and Muscat of Poloskei. Progenies of Seibel 5279 (RF 5, RF 16, RF 48) were susceptible. Kunbarat is a *V. axnurensis* x *V. vinifera* hybrid, and it was resistant against roter brenner.

Valuable and promising varieties from abroad: Orion, Srlva, Vronka, Festivalny,

Fr 996-30 and red hybrids of Seibel 13666 x Merlot from Moldova also were very susceptible.

According to our results the great part of derivatives of Franco-American resistance source are susceptible or very susceptible. It was found only a few resistant hybrids among the progenies of SV 18315.

Among Hungarian varieties originated from *V. amurensis* Kunbarat showed good resistance.

The great part of varieties originated from *V. amurensis* x *V. vinifera* cross showed good resistance. The *V. vinifera* x *V. amurensis* x SV 12375 hybrids were very susceptible or susceptible.

V. axnurensis and (*V. amurensis* x *V. vinifera*)F1 were resistant. (Table 4.) Among varieties originated from (*V. am.* x *V. vin*)BC1 there were susceptible (e.g. Kuleany), medium resistant (Galubok) and resistant varieties (Stepnak). Among the varieties originated from *V. amurensis* and *V. vinifera* BC2 there could be found both resistant and susceptible varieties. Progenies of (*V. am.* x *V. vin*)F1 x BC1 showed medium resistance. Hybrids originated from *V. am* x *V. vin* x SV12375 cross proved to be susceptible.

Conclusion

1. Together with the resistance against downy mildew there is very important to examine the susceptibility to refer brenner. There is necessary to select for resistance against refer brenner disease in the course of crossing program.

2. At choosing of parents it is necessary to consider that the great part of successful resistance sources are close relatives and susceptible.

3. In the course of further breeding work we have to use more intensively *V. amurensis* hybrids.

4. To the successful selectional work against refer brenner I suggest as susceptible control varieties the next: CsFt 194, Festivalnij and Fr. 993-60. They are resistant against downy mildew.

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Table 1.

Resistance of rootstock varieties against roter brenner

Varieties	Degree of resistance (Code OIV476)
Vitis arizonica	9
Vitis riparia	9
Vitis labrusca	3
Vitis aestivalis	9
Vitis Solonis	7
Vitis Berlandieri	9
Rupestris tomentosa	9
Rupestris du Lot	9
Mourvedre x Rupestris 1202 C	9
Riparia portalis	9
Aramon x Riparia 143 B	9
Solonis x Riparia 1616 C	7
Berlandieri x Riparia T5A	9
Berlandieri x Riparia TK5 BB	7
Berlandieri x Riparia T 5C	3
Berlandieri x Riparia SO4	3
Berlandieri x Riparia T8B	9
Berlandieri x Riparia 125 AA	9
Chasselas x Berlandieri 41 B	9
Fercal	7
Riparia x Rupestris 3309 C	9
Georgikon 28	7

Table 2.

Resistance against roter brenner of Hungarian downy mildew resistant varieties

Varieties	Origin	Degree of resistance (Code No. OIV476)
Zalagyöngye	SV 12375 x Csabagyöngye	4
Bianca	SV 12375 x Bouvier	3
CsFT 194	SV 12375 x Csabagyöngye	1
CsFT 195	SV 12375 x Csabagyöngye	4
R 58	SV 12375 x Olimpia	2
R 65	SV 12375 x Magarach	3
R 68	SV 12375 x 623-24	2
R 78	SV 12375 x 658-215	3
R 79	Zalagyöngye x 6829-93	3
Pölöskei musk.	Zalagyöngye x 6829-93	2
Medina	SV 12286 x Medoc noir	3
Göcseji zamatos	SV 12286 x Medoc noir	3
CsFT 61	Seibel 4986 x Olimpia	3
RF 5	Pannonia k. x Seibel 5279	2
RF 16	Gloria Hun. x Seibel 5279	3
RF 48	Csabagyöngye x Seibel 5279	2
Kunleány	(V.am. x V.vin.) F2 x Afuz Ali	3
Kunbarat	(V.am. x V. vin.) F2 x Italia	7

Table 3.

Resistance of varieties originated from *V. amurensis* against roter brenner

Varieties	Origin	Degree of resistance
<i>Vitis amurensis</i>		7
Kaberam	(<i>V. am.</i> x <i>V. vin.</i>) F ₁	5
Bruskam	"	7
Kunlcany	(<i>V. am.</i> x <i>V. vin.</i>) BC ₁	3
Kunbarat	"	7
Kabernet severmüj	"	5
Stepnak	"	7
Galubok	"	5
SK 77-4/5	(<i>V. am.</i> x <i>V. vin.</i>) BC ₂	7
SK 77-5/3	"	7
SK 76-3/3	"	3
SK 77-10/54	"	7
SK 77-12/6	"	3
Gm 64-95-3	"	5
Skif	(<i>V. am.</i> x <i>V. vin.</i>) F ₁ x BC ₁	5
Sarmat	"	5
Kazachka	"	5
Vostorg	"	5
Agat donszkoj	"	5
216-25-5-3	(<i>V. am.</i> x <i>V. vin.</i>) x Franko-am. hybrid	1
C43	"	3
C50	"	5
SK 78-10/265	"	3

Table 4.

Resistance of Franco-American hybrids against roter brenner

Varieties	Origin	Degree of resistance
		Code N ^o OIV 476
Seyve-Villard 12286	Seibel 6469 x Seibel 6905	3
Seyve-Villard 12303	"	5
Seyve-Villard 12309	"	5
Seyve-Villard 12358	"	4
Seyve-Villard 12375	"	3
Seyve-Villard 12390	"	5
Seyve-Villard 18315	Seibel 7053 x Seibel 6905	7
Seyve-Villard 5276	Seibel 5656 x Seibel 4986	5
Seyve-Villard 20365	Pance x SV 12375	4
Seyve-Villard 20473	SV 12129 x Pance	5
Seyve-Villard 23410	-	5
Seyve-Villard 23657	-	4
Seibel 5279	S 788 x S 29	4
Seibel 7053	S 5163 x S 880	3
Seibel 5455	S 4469 x V. Berl	9
Seibel 5450	S 867 x S 4182	9
Seibel 8718	S 5163 x S 880	3
Seibel 8745	S 5163 x S 880	3
Seibel 11803	S 2859 x S 4643	3

SESSION 3 : GENE ANALYSIS

PROBLEMS OF GRAPEBREEDING AND GENETICS

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Grape assortment of the former Soviet Union's viticultural republics is rather large though average yields most cultivars give are low and per capita fresh consumption of grapes is still unsatisfied. Conditions of most wine brands are also low and do not always satisfy consumer demands. This is due to a number of reasons, and one of them is that Euro-Asian grape assortment contains cultivars with good quality of fruit but lacks those resistant to frosts and immune to pests and diseases. As a result of this, the world's total grape area accounting for about 10 million ha fails to supply annually up to 26 million tons of grapes. In addition to economical damage because of such losses of fruit, measures to control pests and diseases are expensive and application of chemicals inflicts much harm to environment. In Armenia, for instance, prior to the total privatization of land, more than 10-15 million roubles (in comparable prices of 1988) were spent for this purpose.

Thus, an urgent need arose to release new cultivars combining useful qualities of Euro-Asian, American and Amur grapes. Using a single methodology, breeders and geneticists of the former Soviet Union developed a program aimed at the creation of frost-resistant cultivars to be cultivated in regions with highly continental climate and those resistant to pests and diseases for regions with wet climate and phylloxera-infested soils. That program was to be fulfilled throughout the grape cultivation area of the former Soviet Union, and this paper will be concerned with some promising results of grape breeding achieved in Armenia and in the Crimea. In Armenia, the Ararat valley and its premountainous area is the main region of commercial viticulture. Own-rooted and irrigated grapes which have to be protected in winter are cultivated there. Cold winter with absolute minimum soil temperature of -34°C is the main limiting factor of viticulture in that region yet fungal diseases are not negligible either. Considerable damage is inflicted to the country's viticulture in years when mildew and oidium outbreaks occur. Viticulture of the north-eastern part of Armenia is typical of phylloxera-infested areas. Grafted vines cultivated there are subject to annual epiphytotic outbreaks of mildew and oidium. In separate years, winter temperatures even in that zone may be as low as -18 and -20°C .

The fulfillment of the above program in Armenia has led to the development of 145 new grape forms. These were developed through classical methods of intra- (*V. vinifera*) and interspecific hybridization. The best plants were then selected from these forms to obtain the superelite. Forty-five of these cultivars are frost-resistant and 32 of them were submitted to the State trials. Depending on their bud and wood frost resistance, they can be divided into four groups: those withstanding frosts up to -26°C , -28°C , -30°C and -32°C . During selection of grape forms to which the status of cultivar could be granted, preference was given to those with late budbreak, at least as late as in European grapes. Their yields vary over the range of 146-273 q/ha, with sugar content of 24-29% and moderate or increased acidity. Some cultivars possessed also mildew and gray rot resistance (rated 1-3). Frost-resistant cultivars, in addition to high coefficient of fruitfulness and good average bunch weight, are capable of abundant fruiting from supernumerary buds and provide desired size of yield even if 70% of buds are injured in years with especially cold winter and after early spring frosts and hailstorms. Such frost-resistant cultivars can be cultivated on slopes of 5° and more, which enables them to withstand most unfavorable winters practically without injury. The winters of 1984-85 and 1993-94 prove that. Over those periods, optimum temperatures for European grapes observed in December, January and February were followed by thaws of up to 17°C during 7th-17th February. As a result of this, the exogenous dormancy of vines was broken while the last ten days of February saw critical temperatures of -20 to -28°C depending on the region. Frost-resistant cultivars located on flat sites showed 50 to 100% of injury depending on the temperature of a microregion and the cultivar, while those cultivated on slopes had wintered virtually without injury under the same temperatures. In these cultivars, even central buds were not injured.

Considerable success has also been achieved in the development of phylloxera-resistant cultivars through intraspecific hybridization (*V. vinifera*). Eleven cultivars were released and submitted to the State trials and two of them were introduced into commercial cultivation in the north-eastern zone of the country where soils are phylloxera-infested.

The grape and wine industry of Armenia faces now numerous problems as the country has adopted new forms of land property, of perennial plantings, too. Breeders have to charge themselves now with the development of cultivars to be in demand of growers-owners (future farmers). Such cultivars should be capable of making efficient use of natural factors and improved conditions of cultivation as well as to provide high yields, good quality of fruit and resistance to pests and unfavorable environmental factors.

With this in mind, breeders of the country set themselves to an enlarged program in the 1980 which aims at step-by-step creation of hypothetically ideal cultivars. Such cultivars are meant to be not inferior to existing ones in yielding capacity, quality of fruit and frost resistance. Besides, they should possess field resistance to phylloxera, mildew, oidium, gray rot and other pathogenic agents and their fruit, both fresh and processed, should contain valuable nutrients and biologically active substances.

Thus, a number of problems had to be solved, with relation to the use of new approaches aimed at the improvement of grape assortment. Among these, to be mentioned are breeding for heterosis, mathematical, evolution and population genetics, physiologo-biochemical, biophysical and biotechnological methods and clonal selection. These approaches can be applied to any agricultural crop and are valuable for grape breeding and genetics in all regions where vines are cultivated.

Under conditions of the Ararat valley, we studied for the first time regularities of heterosis expressivity and inheritance in hybrid grape progeny for such characters as sugar content, berry color, vitamins, amino acids and mineral substances, chlorogenic acid, free catechins as well as for their interrelationships and correlations with resistance to biotic and abiotic factors depending on the genetic nature of the pairs to cross, metabolism of vine organs and photosynthetic intensity.

We proved that, generally, the true heterosis for quantitative characters responsible for quality of fruit in the progeny was expressed in more than 50% of seedlings and in 100% of seedlings in some exerts-combinations. In grapevine, the adaptive heterosis is most difficult to achieve: not more than in 4-6% of seedlings in the progeny, with yield characteristics meeting necessary requirements.

The expressivity of heterotic progenies in grapevine is due to a large amount of variants of genetic characters along with participation of matrix structures and transposition of mobile genetic elements, liability of the structure and organization of mitochondrion and plastid genomes, nucleo-cytoplasmic phenomena, etc. In grapevine, however, the degree of expressivity of a number of characters in different groups and cross-combinations varies considerably: from $H_d = -16$ to $H_d = +48$. In the first case, this is due to hybrid depression caused by accumulation of alleles determining a definite character negatively and in the second case this is due to superdominance because of accumulation of alleles determining a definite character positively, which results in better balanced physiological systems of a new hybrid organism. Nevertheless, it is also probable that a high degree of heterosis may be determined by positive one-purposed nonallelic interaction of genes (complementary epistasis) or unique functional organization of the genotype of a seedling which can provide development and morphogenesis at each step of inheritance following the most efficient route for definite situations.

The regularities we revealed over the period 1965-80 (first step) were confirmed by the development of cultivars possessing "a heterosis complex", i.e. a number of heterotic characters: Megrabuir, Vardanank, Aknalich, Zeitun (which withstand frosts of -28°C , produce yields of up to 300 q/ha with sugar content of up to 28%) and Arataber, Berkanush, Aboviani, Musaler (producing yields of up to 250 q/ha with sugar content of 24-29%) as well as a number of elite forms.

In 1979, a new step of hybridization was undertaken with an intention to increase the effect of heterosis in hybrid progenies of grapevine that were meant to be highly heterotic for a number of economic characters, including complex resistance to pests and diseases. During this step, we crossed to each other best heterotic cultivars and elite forms (as far as definite characters were concerned) which had been released earlier, and such forms and cultivars were also crossed to collection forms and cultivars possessing desired characters which had been introduced in the Ararat and in the north-eastern zones of Armenia, to frost-resistant forms obtained from the Timiriazev Academy of Agriculture and the All Russia Potapenko Institute for Viticulture and Enology, to mildew-resistant forms from the Moldavian Research and Industrial Center "Vierul" and to polyploid forms released by the Department of Distant Hybridization of the botanical Gardens within the framework of the Moldavian Academy of Sciences, to Hungarian forms resistant to mildew and oidium (reciprocal crossing) as well as to such hybrids as Serve Villard, Seibel, Baco, DRX, etc. We also conducted intravarietal hybridization with the same intention.

At this step of investigation, a large grape genofond was studied and important results were obtained. Heterosis for resistance and adaptivity was achieved in 50% and more of seedlings in a number of cross-combinations possessing a true heterotic effect for such characters as resistance to frosts and at least to one pest; a somewhat smaller proportion of seedlings displayed resistance to two pathogenic agents and about 15-36% of seedlings were resistant to all pests against maximum 4-6% achieved at the first step of the breeding process. The heterotic effect was increased for other characters, too, that had been improved already at the first step of the breeding for heterosis. In some cultivars, the heterotic effect for resistance was combined with Muscat flavor of berries. It was also established that no cross-combination containing Megrabuir as a parent was superior to this cultivar in the heterotic effect for vigor which is typical of Megrabuir.

As a result of our investigation, 80 new superelite forms of wine, table and raisin grapes were obtained. After their vegetative progenies had undergone an additional testing, some cultivars (Amaras, Muscat Ijevani, Muscat Dvin, Muscat Nairi, Zoravar, Karmirblur, Chinchin, Debedi, Avarair and a number of others) were submitted to the State trials. All these cultivars combine in one genotype high yielding capacity, resistance to biotic (phylloxera, mildew, oidium, gray rot, acari) and abiotic factors as well as good quality of fruit.

Based on the results obtained, we came to a conclusion that breeding for the heterotic effect was a promising approach. In 1992, The Armenian Institute for Vine Growing, Wine Making and Fruit Farming conducted the forth step of hybridization with an intention to release frost-resistant seedless cultivars with Muscat flavor of berries and possessing a high effect of true heterosis for these characters. The great bulk of this genofond is being studied in the Ararat valley, and six cultivars which had been under the State trials of Armenia and in the ampelographic collection of the institute "Magarach" and about 200 seedlings were plaited in the spring of 1994 on sites owned by the Yalta Experiment Station and the Company "Apis" on the South Coast of the Crimea where they are going to be investigated.

The fact that the institute for Vine and Wine "Magarach" (Ukrainian Academy of Agrarian Sciences) hosted the VIth International Symposium on Grape breeding proves this Institute's breakthrough in the line of the development of cultivars with immunity to biotic factors and complex resistance to abiotic ones. Beginning with 1928, breeders of this Institute have released the total of 45 cultivars. There were tested in the State Trial Net of the former Soviet Union and 12 of them were Included in the State Registers of Ukraine, Russia and Moldova. Among these cultivars, to be mentioned are such as Pervenets Magaracha, Podarok Magaracha, Yubileiny Magaracha, Antei. Magarachsk, Aurora Magaracha, etc. The recent 10-15 years saw recognition and wide distribution of these cultivars all over the Soviet Union. At present, they occupy 10-15 thousand ha throughout the ex-USSR, including 600 ha in the north-eastern part of Armenia where they have been in cultivation since 1984. Besides, in order to enlarge research in the line of breeding for resistance and high yielding capacity, the Institute for Vine and Wine "Magarach" and the Armenian Institute for Vine Growing, Wine Making and Fruit Farming carried out this year reciprocal "saturant" crossings of frost-resistant high-yielding cultivars released in Armenia to phylloxera-resistant cultivars and forms of grapevine released in the Crimea.

At present, breeders of the Institute "Magarach" set themselves a task to develop cultivars especially for the South Coast of the Crimea where frost resistance is not necessary, for the central and northern steppe zones of the peninsula and their counterparts in the viticultural area of Ukraine, for the special zone of the Dnepr with sandy soils as well as for the Ararat, Premountainous and north-eastern zones of Armenia which should combine all desired characters in one genotype.

This hybrid material which will have to be cultivated in the Crimea and that to be cultivated in Armenia will provide genetic resources to release outstanding cultivars even at the beginning of the 21st century. Besides, one has to take into account success achieved in this line by efficient breeding institutions of Germany, Hungary, Moldova, Russia and other countries, and this enables us to expect that series of prototypes for "ideal" grape cultivars are going to be developed, as the general philosophical law of transition from quantity to quality should work for grape genetics, too. Such cultivars once developed, next step forward is to be made by nursery men whose business will be to propagate these cultivars and by technologists who will have to develop new methods of vilification.

Yet breeders should not limit themselves only to applied results whatever promising they may be. Dealing with so complicated a heterozygote as grapevine, fundamental research should also be conducted, which, in turn, will work for a new breakthrough in the breeding activities. Thus, approaches based on particular genetics, for instance, may be helpful in the solution of such problems as rational usage and conservation of genetic resources and their protection from gene erosion, control of hereditary variation of genotypes of grape cultivars, forms or clones, morphoanatomical and physiologo-biochemical analyses of grape ontogenesis and its control, detection and isolation of new genes to be used in grape breeding and biotechnology, control of yielding capacity and quality of fruit in resistant cultivars, improvement of their resistance to obtain perfect immunity, analysis of genotypic structures and chromosome mapping.

Solution of the above problems requires (i) good understanding of the organization and functioning of the genetic apparatus, (ii) detection and cloning of genes controlling definite processes determining the structures of such genes and proteins they control.

Genetic variation is The basis for the development of grape forms possessing high adaptivity, good yielding capacity and resistance to biotic and abiotic stresses in the presence of existing commercial technologies. That is why the principal task of this trend of investigation is the search for regularities of their variation, with analysis of genetic determination of variation being the key problem.

As genetic variation is described by probabilistic regularities, one should not expect large genotypic shifts without the introduction of personal computers into the breeding routine. Moreover, good understanding and usage of chromosome variation will certainly contribute to the general progress of breeding.

At present, much importance is attached to analysis of somaclonal variation in plants obtained via biological methods, in vitro techniques in particular. Understanding of the regularities of this phenomenon is very important both for obtaining general genetic knowledge of the mechanism of variation and for breeding practice. Of considerable importance is also testing of certain pathogenic viruses and viroids.

Development of such methods and creation of adequate forms of selection of necessary genotypes are the principal goals of investigations conducted in the Institute "Magarach". Such investigations are conducted in cooperation with the Institute for Plant Physiology and Genetics of the Ukrainian Academy of Agrarian

Sciences (B.A. Levenko), the Zabolotny Institute for Microbiology and Virology of the Ukrainian Academy of Agrarian Sciences (A.M. Zaichenko, I.G. Rubezniak), the Institute for Breeding and Genetics of the Ukrainian Academy of Agrarian Sciences (Yu. M. Sivolap), the Grimes Institute for Agriculture (V.O. Ostroverkov), the Sevastopol Technological. University (Yu.I. Tomin), the Annenian Institute for Vine Growing, Wine Making and Fruit Farming (K.S. Pogonian), the Erevan University (R.M. Oganessian, A. Drampian) , etc.

At present, grape breeders make efficient use of induced mutation and recombinations, heterosis, polyploidy, immunogenetics, cell engineering, distant hybridization, protein- and DNA-markers in their work. Even partial usage of these methods has led to a 50% increase of yielding capacity of these cultivars, and one may expect that such methods, when improved as a result of acquired knowledge in the field of molecular genetics, will provide a strategic breakthrough with refer to the development of grape cultivars immune to biotic factors and complex resistant to abiotic ones.

GRAPE BREEDING FOR SEEDLESSNESS

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N. G. PAVLIUCHENKO

Obligate seedlessness of grapes is a valuable economical character in cultivars destined both for the fresh consumption and production of raisins, juice, wine and other products.

Summing up the discussions of International conferences and data of leading foreign researchists, it can be concluded that the problem of grape seedlessness is the greatest concern in the USA, Italy, the former Soviet Union, Australia, Israel, Spain, Bulgaria and Yugoslavia. Based on the analysis of reports and published materials, visits to vineyards and exchange of information, the following changes in the principal approaches to the solution of this problem may be revealed.

1. In 1930-50s, seedlessness was considered valuable mainly with refer to the production of raisins.

Onward, it came to be regarded as useful for table cultivars too. Currently, studies of seedlessness are conducted based on cultivars destined for the production of wines and some other products.

2. In the beginning of breeding for seedlessness, new cultivars were developed through the hybridization based mainly on the species *Vitis vinifera* L. and, precisely, within the ecologo-geographical group *Convar. orientalis* Negr., and to a lesser extent, within the group *Convar. occidentalis* Negr. This determined the geography of the breeding research as well as the ways and approaches to solve this problem.

3. At present, the breeding programs of many viticultural countries aim to develop seedless cultivars resistant to unfavorable biotic (pest and diseases) and abiotic (low winter temperatures) factors. This caused the breeders to practise interspecific hybridization, which allowed to fulfil such breeding programs in more northerly regions. On all taxonomic levels (intra- and interspecific hybridization), the parent pairs contained a seeded female form and a seedless male form.

4. Due to the progress in biotechnology, especially in the tissue culture techniques, and with a possibility to grow a seed embryo under "in vitro" conditions, a new method of breeding for seedlessness has been developed, i.e. the obtention of viable progeny through the crossing of seedless cultivars with each other followed by the "in vitro" cultivation of seed embryos. This approach suggests absolutely new prospects and allows to treat the genetics of seedlessness in grapes in a new way.

During our investigations, we studied 5500 hybrid seedlings of 85 cross-combinations obtained through the intra- and interspecific hybridization, with a seeded cultivar entering as a female parent in all the crossing and a seedless cultivar as a male one. We also tried to use such female parents which were prone to the expression of stenospermocarpy.

The percentage of seedless forms in the hybrid intra- and interspecific progenies depends to a considerable extent on the assignment of the initial forms to different ecologo-geographical groups within the *Vitis vinifera* L. species and genetic peculiarities of the parental forms. Of 85 interspecific cross-combinations, only 43 (50,6%) gave seedless forms.

The highest percentage of cross-combinations in which seedless seedlings were obtained was found in the group of populations with hybrid cultivars belonging to the ecologo-geographical group *Convar. orientalis* Negr. used as female parents (76,5%). Then the group of hybrid families comes in which ancient indigenous table and seedless raisin cultivars belonging to the same ecologo-geographical group were used as female parents (58,1%).

The lowest percentage of cross-combinations containing seedless forms (16,7%) was found when cultivars of the ecologo-geographical group *Convar. occidentalis* Negr. entered as female parents.

Within the above ecologo-geographical groups, the percentage of seedless seedlings in different cross-combinations differed considerably depending on genetic peculiarities of cultivars as original parental forms.

We compared the parental forms based on the pattern of the transmittance of seedlessness to the progeny, and high influence of the female parent was observed. This caused us to conduct investigations aimed at the following: of a large amount of cross-combinations obtained through the crossing of seeded cultivars with seedless ones, we selected those which yielded the highest percentage of seedless forms.

The analysis of these cultivars revealed that, as a rule, they were highly capable of producing stenospermocarpous berries in the bunch as well as of forming large weight of the berry caruncle per unit weight of the seeds developed in the berry. Seeded cultivars which had been used in this case as female parents were pollinated with pollen of seeded cultivars belonging to this group under conditions of strict isolation after the flowers had been castrated. Of the hybrid seedlings which started bearing six seedless forms were revealed. This enables us to conclude that a number of seeded genotypes possess one or several genes whose recessive alleles, when combined, led to the formation of seedless forms. It seems probable that this fact supports the hypothesis suggesting the initial formation of seedless forms in seeded varieties.

Analysis of the morphology and anatomy of seeds and seed rudiments, their size and gustatory perceptibility during the tasting of fresh berries produced by hybrid seedlings released through the intra- and

interspecific hybridization shows that, within each cross-combination, there exists a considerable variation in seedlessness, from large and normally developed seeds solid skin to soft seeds and seed rudiments with different degrees of development. This caused us to work out their classification to be used by grape breeders and growers as a special reference. This classification is widely used now in the breeding for seedlessness. If a breeder intends to develop a large-berried seedless cultivar, of great importance is to reveal the pattern of inheritance of seedlessness by the hybrid progeny and the correlation of this pattern of inheritance with seedlessness.

Hybridological analysis of intra- and interspecific seedlings reveals that seedlessness is inherited by the hybrid progeny obtained through the crossing of seeded cultivars with seedless ones according to the general laws of genetics. As for the correlation between seedlessness and berry size, this is as follows: of 18 intraspecific (*Vitis vinifera* L.) cross-combinations, only seven displayed positive correlation between seed weight and berry size. This result indicates that, theoretically, it is possible to develop large-berried and seedless hybrid cultivars. In interspecific hybrids, the correlation between berry weight and seed weight is slightly different. Depending on the cross-combination, the coefficient of correlation varies from $r = 0,55$ (cross-combination Chambourcin x Kishmish unikalny) to $r = 0,84$ (hybrid progeny of SV 12-375 x Korinka russkaya). Seed amount and seed weight are negatively correlated, and this negative correlation varies from $r = 0,13$ (Chambourcin x Kishmish unikalny) to $r = 0,46$ (population of SV 12-375 x 3-10-9-8-1).

Within the group of seedless seedlings with different degrees of the seed rudiment development, the combination of small size of seed rudiments (Categories I and II) with small berry size occurs, as a rule, more frequently, which indicates that these two characters tend to correlated with each other. However, this trend ought not to be regarded as a regularity which, theoretically and practically, would have rendered it impossible to develop large-berries seedless grape forms through the inter- and intraspecific hybridization. On the contrary, large-berried seedless cultivars have been released both in our country and abroad.

Another important problem of grape breeding for seedlessness is that of evaluation of male seedless parents depending on of different forms of obligate seedlessness: parthenocarpy or stenospermocarpy. In other words, we may ask ourselves whether there is some preference for the seedlessness to the hybrid progeny of one of these forms of seedlessness. Results of our investigations and finding of other researchists indicate that seedlessness can be transmitted to the hybrid progeny both by parthenocarpous and stenospermocarpous seedless cultivars.

However, we think the stenospermocarpous form of obligate seedlessness to be of greater importance for practical breeding. As a rule, a higher percentage of seedless forms in the hybrid progeny is found in cross-combinations where seeded cultivars capable of producing stenospermocarpous berries in the bunch entered as initial female parents, these stenospermocarpous berries being similar to normally developed berries of a given cultivar with refer to their shape and size. Besides, it is just stenospermocarpous cultivars which are mostly used in breeding as donors of seedlessness. Progress in breeding for seedlessness proves that seedless cultivars belonging to this group are very promising.

We may also ask ourselves whether the category of seedlessness of a seedless cultivar used as a donor is of any importance for the transmittance of seedlessness to the hybrid progeny; in other words, whether we may expect that when using a seedless cultivar with larger seed rudiments as a donor of seedlessness, seedless hybrid forms of the Categories of seedlessness I and II can be obtained in the hybrid progeny. Hybridological analysis of a large amount of seedlings released through the intra- and interspecific hybridization provides us with a positive answer.

Good nutritive and dietary value of newly bred grape cultivars are their important conomic characteristics. Biochemical investigations of grapes conducted by M.V. Melkonian, L. A. Maistrenko and other researchists indicate that seedless cultivars are superior to seeded ones with refer to their content of many important components.

L. A. Maistrenko studied the chemical composition of the juice of 23 seedless cultivars cultivated in the Lower pre-Donarea. The results of this research indicate that the seedless cultivars possess higher sugar content and Vitamin C content, especially in berries of the cultivars belonging to the Categories of seedlessness I and II compared to seeded cultivars. The juice of these cultivars was rather high in pectin (1,14-2,83%), calcium (0,07-0,2%) and zinc (17,56-19,22 mg/kg). The juice of seedless cultivars and hybrid forms contained 16 amino acids, 7 indispensable ones among them. These interspecific cultivars and hybrid forms are not inferior to *Vitis vinifera* L. grapes with refer to the chemical composition of juice and as far as some other parameters are cocerned, they are even their superiors.

Currently, in breeding for seedlessness, special cocerns relate also to their resistance to low winter temperature, pests and diseases. It is well known that these characters are either absent from *Vitis vinifera* L. cultivars or expressed to a small extent. That is why new cultivars can be lended these characters only through the interspecific hybridization. Research in this direction is now being conducted by the All-Russia Potapenko Institute for Viticulture and Enology in cooperation with the Department of Viticulture of the Moscow Timiriazev Academy of Agriculture.

Breeding resistant seedless cultivars for north areas of commercial grape cultivation is conducted in two direction: the development of super-early and early-ripening cultivars with high sugar content to be used for the production of raisins, wine, juice and brandy on the one hand, and that of large-berried seedless cultivars destined for the fresh consumption and the production of raisins.

Euro-Amur and Euro-American seeded cultivars are used as female parents and donors of resistance to unfavorable environmental factors while seedless *Vitis vinifera* L. cultivars and those of interspecific origin enter as male parents.

In breeding wine seedless cultivars, we used SV 12-375 and Chambourcin as female parents. The total of 409 seedlings of six cross-combinations was studied.

Cultivars Korinka russkaya and Rusbol proved to be the best donors of seedlessness. When Korinka russkaya used as a male parent was crossed with cultivars Rusmol, Druzhba and Kodrianka, seedless and soft-seeded forms accounted for 36,4-27,5%. Crosscombinations with Rusbol cultivar yielded 14,8% of seedless seedlings belonging to the Categories of seedless I-III and 18,5% of soft-seeded forms.

The best combination of seedlessness and resistance to frost and mildew observed in the cross-combination Chambourcin x Kishmish unikalny: 15,1% of seedless seedlings, 82% of those with bud preservation of 51-100% and 40% of those with mildew resistance rated 1-2.

Hybrid seedlings of cross-combinations SV 12-375 x Korinka russkaya, SV 12-375 x 3-10-9-8-1 were distinguished for good frost resistance and yielded 92% and 93% of frost-resistant seedlings, respectively.

Hybridological and genetic analysis of seedlings obtained through the interspecific crossings of resistant cultivars with susceptible European cultivars and mildew-resistant interspecific hybrids reveals that resistance to mildew is a dominant character and is inherited as a simple one in monohybrid crossings, with a ratio 3:1. Chambourcin seems to be best donor of this quality.

When crossed with Kishmish unikalny (Amur-European seedless hybrid), Chambourcin yielded 40% of seedlings with good resistance to mildew (rated 1-2) and 55,3% of those with mildew resistance (rated 3). The population of Chambourcin x Rosovy biser (European cultivar) yielded 48,3% of hybrid forms with good resistance to leaf injuries (rated 1-2) and 46% of resistant forms (rated 3). A number of promising cross-combinations was also revealed and they were recommended for further crossings.

Genetic analysis yielded similar results with refer to the pattern of inheritance of resistance to oidium which is a dominant character and is inherited as mildew resistance with a ratio of 3:1. The following cross-combinations yielded the highest percentage of seedlings resistant to oidium (rated 1-2): SV 12-375 x Mechta (71,7%), SV 12-375 x Surpriz (57,7%), Chambourcin x Rosovy biser (48,3%), SV 12-375 x Korinka russkaya (36,6%) and SV 12-375 x hybrid 3-10-9-8-1 (32,4%).

The results of our investigations which were aimed to reveal the pattern of combining seedlessness and resistance to mildew and oidium enable us to conclude that these character are inherited by the hybrid progeny irrespective of each other. However, as several combinations prove it, they may be comined in one genotype. The cross-combination SV 12-375 x Korinka russkaya showed the best combination of seedlessness and complex resistance to the above fungal diseases. This cross-combination yielded 4,5% of seedless seedlings belonging to the Categories I-III, 18,6% of those with resistance top mildew rated 1-2, and 71,7% of those with good resistance to oidium.

The above-mentioned refers to the methodology of grape breeding for seedlessness as well as to theoretical and methodological principles of combining seedlessness and other valuable economic and biological properties in the hybrid progeny. This is meant to be of use for the development of new seedless cultivars with characteristics fixed in a breeding program. Currently, breeders all over the world display an increasing interest to seedless grape cultivars, and the seedless assortment has been considerably enlarged and improved. Due to great effort of breeders, the world's seedless assortment of grapes has been enriched with many valuable cultivars. Australia, for instance, possesses many new seedless cultivars, such as Marrow seedless, Nora, Blanche seedless, Septennial seedless, Red Globe, etc. A number of new valuable seedless cultivars have been released in the U.S.A.: Flame seedless, Fresno seedless, Fresno 32-68, etc.

Based on the data found in the literature, we attempted to generalizze practical results of breeding for seedlessness. With this intention, we collected brief characteristics of 65 seedless grape cultivars released recently. Of course, our information is not complete and we invite our foreign colleagues to join our efforts and to collect reference material in relation to this problem as it was suggested by the 73rd OIV General Assembly (San Francisco, 1993).

With refer to the practical results of our research, they are as follows:

1. Breeding for seedlessness through the intra- and interspecific hybridization

In Uzbekistan, large-berried seedless table and raisin grape cultivars were obtained through the intraspecific hybridization: Kishmish Hishrau, Kishmish Irtysnar, Kishmish Zarafshan, Kishmish Sogdiana, Kishmish samarkandski, etc. These cultivars are under the State trials in the countries of Middle Asia. Kishmish Hishrau was included in the Register of cultivars for commercial cultivation in Uzbekistan and Turkmenistan. In the Federative Republic of Russia, breeding through interspecific crossings is only at the initial stage of selection and study of hybrid seedlings and the practical results include only to the development of 11 elite grape forms, the obtention of promising cross-combinations and donors of seedlessness and resistance to pests, diseases and low winter temperatures. As a result of cooperation with Bulgarian breeders (the Institute for Vine and Wine in Pleven), a mid-ripening seedless cultivar, Rusbol, was developed through the interspecific hybridization (SV 12-375 x Sverhranni bessemianny) in 1990 and transmitted into the State trial system. This cultivar withstands frosts of -25 50 0C and its mildew resistance is rated 3. Basic vineyards of this cultivars account for 2 ha (five farms of the Rostov region). From cross-combinations SV 12-375 x Mechta, a promising seedling was selected (III-14-5-II) which was transferred to the State trial system of the Federative Republic of Russia under the name of Kishmish Novocherkasski.

2. Within the framework of the investigation and testing of cultivars, a collection of seedless grapes containing specimens of 50 cultivars was established.

This collection contains cultivars and elite hybrid forms whose valuable and economical characteristics allow to recommend them for the production of raisins in the area of nonprotected viticulture of the Lower pre-Don region (Rusbol, Hybrid 32, Korinka russkaya, Romulus) and in the area of protected viticulture (Kishmish luchisty, Belgradski bessemianny). Large-berried Bulgarian hybrids V-6 and VI-4 and cultivar Bessemianny Magaracha are recommended for fresh consumption. Cultivars Rusbol and Kishmish Novocherkasski can also be used for the production of white dry wines.

SESSION 4 : GENETIC TRANSFER, MARKER-AIDED SELECTION

PLANT BREEDER'S RIGHTS AND PATENT WITH RESPECT TO TRANSGENIC VARIETIES

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INTRODUCTION

Industrial property rights are very important for the purpose of refunding investments. They stimulate activities for the development of new progressive ideas, products and processes for the benefit of mankind. In case of plant varieties the grant of plant breeder's rights has proved to be such a stimulus for plant breeders. Plant breeder's rights are clearly separated from the common patent legislation in order to take into account the particular features of a plant variety and in order to balance the interests of the breeder on the one hand and the interests of the farmers and further consumers on the other hand.

In view of the new development in plant breeding especially in biotechnology (e.g. transgenic varieties) workable interfaces between plant breeder's rights and patent rights exist by overlapping.

The following comparison between patent and plant breeder's rights shall elucidate necessary to plant breeder's the differences between the two property rights

2 BASIC RULES FOR PROTECTION

2.1 Plant breeder's rights

The basic rules for pbr are formulated in the International Convention for the Protection of New Varieties of plants, the so-called UPOV convention, which has been revised in 1991. The 25 contracting states are about to prepare the adoption of the revision in their national variety protection legislation.

2.2 Patent

Patent Protection is available under the European Patent Convention of 1973, harmonizing the patent systems of the contracting states. Patent offices with a long tradition also exist in the United States of America, in Japan and elsewhere.

3 OBJECT OF PROTECTION

3.1 Plant breeder's rights

In principle pbr can be granted for plant varieties of all genera and species. This is already common practice in Germany with the consequence that plant varieties cannot be patented.

It does not make any difference to the grant of pbr whether a variety is the result of systematic breeding activities or whether it has been discovered, for instance the mutant of a vine variety with a different colour of the berries.

3.2 Patent

According to the European Patent Convention a patent can be granted among others for micro-organisms, microbiological or essentially technical processes and products thereof, genes and gene constructs. This means that transferable artificial genes expressing, for instance, fanleaf virus resistance in grapevine can be patented. European patents shall among others not be granted for discoveries, plant and animal varieties and essentially biological processes for the production of plants and animals. In this context it is worth mentioning that some contracting states of the UPOV Convention like the United States of America, Italy and Hungary grant pbr in the form of special plant patents. However, the term patent is somewhat misleading because these patents are granted according to the rules of the UPOV Convention regularizing pbr.

Plant varieties are excepted from patentability. Still patents have already been granted for plants and parts of plants characterised only by a transgene expressing resistance against Phosphinothricin ("Basta"-resistance).

4 SCOPE OF PROTECTION

4.1 Plant breeder's rights require the prior authorisation of the holder of the right before any propagating material of a protected variety can be produced, conditioned, offered, sold, exported, imported or stocked for one of these purposes. The scope of protection includes also varieties which cannot be clearly distinguished from the protected variety and varieties which are essentially derived from the protected variety

(e.g. a transgenic variety) unless the protected variety is itself a derived variety. It has to be emphasized that a clearly distinguishable derived variety can itself be protected as well.

With the revised convention the former farmers privilege allowing farmers to use their own harvest for propagating purposes free of charge has been restricted. In future farmers will be obliged to pay an adequate compensation to the holder of pot if they should use their own harvest. Moreover the Contracting parties can restrict farmer's privilege to certain species.

No authorisation of the holder of the right is required if the protected variety is used as initial material for the creation of a new variety (breeder's privilege) unless the new variety is essentially derived from the protected variety.

4.2 Patent

In contrast to pot patent claims can be freely formulated by the applicant for instance on an artificial resistance gene, its gene structure, vectores containing the gene and plants or parts thereof containing the gene. Still a claim will be exhausted by marketing the object of a patent .

5 CONDITIONS OF PROTECTION

5.1 Plant breeder's right

The grant of pbr requires the following criteria to be fulfilled:

5.1.1 Novelty

At the date of filing the application, commercialisation with the consent of the breeder must not date back more than one year in the State where the application was filed and in the case of vine varieties not more than six years in any other State.

5.1.2 Distinctness

The variety has to be clearly distinguishable from any other variety whose existence is a matter of common knowledge.

5.1.3 Uniformity

The variety has to be sufficiently uniform in its relevant characteristics taking into account the particular features of its propagation.

5.1.4 Stability

The variety must be stable in its relevant characteristics after repeated propagation.

5.1.5 variety denomination

The variety needs a denomination which enables a clear identification.

5.2 Patent

Patent protection for disclosed inventions is available if the following criteria are fulfilled

5.2.1 Novelty

The invention must not be state of the art and must not be madeavailable to the public by no means before the date of filing the application.

5.2.2 Industrial application

It must be possible to make use of the invention in some kind of industry .

5.2.5 Inventive step (non-obviousness)

The invention must not be obvious to a person skilled in the art.

5.2.4 Repeatability

The invention must be disclosed in such a way that a person skilled in the art is able to repeat the procedure.

5.3 Comparison of pbr and patent

Plant varieties would hardly be able to fulfill all criteria for patent protection:

Novelty in the sense of "not being state of the art" and repeatability are not applicable to plant varieties because the factor chance will influence the composition of a genome to a very high extent. Inventive step, rsp. non-obviousness, would concern the value of a variety, but the value is very much depending on local conditions. So a general rule cannot be given. Moreover mutations would be excluded from patentability if they were not inventions but discoveries. Industrial application can be expected to be self-evident for plant varieties.

6 TRANSGENIC VARIETIES

Transgenic varieties ace the result of new developments in the field of biotechnology. Still no application for a breeder's right for a transgenic variety has been filed in Germany up to now. One of the reasons might be that we are still lacking clear rules for the protection of biotechnological inventions within the patent system. However the European Union is about to elaborate such rules. The current draft is taking into account the necessary separation of pbr and patent as far as plant varieties are concerned. This is necessary in order to avoid conflicts between the two property right systems.

Therefore in the case of transgenic varieties pbr and patent have to be considered simultaneously:

6.1 Plant breeder's rights and patent

In principle pbr can also be granted for transgenic varieties even if the transgene or the process for isolating, modifying or transferring the gene should be patented. Consequently propagating material of such a transgenic variety can be marketed without consent of the holder of the patent. However, royalties for the use of the patent will depend on an agreement between breeder and holder of the patent.

6.2 Examination of transgenic varieties

Before a transgenic variety can be released the relevant security aspects have to be considered. Therefore the applicant will have to submit a release permit together with the application form. The applicant will have to declare that his variety has been developed by means of genetic engineering and he will have to inform about the initial variety, the transgene and its expression, and about the method by which the transgenic variety can be characterized.

With this information important reference varieties can be chosen and methodical aspects can be considered in order to include the transgene and its expression in the official variety tests. In case the transgene or its expression should be used as an additional characteristic for the description of a variety, it has to be uniform and stable like all other characteristics which are used: Depending on the way of transferring a gene, for instance by a particle gun, a lack of homozygosity might occur combined with interactions between initial genome and transgene or marker or regulatory ANA sequences like promoter sequences.

The result could be segregation influencing uniformity and consequently also stability. In inbred lines of maize for example selfing or backcrossing could be a solution to stabilize the genome. Vegetatively propagated species like vine seem to be less problematic.

6.3 Essentially derived transgenic varieties

Distinction of a variety might be based only on a transgene or its expression, e.g. fanleaf resistance transferred to the variety Keener or White Riesling. To the grant of pbr it is irrelevant whether the initial variety is protected or not. However, according to the dependency right system laid down in the new UPON Convention of 1991, the scope of protection of a non-derived initial variety will also extend to a transgenic variety which is essentially derived from the initial variety even if the transgenic variety should be protected itself. Consequently royalties have to be paid to the owner of the initial variety if the transgenic variety should be commercialised. It is important to mention that controversies concerning dependency rights will be decided by the court and not by testing authorities. Still no comprehensive rules for dependency are available as long as no precedents have been set.

6.4 Breeder's privilege

In principle breeder's privilege is also valid in the case of a protected transgenic variety carrying a patented transgene. However, it is still discussed if the patent claim for the transgene will be exhausted after its first release or if the holder of the patent can prohibit the use of breeder's privilege as far as the patented transgene is concerned. Besides it is discussed if a compulsory license system would work in case the holder of the patent is not willing to permit the use.

6.5 Farmer's privilege

In the future part system of the European Union farmer's privilege to use his own harvest as seed will be combined with the payment of an adequate compensation to the holder of pbr. If this variety should carry a patented transgene the holder of the patent would participate in the royalties.

7 FINAL REMARKS

According to the conception of the European Union patent protection for a gene covers all generations of plants and parts thereof containing this gene with the exception of consumer material produced thereof. It remains to be seen whether plants and parts thereof can always be clearly separated from plant varieties.

INTEGRATION OF MOLECULAR TECHNIQUES AND CONVENTIONAL BREEDING

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Introduction

In Australia the production of grapevines covers a wide range of latitudes from the early ripening table grape regions in the tropics (Possingham et. al. 1990) to the cool climate wine grape regions in Tasmania. However the main areas of production are in the hot irrigated districts of the Murray Valley, Darling Basin which produce almost all the table and drying grapes and about 80% of the winegrapes.

Since the mid 1960's the CSIRO Division of Horticulture has maintained a vine breeding program which aims to develop new wine, drying, table and rootstock selections for the Australian environment, industry and consumer needs. A range of selections from conventional crosses between *Vitis vinifera* or disease resistant species or species hybrids have been produced. Most recently CSIRO has begun using new techniques of molecular biology designed to integrate into and increase the power of our genetic improvement program based on conventional breeding and selection.

Varietal Identification

Conventional breeding has been complimented by the introduction, development and evaluation of a major germplasm collection of over one thousand varieties, which include a range of wine, raisin, table, juice and rootstock varieties, as well as inter-specific hybrids and *Vitis* species. The first of the molecular techniques to be completed and utilised has been a DNA typing program for grapevines (Thomas and Scoff, 1993, Thomas et. al. 1994). This program was designed to identify grapevine varieties by measuring characters of the DNA of a grapevine in an objective way in a laboratory test to supplement the somewhat subjective techniques of ampelography which also require a high level of skill and training.

The ability to characterise a genotype by DNA typing is independent of the type of material available, e.g. leaf, root or berries and of the environment in which the material was grown. DNA is extracted from grapevine tissue and a number of genetic loci are examined and characterised. The genetic loci chosen for this purpose are microsatellite DNA loci and the size or length of any one microsatellite allele at one locus will vary from variety to variety. Thus the result of an individual DNA measurement at a single genetic locus can be seen as an electrophoretic band pattern where each of the two bands represent one of the alleles from the individual parents of the original cross. These alleles can be scored directly for length by numerical measurement, or by giving them an identifying letter.

Each microsatellite locus chosen for grapevine identification has several alleles (5 to 14) consequently the loci are extremely informative for either genetic marking or for DNA typing and the inheritance of the alleles follows normal Mendelian patterns. Since the length of DNA in each allele is measured directly the analysis can be easily semi-automated using fluorescent dyes to identify the alleles. The results are then directly scored, analysed by computer and compared with or entered into an electronic database.

It is also planned to include ampelographic descriptors in this database so that both technologies can be used for grapevine identification. In addition, worldwide access for the database is planned and negotiations are under way to establish a linkage around the world such that major laboratories, both research and industrial, do not need to set up separate analytical procedures and databases. The technology offers a worldwide standard of identification of varieties and has already been found to give identical and reproducible results in laboratories in different parts of the world. This allows, for the first time, direct and objective comparisons of grapevine identifications made in different countries without the almost impossible task of having to reconcile ampelographic descriptors in different environments.

With over 100 grapevine varieties including scions, table grapes, wine grapes, rootstocks and related *Vitis* species entered in the database so far, the DNA typing technology has shown that all major grapevine varieties

ate genetically distinct and have arisen as a result of individual zygotic events. Thus they can be viewed as formal varieties in an agricultural sense.

On the other hand the technology has not been able to show any differences between clones of Pinot including Noir, Gris and Blanc, nor clones of Sultana and Cabernet Sauvignon (Thomas et. al. 1993). It seems clear that these clones are the result of somatic mutations, having been infected by systemic diseases, such as grapevine leafroll virus (Thomas et. al.), or, in some cases, by the existence of a chimera, e.g. Pinot Meunier (Skene and Barlass, 1983). Because of the uncertainty of the mechanisms and origins of these types of clonal variation it is unlikely that current nucleic acid technology will provide a method for grapevine clonal identification. Before this can be achieved the origins of clonal variation will have to be defined.

Breeding

Winegrape breeding in Australia (Clingeffer 1984) is focusing on the selection of suitable varieties for the hot irrigated districts. The aim is to produce high yielding, late ripening selections which compared with traditional, poorly adapted European varieties, have a better sugar/acid balance, organic acid composition, flavour and, in the case of red types, better colour. Evaluation is based on yield and juice characteristics supported by small scale winemaking, sensory evaluation of wines, measurement of their organic acid composition and, in the case of red wine spectral analysis. Successful CSIRO winegrape introductions at Tarrango and Taminga. Tarrango (Antcliff 1975) is a cross of the Portuguese variety Touriga and Sultana. It is a black, high yielding, late ripening selection with a low pH (3.2-3.4), a good sugar acid balance (a titratable acidity of 7.0 g per L at 20 °Brix) and an organic acid composition characterised by a high tartrate/malate ratio (Kerridge et. al. 1987). The wine has an attractive, stable bright colour associated with its low pH, somewhat similar to the French Beaujolais style. Taminga (Antcliff 1982) was selected from a cross between an unnamed Merbein selection (Farana x Sultana) and Traminer. Compared with Traminer it yielded higher, ripened three to four weeks later, had a better must composition, with a lower pH and a good sugar acid balance, and developed wines with more character (Kerridge et. al. 1987b). Currently a range of red wine selections are being evaluated in both the hot and cooler districts for heavier wine styles.

Emphasis in breeding raisin and tablegrape types (Clingeffer, 1985) has been placed on development of improved seedless varieties. To achieve this goal in-ovule embryo culture from seedless x seedless genotypes has been added to the breeding program (Barlass et al. 1988). Diversity has been increased through the importation of pollen from seedless types not available in Australia. The most successful new raisin variety is the small berried, black seedless selection Carina, a cross between Shiraz and Sultana (Antcliff 1975). It is both more productive and more tolerant of wet humid conditions than the traditional Zante currant and accounts for about half of the currant production in Australia. Merbein Seedless, a Farana x Sultana cross was released in 1981 (Antcliff 1981) as a possible replacement for Sultana. It is being adopted by some growers because of its high productivity, early ripening and adaptability to trellis drying and total mechanisation. Some currently unnamed releases for raisin production include a small berried Sultana type and a large berried, seedless muscat selection.

New tablegrape selections include the very early ripening white, Fresno Seedless jointly released by CSIRO and the USDA (Clingeffer and Ramming 1990) and the CSIRO variety, Marroo Seedless (Clingeffer and Possingham 1988). Attributes of Marroo Seedless include a large, black, crisp and seedless berry with a pleasant sweet taste, a uniform bunch shape, good vigour, high yield and resistance to downy mildew. It is now being grown in most of the major tablegrape producing countries. Marroo Seedless is a cross of Carolina Blackrose and Ruby Seedless. Thus it is a complex hybrid with a pedigree that encompasses 100 years of grape breeding. The downy mildew resistance trait can be attributed to its grandparent, Villard Blanc, a complex 8 species French hybrid. Like its male parent Ruby Seedless, it is however very susceptible to powdery mildew.

Molecular techniques designed to integrate into the breeding program include the identification of promoters and genes that control factors of berry quality including colour, flavour, sugar accumulation, and ripeness characteristics and factors that affect grapevine cultivation including disease resistance. Experiments to that end are now well under way. For example studies on the browning of sultanas has shown that the enzyme polyphenoloxidase plays a significant role in colour development of dried grape (Rathjen and Robinson, 1992) and indeed of many other vegetables. The gene that controls synthesis of the enzyme has been isolated from grapevine and when a suitable transformation system becomes available, we plan to down-regulate its activity. This is particularly important in Sultana, where premium price is paid for light, golden fruit which is the result of drying grapes in the presence of low polyphenoloxidase activity.

Disease Resistance

As mentioned above a major aim of the program is to develop disease resistant varieties which will facilitate a reduction in chemical use. The fungal diseases of interest are anthracnose, powdery mildew, downy mildew and Botrytis bunchrots which all have their origins in the American continent. While there are varying degrees of susceptibility between various *V. vinifera* varieties there appears to be little prospect to breed disease

resistant varieties without broadening the genetic base. - Consequently a diverse range of disease resistant genotypes other than *Vitis vinifera* are used in the CSIRO breeding program (Possingham et. al. 1990). In some cases, crosses over a number of generations have produced very complex and involved breeding lines. Genotypes used include *V. rotundifolia* (DRX55), *V. labrusca* hybrids, other species such as *V. cinerea*, *V. caribea*, *V. longi* and *V. aestivalis* and complex species hybrids such as Chambourcin, Muscat St. Vallier, Villard Blanc, Aurelia, Carolina Blackcrose, Lady Patricia, Illinois 271-1, S14664, SV12-309 and SV12-303. Prior to planting in the field the seedling progeny from disease resistant crosses are left unsprayed in the glasshouse and screened for resistance. This technique appears to be suitable for identifying selections resistant to powdery mildew. Susceptible types are eliminated. For screening resistance to downy mildew, in vitro dual culture techniques have proved useful (Barlass et. al. 1986).

Molecular techniques are showing potential to provide new methods for the selection of disease resistant types based on the understanding of the molecular mechanisms involved. For example the cell walls of powdery and downy mildew fungi contain chitin's and β -glucans. It has been suggested that the levels of endogenous chitinases and glucanases in plant tissues is correlated with the degree of resistance against infection by fungi such as powdery mildew. Consequently in the presence of the enzyme fungal growth is being inhibited by digestion of fungal cell wall material. Such a correlation has been found for grapevines ranked according to their degree of resistance to downy and powdery mildew and their endogenous activities of chitinase and β -glucanase (Giannakis, Skene and Scoff, unpublished results). This difference in susceptibilities of grapevines to infection is illustrated by comparing the sensitive grapevine variety Sultana with the resistant complex species hybrid, Seyval. Forty eight hours after inoculation of leaves of both varieties with fungal spores, the rate of spore germination was very similar. However, eight days after inoculation Sultana leaves were totally overgrown with powdery mildew hyphae while Seyval leaves showed no obvious signs of infection except under a microscope.

The chitinase and glucanase enzymes were purified to homogeneity from Seyval and Sultana and tested in a bioassay which measured the ability of the enzymes to digest the tips of hyphae from germinating powdery mildew spores. Extracts of these enzymes from Sultana were far less effective than those from Seyval. In the case of the purified enzymes the combination of chitinase and glucanase together was extremely effective in causing lysis of the fungal cell tip.

The current research program is also focussing on identifying genes that control these enzymes and whether there are methods to influence their activity and thus improve the disease resistance of existing varieties. In the longer term these genes will be isolated with a view to regulating their activity in transgenic grapevines. Similar studies are also focussed on genes directly concerned with colour, sugar and flavour development in grapevine berries.

Conclusion

In summary conventional genetic breeding has the power to dramatically improve the quality of drying, table and wine grapes but there will always be certain characters that are difficult or impossible to access in this way. Modern techniques of genetic manipulation add a new tool to the plant breeder's collection to achieve improved grapevine varieties.

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At this stage, the table grape variety "Russky ranny", "Vostorg", "Agat donskoy" were obtained, which endure frosts up to -24, -26 C, as well as wine grape varieties "Kazachka", "Sarmat", "Askay", "Skif", with the frost resistance up to -27, -29 So 0C.

4) The breeding of varieties with group resistance to frost, mildew and phylloxera. Due to the appearance of phylloxera in the main viticultural areas of Russia, the problem of breeding phylloxera resistant varieties has arisen. As *V.amurensis* and its hybrids are not resistant to phylloxera, in the last hybridization stage Franco-American hybrids were involved (1972 - 1993).

Complex hybrids, combining genomes of *V.vinifera*, *V.amurensis* and of American species show high resistance to frost, mildew and phylloxera; some of them can be used in own-rooted culture.

In collaboration with the Research Institute of grape and wine in Pleven (Bulgaria)

- grape varieties "Druzhba", "Slava", "Rusbol", "Muskat aksaysky", "Razdorsky bely" were obtained; together with the Institute of grape and wine in Kechkemet (Hungary)

- "Rusven" variety; with the Institute of grape and wine in Moldova - "Rusmol" and "Grushevsky bely".

Euro-Amur varieties, created in Russia, are the most adapted to the short vegetation period in the northern viticultural areas and possess a number of significant advantages, as compared with the standart *V.vinifera* varieties: 1) higher frost resistance, enabling the cultivation without protective covering; 2) high regeneration ability of over - and under - ground organs, which allows recovery of high productivity level the next year after a severe frosty winter (e.g. in 1968/69, 1971/72, the same we expect for 1993/94); 3) higher cold tolerance, that is the ability to produce a standard grape yield during the years with the heat supply much below the average; 4) higher sugar accumulation ability, enabling the production of high quality sweet wines.

Howere, the lack of phylloxera resistance and insufficient fungus resistance of these hybrids have made us use Franco-American hybrids in the hybridization process.

In the month of may 1994 a grape selection centre was founded of the Potapenko Research Institute with the aim of coordination of grape selection work.

Analysis of the practical results of the selection showed, that the greatest number of resistant varieties, submitted for the State variety trial, are table and white wine varieties. Percentage of red wine, seedless and rootstock varieties is very poor. The frost resistance level of -25, -27 So 0C has been achieved, the situation concerning the mildew resistance is more or less normal (though the resistance of the majority of the varieties is 3 - 3.5 by Gusfeld), there are very few varieties tolerant to phylloxera and suitable for own-rooted culture, and there are practically no oidium resistant varieties ("Pifos", "Slava" and "Blanka" are relatively resistant).

At the selection centre a number of priority research programs were elaborated:

- breeding of resistant red wine varieties - analogues for "Cabernet", "Saperavi", "Tsymliansky cherny", "Krasnostop Zolotovskiy", suitable, first of all, for sparkling and vintage wine production;

- the creation of Muscat varieties with high sugar content, suitable for sparkling and sweet wine production (Muscat program);

- breeding of large berry varieties (a berry weight 20 g and more), of coloured (mostly red) varieties with unusual berry shape.

In conclusion I would like to say a few words about the problem of the common worry - the quality of products, obtained from the resistant grapes.

As to the table and seedless varieties, consumed fresh or dried, there are no problems and no discussions. Anxiety arises in connection with the wine grapes and the corresponding wines. Discussions are evoked by the problems of harmfulness of Euro-American hybrids (and Euro-Amur ones as well), containing 3 - 5 diglucosid malvidin.

In our work we pay due attention to this aspect. In collaboration with Rostov medical Institute, Don Agricultural Institute, the comparative study of the effects of consumption of wines and juices, produced from the grape of various species (including Amur species), was carried out on white rats and hens.

The results (I.N.Martynov) showed no significant differences between wines and juices of European, Euro-American and Euro-Amur grapes as to their influence on the animal organism.

The promising prospects of selection of the resistant grapes are supported by the fact of their suitability for the production of wines of any type, competitive to *V.vinifera* varieties.

A new variety presupposes a new cultivation technology, unusual production. The success in breeding of new resistant varieties should not be opposed to classical varieties, they are to be combined rationally in the assortment of every particular viticultural area.

TRANSFORMATION GENETIQUE D'EMBRYONS SOMATIQUES DE VIGNE PAR *AGROBACTERIUM TUMIFACIENS* ET REGENERATION DE PLANTS TRANSGENIQUES PRODUISANT LA PROTEINE CAPSIDIALE DU VIRUS DE LA MOSAÏQUE CHROMÉE DE LA VIGNE (GCMV)

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RESUME

Des plants transgeniques de la variete pone-greffe 110 Richter ont ete regenes a partir de cals embryogenes co-cultives avec la souche *Agrobacterium tumefaciens* LBA 4404 pKVHG 2+, hebergeant un vecteur binaire portant les genes codant pour la resistance a l'hygromycine (HPT), la resistance a la kanamycine (NPT II), la S-glucuronidase (GUS) et la proteine capsidiale du virus de la mosaïque chromée de la vigne (CP-GCMV). Le taux de transformation le plus eleve a ete obtenu en selectionnant les cals sur un milieu additionne de 16 µg/ml d'hygromycine. Une reaction GUS positive est observee sur les embryons somatiques issus des cals selectionnes ainsi que sur les plantes regenees. In vitro, celles-ci manifestent une forte resistance aux deux antibiotiques en microbouturage et micropropagation axillaire. Des taux eleves de production de la CP-GCMV ont ete detectes par tests ELISA et par *Western Blot* dans les embryons somatiques transformes ainsi que dans les feuilles et les racines des plantes regenees. La presence dans les plantes transformees des genes portes par le plasmide pKVHG2+ a ete anise en evidence apres amplification de leur ADN par PCR (*Polymerase Chain Reaction*). Lente integration effective dans le genome vegetal a ete confirmee par *Southern Blot*.

INTRODUCTION

Les nepovirus sent un groupe important de virus phytopathogenes caracterises par leurs particules polyedriques et leurs aptitudes a etre transmis par des trematodes du sol appartenant aux genres *Xiphinema* et *Longidorus*. Certains d'entre eux ont un impact economique important sur la vigne, en particulier le court-noue (GFLV) et la mosaïque de L'Arabette (ArMV). Le virus de la mosaïque chromée de la vigne (GCMV) a une distribution limitee a quelques pays d'Europe centrale et orientale. Cependant, dans les regions ou il est endemique, il peut causer de serieux dommages sur vigne en induisant des symptomes tres similaires a ceux du court-noue (Martelli et Quacquarelli, 1965). D'autre part, le virus des anneaux noirs de la tomate (TBRV), autre nepovirus pathogene pour la vigne, identifie en Europe septentrionale, est etroitement apparente au GCMV (Dodd et Robinson, 1984). Cette parente se traduit en particulier par des phenomenes de protection croisee mis en evidence sur *Chenopodium quinoa* (Bretout, 1987). Le genome du GCMV a ete clone et completement sequence (Le Gall et al., 1988; Brault et al., 1989). Un gene hybride, dont l'expression conduit a une production elevee de la proteine capsidiale du virus (CP-GCMV) a ete construit et transfere dans le genome de plants de tabac (Brault, 1990). Les plants de tabac transgeniques produisant la CP-GCMV presentent un niveau significatif de resistance au virus (Brault et al., 1993). Depuis l'article original decrivant la resistance au virus de la mosaïque du tabac (TMV) de plants de tabac transgeniques produisant la CP-TMV (Powell-Abel et al., 1986), de nombreux resultats similaires ont ete obtenus chez les plantes cultivees. La protection de plants de tabac transgeniques contre le GFLV et l'ArMV a egalement ete observee (Bardonnnet et al., 1994; Bertioli et al., 1992).

La vigne s'est jusqu'a une date recente montree recalcitrante a la transformation genetique. Mullins et al. (1990) ont ete les premiers a obtenir des plantes transgeniques exprimant le gene de la B-glucuronidase apres co-culture d'embryons somatiques de la variete *Rupestris* du Lot avec une souche d'*Agrobacterium tumefaciens* recombinante. Plus recemment, un certain nombre de resultats positifs ont fait l'objet de communications ou de publications (Krastanova et al., 1993; Mauro, 1993; Nakano et al., 1994; Pert et al., 1994). Dans cette communication, nous presentons l'obtention de plants transgeniques de la variete porte-greffe 110 Richter exprimant les genes de resistance a l'hygromycine (HPT) et a la kanamycine (NPTII), le gene GUS et le gene de la CP-GCMV.

MATERIAL ET METHODES

Les cats embryogenes de 110 Richter (*Vitis Berlandieri* x *V. rupestris*) ont été obtenus à partir d'anthers mises en culture sur ce milieu MS 1/2 additionné de NOA (5µM) et de BAP (1µM). Ils ont été entretenus sur ce milieu appelé NB, à l'obscurité et à 28°C, pendant plus d'un an, de manière à être parfaitement stabilisés. Quinze jours avant la co-culture, ils ont été transférés du milieu NB sur un milieu sans hormones afin d'induire la formation d'embryons somatiques.

Le vecteur binaire pKVHG2+, construit et décrit par Brault (1990) a été introduit dans *Agrobacterium tumefaciens* souche LBA4404 par électroporation. Il porte le gène NPT II sous promoteur nopaline synthase (NOS), ainsi que les gènes CP-GCMV, HPT et GUS sous promoteur CaMV 35S (Fig.1). Les bactéries sont cultivées à l'obscurité (28°C) sur milieu YES semi-solide additionné de 50 µg/ml de rifampicine, 200 µg/ml de neomycine et 500 µg/ml de streptomycine.

Pour les besoins de la co-culture, les colonies bactériennes, obtenues à partir de cultures fraîchement repiquées (24 heures), ont été mises en suspension dans un milieu MS 1/2 liquide (pH 5.2) additionné de 50 µM d'acetosyringone, et diluées jusqu'à une densité optique (630 nm) de 1, correspondant approximativement à 10x9 bactéries par ml. Les cats embryogenes (10 g de matière fraîche) ont été immergés dans 50 ml de suspension bactérienne pendant 30 minutes après un passage sous vide (700 mm Hg) de 5 minutes. Après inoculation, les cats ont été transférés dans des boîtes de Petri de 90 mm contenant du milieu MS 1/2 semi-solide (pH 5.2) recouvert de papier filtre stérile. La co-culture s'est poursuivie pendant 48 heures à l'obscurité (28°C). À la fin de celle-ci, les cats ont été rincés avec du milieu EMS 1/2 liquide (pH 6.0), soigneusement séchés sur papier buvard stérile, et fragmentés en explants d'environ 20 mg. Ceux-ci ont été transférés dans des boîtes de Petri de 55 mm sur un milieu MS 1/2 sans hormones, additionné de 400 µg/ml de cefotaxime, et de différentes concentrations d'hygromycine (0,8,12,16,20 µg/ml).

RESULTATS

I. Sélection d'embryons somatiques et de cats embryogenes résistant à l'hygromycine Six semaines après la co-culture, des embryons somatiques différenciés ont été observés sur de nombreux explants : 63, 8 et 1 embryons ont été isolés à partir de 29, 7 et 1 explants cultivés sur les milieux contenant respectivement 12, 16 et 20 µg/ml d'hygromycine (cf. Tableau I). Sur les 300 explants cultivés sur ces milieux, 30 ont été perdus par des pollutions et 227 étaient totalement nécrosés dès la 3^e semaine de culture. Comparativement, sur les mêmes milieux sélectifs, le nombre d'embryons somatiques développés sur 60 explants témoins non co-cultivés (55 explants totalement nécrosés) était respectivement de 5,1 et 0. Sur le milieu contenant 8 µg/ml d'hygromycine, le nombre (240) d'embryons somatiques observés sur 80 explants co-cultivés (20 explants perdus par pollutions) n'était pas significativement différent du nombre (51) d'embryons somatiques observés sur les 20 explants utilisés comme témoins. Une concentration de 8 µg/ml d'hygromycine ne semble donc pas suffisante pour inhiber la croissance et le développement d'embryons non transformés.

20 des 63 embryons somatiques isolés à partir des explants cultivés sur 12 µg/ml d'hygromycine ont été testés pour l'activité GUS, selon la technique histochimique proposée par Jefferson (1987) et légèrement modifiée. 80 % d'entre eux ont montré une coloration bleue sous forme de petites taches ou de larges secteurs, généralement localisés sur les hypocotyles et les cotylédons. Les 43 embryons somatiques qui n'ont pas subi le test GUS, ainsi que les 9 embryons somatiques isolés à partir des explants cultivés sur 16 et 20 µg/ml d'hygromycine ont été mis à germer : 11 et 4 plantes ont pu être ainsi régénérées. Aucune d'entre elles n'a manifesté d'activité GUS dans leurs jeunes feuilles. Toutes les tentatives pour induire une embryogenèse somatique secondaire à partir des embryons somatiques qui n'avaient pas germé ont été vaines. La survie de ces embryons somatiques sur des milieux hautement sélectifs est difficilement explicable. Elle pourrait être due à une detoxification partielle de l'antibiotique par de larges secteurs de cellules épidermiques transformées au sein de structures chimeriques. De toute évidence, les cellules méristématiques de ces embryons somatiques, probablement trop différenciés au moment de la co-culture, n'ont pas été transformés.

Trois mois après la co-culture, la présence de petits cats embryogenes a pu être observée au sein des tissus nécrosés de certains explants cultivés sur 12, 16 et 20 µg/ml d'hygromycine. Au cours des jours suivants, beaucoup de ces cats ont brunis et cessé toute croissance, du fait de la présence probable de substances toxiques sécrétées par les tissus nécrosés environnants.

Neanmoins, 39 petits cats embryogenes survivants ont pu être isolés et transférés sur un milieu NB contenant 100 µg/ml de cefotaxime, mais dépourvu d'hygromycine afin de stimuler leur croissance. Après un mois, 24 des 39 explants transférés avaient repris leur croissance et produit des cats bien développés. Ceux-ci ont été fragmentés et transférés à nouveau sur un milieu NB sélectif contenant 16 µg/ml d'hygromycine, mais dépourvu de cefotaxime. 6 des 9 cats isolés à partir des explants cultivés sur 16 et 20 µg/ml d'hygromycine se sont révélés uniformément résistants à l'hygromycine. Dans le cas des explants cultivés sur 12 µg/ml d'hygromycine, la proportion de cats résistants n'a été que de 3 sur 15.

Sur chacun des 9 cals résistants à l'hygromycine, des fragments de tissus embryogènes ont été prélevés et soumis au test GUS. Tous ont montré une réaction positive homogène. Aucun développement bactérien n'a été observé sur des fragments de ces cals, placés sur un milieu YES dépourvu d'antibiotiques et cultivés à 28 °C pendant une semaine, indiquant que la coloration GUS n'était pas due à la présence de bactéries résiduelles dans les tissus potentiellement transformés. De plus, un niveau élevé de production de la protéine capsidiale du virus a été observé au cours des tests effectués sur ces cals en utilisant la technique DAS-ELISA classique (Clark et Adams, 1977) et un sérum polyclonal anti-GCMV obtenu à partir de particules purifiées du virus. Enfin, le transfert des cals sur un milieu contenant 16 µg/ml de kanamycine, a montré que ceux-ci étaient également résistants à cet antibiotique.

2. Régénération et analyse des plantes transgéniques

Les cals embryogènes transformés ont été transférés sur un milieu MS I/2 dépourvu de substances de croissance et d'antibiotiques. En quelques semaines, 5 des neuf cals ont produit de nombreux embryons bien développés. Après un passage temporaire de 7 jours sur un milieu contenant 2 µM/ml de BAP, ceux-ci ont été capables de germer et de donner des plantules racinées avec des pourcentages variant de 10 à 40% selon les clones cellulaires. Les 4 autres clones cellulaires n'ont pas pu être régénérés et ont été perdus.

Les plantules cultivées *in vitro* ont été testées pour l'activité GUS (méthode histochimique et mesure quantitative par fluorométrie), pour la production de la Co-GCMV, ainsi que pour la résistance à l'hygromycine et à la kanamycine, en microbouturage sur un milieu sans substances de croissance et en micropropagation axillaire sur un milieu contenant 4 µM/ml de BAP. Dans tous les cas, les plantules testées ont manifesté les caractères attendus.

Pour les tests ELISA, 5 plantes *in vitro* ont été utilisées pour chaque clone (Tableau III). Des différences ont été observées entre clones. Les densités optiques moyennes mesurées à 405 nm variaient de 0.3 à 1.2 chez les clones transgéniques et étaient toujours inférieures à 0.15 chez le clone témoin 110 R non transformé. Le témoin positif était constitué d'une lignée de tabac transgénique (2.T1) présentant un très haut niveau de production de la protéine capsidiale du GCMV. La DO moyenne observée à 405 nm (1.8) était comparable à celle observée sur un plant de vigne fortement infecté par le GCMV.

La résistance aux antibiotiques révélée chez les plantules transgéniques en micropropagation axillaire est extrêmement marquée (Tableau II). Sur les plantules témoins, le développement des bourgeons axillaires est fortement inhibé à partir de 0.5 µg/ml d'hygromycine et 10 µg/ml de kanamycine. Les plantules transgéniques se multiplient sans difficultés sur des milieux contenant 10 µg/ml d'hygromycine ou 50 µg/ml de kanamycine. Cette résistance aux antibiotiques se manifeste également fortement sur les plantules microbouturées.

Après acclimatation en serre, les plantes transgéniques présentent un aspect, une croissance et un développement tout à fait comparables à ceux de plantes témoins non transformées. Les tests GUS histochimiques et fluorométriques (Fig.2) ont confirmé l'expression du gène dans tous les organes de la plante (tiges, pétioles, limbes et racines). Plusieurs séries de tests ELISA ont été pratiquées à différentes époques sur des plantes en croissance active et ont montré que les plantes transgéniques produisaient la CP-GCMV dans les jeunes feuilles, les feuilles adultes et les racines. En particulier, ces tests ont été réalisés sur trois clones, 15 mois après leur acclimatation en serre, avec 4 plantes par clone (Tableau III). Les résultats obtenus sur les plantes en serre ne semblent pas en accord avec ceux obtenus sur les plantes *in vitro*, puisque le clone 2 qui réagissait le plus faiblement dans ces conditions, est celui qui produit en serre la plus grande quantité de protéine capsidiale à son niveau le plus élevé quel que soit le type d'organe testé.

L'expression correcte du gène de la CP-GCMV, mise en évidence par les tests ELISA sur cals, embryons somatiques, plantules *in vitro* et plantes en serre, a été vérifiée par *western blot* (Fig 3) sur des embryons somatiques de deux clones, selon la technique décrite par Brault et al (1993)

Lorsque des extraits de feuilles de vitroplants sont analysés, la présence de quantités élevées d'une protéine végétale (Rubisco ?) dans la même région du gel d'électrophorèse, interfère avec la détection de la protéine capsidiale. Le *Western blot* a donc été effectué sur des tissus embryonnaires dépourvus de cette protéine. Comme témoin positif, 50 ng de particules virales purifiées ont été ajoutées à un extrait protéique correspondant à 20 mg de tissus d'embryons somatiques non transformés. Un extrait similaire a été utilisé comme témoin négatif. La concentration en protéine capsidiale est plus élevée chez le clone 5 que chez le clone 3, ce qui semble en accord avec les résultats des tests ELISA sur plantules *in vitro*. La concentration en protéine capsidiale observée dans les embryons somatiques du clone 5 est comparable à celle de l'extrait témoin positif. Un extrait de la lignée de tabac transgénique 2.T1 a été testé en parallèle. Étant donné que cette lignée est celle qui exprime le plus fortement le gène de la CP-GCMV, et qu'à la différence des plants de vigne transformés, elle est homozygote pour le transgène, les résultats du *Western Blot* semblent indiquer que le niveau de production de la CP-GCMV est du même ordre dans les tissus embryonnaires de vignes transformées et dans les tissus foliaires de tabacs transformés.

La presence des genes du plasmide pKVHG2+ dans les plantes transformees a ete demontree apres amplification de leur ADN par la technique PCR (*polimerase chain reaction*).

Cette technique a ete appliquee aux genes GUS, NPT II et Co-GCMV, pour lesquels des amorces nucleotidiques specifiques etaient disponibles. Le gene HPT' etant situe sur le plasmide entre le gene GUS et le gene CP-GCMV, il est tres probable qu'il a ete transfere conjointement dans les plantes.

L'integration effective des transgenes dans l'ADN genomique des plantes a ete confirme par *Southern blot* (fig 3) Une sonde cDNA, derivee de sequences aleatoires du plasmide pKVH 2+, a ete hybridée avec l'ADN extrait des plantes transgeniques et digere par l'enzyme de restriction Hind III. Cette enzyme excise la cassette d'expression du gene de la CP-GCMV ses deux extremités et laisse deux fragments de bordure, constitue chacun de l'une des deux sequences complementaires du T-DNA plasmidique et d'un fragment de l'ADN genomique vegetal. La taille de ces deux fragments de bordure est fonction de la distance entre l'extremite du T-DNA et le plus proche site Hind III sur l'ADN des plantes transformees. Elle depend donc de la position de l'insertion des genes transfères dans cet ADN. L'utilisation de l'enzyme Hind III et d'une sonde representative de tout le T-DNA du plasmide, permet ainsi de visualiser sur l'electrophoregramme la cassette d'expression du gene CP-GCMV qui est presente dans les 5 clones transgeniques analyses ainsi que dans la lignee de tabac transgenique 2.TI, et est absente chez le clone 110 R temoin non transforme. Elle permet egalement de visualiser la presence d'autres fragments dont les profils electrophoretiques caracterisent les clones transformes de maniere independante.

Dans le cas d'insertions multiples, le nombre de celles-ci est la moitie du nombre de bandes revelees par l'electrophorese. Dans le cas des 3 clones analyses, il n'est pas superieur a 2. Un *Southern blot* similaire a montre que les plantes regenerees a partir de quatre sous-clones derives par fragmentation du meme clone cellulaire (clone 5), presentaient toutes le meme profil et pouvaient donc etre considerees comme derivant d'un evenement unique de transformation.

Tous ces resultats ont ete confirmes apres digestion de l'ADN par l'enzyme BamH I, au lieu de l'enzyme Hind III : L'observation d'un type different de profil electrophoretique pour les clones analyses, permet d'aboutir aux memes conclusions.

CONCLUSIONS

En co-cultivant des cals embryogenes de la variete porte-greffe 110 Richter avec une souche d'*agrobacterium tumefaciens* recombinante, nous avons pu obtenir des plants de vigne transformes exprimant quatre genes differents, dont l'un (CP-GCMV) est susceptible de presenter une importance economique. La voie embryogene semble etre la plus prometteuse pour transformer la vigne. Mullins et c/., (1990) ont ete les premiers a obtenir des plants de vigne transformes a partir d'embryons somatiques co-cultives avec *Agrobacterium tumefaciens*, mais en regenerant des bourgeons et des axes caulinaires a partir de ceux-ci. En revanche, Colby et al/., (1991) n'ont pas abouti en utilisant la voie de la caulogenese adventive a partir d'explants foliaires. Plus recemment, Krastanova et c/., (1993) ainsi que Mauro (1993) ont rapporte avoir obtenu des plants de porte-greffes transformes a partir de cals ou de suspensions cellulaires embryogenes. Enfin, Nakano et al/., (1994) ont obtenu des plants de vigne (*Vitis vinifera* L.) transformes apres co-culture de cals embryogenes avec *agrobacterium rhizogenes*.

Nous avons utilise un gene de resistance a l'hygromycine comme agent de selection. Les exemples d'utilisation d'un tel gene en transformation sont encore rares mais commencent a se multiplier. Ainsi, Pert et al., (1994) ont recemment rapporte avoir introduit le gene *Suc 2* de l'invertase dans des varietes de raisin sans pepins, en utilisant egalement la voie embryogene et l'hygromycine a 20 µg/ml comme agent selectif. Ils signalent que la kanamycine, meme a fortes concentrations, ne semblait pas efficace, ce que nous avons pu egalement observer au cours d'essais preliminaires. Des experiences complementaires sont donc necessaires pour comparer les avantages et inconvenients respectifs de l'hygromycine et de la kanamycine pour la transformation genetique de la vigne. L'utilisation d'une construction telle que le plasmide pKVHG 2+, ou sa forme pKHG 4, ne portant pas le gene de la CP-GCMV, devrait permettre une telle comparaison.

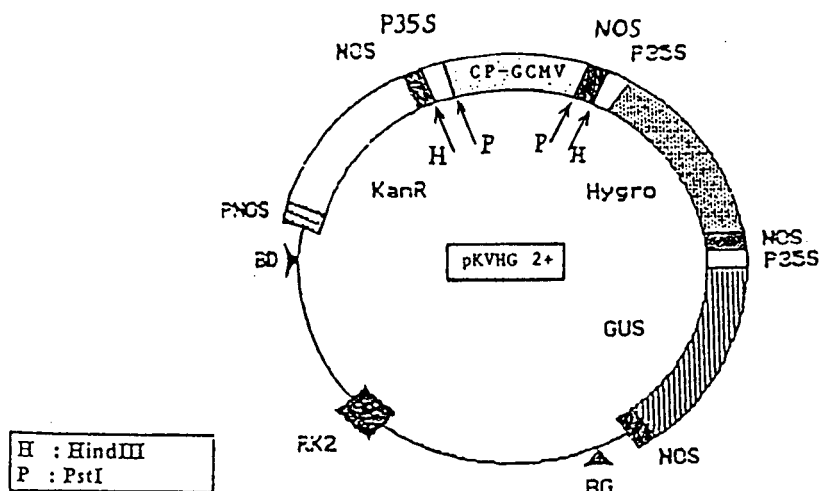
Le taux de transformation observe est relativement faible, puisque a partir de 4 g de cals embryogenes co-cultives et selectionnes sur des milieux a 16 et 20 µg/ml d'hygromycine, nous n'avons pu obtenir que cinq clones cellulaires transformes de maniere independante, a partir des dizaines de plantes transgeniques ont pu etre regenerees, mais qui se sont revelees genetiquement identiques notamment dans le cas du clone 5. Cependant, le taux de transformation est sensiblement plus eleve si on considere uniquement les explants cultives sur 16 µg/ml d'hygromycine (4 clones transformes obtenus a partir de 1,6 g de cals embryogenes). Bien que cette concentration d'antibiotique puisse etre considerée comme optimale, il est evident que des efforts doivent etre faits pour ameliorer l'efficacite de la transformation et des procedures de regeneration. En particulier, il semble indispensable d'utiliser des cals embryogenes aussi peu differencies que possible, malgre les difficultes que representent leur manipulation au cours de la co-culture. D'autre part, il faut proceder tres rapidement a l'isolement des amas cellulaires potentiellement transformes lorsque ceux-ci apparaissent sur les explants cultives sur des milieux aussi selectifs que ceux a 16 ou 20 µg/ml d'hygromycine, afin de les extraire d'un environnement de tissus fortement necroses, defavorable a leur survie. Les plantes transgeniques obtenues

sent actuellement en cours d'évaluation pour lentesistance ou tolerance an GCMV, en utilisant le microgreffage *in vitro* on la greffe en vert enserre. Le vecteur du GCMV qui est probablement le nematode *Xiphinema vuittenezi* a ete identifie depuis longtemps dans les vignobles septentrionaux, notamment en Champagne et en Alsace (Dalmasso, 1970), alors que pour l'instant le GCMV n'jamais ete trouve sur vignes dans ces regions. Mais la similitude des symptomes du GCMV avec ceux du court-noue (GFLV) pourrait laisser penser que sa presence n'est pas aussi improbable que certains l'affirment, d'autant plus que le TBRV, virus apparente au GCMV, mais transmis par *Longidorus attenuatus* et *L. elongatus*, est connu depuis longtemps en Allemagne et a ete recemment identifie dans les vignobles septentrionaux francais.

Quoi qu'il en soit, bien que des elevages de *Xiphinema vuittenezi* ou de *Longidorus attenuatus* et *L. elongatus*, susceptibles de transmettre egalement le GCMV, soient disponibles au departement de zoologie et nematologie du Scottish Crop Research Institute de Dundee (D. Brown, conun. person.), le fait de mettre en contact le virus et son vecteur dans des zones viticoles (Midi mediterraneen ou facade atlantique), ou ni l'un ni l'autre ne semblent exister pourrait susciter des critiques justifiees. Dans ces conditions, les tests de resistance an GCMV utilisant les vecteurs naturels de transmission, ne pourront se faire que dans des pays ou vecteurs et virus sent endemiques, ce qui implique par consequent une collaboration avec les chercheurs de ces pays.

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- H : HindIII
 P : PstI
- BD : Bordure droite du T-DNA
 BG : Bordure gauche du T-DNA
 KanR : Gène de la néomycine phosphotransférase (NPT II)
 CP-GCMV : Gène de la protéine capsidiale du GCMV
 Hygro : Gène de résistance à l'hygromycine (HPT)
 GUS : Gène de la glucuronidase (GUS)
 RK2 : Origine de réplication dans *E. coli* et dans *Agrobacterium*
 NOS : Terminateur du gène de la nopaline synthase
 PNOS : Promoteur du gène de la nopaline synthase
 P35S : Promoteur 35S du CaMV

Figure 1 - Schéma du plasmide pKVHG 2+

CONCENTRATION EN HYGROMYCINE	12 µg/ml		16 µg/ml		20 µg/ml	
CO-CULTURE	pKVHG 2+	Témoin	pKVHG 2+	Témoin	pKVHG 2+	Témoin
NONBRE DE :						
EXPLANTS	90	20	80	20	100	20
EXPLANTS TOTALEMENT NECROSES	61	16	69	19	97	20
EMBRYONS SOMATIQUES ISOLES	63	5	8	1	1	0
EMBRYONS REGENERES EN PLANTES	11	2	3	0	1	0
PLANTES GUS +	0	0	0	0	0	0
CALS EMBRYOGENES ISOLES	23	4	14	1	2	0
CALS EMBRYOGENES DEVELOPPES	15	2	8	0	1	0
CALS EMBRYOGENES HYG ^R - GUS +	3	0	5	0	1	0
CALS AYANT DONNE DES PLANTES TRANSFORMEES	0	0	4	0	1	0

TABLEAU 1 : EFFET DE LA CONCENTRATION EN HYGROMYCINE SUR LA PRODUCTION DE PLANTES TRANSGENIQUES DE LA VARIETE PORTE-GREFE 110 RICITER APRES CO-CULTURE DE CALS EMBRYOGENES AVEC LA SOUCHE AGROBACTERIUM TUMEFACIENS LBA 4404 pKVHG 2+

TABLEAU II - Effet de l'addition d'antibiotiques sur le comportement du 110 R, transformé (RT) ou non (R), en multiplication axillaire

	110 R		110 RT 5	
	PVEG	NP5	PVEG	NP5
K 0	258 a	5.3 a	273 a	5.7 a
K 10	63 b	1.5 b	238 a	5.8 a
K 25	37 b	1 b	209 a	4.8 ab
K 50	25 b	1 b	179 a	3.1 b

11 0	390 a	5.8 a	354 a	6.3 a
11 0.5	117 b	3.3 b	288 ab	5.3 ab
11 1	68 bc	1.8 c	296 ab	5.5 ab
11 2	42 bc	0.8 d	210 bc	4.4 bc
11 5	9 c	0.3 d	268 bc	5.5 ab
11 10	1 c	0.1 d	183 c	3.9 c

Concentrations en kanamycine (K) ou en hygromycine (H) en µg/ml - Comparaison par type d'antibiotique et par génotype.
 110 RT : 110 R transformé par la souche LBA4404 pKVHG 2+ (cf. n°5) - Notations effectuées à 30 jours
 PVEG : poids de la végétation néoformée (mg) par explant - 12 explants par traitement
 NP5 : nombre de pousses d'une longueur supérieure à 5 mm

Tableau III - Résultats des tests ELISA* effectués en 1994 sur les clones de 110 R transformés par la souche LBA4404 pKVHG 2+

Avr-94		Valeurs individuelles					Moy
Vitro-plants (2 mois)**	110 RT 2	326	224	401	312	205	293
	110 RT 3	471	338	552	391	312	412
	110 RT 5	649	579	808	891	782	742
	110 RT 10	1153	1087	1261	1159	988	1126
	110 RT 11	1243	1351	1016	1078	1248	1187
	110 R	117	109	117	118	108	114
Plants en serre (12 mois)	110 RT 2	1182	1000	1280			1154
	110 RT 3	706	542	663			637
	110 RT 5	947	1131	1065			1048
	110 R	115	111	121			116
	H15-3 GCMV	1539					1539
	T CP+	1880	1673	1722			1758

Jun-94		Valeurs individuelles					Moy	
Plants en serre (3 mois)	110 RT 10	1144	739	662	776	581	1296	866
	110 RT 11	962	739	636	809	882	770	800
Plants en serre (15 mois)	110 RT 2 FA	1319	1069	589	1000			994
	FJ	1208	700	507	668			771
	R	1466	1689	633	1226			1253
	110 RT 3 FA	666	775	357	804			651
	FJ	508	504	687	559			571
	R	660	720	459	443			565
	110 RT 5 FA	216	626	767	592			413
	FJ	420	471	524	479			473
	R	522	317	413	205			364
	110 R FA	176	185	162				174
	FJ	173	176	199				183
	R	201	189	nd				195
T CP+	1768	1838	1860	1911			1836	

T CP+ : tabac transformé par le pKVHG 2+ et produisant la CP-GCMV * 1000 x DOX405nm à 10 min
 110 R : témoin ; 110 R non transformé et non infecté de GCMV ** () âge du matériel testé
 H15-3 GCMV : variété contaminée par le GCMV nd : non déterminé
 FA : feuilles adultes de la tige principale
 FJ : jeunes feuilles portées par les entrecoeurs
 R : système racinaire (sauf parties basales)

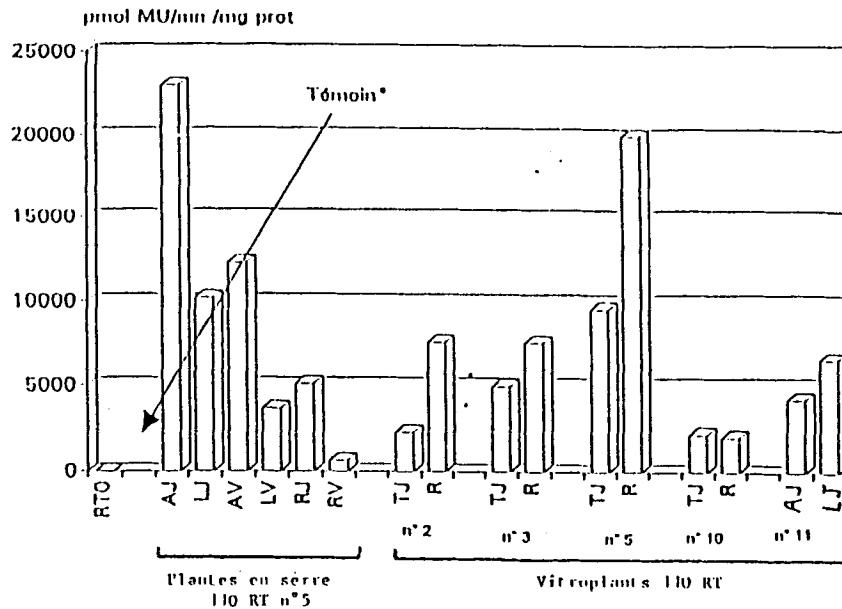


FIGURE 2. - Niveau d'activité GUS dans les plants et vitro-plants de 110 R transformés (Test à fluorométrique)

- AJ : Tiges et pétioles des 3 plus jeunes articles
- LJ : Limbes correspondants
- AV : Tiges et pétioles des 5 au 8ème articles suivants
- LV : Limbes correspondants
- IJ : Pointes racinaires (30 à 40 premiers mm)
- TJ : AJ + LJ
- RV : Troncs racinaires
- R : Système racinaire en totalité
- * : Témoin AJ + LJ de vitro-plants de 110 R non transformé

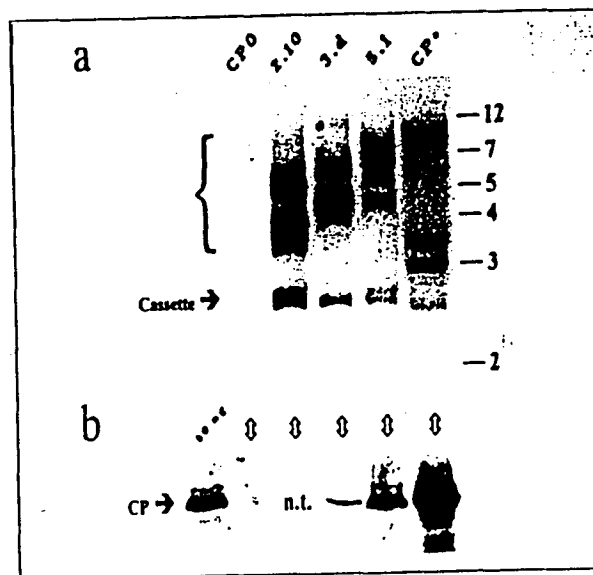


Fig. 3 : CARACTERISATION MOLECULAIRE DES VIGNES TRANSGENIQUES

- a) Southern blot effectué sur un clone non transformé (CP 0) et trois clones transformés (2.10, 3.d, 5.1) de 110 Richter, ainsi que sur la lignée de tabac transgénique 2.T1 (CP+). Les positions de la cassette d'expression de la CP-GCMV et des fragments de bordure (accolade) sont indiquées sur la gauche, et celles des marqueurs de taille de l'ADN sur la droite.
- b) Western blot effectué sur un clone non transformé et deux clones transformés de 110 Richter, ainsi que sur la lignée 2.T1. 50 ng de particules purifiées de GCMV ont été diluées dans un extrait du clone non transformé et utilisés comme témoin de concentration. La position de la CP-GCMV est indiquée sur la gauche. (n.t. : non testé)

SEARCH FOR MOLECULAR MARKERS AND THEIR APPLICATION IN CULTIVAR IDENTIFICATION AS WELL AS MARKER ASSISTED SELECTION

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Introduction

What are molecular markers?

Molecular markers are indicators of genetic differences, that means they exhibit DNA sequence variation between two or more organisms. Such markers may be generated by two different techniques: RFLP- and PCR technology. The first method, detection of restriction fragment lengths polymorphisms (RFLP) relies on the search for ANA sequence variation reflected in variable restriction patterns. However, in order to detect these, specific "polymorphic" probes need to be labeled and hybridized to the sample DNA. The trial of several probes, the large amounts of restriction enzymes needed and the high amount of genomic DNA required for this type of analysis renders it quite costly and tedious.

The second technique is a special variation of the polymerase chain reaction (PCR). Short oligonucleotides are offered in hybridization reactions with genomic DNA. If they can find complementary sequences, they will bind to the sample DNA. If two such oligonucleotides have been bound in opposite orientations and in a distance not too far from each other, they will serve as primers for DNA synthesis. In repeated cycles of polymerization the DNA in-between the binding sites may be amplified (PCR).

The amplification results in one or several non-specified products, which are characterized for their size by gel electrophoresis. Very short random oligonucleotide primers have a statistical probability to bind at multiple sites in a complex eucaryotic genome, such as that of grapevine. Usually a sample DNA tested will yield several products generating specific pattern of bands with a single primer. This technique is called "random amplified polymorphic DNA" (RAPD). RAPD analysis requires only very little genomic DNA (about 1/500 to 1/1000 of the DNA amount for RFLP analysis) and therefore can be performed with very small tissue samples.

However it is noteworthy to mention, that both techniques yield in DNA bands representing only a tiny small part of the whole genome in individual assays of oligonucleotide primers or probe/enzyme combinations. In order to obtain data allowing thoroughful genetic analysis, these assays have to be multiplied. RAPD-PCR can easily be done with large sets of multiple primers. Therefore it is the technique of choice for our studies .

Why do we want molecular markers? Are they really useful?

Grapevine is a very old crop grown since many thousand years and distributed all over the world. The clear identification of cultivars or determination of their ancestry is therefore sometimes difficult. However this knowledge is of basic importance for breeders and others involved in viticulture. The classical morphological determination through ampelography is very useful but sometimes hampered by influences of environmental factors or the developmental stage of plants. The same holds true for analysis of pollen wall proteins or isoenzymes. Only direct genetic analysis can be free of such variation. In addition, since PCR techniques require very little material, they may be performed on small tissue fragments readily available from a young seedling. Quite in contrast, formation of all the morphological characters including blooming and fruit set will require two to three years of plant development. Molecular markers therefore can be screened much faster. If specific markers can be correlated with certain desired traits such as disease resistance, they may be used in marker-assisted selection allowing to cut down the long time required to select a desired genotype in the progeny of a certain cross. Furthermore, such markers could be employed to select the parents for crosses in breeding programs. Finally they represent the initial step in the efforts to map and analyse the respective gene(s) responsible for the desired trait.

Materials and methods

We prepare genomic DNA from grapevines grown in the living collection of Geilweilerhof according to the method of Thomas et al., 1993. Usually young leaves of field-grown plants are used as the starting material. In vitro plants (roots omitted), suspension cultures or callus tissue may also be used. For RAPD-PCR the DNA is diluted to 20 ug/ml in To (10 mM Tris-Cl, 1 mM EDTA pH 8.0) and stored frozen. The standard reactions include 10 mM Tris- CL, 50 mM KC1, 1.5 mM NgCl₂, pH 8.3; 100 uM each of dATP dGTP, dCTP and TTP, 20 ng DNA, 30 ng primer (Operon technologies Inc. Alameda, CA, USA) and 1 U of Tag DNA polymerase (Boehringer Mannheim) . Total reaction volume is 50 ul, overlaid with 35 ul of mineral oil (Perkin Elmer

Cetus, Norwalk, Ct, USA). Temperature cycling is performed in a Hybaid TR3-CM cyler (MWG Biotech, Ebersberg, FRG) programmed as follows: Initial denaturation at 94°C for 5 min. followed by 45 cycles of 1 min. denaturation at 94°C, 1 min. annealing at 36°C and 1.5 min. DNA synthesis at 72°C. Heating from 36 to 72°C is performed with the ramming function set to 2°C/sec.. After cycling, the reaction mix is extracted with 35 ul of chloroform to remove the oil. 5 to 10 ul of the products are analysed on 1.5 to 2% agarose gels prepared in TBE buffer (89 mM Tris-borate, 2 mM EDTA pH 8.3) and stained with Ethidium bromide (0.5 ug/ml) for 10 min. Photographic records are taken on Polaroid 665 film. In the case of hybridizations, the PCR products (or cut genomic DNA) are transferred to nylon membranes (Hybond N, Amersham-Buchler, Braunschweig, FRG) by capillary transfer (Sambrook et al., 1989) in 20xSSC (3M NaCl, 300 mM sodium citrate, pH 7.0) and reacted with digoxigenin-dUTP- (Boehringer Mannheim, Mannheim, FRG) -labeled probes as specified by the supplier.

Detection of hybridized probes uses chemiluminescence of anti-digoxigenin Fab fragments coupled to alkaline phosphatase processing Lumigen TM PPD (Boehringer Mannheim, Mannheim, FRG). Comparison of the various RAPD patterns was assisted by scanning gel photographs on a modified Digiblot Watanabe WX 4671 (Watanabe. GmbH, Herrsching, FRG) and transferring them into synthetic gel pictures. Similarity coefficients were calculated according to the formula $SD = \frac{\text{no. of bands in A common to A and B} + \text{no. of bands in B common to A and B}}{\text{no. of total bands in A} + \text{no. of total bands in B}}$. complete identity yields SD = 1, complete divergence results in SD = 0. SD calculations were assisted by the computer program "Rapid" developed by R. Blaich at our institute. The resulting SD values were further grouped into clusters with the help of a program developed by J.Tschammer.

Results

Characterization of wild species Wild species of *Vitis* are important resources for breeding purposes as they often carry resistance genes to diseases or abiotic stress factors such as frost or draught. Understanding their phenetic relationships, correlation of specific genotypes with the geographic origin (Eurasia or America) or the separation of *v. rotundifolia* with its increased chromosome number ($2n = 40$) from other *mitts* spec. ($2n = 38$) can create basic knowledge for the breeder. Therefore we started to analyse a set of 12 species (Table 1) with RAPD markers. Two plants of each species were used to prepare ANA and both samples were amplified with a set of primers.

Fig.1 shows an example of the results obtained. With primer 0P-F16 distinct banding patterns are obtained which unequivocally differentiate the wild species (Fig.1a). Both independent RAPD assays are identical, showing the good reproducibility. The only exception is *v. labrusca* vat. *Isabella*, where both individual plants exhibit related, but different banding patterns. This was observed repeatedly with at least 10 different primers and hence indicates genetic variation within that species. In few cases only, the duplicates may vary in concentration of amplification products, but still yield the same overall pattern. In this instance, repetition with freshly prepared ANA is required. However, the profiles can be used to calculate similarity indices (SD) providing the data for further comparison such as cluster analysis. In order to use the Rapid program, standardized migration distances and intensities of individual bands are stored in a computer file. This creates a synthetic gel picture as shown in Fig.1b. These data are then analysed for SD values.

Concerning the wild species shown in Fig.1a It seems noteworthy to mention, that *V. vicifolia* and *V. thunbergii* show some relatedness, but are not identical as stated earlier (Galet, 1988). *V. rotundifolia* (*Muscadinia*) is clearly very different from the other *Vitis* spec. (*Euvitis*) analysed.

Vitis wild species investigated by RAPD analysis

Species	Origin
<i>V. aestivalis</i> Mchx.	America (Northern US, Canada)
<i>V. amurensis</i> Rup.	Eurasia (Siberia)
<i>V. berlandieri</i> Colombard	America (Texas, Mexico)
<i>v. doaniana</i>	America (New Mexico, Texas)
<i>v. ficifolia</i>	Eurasia
<i>V. girdiana</i>	America (California, deserts)
<i>V. labrusca</i> <i>Isabella</i>	America (North-East US)
<i>V. pisaetzii</i>	Eurasia (Caucasus, China)
<i>V. riparia</i> Michx.	America (US, Canada)
<i>V. rotundifolia</i> 351	America (South-East US)
<i>V. rupestris</i> du Lot	America (Southern US)
<i>V. thunbergii</i>	Eurasia (Japan, Korea, China)

Fig. 1a. RAPD profiles of *Vitis* spec. amplified with primer OP-F16 (sequence GGAGTACTGG).

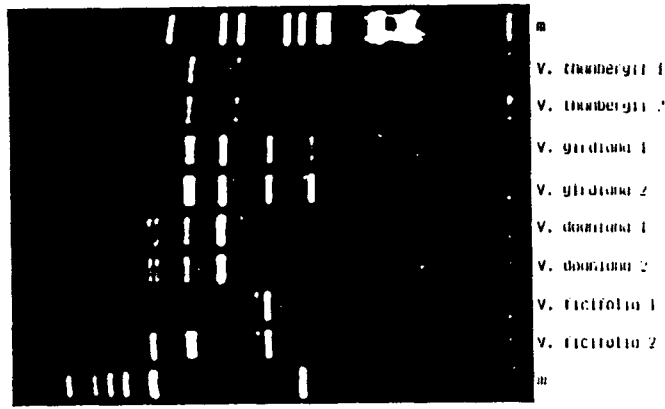
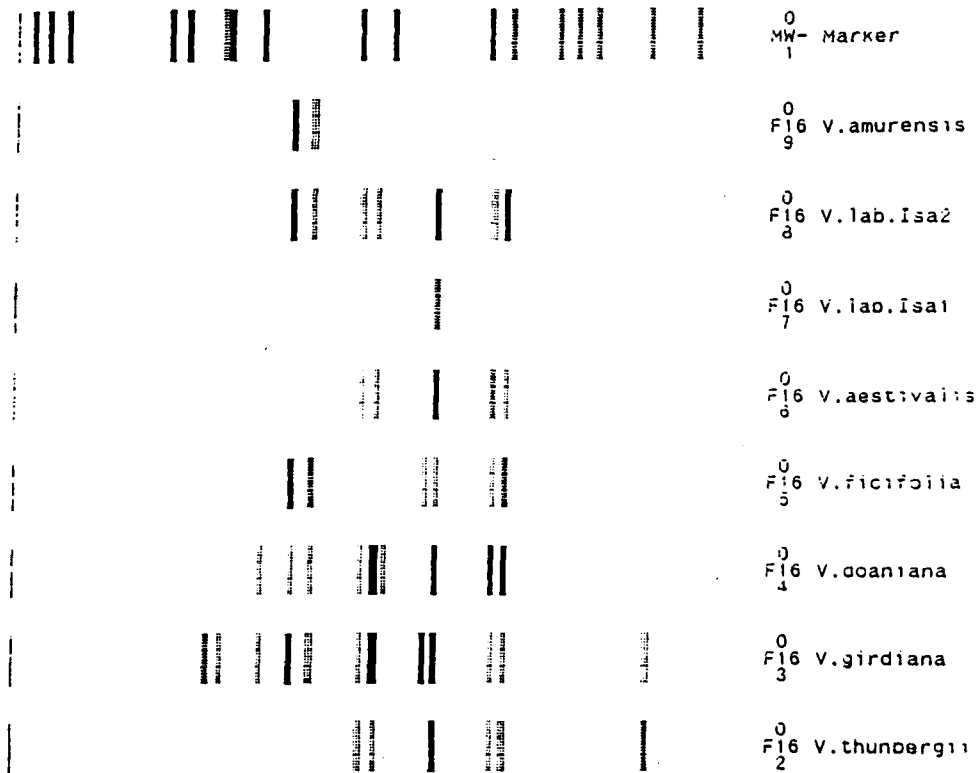


Fig. 1b. Synthetic gel picture of *Vitis* spec. amplification products obtained with primer OP-F16 and table of corresponding SD values.



* Table of Similarity Coefficients *

SPEC. / VAR.	#	2	3	4	5	6	7	8	9
F16 <i>V. amurensis</i>	9	0.000	0.286	0.364	0.500	0.000	0.000	0.444	1.000
F16 <i>V. lab. Isa2</i>	8	0.769	0.737	0.875	0.769	0.933	0.250	1.000	
F16 <i>V. lab. Isa1</i>	7	0.286	0.154	0.200	0.286	0.333	1.000		
F16 <i>V. aestivalis</i>	6	0.909	0.588	0.714	0.545	1.000			
F16 <i>V. ficifolia</i>	5	0.500	0.667	0.667	1.000				
F16 <i>V. doaniana</i>	4	0.667	0.762	1.000					
F16 <i>V. girdiana</i>	3	0.667	1.000						
F16 <i>V. thunbergii</i>	2	1.000							

Figure 1b *Vitis* spp.: RAPD Patterns with Primer OP-F16

Although there are only few rootstock varieties commonly used in the winegrowing areas of Germany (as in other countries), these represent a special identification problem: After grafting, morphological characters useful for ampelography are scarce. Therefore we tested 20 different oligonucleotides for their capability to differentiate five rootstock varieties (Table 2). Although these all are of interspecific origin (*V. Berlandieri* x *V. Riparia* resp. *V. riparia* x *V. Cinerea*) and hence were expected to be genetically quite heterogeneous, their identification demands the use of several primers in differential analysis. So far, out of 20 primers none was capable to discriminate all of the five rootstock varieties (Fig.2).

Table 2
Rootstock varieties analysed by RAPD-PCR

SO4	<i>V. berlandieri</i> x <i>V. riparia</i>	Teleki Selektion Oppenheim 4
5C	<i>V. berlandieri</i> x <i>V. riparia</i>	Teleki 5C Geisenheim
5BB	<i>V. berlandieri</i> x <i>V. riparia</i>	Teleki Kober 5BB
125AA	<i>V. berlandieri</i> x <i>V. riparia</i>	Teleki Kober 125 AA
Börner	<i>V. riparia</i> x <i>V. cinerea</i>	

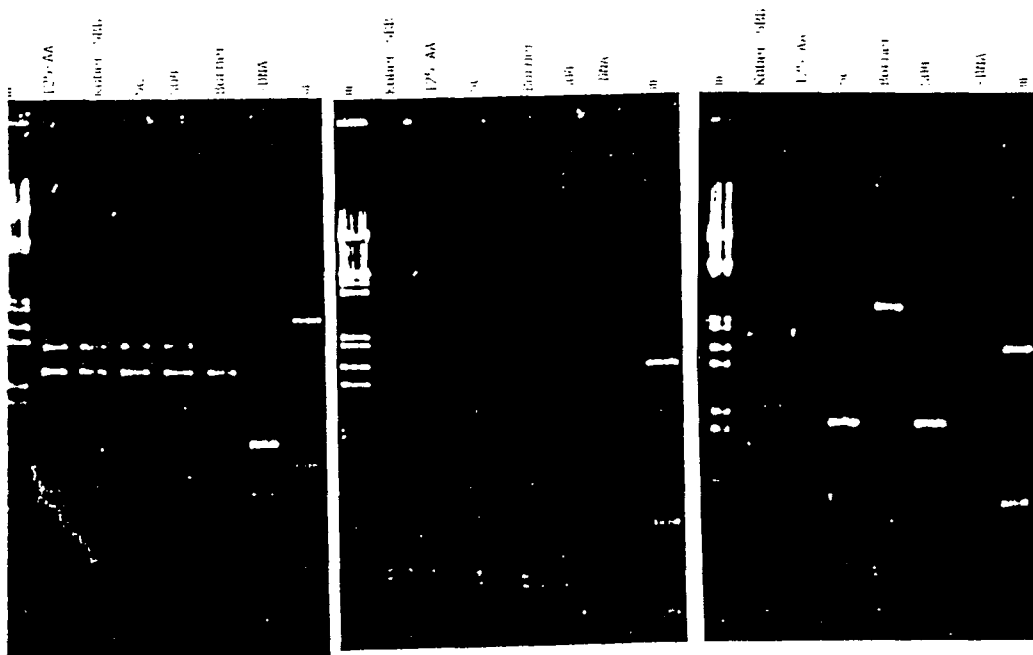


Fig. 2. RAPD analysis of rootstock cultivars with primer OP-F13 (sequence GGCTGCAGAA) (left), OP-F17 (sequence AACCCGGGAA) (middle) and OP-F18 (sequence TTCCCGGTT) (right).

Studies on "classical" *V. vinifera* cultivars

More than twenty oligonucleotides were employed to try differentiation between the closely related cultivars of the so-called "Burgundy"- or "Pinot"-family. None of them was able to discriminate between Pinot noir, Pinot gris, Pinot meunier and Pinot blanc (SO primers tested). Chardonnay and Chardonnay musque were always found to be rather identical (and similar to Auxerrois), as well as Aligote and Aligote vert. Chardonnay is clearly distinguishable from Pinot blanc. The variety Morillon was found to be different from Chardonnay or Pinot blanc, however two Morillon samples from two localisations also differed from each other. From its genetic similarity, Morillon does not seem to belong to the "Burgundies".

The same seems to hold for Saint Laurent, although this variety is thought to be derived front Pinot noir by mutation. In contrast, the variety Affenthaler seems related to the "Burgundies". The data obtained front 20 primers tested with the Pinot-family have been sunrmarized into the cluster dendrogram shown in fig.3.

Comparison of the genetic similarity within other grapevine cultivars could he used to shed some light on the origin of Mueller-Thurgau, formerly believed to be derived from the cross Riesling x Silvaner. RAPD analysis indicated that Riesling might well be the "mother", but Silvaner most likely is not the "Father" of Mueller-Thurgau (Buescher et. al. , 1994). This example shows that RAPD-analysis can be used to clarify doubtful origins.

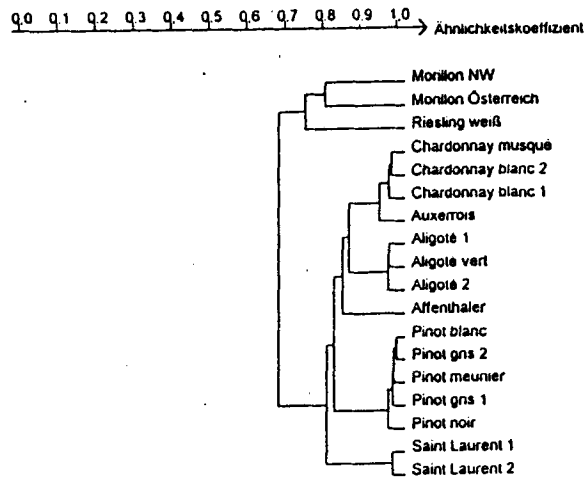


Fig.3.. Dendrogram of similarities inbetween the "Burgundy"- or "Pinot"-family of grapevine cultivars. Clusteranalysis was performed on data derived from amplifications with 20 different primers (OP-F1 to OP-F20).

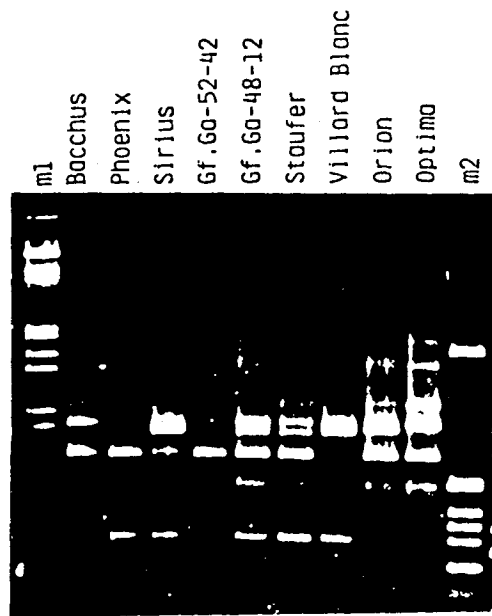


Fig. 4. RAPD-Analysis of newly bred varieties from Geilweilerhof with primer OP-F04 (sequence GGTGATCAGG).

Phoenix	Bacchus x Villard blanc
Sirius	Bacchus x Villard blanc
GfGa 52-42	Bacchus x Villard blanc
GfGa 48-12	Bacchus x Villard blanc
Staufer	Bacchus x Villard blanc
Orion	Optima x Villard blanc

Characterization of newly-bred cultivars with interspecific hybrid ancestry.

At Geilweilerhof, grapevine breeding is mainly aimed at the development of new fungus-resistant cultivars maintaining the quality of traditional wines for taste, yield and adaptation to the climatic factors in the german wine-growing areas. In this context, we are studying the new lines, for example those listed in Table S derived from the cross Bacchus x Villard blanc or optima x Villard blanc. Differentiation of these newly bred varieties is easily obtained applying the RAPD technique (e.g. see Fig. 4 with amplification products of primer OP-F04).

As the samples represent F₁ progeny from well-defined parentage, they are also very useful for genetic analyses. Most individual bands of the RAPD profiles seem to behave as independently segregating markers, just like chromosomes. This is to be expected with the high chromosome number of *Vitis* and the low number of bands per primer (1 to 15) in informative RAPD patterns. However compiled data from multiple analyses with a plenitude of primers need to be checked for linkage in the future. This also should enable the beginning of marker mapping by frequencies of genetic recombination.

As all progeny has been carefully selected for resistance to fungal diseases (mainly *Oidium*), these studies provide the possibility to detect markers correlating with that phenotype for "marker assisted selection". In fact, several putative marker candidates were found that way, comparing the small family *Bacchus* (s), *Phoenix* (r), *Sirius* (r) and *Villard blanc* (r) in amplifications with 60 different random primers. One of them, maintained its correlation with the character of resistance when amplification was extended to about 50 susceptible and resistant grapevine varieties.

Its amplification products as well as other interesting RAPD bands have been cloned in bacterial vectors. They can now be multiplied easily and used as molecular probes in hybridizations (cf. Fig. 5). Besides their use as RFLP-probes in genomic hybridizations, they may be applied to test the apparent identity of same-sized amplification products obtained with that primer from other samples. In this way they can be applied to check transferability of such markers from one family to another, prerequisite for a wide use in breeding schemes in the future. Further, such cloned amplification products can be sequenced and used to design longer, allele specific primers less difficult to use in PCR reactions and to start gene analysis in the *Vitis* genome. These studies are now under way.

Conclusions and Discussion

The application of molecular techniques allows to discriminate grapevine cultivars on the genetic level. In the case of morphologically very similar varieties, these methods can give good indications for nonidentity. In case of doubtful origin or relatedness, the molecular techniques can clarify the situation. However the Pinot-variants, probably arisen by spontaneous point mutations in berry colour from each other, have so far not been distinguishable. The more important feature of molecular markers is their potential to enable genetic analysis of grapevines, a plant recalcitrant to classical genetics due to its strong heterozygosity, high level of inbreeding depression and long generation cycle. So the molecular markers can find their practical application as early indicators in breeding schemes, but furthermore represent the first step towards detailed genetic analysis of that ancient crop. Their mapping and correlation with specific characters will finally lead to the analysis of the genes involved in desired traits such as disease resistance and understanding of the cellular mechanisms involved. This should help us to produce wine in an ecologically safe way in the future.

Domina (s) x Chambourcin (r)

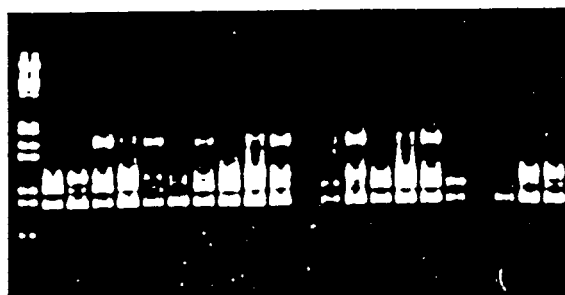


Fig. 5. Hybridization of a 820 bp marker from *Villard blanc* amplification with F₁ progeny (21 individual seedlings) of the cross *Domina* x *Chambourcin* amplified with the corresponding primer.

Acknowledgements

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SESSION 5 : CLONAL SELECTION

MODELING OF RADIO SENSITIVITY OF GRAPE CLONES TO X-RAY IN ORDER TO DE-TERMINAE THE DOSES THAT POSSIBLY BRING ABOUT MUTATIONS

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Abstract

Having exposed the buds of six grapevine varieties that are important in Hungary to X-ray irradiation, the growth were studied. In the growth up to 100 Gy either a stimulation (*Chardonnay clone type*, Harslevelu K. 9, Irsai Oliver K. and Muscat Ottonel clone type or a deterioration (Cabernet Sauvignon E 153.) or no influence Koviadinka. K. 8.) were observed. With more than 100 Gy being applied to buds in the growth a deteriorating effect manifested itself. A multihit model was set up using eqyations of $Y'=a+bD+cD^2$ and $Y'= 1-(1-e^{-kD})^n$. The LD_{50} and LD_{37} values varied with a range of 38+320 Gy and 47+328 Gy. The assumed sensitive volumes were $3.08+2128 \mu^3$. The LD50 values are to be applied for further mutation work.

Introduction

It is well known the variability of cultivated grape varieties, although the background of this is not understood, yet (ALLEWELDT-SPIEGEL-ROY ET AL., AL., 1990; PIRES-ROMMER ET AL., 1988; RATHJEN-ROBINSON, 1992; STRAVRAKAKIS, 1988). At the same time in the countries growing grape, the cultivation of grapes has an economical merit. However the time for developing of a clone takes about 20-25 years.

The mutability of the varieties would be useful in order to carry out an effective selection work (BOTTA-VALLANIA ET AL., 1989; DONINI-MANINI ET AL,1991; CARAMIELLO-SINSCALCO, ET AL., 1989).

In addition consider the irradiation as one of the tools helping to understand this mutability.

For the ut supra outlined purpose we have been carrying out experiments with X-ray irradiation involving six varieties (some of which are clones) important in Hungarian conditions (see Material and Methods).

As a first step we determined the radiosensitivity of these varieties in average of the plants developed from twelve buds, using 400 Gy dose as a threshold one In this respect Harslevelu K. 9. appeared to be the most sensitive, with Cabernet Sauvignon E.153 being the most tolerant (HAJDU-KOROSI ET AL., 1991).

In the second step within these varieties sensitivity of twelve bud levels were also established. A general conclusion can be drawn that the basipetal buds were the most sensitive to X-ray taking consideration of every investigated varieties (HAJDU-KOROSI ET AL., 1994)

In the third step based upon the bud resistant study we have chosen the buds in which we have postulated a model.. According to this multihit model the radio sensitivity constants, LD_{50} , LD_{37} were calculated and sensitive volumes hypothesized.(WEBER, 1987; FABRIKANT. 1972: KUDRAJSOV-BERENFEL'D, 1982). These results will be presented in this lecture

Materials and method

For the experiments the following varieties and/or clones were involved:

Cabernet Sauvignon E153, *Chardonnay clone type*, Harslevelu, K. 9., Irsai Oliver K.11., Kovidinka K 8., Muskat Ottonel clone type.

For the irradiation X-ray apparatus Liliput 140, Medicor, Hungary, was used and the irradiation was performed at the Central Laboratory of the Agricultural University, Godollo. The doses applied were 10 Gy, 50 Gy, 100 Gy, 200 Gy, 400 Gy (120 kV, 4.5 mA).

According to our earlier bud radiosensitivity study for the varietal clones involved, the bud levels considered to be the most appropriate for further modeling are presented in Table 1 (HAJDU-KOROSI ET AL., 1994)

Table 1.

The bud levels chosen for the empirical modeling

Variety/clone	Bud level
Chardonnay clone type	10 th
Hárslevelű K. 9.	7 th
Irsai Olivér K.11.	10 th
Muscat Ottonel clone type	9 th
Kövidinka K. 8.	8 th
Cabernet Sauvignon E. 153.	10 th

For every variety the buds were irradiated with ten repetitions. Therefore for evaluation every dose and the control ones 10 repetitions were available.

The cuttings that were collected in 1992 in the experiment after the irradiation, were put in pots which contained an appropriately moistened mixture of peat and perlite (3:1 v/v). Then they were put in a greenhouse and allowed to develop and grow for three months.

After that the shoot and root dry weight

were evaluated using statgraf ver. 4.0.

By means of empirical modeling summarized in the works of WEBER (1987), FABRIKANT (1972) AND KUDRJASOV (1982) the plant production as affected by irradiation in relation to the non irradiated ones was modeled. Then the radiosensitivity constant's, LD₅₀, LD₃₇ values were calculated and the target volumes were postulated. LD₅₀ = reduction in the relative growth to 50 %, LD₃₇ = reduction to in the relative growth to 37 %. The models set up were based on the following equations

$$Y' = a + bD + cD^2$$

$$Y' = 1 - (1 - e^{-kD})^n$$

where Y' = plant production (DW, g) or irradiated plant production in relation to non irradiated ones

D = dose (Gy)

b, c, k = radiosensitivity constants

n = target numbers

Results

There was a sharp decrease in the growth of Cabernet Sauvignon F. 153. as a result of doses up to 50 Gy (Fig. 1). Under the effect of 400 Gy dose, the growth totally deteriorated. In contrast to that of Cabernet Sauvignon E. 153., up to 50 Gy a so called radio-stimulation was brought about by X-ray irradiation (Fig. 2.) A plate in the enhanced growth appeared between 10-50 Gy. even at 200 Gy dose. the extent of the dry matter formation was not below the control values.

For the Harslevelu K. 9 under the influence of the irradiation the same trend was observed as for the Chardonnay clone type (Fig. 3). The exception was that already 200 Gy seemed to be lethal.

For Irsai Oliver K. 11. variety the 10 Gy dose was proven to be positive, 50 Gy lowered the growth by about 50 %, while doses a 100 Gy doses stopped the growth altogether (Fig. 4)

The dry matter formation of Kovidinka K. 8. was not affected by an X-ray dose range of 10 +100 Gy (Fig. 5). Nevertheless ≥ 200 Gy doses irreversibly deteriorated the genes responsible for growth and development to take place.

For the Muscat Ottonel clone type a 10 Gy dose set off a slight positive effect, and in the meantime 50 Gy did not cause any changes in the growth (Fig. 6.)

At the 10th bud level Irsai Oliver K. 11. and Cabernet Sauvignon E. 153. can be characterized as having high sensitive constants and volumes (Table 2.).

Chardonnay clone type also at 10th bud level appeared to be the most resistant with a sensitivity to irradiation volume of 3.08 μ³.

According to the models set up for our further radio-mutational work the D₅₀ doses are to be postulated.

In this respect for Chardonnay clone type 320 Gy (10th bud), for Harslevelu K 9. 90 Gy (7th bud), for Irsai Oliver K. 11. 58 Gy (10th bud), for Muscat Ottonel clone type 81 Gy (9th bud), for Kovidinka K. 8. 145 Gy and for Cabernet Sauvignon E 153. 38 Gy are supposed to be doses with that mutational analysis to be carried out.

Conclusions

- = Under the influence of X-ray irradiation in the growth a radio-honeisis appeared for Chardonnay clone type, Harslevelu K. 9.. Irsai Oliver K. 11. and to a lesser extent for Muscat Ottonel clone type.
- = For Cabernet Sauvignon E. 153. every applied dose had a deteriorating effect on the growth.
- = Up to 100 Gy dose the X-ray irradiation did not influence the dry matter production of Kovidinka K. 8. .

- = The arranged empirical models using $Y' = a + bD + cD^2$, $Y' = 1 - (1 - e^{-kD})^n$ equations more than 0.5 correlation coefficient described the relative growth (in relation to non irradiated plants) of plants developed from irradiated buds.
- = It generally can be stated that the higher the LD_{50} doses, the lower the assumed sensitive volumes at given bud levels.
- = The LD_{50} and LD_{37} values varied with a range of 38 + 320 Gy and 47 + 328 Gy. The assumed sensitive volumes were $3.08 + 21.28 \mu^3$.

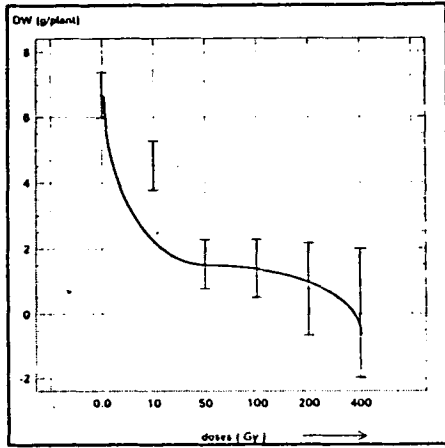


Fig. 1
Effect of X Ray irradiation on the dry matter production of Cabernet Sauvignon E. 153., developed from the 10th bud

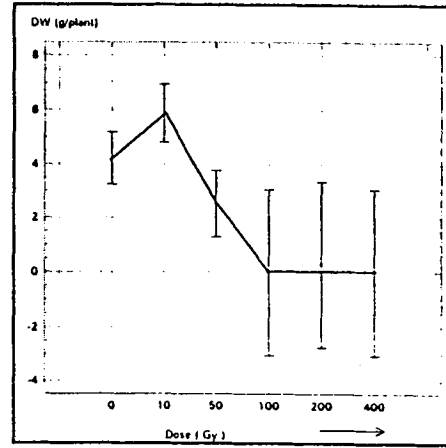


Fig. 4.
The effect of X-ray irradiation on the dry matter production of Isai Oliver K. 11., developed from the 10th bud

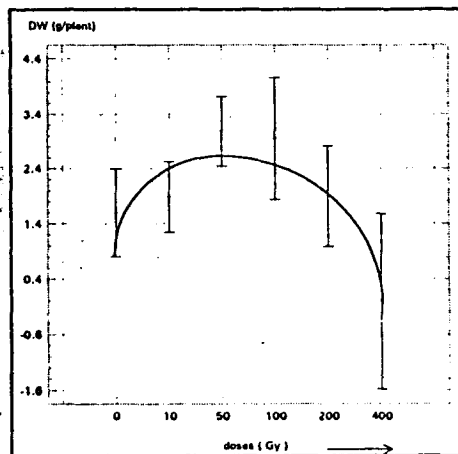


Fig. 2.
Effect of X-ray irradiation on the dry matter production of Chardonnay clone type, developed from the 10th bud

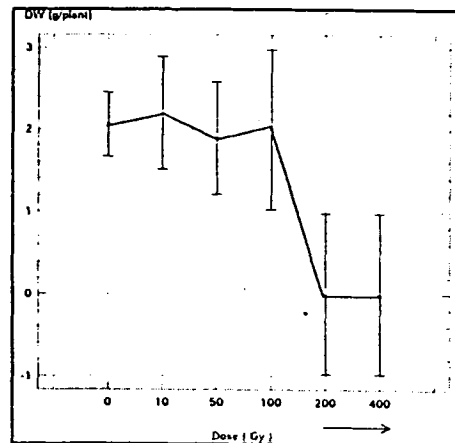


Fig. 5.
Effect of X-ray irradiation on the dry matter production of Kóvidiána K. 2., developed from the 8th bud

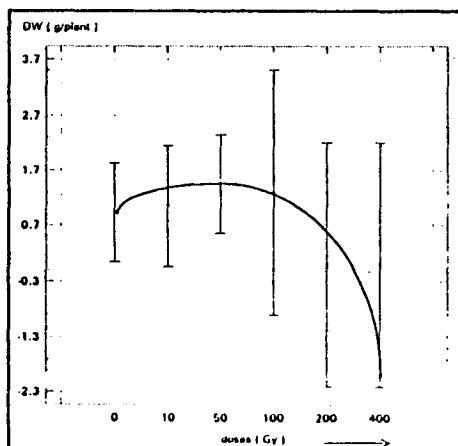


Fig. 3.
Effect of X-ray irradiation on the dry matter production of Hárslevelő K. 9., developed from the 7th bud

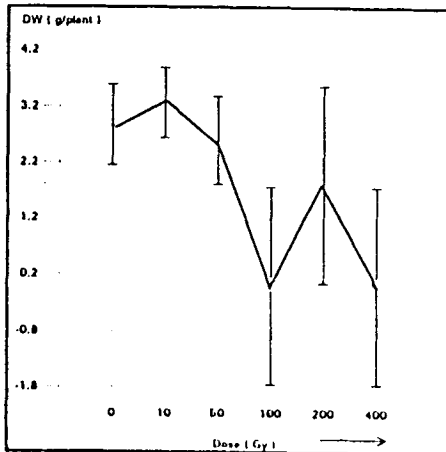


Fig. 4.
Effect of X-ray irradiation on the dry matter production of Muscat Ottomai clone type, developed from the 9th bud

Table 2

Radiobiological parameters characterizing the growth of the grapevine developed from X-ray-irradiated buds

Varieties/clones	Bud level	Radiosensitivity constant	St. error	correlation coefficient of the model (r)	LD ₅₀ (Gy)	LD ₃₇ (Gy)	Sensitive volumes (μ^3)
Chardonnay clone type	10 th	0.0072 -0.0038	0.0041 0.0009	0.5233	320	328	3.08
Hárslevelű K.9.	7 th	0.1367	0.0092	0.5900	90	118	8.47
Irsai Olivér K.11.	10 th	0.1583	0.0091	0.6830	58	72	13.89
Muscat Ottonel clone type	9 th	0.0424	0.0067	0.8402	81	90	11.11
Kövidinka K. 8.	8 th	0.0621	0.0082	0.9547	145	160	6.25
Cabernet Sauvignon E. 153.	10 th	0.1457	0.0087	0.8337	38	47	21.28

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SESSION 5 : CLONAL SELECTION

CONTROLES DE LA CONSERVATION DE L'ETAT SANITAIRE EN SELECTION CLONALE

M. LEGUAY . ONIVINS

La sélection clonale de la vigne s'est attribuée deux objectifs :

Une amélioration de l'état sanitaire du vignoble et une amélioration du potentiel génétique (quantité et qualité) du matériel végétal utilisé. L'amélioration sanitaire porte essentiellement sur des viroses ou des maladies de type viral, dont la transmission par le matériel végétal est connue. Figurent en premier le court noué et l'enroulement.

Afin de permettre la mise à disposition des viticulteurs d'un matériel d'une qualité reconnue, la France, puis l'Union Européenne ont mis en place une réglementation de certification définissant un cahier des charges qui permet de limiter les risques de recontamination.

La plupart des exigences portent malgré tout sur des opérations en amont du matériel végétal distribué, et les contrôles sont réalisés en général a priori. Les tests sanitaires et la méthodologie présentés correspondent à des contrôles a posteriori.

1) LES REGLES DE LA CERTIFICATION

L'application au secteur des bois et plants de vigne des règles élémentaires de la virologie s'est traduite par la mise en place d'une réglementation qui s'est efforcée de trouver un compromis entre la rigueur nécessaire des règles sanitaires permettant de multiplier du matériel végétal à l'abri des contaminations, et les contingences pratiques qui autorisent une production de qualité à des professionnels dans des conditions de culture en plein champ et à une échelle économiquement viable.

De ce fait, il serait vain de vouloir nier les possibilités ou les risques de recontamination sur le terrain : seules les productions de laboratoires ou éventuellement hors sol peuvent être susceptibles d'apporter ces types de garanties. Le système mis en place s'appuie sur une limitation du nombre de générations entre le matériel initial sélectionné, testé, reconnu indemne et conservé par l'établissement de sélection à l'abri des recontaminations, et le matériel végétal mis à disposition du viticulteur.

Chaque génération, du fait d'une implantation sur le terrain en plein champ d'une part, des manipulations et des risques d'introduction de matériel étranger d'autre part, présente un risque certain de voir l'état sanitaire se dégrader.

La réglementation a été mise en place afin de limiter les causes de dégradation de cet état sanitaire, à la lumière des connaissances scientifiques du moment :

des règles strictes de séparation du matériel végétal pour éviter les mélanges, d'isolement du matériel sélectionné par rapport aux parcelles voisines pour les vignes mères, ou aux lots voisins en pépinière, l'assurance que le sol où seront établies les vignes mères ou les pépinières ne présente qu'un risque minimum par l'absence de cultures de vigne ou d'autres plantes hôtes des nématodes vecteurs, et/ou après désinfection et respect d'un assolement.

Cette réglementation a été mise en place en France au milieu des années 60, a été révisée en 1970-71 à la suite de la directive communautaire de 1968 et a été réactualisée en 1980-81, mais sans en modifier fondamentalement ni l'esprit, ni l'essentiel des méthodologies.

Un bilan peut donc être envisagé après maintenant 30 ans de mise en application.

2) LES CONTROLES

a) Contrôles documentaires

Ils permettent le suivi administratif du matériel végétal afin de s'assurer de la qualité et de la quantité du matériel mis en oeuvre, que ce soit au niveau des boutures utilisées pour la fabrication des plants ou des plants utilisés pour la réalisation des plantations de vignes mères.

b) Contrôles visuels

A la suite de la plantation des vignes mères, un contrôle visuel permet de s'assurer de l'exactitude du plan de la parcelle et de l'identité variétale, et les contrôles ultérieurs permettront de s'assurer de l'absence de symptômes des maladies.

Chacun connaît les faiblesses de l'observation des symptômes, mais l'expérience a montré que les contrôleurs connaissant bien les cépages sont susceptibles d'apprécier avec beaucoup de sensibilité des manifestations anormales qui permettent d'écarter momentanément ou définitivement les souches ou les parcelles en cause. L'observation de symptômes en pépinière est plus aléatoire, car leur présence est en général synonyme d'infestation massive.

Ces contrôles restent nécessaires notamment vis à vis de l'identité variétale, de l'importance des lots, et pour veiller aux erreurs de manipulation.

c) Les contrôles par tests sanitaires

Le développement en laboratoire des tests ELISA, l'amélioration de leur fiabilité et de leur sensibilité, la facilité de leur emploi, ont permis leur mise en application pratique comme techniques de contrôles de routine :

Après une période expérimentale une méthodologie a été retenue et les tests sanitaires sont maintenant utilisés en France depuis 1990 à grande échelle par le service de contrôle ONIVINS, pour les viroses du court noué (GFLV et AMV). Et un travail préparatoire est en cours pour une extension éventuelle à l'enroulement mais cette dernière maladie possède l'avantage de bien extérioriser les symptômes à condition d'effectuer les contrôles en fin de végétation, et les tests sérologiques ne sont pas satisfaisants pour tous les sérotypes de cette maladie (GLRV).

3) METHODOLOGIE DES CONTROLES SANITAIRES

Les contrôles sont effectués avec une méthodologie différente selon la catégorie du matériel végétal considéré

a) matériel initial

A l'intérieur des établissements de sélection, le matériel initial du clone mis en conservatoire fait l'objet d'un prélèvement sur feuille et sur bois après vérification de son identification et de sa localisation.

Les tests sont réalisés sur les prélèvements de feuilles d'une part (court noué) et sur boutures (ensemble des viroses pour lesquelles le clone a été testé).

S'agissant d'analyses lourdes, peu de clones sont analysés chaque année : ils sont choisis selon la suspicion concernant leur descendance ou de façon aléatoire.

b) matériel de base

Après une période pendant laquelle les tests ont porté sur les plants mis en pépinière, il s'est avéré plus judicieux d'effectuer l'inventaire sanitaire de l'ensemble des vignes mères productrices de matériel de base.

Ainsi, l'ensemble de ces parcelles a fait l'objet de prélèvements exhaustifs à raison d'une feuille par souche, une analyse étant effectuée en regroupant 20 feuilles maximum correspondant à 20 souches consécutives.

Il est tenu un plan précis des prélèvements, le nombre de 20 feuilles a été retenu après expérimentation par l'INRA de Colmar, celle-ci ayant démontré que le prélèvement d'une feuille sur une souche contaminée était suffisant pour rendre le test positif malgré la présence de 19 autres feuilles provenant de pieds sains.

Les lots ayant fait l'objet de réactions positives font l'objet d'un deuxième prélèvement où sont testés les souches une à une afin de s'assurer de la densité de la présence de la maladie.

Compte tenu de la densité moyenne de la plantation le testage d'un ha de vignes de prémultiplication nécessite le prélèvement de 4000 feuilles en moyenne qui nécessiteront 200 tests sanitaires ELISA.

Il est envisagé de renouveler cet examen sanitaire avec une périodicité de 5 à 10 ans.

c) Vignes mères productrices de matériel certifié

Compte tenu de la différence importante des surfaces, le testage souche par souche n'est pas possible du fait du coût qui deviendrait prohibitif.

Durant l'été 1994 les parcelles productrices de matériel de base couvrent respectivement 48.23 ha pour les vignes mères de porte greffe et 66.31 ha pour les vignes mères greffons contre respectivement 2237.04 ha et 1700,23 ha pour les vignes mères productrices de matériel certifié.

La première approche consiste à déterminer l'identité des parcelles qui doivent être testées en priorité : il s'agit de faire une évaluation du risque en s'appuyant sur des considérations telles que l'âge de la parcelle, la pression de recontamination liée à l'environnement (état sanitaire des parcelles du voisinage, érosion possible ...), ou le doute pesant sur l'état sanitaire des plants mis en oeuvre, ou celui des plants produits à partir de celle-ci..

Ces parcelles sont soumises à une pression de contamination plus importante que celles produisant du matériel de base, du fait de précautions moins importantes au moment de leur plantation (durée du repos du sol et isolement moins sévères). Certaines peuvent même montrer des symptômes. La première opération consiste donc à parcourir rapidement la parcelle afin de s'assurer de la présence ou non de symptômes. Dans ce cas et afin de confirmer l'observation visuelle, un test par souche présentant des symptômes est effectué, à raison de 6 à 10 feuilles prélevées sur chacune. La probabilité d'avoir une réaction positive est très élevée, et le nombre de souches à prélever est limité, les décisions quant à l'avenir de la parcelle étant facile à prendre.

Dans les parcelles ne présentant pas de symptômes, les prélèvements sont effectués sur des rangs déterminés, selon la densité de plantation. Lorsque les rangs sont peu espacés (1 m), à raison de un rang sur trois, ou un rang sur deux pour des écartements de 1,5 à 3 mètres, ou sur chaque rang en cas de vigne à plus de 3 m d'écartement. Sur chaque rang prélèvement systématique d'une feuille une souche sur trois, ce qui correspond à une souche au moins pour 10 m² environ. Chaque feuille est ensuite groupée par 20 au maximum

d) Environnement

Les éventuelles parcelles de vigne implantées à proximité immédiate, en dehors de la zone d'isolement, mais en bordure de celle-ci, font également l'objet de prélèvements, qu'elles aient été constituées avec des plants certifiés ou standard. De même, lorsque les tests visent une parcelle clonale incluse dans une parcelle en comprenant plusieurs, de variétés identiques ou différentes, les parcelles élémentaires voisines font l'objet d'un prélèvement.

Ainsi, toute présence de l'agent infectieux, dans la parcelle, ou dans son environnement immédiat doit pouvoir être détecté avec une bonne probabilité.

e) Discussion

Des tables statistiques permettent de déterminer le nombre de souches à prélever connaissant le taux de recontamination que l'on cherche de mettre en évidence.

Cependant ces tables s'appuient sur des prélèvements au hasard d'une part, et une répartition aléatoire de la maladie d'autre part.

Or au vignoble les souches sont présentées par rangs et un prélèvement aléatoire est pratiquement impossible à effectuer, alors que procéder d'une manière systématique avec un pourcentage précis permet de bien quadriller les parcelles. Par ailleurs l'expérience montre que la maladie touche en général des souches groupées, ce qui tient au mode de dissémination.

Un certain nombre de précautions doivent être prises : les feuilles doivent être ramassées sans rosée et en évitant des températures élevées.

Les prélèvements sur bois aoûté ou sur rameaux sont possibles, mais ils ne permettent pas d'avoir la même efficacité relativement au nombre de ceps échantillonnés, par test unitaire.

f) Suites données

La mise en évidence de la présence de la maladie dans une parcelle productrice de matériel de multiplication végétative ne nous permet plus de l'utiliser à cette fin.

Il est relativement aisé de radier du contrôle et de ne plus admettre la récolte de greffons sur une vigne mère lorsque celle-ci peut continuer à être utilisée comme vigne à fruit, la récolte de raisins étant en général suffisante pour amortir la plantation et son coût d'entretien.

Par contre pour les vignes mères de porte greffe, aucune production autre que celle des boutures n'étant envisageable, la seule issue est l'arrachage.

4) QUELQUES RESULTATS

a) matériel initial

Peu de contrôles ont été réalisés à ce jour mais aucun n'a donné de réponse positive.

b) matériel de base

Depuis 1990 l'ONIVINS s'est engagé dans un programme de tests important qui a permis la réalisation sur 5 campagnes de végétation de plus de 30000 tests.

L'intégralité des parcelles productrices de matériel de base a été testée ainsi que leur environnement ; moins de 1 ha de parcelles a été trouvé réellement contaminés, mais plus d'une dizaine d'ha (5,30 ha pour les vignes mères de greffons et 6,30 ha pour les vignes mères de porte greffe) ont été éliminés de la production du matériel de multiplication, une grande sévérité concernant l'état sanitaire de la proximité a été retenue du fait du caractère multiplicateur qui serait observé à la génération suivante (production de matériel certifié). En relation avec l'ENTAV, établissement de sélection et conservatoire du matériel initial, des contrats sont passés avec les prémultiplicateurs afin d'effectuer un suivi régulier de l'état sanitaire par prélèvements et il est envisagé une évolution technique permettant d'assurer plus de garanties :

- en limitant la durée d'admission au contrôle de ces parcelles
- en réduisant la surface unitaire
- en encourageant une production hors sol, par production herbacée ou par cultures hydroponiques

c) matériel certifié

Seulement les parcelles jugées prioritaires ont fait l'objet du travail engagé lors de ces premières campagnes. Les résultats ne sont donc pas significatifs de l'ensemble de la surface des vignes mères puisque les parcelles jugées sans risque ont été écartées de cette première approche.

Les surfaces concernées couvrent environ 220 ha, dont 120 ha de vignes mères de greffons et 100 ha de vignes mères de porte greffes, ainsi que leurs abords immédiats.

En cumulant les tests affectés au premier passage et ceux correspondant à la confirmation de la localisation, le taux de réaction positive varie entre 1,5 et 3,1 % selon les années, en cumulant les deux virus AMV et GFLV. Pour les parcelles se révélant contaminées présentant des taux de contamination très variables, qui se situent entre moins de 1 pour mille, et 2 à 3 % des souches. Ces résultats ont conduit à éliminer de la multiplication 40 ha soit jugés contaminés, soit par précaution, l'environnement immédiat ayant réagi : 20 ha de vignes mères de greffons ont été écartés et 20 ha de vignes mères de porte greffes ont dû être arrachés..

d) Sources de contamination

Des qu'un résultat positif est trouvé dans une parcelle, en dehors de la sanction qui frappe celle-ci, est menée une enquête tendant à trouver une explication de la contamination.

Les raisons évoquées sont par ordre de priorité :

- recontamination par le sol même après désinfection lorsque la durée de repos du sol était insuffisante, derrière une culture de vigne ou ancienne friche partant de la vigne :

La durée réglementaire de 6 ans ou 12 ans montre les limites de la désinfection, essentiellement par rapport à une culture de vigne.

- érosion ou transport de terre ayant apporté sur le sol de la parcelle des nématodes contaminants.

La position géographique de la parcelle vis à vis de son environnement est très importante.

- remplacement de manquants effectués avec des plants dont l'origine est douteuse : une vigilance accrue est à apporter sur ce point précis en particulier dans les cas de reprise difficile de la plantation, la totalité des plants utilisés devant appartenir à la catégorie matériel de base.

- erreur de manipulation ayant entraîné un approvisionnement en plants contaminés, mélangés ou substitués à la fourniture prévue. Ce cas a été confirmé par la présence de mélanges variétaux.

5) CONCLUSION

La mise en oeuvre de tests sanitaires a posteriori, autorisée par le développement des techniques de laboratoires permettant sur un échantillonnage précis, et avec un coût acceptable d'obtenir avec une bonne fiabilité des résultats prouvant la présence de l'agent infectieux dans les vignes productrices de matériel de multiplication, s'avère un outil complémentaire, précieux pour les organismes de contrôle de la qualité du matériel végétal. La méthode utilisée en France a privilégié les tests réalisés sur les vignes mères plutôt que ceux réalisés en pépinière ; ces tests permettent d'écarter de la multiplication les parcelles jugées douteuses et donc d'assurer pour la production de plants un état sanitaire qui sans prétendre être absolu, ~~puisque la perfection demeurera impossible~~, permet toutefois une amélioration significative par rapport aux risques bien réels de la recontamination sur le terrain.

D'ores et déjà, des conclusions sont tirées de ces tests compte tenu des surfaces importantes qui en ont fait l'objet et qui tendent à modifier le contenu du cahier des charges et la réglementation de certification dans le sens d'une plus grande rigueur sur le choix des sites propres à l'implantation des parcelles de multiplication, une limitation de leur surface, une attention particulière à la présence ou à l'absence de vignes lors des années qui ont précédé la plantation de ces parcelles, leur situation géographique, et la présence dans l'environnement de plantes hôtes éventuels de nématodes vecteurs. Une réduction de la durée d'exploitation de ces parcelles est également envisagée.

En tout état de cause, la production hors sol doit être encouragée et si elle n'est pas envisageable à grande échelle pour des raisons pratiques et économiques pour la production de matériel certifié, elle devrait être envisagée pour la production de matériel de base.

M. LEGUAY 25/08/94

SESSION 5 : CLONAL SELECTION

CHANGES IN FIELD PERFORMANCES OF CLONES OF THE GRAPEVINE CV NEBBIOLO AFTER VIRUS ELIMINATION BY HEAT THERAPY*

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Summary: In order to better clarify the influence that the presence of GFLV, GFKV, GLRaV I and III and GVA may have on vine morphology, field performances and grape quality, two trials have been carried out comparing the progeny behaviours of original virus-infected and heat-treated virus-free clones of 'Nebbiolo'. When the sanitation involved GLRaVs and GVA the changes in vine performances were not evident. Sanitation induced an increase in vegetative vigour and in total berry skin anthocyanin amount, but all the other parameters (yield, soluble solids, acidity) were not affected. Regardless of the virological status, a strong clonal influence on vine field performances has been detected. When sanitation involved GFLV and GFKV, the virus elimination resulted in a dramatic modification of bud burst, vigour, leaf surface, cane internode length and yield that were all increased. Despite the much heavier crop only juice acidic parameters were higher, meanwhile the soluble solids were not modified. GFLV elimination induced a strong leaf morphology modification, but shoot malformations, often considered as a symptom of GFLV, were still present in the GFLV-free material.

Key words: grape, clone, virus, heat-therapy, morphology, yield, must quality, phenolic compounds.

Introduction

'Nebbiolo' is regarded as one of the best Italian grapevine cultivars in terms of wine quality. Unfortunately, virus symptom observations and indexing, intensively carried out during a clonal selection project, indicated that in the 'Nebbiolo' population there is a widespread presence of grapevine leafroll (GLR), rugose wood and grapevine fanleaf virus (GFLV). The GFLV occurrence is particularly high (100 %) in 'Nebbiolo Michet', which is regarded by local growers as the biotype of highest quality. Consequently, the use of virus-free clonal material of 'Nebbiolo' is becoming more and more common and in the case of the biotype 'Nebbiolo Michet' artificial sanitation (usually by heat therapy) is needed to obtain GFLV-free material.

In recent years some grower concerns arised about the risk that the quality of the grape obtained by virus-free clonal vines, reputed more vigorous and yielding than the original ones, could be reduced. So far, experimental data on this subject are limited.

It is generally reported that the virus-free status induces an increase of vine vigour and, to a lesser extent, yield. In a warm climate these modifications of vegetative parameters did not penalized the berry juice composition, i.e. soluble solids and acidity (Woodham et al. 1984a,b; Conradie et al., 1989; Mc Carthy, 1988). In this respect, more doubtfull are the results obtained in cool climate area, such as in many areas of Europe (Basler and Brugger, 1981; Grenan e Valat, 1992; Balthazard, 1993; Mannini et al., 1993; Mannini et al., 1994). In addition, a negative correlation between vegetative vigour and grape or wine quality has been frequently reported (Smart et al., 1985; Schneider et al., 1989; Morrison and Noble, 1990), so it could be expected that the virological status affecting the vegetative vigour may have a similar negative effect.

In order to better clarify the influence that the presence of viruses and namely grapevine fanleaf virus (GFLV), grapevine fleck virus (GFKV), grapevine leafroll-associated closteroviruses (GLRaV I and III) and grapevine virus A (GVA) may have on vine morphology, field performances and grape quality, two trials were carried out comparing the progeny behaviours of original virus-infected and heat-treated virus-free clones of 'Nebbiolo' and 'Nebbiolo Michet'.

* Contribution no. 305 of the Centro Miglioramento genetico e Biologia Vite, CNR, Torino.

Material and methods

Virus infected clones of 'Nebbiolo' and 'Nebbiolo Michet' were heat-treated in a thermotherapy chamber with artificial lighting at about 37°C for periods varying between 70 and 140 days followed by in vitro

culturing of 0,5 cm terminal shoot tip explants. Established daughter vines were repeatedly indexed for infectious diseases by ELISA for GFLV, GFKV, GVA and GLR-associated viruses (GLRaVs). The virological status of the original (MP) and heat-treated (HT) material of two clones of 'Nebbiolo' and three clones of 'Nebbiolo Michet' is shown in tables 1 and 2.

Both original and heat-treated material was propagated on virus-certified rootstocks and experimental vineyards with a complete randomized block design were established in the years 1989 and 1990 at Barolo and Barbaresco (northwest Italy). Both of the vineyards are on hillsides and the vines are single cane-pruned (Guyot) and vertical trained. The soils are loamy with a pH of 7.8 and an average total carbonate content of 20%. Spacing is 3.00x0.80 m for 'Nebbiolo' and 2.50x0.70 m in the case of 'Nebbiolo Michet'.

Three and two year data respectively for 'Nebbiolo' (1991-93) and 'Nebbiolo Michet' (1992-93) on the main agronomical parameters (pruning weight, potential fertility, yield, number of clusters per vine and mean cluster weight) and must composition (soluble solids, pH, titratable acidity, tartaric and malic acid, potassium) have been collected. Tartaric and malic acids were measured by HPLC and cations by atomic absorption spectrophotometry.

At the harvest (in 1992-93 for 'Nebbiolo' and only in 1993 for 'Nebbiolo Michet') samples of 300 berries were collected from each plot and skin phenolic content (mg/kg of berries) determined by spectrophotometry (Di Stefano, Cravero, 1991). Total flavonoids and non anthocyanic flavonoids were expressed as (+) catechin and anthocyanin pigments as malvine. In addition, some phenological parameters such as the bud burst index (after Eynard et al., 1978) and the % of veraison were recorded.

In 1993, sampling of 130 adult leaves for each treatment (MP and HT) was carried out on the vines of a 'Nebbiolo Michet' clone and the main phyllometric parameters measured by using a computerized graphic digitizer (Schneider, Zeppa, 1988). The phyllometric parameters most characterizing the leaf morphology were chosen according to Galet (1985) and Schneider (1988), as shown by figure 1. On the same vines, shoot characteristics such as average internode length and diameter, and the frequency of some aspects reputed to be virus symptoms like shoot fasciations, shoot double buds and cane blind buds were recorded.

All data were processed by analysis of variance (ANOVA) and the phyllometric data also by MANOVA.

Results

When the sanitation involved GLRaVs and GVA, as in the case of 'Nebbiolo', despite the fact the original infected plants showed typical and evident leaf rolling and reddening and the vegetation of the heat-treated plants was totally symptomless (although the clone A remained infected by GVA) changes in vine performance were not very evident. Sanitation (even partial) induced a slight but significant increase in vegetative vigour as shown by higher winter pruning weight (tab. 3). All the other parameters (yield, soluble solids, acidity) examined over a three year period, were not affected (tab. 4), except for phenolic compounds. GLRaVs elimination, in fact, seems to have a profitable effect on the grape phenolic profile increasing the amount of berry anthocyanins (tab. 5). The result could be related to the significant anticipation of veraison for the heat-treated clones compared to the original ones. Regardless of the different virological status, a strong clonal influence on vine field performances has been detected (tab. 3 and 4), and the virus presence seems only to mask the clonal differences although no significant interaction has been found.

When sanitation involved GFLV (and GFKV), as in the case of 'Nebbiolo Michet', two years of data show that the virus elimination resulted in a dramatic modification of vigour and yield (tab. 6-8). In terms of vigour, the virus-free vines showed an amazing increase in all the parameters measured: leaf area, shoot internode length and diameter and winter pruning weight. In addition, bud burst was earlier and the numbers of blind buds (quite frequent in the 'Nebbiolo Michet' biotype) reduced compared to the non-treated vines. In terms of yield, the increase was important (1/3 more in virus-free vines) and determined by heavier clusters and, even more, by better bud break on the cane (and the consequent increase of bearing shoots per vine). The potential fertility per shoot, i.e. number of clusters per shoot at bloom, in fact, was slightly lower in the heat-treated material probably as a consequence of increased vegetative growth. Despite the much heavier crop, only juice acidic parameters were modified, not the amount in soluble solids that was practically identical in both infected and virus-free vines (tab. 7). Titratable acidity, tartaric and malic acids were higher, and the pH lower in the juice of virus-free material. This result is not necessarily a penalization of quality for grapes like 'Nebbiolo', which give origin to wines that require a period of ageing before release on the market. Moreover, a good acidity, maintaining a correct tartaric acid/malic acid ratio, may better contribute to the structure of wines like Barolo and Barbaresco (100% 'Nebbiolo' grape) characterized by an important presence of alcohol (over 13^o), glycerol and polyphenols. With regard to phenolic profile the preliminary results (only one year of observations on few replications) seem to confirm a beneficial effect of sanitation (as already seen with GLRaVs) although further investigations are needed.

GFLV elimination induced strong leaf morphology modifications: dimensions, shape, lateral sinus depth, tooth height and petiolar sinus opening (tab. 9). These results confirm the suspects that several ampelographical characteristics that grapegrowers consider typical of the 'Nebbiolo Michet' biotype are related to GFLV infection more than genotype. On the contrary, shoot malformations (fasciations), often considered as a symptom of GFLV, were still present in the GFLV-free material (tab. 8). Yet the shoot double buds, highly present in the infected material, were practically absent in the heat-treated plants.

Conclusions

In general, the results obtained under our experimental conditions show that GFLV elimination induced a strong modification of vine behaviour from the earliest years of vineyard life, while the closterovirus (GLRaVs) sanitation had a weaker effect and, so far, only a few parameters were influenced.

In the first case, the changes in field performances, particularly the increase in vigour and yield, has been so evident that the heat-treated material must be carefully monitored before release to the nurseries in quality-wine cool-climate areas like northwest Italy. Although the quality of grapes was not reduced in our trial, growers must be aware that the changes in vine vegetative behaviour of GFLV-free 'Nebbiolo Michet' clones will involve necessarily a modification of vineyard management in the new plantings. Row spacings, winter and green pruning, grape thinning and so on, shall be properly modified to better fit the potentiality of GFLV-free material.

In the case of GLRaVs, sanitation has induced only a slight increase of vigour and an improvement of the amount of berry skin phenolic compounds, particularly anthocyanins. This effect on phenolic profile, if confirmed, could have great importance for the quality of red wines.

Clonal (i.e. genetic) differences in agronomical performances have been detected regardless of the virological status, although phyllometric evaluations have evidenced that some ampelographical characteristics so far considered clonal are, on the contrary, related to GFLV infection.

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Tab. 1 - Virological status of original (MP) and heat-treated (HT) 'Nebbiolo' clones (A and B).

CLONE	ELISA				
	GFLV	GLRaV I	GLRaV III	GVA	GFKV
A MP	-	+	-	+	-
A HP	-	-	-	+	-
B MP	-	+	+	+	-
B HT	-	-	-	-	-

Tab. 2 - Virological status of original (MP) and heat-treated (HT) 'Nebbiolo Michet' clones (C, D and E).

CLONE	ELISA			
	GFLV	GLRaVs I and III	GVA	GFKV
C MP	+	-	-	+
D MP	+	-	-	+
E MP	+	-	-	+
C HT	-	-	-	-
D HT	-	-	-	-
E HT	-	-	-	-

Tab. 3 - Agronomical data of original virus-infected (MP) and heat-treated (HT) 'Nebbiolo' clones (A and B). Averages 1991-93.

DATA	CLONE A		CLONE B		Signif. F	
	MP	HT	MP	HT	clone	treat.
Pruning weight (g/vine)	752	1088	895	1034	n.s.	**
Potential fertility cluster no./shoot	1.19	1.36	1.02	1.04	***	n.s.
Yield (g/vine)	2179	2347	1629	1628	***	n.s.
Cluster no./vine	8.81	9.19	7.66	6.75	**	n.s.
Average cluster weight (g)	239	254	211	239	*	n.s.

n.s.= not significant; * p<0.05; ** p<0.01; ***p<0.001

Tab. 4 - Must composition of original virus-infected (MP) and heat-treated (HT) 'Nebbiolo' clones (A and B). Averages 1991-93.

DATA	clone A		clone B		Significance F	
	MP	HT	MP	HT	clone	treat.
Soluble solids (°Brix)	21.43	21.50	21.42	21.64	n.s.	n.s.
Titrateable acidity (‰)	8.12	8.51	7.74	7.70	***	n.s.
pH	3.09	3.10	3.13	3.12	n.s.	n.s.
Tartaric acid (‰)	6.49	6.28	6.08	6.30	n.s.	n.s.
Malic acid (‰)	3.92	3.77	3.79	3.62	n.s.	n.s.
Potassium (‰)	1.22	1.22	1.36	1.35	**	n.s.

n.s.= not significant; ** p<0.01; ***p<0.001

Tab. 5 - Berry skin phenolic profile of original virus-infected (MP) and heat-treated (HT) 'Nebbiolo' clones. Averages 1992-93.

	MP	HT	Signif. F
Total flavonoids (mg/kg)	1024	1154	n.s.
Anthocyanins (mg/kg)	717	825	**
Non anthocyanic flavonoids (mg/kg)	566	635	n.s.

n.s.= not significant; ** p<0.01

Tab. 6 - Agronomical data of original virus-infected (MP) and heat-treated (HT) 'Nebbiolo Michet' clones (averages 1992-93).

	MP	HT	Signif. F
Pruning weight (g/vine)	514	1154	***
Potential fertility cluster no./shoot	0.85	0.62	**
Yield (g/vine)	1087	1563	*
Average cluster weight (g)	255	291	n.s.
Cluster no./vine	4.50	5.55	n.s.

n.s.= not significant; * p<0.05; ** p<0.01; ***p<0.001

Tab. 7 - Must composition of original virus-infected (MP) and heat-treated (HT) 'Nebbiolo Michet' clones (averages 1992-93).

	MP	HT	Signif. F
Soluble solids (°Brix)	23.64	23.62	n.s.
Titratable acidity (‰)	8.00	9.33	**
pH	3.13	3.06	**
Tartaric acid (‰)	6.63	7.41	*
Malic acid (‰)	2.58	3.41	**
Total flavonoids (mg/kg)	1428	1602	-
Anthocyanins (mg/kg)	1011	1198	-
Non anthocyanic flavonoids (mg/kg)	832	897	-

n.s.= not significant; * p<0.05; ** p<0.01; • = 1993

Tab. 8 - Leaf and shoot parameters of vines obtained from a virus-infected 'Nebbiolo Michet' clone (MP) and from its heat-treated progeny (HT).

	MP	HT	Signif. F
Shoot internode length (cm)	10.1	13.5	***
Shoot diameter at 50 cm of height (mm)	8.23	8.94	**
Leaf area (cm ²)	141	197	***
Bud burst index	5.71	6.46	**
Blind buds (%)	32.1	22.3	*
Shoot fasciations/vine	6	5.6	n.s.
Shoot double buds/vine	7	0.2	***

n.s.= not significant; * p<0.05; ** p<0.01; ***p<0.001

Tab. 9 - Main phyllometric parameters of original virus-infected (MP) and heat-treated (HT) 'Nebbiolo Michet' clone.

Phyllometric parameters	F Value	Signific. F
Length x Width (cm ²)	64.66	***
Length/Width	17.76	***
OP/ON1	121.45	***
ON2/ON1	9.00	**
ON4/ON1	12.92	**
OS/ON2	1.10	n.s.
OI/ON3	5.72	*
Angle α (rad)	8.61	**
Angle ϵ (rad)	15.01	***
Angle τ (rad)	51.22	***
Angle δ (rad)	0.90	n.s.
Angle π (rad)	171.39	***
TH/TB	22.65	***
MANOVA (all parameters)		***

n.s.= not significant; * p<0.05; ** p<0.01; ***p<0.001

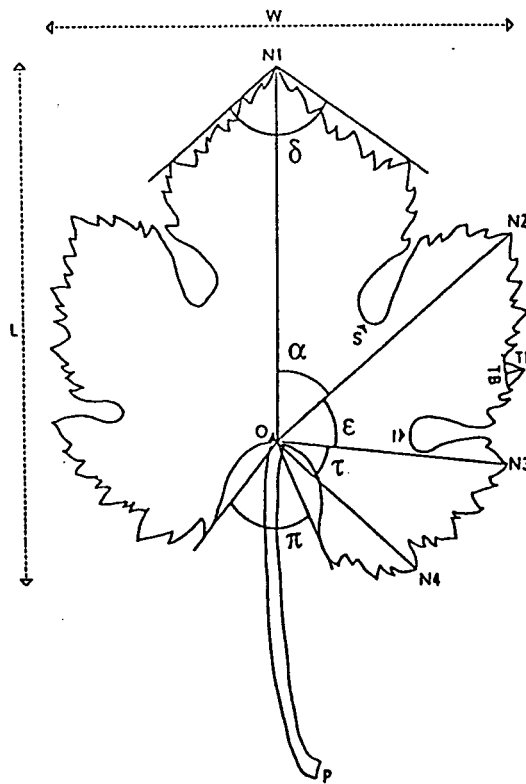


Fig. 1 - Leaf parameters measured for phyllometric evaluations.

ROOTSTOCK EVALUATION FOR LOW JUICE pH

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Abstract

The pH of grape juice or wine is a the major quality factor. Research has demonstrated that rootstocks can affect grape juice pH by changing grape juice K⁺ concentration. Field and glasshouse studies indicated that this effect of rootstocks on scion grape juice pH and K⁺ levels originate from different K⁺ take rates of rootstock varieties. At least with the varieties tested, these genotypic differences in K⁺ uptake only occurred at high levels of K⁺ supply, and not at low levels of K⁺ supply. This indicates that like in the roots of most other plant species, a dual K⁺ uptake mechanism exists in roots of grapevines, and that only the mechanism operational at high K⁺ levels is different in the rootstock varieties studied. It therefore appears feasible to breed rootstock varieties which combine high K⁺ efficiency at low K⁺ supply with restricted K⁺ uptake at high supply. This model is currently verified under German conditions on a range of rootstock varieties.

Introduction

The choice of rootstocks is commonly based on their resistance against phylloxera and nematodes, their suitability to soil types and the effect on vigour and fruitfulness of the scion. The use of rootstocks to affect wine quality is not part of the decision making process due to the lack of knowledge of the interaction between rootstocks and certain quality parameters. In particular grape juice pH or wine pH appears to depend on the chosen rootstock. Hale (1977), Hale and Brien (1978) and Cirami et al. (1984) have each reported significant rootstock effects on grape juice pH and composition. These studies have shown that grapes of the varieties Sultana and Shiraz grafted on to rootstocks like the *Vitis champinii* varieties Dog Ridge or Ramsey have higher grape juice pH than ungrafted vines of these varieties.

From 1985 to 1991 the physiological basis for the rootstock effect on scion grape juice pH was investigated in order to develop a screening method for early selection of rootstock hybrids. This paper represents a summary of the various aspects of these studies (Ruhl 1989, 1990, 1991, 1992a, 1992b, Ruhl et al., 1988, Ruhl and Walker, 1990) and an outlook on the rootstock work currently conducted in this field at Geisenheim.

Rootstock effects on grape juice composition of several scion varieties

Berry samples were collected at harvest from rootstock trials involving both Shiraz (Syrah) and Chardonnay at Nuriootpa (South Australia) and Ruby Cabernet at Loxton (South Australia). Grape juice extracted from these berry samples was analysed for pH, total soluble solids (Brix), potassium (K), sodium (Na), chloride (Cl), tartrate and malate (Ruhl et al. 1988).

Rootstocks in each trial significantly affected scion grape juice composition and pH (Table 1). Ungrafted vines of Ruby Cabernet and Shiraz produced grapes with low pH, while ungrafted Chardonnay vines produced grapes with a pH higher than that of grapes from grafted vines. This strongly indicated that the use of rootstocks does not necessarily lead to higher must pH. Comparisons between the rootstock varieties used in this study revealed that some rootstocks were associated with much higher scion grape juice pH than others: scion varieties on *Rupestris du Lot* (*Rupestris* St. George), and in particular on the *Vitis champinii* selections Dog Ridge, Harmony and Freedom had high scion grape juice pH, while scion varieties on 1202C, 5A Teleki, SQ4, Richter 110, 101-14 and 140 Ruggeri (140R) had low grape juice pH readings.

A link between potassium concentration and pH of grape juice has been reported by several investigators (Wejnar 1971, Mattick et al. 1972, Somers 1975 and Morris et al. 1980). The observations from the trials at Nuriootpa and Loxton support these findings, showing a correlation between pH and potassium concentration in grape juice of scions grafted on different rootstocks. It can therefore be concluded that the rootstock effect on grape juice pH originates from different potassium concentrations in scion grape juice.

The data (Table 1) also indicated that grape juice with high pH and high K⁺ contents generally had increased malate concentrations, which is in agreement with observations reported by Hale (1977).

Potassium uptake of rootstock varieties in relation to their pH effect

To identify the physiological reasons for the rootstock effect on grape juice K⁺ concentration, K⁺ concentrations in the petioles (opposite bunches collected at flowering) of ungrafted rootstocks in the germplasm collection of the Division of Horticulture at Merbein were analysed to estimate K⁺ uptake and/or root-to-shoot transport of the various rootstock varieties (Ruhl, 1989). Comparing these data with grape juice pH readings of the Chardonnay rootstock trial at Nuriootpa and the Ruby Cabernet trial at Loxton gave in both cases significant correlations (Fig. 1), indicating that the higher scion grape juice pH readings of certain rootstock varieties appear to be the result of higher K⁺ uptake and/or root-to-shoot transport.

Potassium distribution in grapevine rootstocks

To investigate the physiological reasons for different K⁺ uptake and/or root-to-shoot transport, the rootstock varieties Ramsey, Dog Ridge and 140R were grown as ungrafted plants in nutrient solutions with three different levels of K⁺ supply: low = 0.1 mM, medium = 1.0 mM and high = 10 mM K⁺. Potassium concentrations were determined in apical and basal laminae, petioles and roots (Ruhl 1992b).

In the high K⁺ treatment Dog Ridge had the highest K⁺ concentrations in laminae and petioles, while 140R had the lowest laminae and petiole K⁺ concentrations (Table 2). Low K⁺ supply gave similar K⁺ concentrations in the roots of all three varieties. Root K⁺ concentrations in all three rootstocks increased as K⁺ supply rose, but was most pronounced in 140R roots (5.28 % K⁺ in the dry matter), compared with Dog Ridge (4.50 %) and Ramsey (3.80%). The levels in both Dog Ridge and Ramsey were not significantly different from the K⁺ concentrations in the medium K⁺ supply level. This indicates that most of the potassium taken up by the roots of Dog Ridge and Ramsey grown at high K⁺ supply was passed across the roots and translocated to the shoot, while in 140R some K⁺ was accumulated in the roots.

When these plants were being harvested, bleeding sap was collected from shoot cut-offs to determine the xylem sap K⁺ concentration. As a measure for the root vacuole sap concentration, sap was squeezed out of the root subsamples using a strong garlic press. The differences in K⁺ distribution between varieties in response to K⁺ supply are also reflected in the different K⁺ concentrations of expressed root and xylem sap (Table 3). No differences could be detected between the varieties in the xylem sap at low and medium K⁺ supply, but at the high K⁺ level Dog Ridge vines had the highest and 140R vines the lowest K⁺ concentrations in their xylem sap. In contrast, the highest K⁺ concentrations were measured in the expressed root sap of 140R vines.

At low and medium K⁺ supply (0.1 and 1.0 mM) K⁺ concentrations measured in the xylem sap were higher than K⁺ concentrations in the nutrient solution, indicating an active transport system. But at high K⁺ supply (10 mM) K⁺ concentrations observed in the xylem were lower, and in the case of 140R even 60 % lower. It can therefore be assumed that at such high levels of K⁺ supply no active transport is required and that K⁺ uptake is passive, possibly using K⁺-channels as proposed by Cheeseman and Hanson (1979) and Kochian et al. (1985) for corn roots. The existence of two different systems for K⁺ uptake at high and low levels of K⁺ supply could also explain, why uptake up to ca. 1 mM K⁺ supply is similar in 140R and *V. champinii* types like Dog Ridge and Ramsey, but different at higher K⁺ supply levels. The existence of two, more or less independent, systems of K⁺ uptake could provide breeders with the option of selecting rootstocks which combine high K⁺ uptake efficiency at low supply with restricted uptake at excessive K⁺ levels, and are therefore adaptable to a wide range of K⁺ supply.

Evaluation of rootstock hybrids

Based on this model and the close correlation between K⁺ concentration in petioles of ungrafted rootstocks growing in the field at Merbein and the effect of these rootstocks on grape juice pH (Fig. 1), in 1987/88 1200 hybrids were screened for K⁺ accumulation. Of these hybrids 120 with low K⁺ accumulation were selected and rooting and grafting studies conducted in 1989. Fifty promising hybrids, plus 5 standard rootstocks (Ramsey, Dog Ridge, Freedom, 140R and 1103P) were grafted with Shiraz and planted in a replicated field trial at a nematode infested site at Koorlong, Vic.

As part of the rootstock breeding program at Geisenheim, the effect of rootstocks on grape juice pH is currently being investigated and these findings will then be used for the screening of rootstock hybrids.

Acknowledgements

I thank M.G. McCarthy, R.M. Cirami and P.R. Nicholas from the Department of Agriculture South Australia and J.R. Whiting from Department of Agriculture Victoria for access to their rootstock field trials, Prof. Dr. Dr. h.c. H. Marschner, Institut für Pflanzenernährung der Universität Hohenheim and Dr. M. T. Treeby for valuable discussion, the staff of the Division of Horticulture at Merbein for their support and, in particular Mrs. April Fuda for her dedicated assistance during these studies. The Australian Grape and Wine Research Council provided financial support for this work.

Table 1: Effect of different rootstocks on the composition of grape juice

Rootstocks	pH	Berry wt (g)	Brix	K (mg/L)	Na (mg/L)	Cl (mg/L)	Tartrate (g/L)	Malate (g/L)
<i>Ruby Cabernet, Loxton</i>								
ungrafted	3.43	1.12	20.0	1.236	99	85	6.3	2.7
140R	3.43	1.31	20.1	1.291	87	36	5.9	3.8
101-14	3.44	1.40	21.6	1.314	92	32	5.7	4.2
Ramsey	3.47	1.36	19.0	1.318	90	39	5.8	4.6
K51-32	3.49	1.39	21.0	1.408	92	46	5.6	4.7
K51-40	3.49	1.39	19.7	1.424	90	71	5.9	4.6
Schwarzmann	3.56	1.27	20.1	1.424	101	39	5.9	4.1
Freedom	3.56	1.24	21.1	1.505	113	89	5.7	4.0
sd (P=0.05)	0.06	0.09	1.1	98	14	21	0.5	0.4
<i>Shiraz, Nuriootpa</i>								
ungrafted	3.56	1.04	23.0	2.098	48	378	5.7	2.2
1.613	3.55	0.96	22.2	1.997	46	241	6.0	2.6
Ramsey	3.62	1.22	20.0	1.962	35	92	4.6	3.2
Schwarzmann	3.67	0.87	22.5	1.973	44	64	5.4	2.6
Harmony	3.75	1.10	22.6	2.200	99	117	4.7	3.2
Dog Ridge	3.75	1.07	21.0	2.496	78	320	4.6	4.3
sd (P=0.05)	0.10	0.13	1.7	246	44	96	0.6	0.4
<i>Chardonnay, Nuriootpa</i>								
ungrafted	4.01	0.82	23.7	2.395	90	401	3.3	1.9
1.202	3.71	0.94	24.7	1.665	182	206	3.6	1.5
SA Teleki	3.77	0.97	24.3	1.697	64	64	3.4	2.2
SO4	3.78	1.05	23.7	1.712	69	89	3.3	2.4
110 Richter	3.79	0.90	24.6	1.607	53	67	3.5	2.0
101-14	3.81	1.01	24.3	1.743	51	50	3.2	2.2
K51-32	3.82	1.03	24.9	2.020	32	138	3.0	2.8
1.616	3.83	0.95	25.8	1.782	87	85	3.3	2.1
5BB	3.83	1.03	24.3	1.876	58	50	3.6	2.3
Schwarzmann	3.84	0.98	24.7	1.732	87	60	3.5	2.2
Ramsey	3.85	1.11	24.0	1.821	101	117	3.3	2.5
ARG1 (AxR1)	3.90	0.81	25.5	2.231	120	330	3.2	1.5
420A	3.91	0.90	25.1	1.938	69	142	3.0	1.8
Harmony	3.91	1.03	24.9	1.981	184	67	3.8	2.8
Rup. du Lot	3.92	0.85	25.0	1.728	258	75	4.0	1.8
1.613	3.92	0.98	24.7	2.083	76	110	3.6	2.3
sd (P=0.05)	0.10	0.09	0.9	296	51	82	0.5	0.4

plant part	K ⁺ supply mequ/L	Potassium		
		Dog Ridge	Ramsey	140R
Apical Laminae	0.1	0.93 ^A	0.71 ^A	0.74 ^A
	1.0	2.17 ^{BC}	1.97 ^B	1.47 ^A
	10.0	3.39 ^E	2.74 ^D	2.41 ^{CD}
Basal Laminae	0.1	0.74 ^A	0.56 ^A	0.61 ^A
	1.0	1.87 ^{CD}	1.75 ^C	1.19 ^B
	10.0	3.16 ^F	2.53 ^E	1.90 ^{CD}
Petioles	0.1	1.38 ^A	0.52 ^A	0.82 ^A
	1.0	3.65 ^{BC}	3.99 ^{BCD}	3.33 ^B
	10.0	5.93 ^F	5.48 ^{EF}	4.75 ^{DE}
Root	0.1	0.94 ^A	0.55 ^A	0.73 ^A
	1.0	4.54 ^D	2.98 ^B	3.76 ^B
	10.0	4.50 ^{CD}	3.80 ^{BC}	5.28 ^E

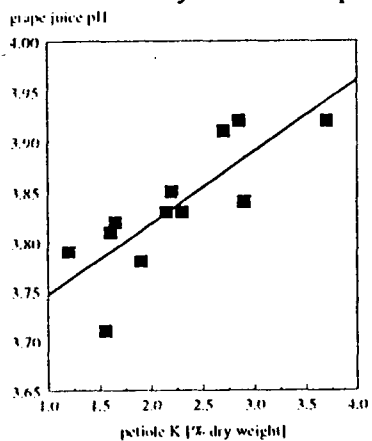
Table 2: Effect of different potassium supply on cation contents in plant parts of three different grapevine rootstock varieties. Data are means of five replicates each and expressed in % dry weight. Means followed by the same letter are not significantly different at P=0.05.

Table 3: Effect of different K⁺ supply on K⁺ content in nutrient solution, xylem and expressed root sap of three different grapevine rootstock varieties. Data are means of five replicates each and expressed in mequ/L. Means followed by the same letter are not significantly different at P=0.05.

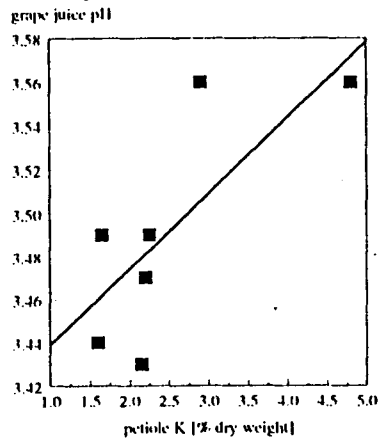
	K ⁺ supply in nutrient solution mequ/L	Rootstocks		
		Dog Ridge	Ramsey	140R
K ⁺	<i>EXPRESSED ROOT SAP</i>			
	0.1	7.3 ^A	4.7 ^A	9.7 ^A
	1.0	30.0 ^{BC}	25.2 ^B	42.7 ^E
	10.0	36.1 ^{CDE}	32.6 ^{BCD}	40.2 ^{DE}
K ⁺	<i>XYLEM SAP</i>			
	0.1	0.55 ^A	0.22 ^A	0.42 ^A
	1.0	2.08 ^B	1.87 ^B	1.53 ^B
	10.0	8.41 ^E	5.43 ^D	3.97 ^C

Fig. 1: Relationship between petiole potassium concentration of rootstocks in the germplasm collection at Merbein and the grape juice pH of the scion varieties Chardonnay and Ruby Cabernet. The asterisks indicate significant differences ($P < 0.05$)

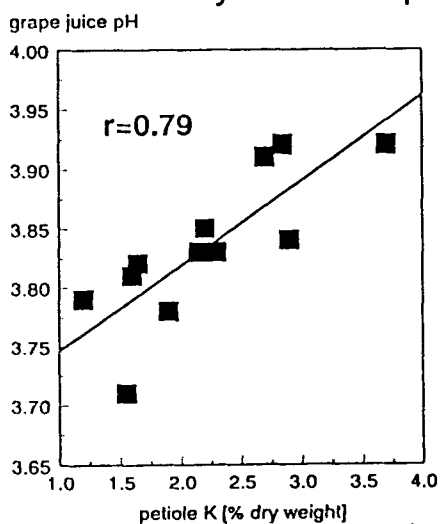
Chardonnay - Nuriootpa



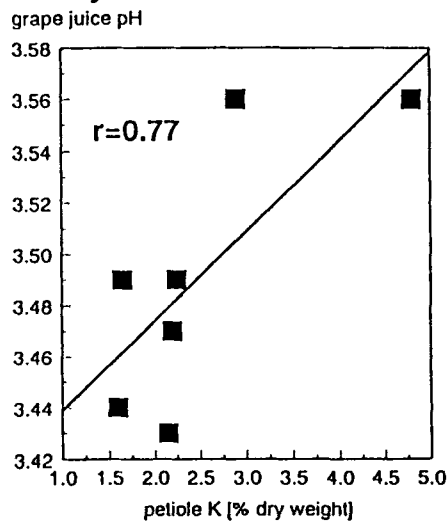
Ruby Cabernet - Loxton



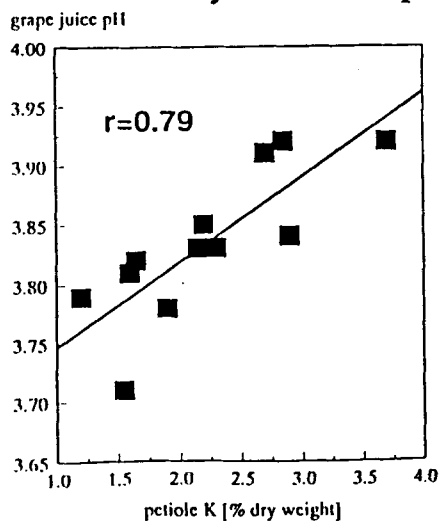
Chardonnay - Nuriootpa



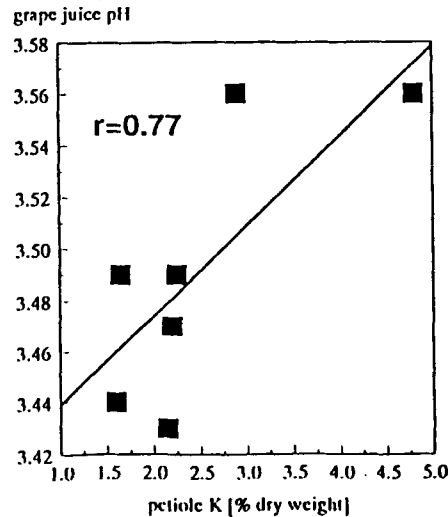
Ruby Cabernet - Loxton



Chardonnay - Nuriootpa



Ruby Cabernet - Loxton



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SESSION 5 : CLONAL SELECTION

EVALUATION OF THE CLONAL SELECTION THROUGH AN INTEGRATED STUDY OF THE PRODUCTION'S QUALITY IN SOME CULTIVARS OF *Vitis Vinifera*

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Abstract

Over 4 years (1990-93), several clones of 3 cultivars of *Vitis vinifera* were compared in order to identify an integrated method for evaluating the clonal selection quality. 13 clones of cv. Chardonnay, 9 clones of cv. Pinot noir, and 5 clones of cv. Sauvignon blanc selected in different areas were investigated.

All the clones were grafted on SO4 and grown at 250 m a.s.l. in the same farm.

During ripening time grapes were sampled weekly and sugar in berries was determined; at harvest time yield per vine was recorded. Clones per each variety were harvested together and vinified by using standard technology. Wines were sensorially analysed by not structured schedule after the definition of the characteristic aroma profile of the wines. In order to study the sugar accumulation in berries a quadratic model with plateau ($y = a + bx - eke$) has been applied and such a function resulted strictly significant for all varieties. Sugar accumulation rates (first derivative) allowed to characterize the clones. For each variety clones were grouped by Cluster Analysis.

The groups of clones obtained were related to wine sensorial data and a strong relation between both informations was showed.

The present work provides evidence to demonstrate that it is possible to mix viticultural and oenological data able to inform us about the adaptability of the specific clones to the environmental conditions. Moreover, the study of year variability allows to choose groups of clones to optimize the productive and organoleptic characteristics of the vineyard.

Key words: *Chardonnay*, *Pinot n.*, *Sauvignon b.* clonal selections, berry ripening modelling, wine sensorial evaluation.

Introduction

For many years the selection programs of clones aimed for increased yield and improved quality measured typically by the grape sugar levels. Yield, total soluble solids (TSS), pH, titrable acidity (TA) have been used to discriminate between clones (Whiting *et al.* 1990 and Ewart *et al.* 1993). With the constant need to improve wine quality, yield alone is insufficient, and must chemical composition only hints at the quality of the wine from these improved selections. Recently, in order to characterize the clonal properties it was proposed to model the sugar accumulation rate in berries (Stefanini *et al.* 1993b). It was opinion of the authors that the speed of sugar accumulation might affect not only grape characteristics but also wine quality.

Shoffling and Faas (1990) as well as Iacono *et al.* (1989) indicated that sugar level is insufficient as a selection criterion for wine quality, and they proposed microvinification and sensory analysis for wine evaluation. Iacono *et al.* (1990, 1993a) indicated that wines might be successfully evaluated through descriptive cards. Such a procedure allows to characterize the sensorial profile of the wines and the scores of each wine might be analysed by the most common statistical procedures. One of the problem encountered in establishing clonal differences as measured by wine quality is the variability introduced by the judge assessment. As previously described (Iacono *et al.* 1993b) judge variability may be reduced by data standardization that allows to consider as 1 and ± 1 respectively the mean and the standard deviation of each scoring.

This paper examines the chemical analysis of must, the parameters of berry ripening models, crop per vine, and sensory characteristics of wines from several clonal selections of *Chardonnay*, *Pinot n.*, and *Sauvignon b.* or. over 4 years. A method to integrate agronomic and oenological knowledges is investigated and proposed.

Materials and methods

Experimental design

Over 4 years (1990-1993) 13 clonal selections of cv. Chardonnay, 9 of Pinot noir, and 5 of *Sauvignon blanc* grafted on SO4 have been investigated (Tab. I). The vineyards were located at 250 m a.s.l. in the experimental farm of the Istituto Agrario di S. Michele all'Adige where all vine training, irrigation, and spraying operations were done by the grower as part of routine procedures on the remainder of the vineyard. *Chardonnay*

and *Sauvignon* or. vines were trained on a one armed "Trentino Pergola", whilst *Pinot n.* vines on Guyot. A randomised block design of 6 replicates of 8 vine plots was used.

During ripening time, a weekly sample of 400 g of berries each was picked in order to study sugar accumulation rate in berry. Yield per vine was measured on 6 plants and clones were harvested together. The grapes were processed by the standard techniques for small-lot winemaking used at the experimental winery of the same institute.

Measurements

Most analyses were carried out on samples after pressing and included TSS concentration (measured as "Brix"), TA, and pH, but in the present paper only data about sugar are considered. All wines were assessed prior to the tasting and were considered to be of commercial standard with no obvious defects. Judges were from the Istituto Agrario di S.Michele all'Adige, all of whom had extensive experience in scoring wine. The wines were judged once in the year of production and the composition and size of the panel was unvaried from year to year. The wines were scored for quality using a descriptive card (Iacono *et al.*, 1990) for each wine tipology (Tabb. 2a, 2b, and 2c). For the definition of the descriptors the wine aroma terminology proposed by Noble *et al.* (1987) has been used.

Statistical analyses

Non Linear regression procedure was used for modeling sugar accumulation rate in berries (Stefanini *et al.*, 1993b). Chemical and estimated data were analysed by 2-way analysis of variance with interaction (ANOVA) and differences between means were determined using Duncans Multiple Range Test ($P < 0.05$). A non parametric procedure was used for testing categorical data (i.e. date of veraison and harvest). Cluster Analysis was used to create groups of likeness for clones.

Sensorial data were analysed by Factorial analysis. Every single factor was then regarded as a new variable containing informations about more than one descriptor at the same time (Iacono *et al.* 1993b). All data analyses were performed using SAS (Statistical Analyses System, Cagney, NC, U.S.A.)

Results

Agronomic evaluation of clonal selections

The quadratic model with *plateau* significantly fitted sugar accumulation in berries ($R^2 = 0.850^{***}$, 0.932^{***} , and 0.901^{***} respectively for *Chardonnay*, *Pinot n.*, and *Sauvignon b.*). By using the parameters of the quadratic functions ($y = a + bx - cx^2$) it is possible to estimate the date of veraison (when TSS = 3 °Brix), the date of maximum TSS level, the maximum TSS level (*at plateau*), and sugar accumulation rate (S.A.R.) at any moment (first derivate). Such parameters, together with TSS at harvest and yield/vine, have been used for Cluster analysis in order to create groups of likeness for each variety.

Chardonnay clones were associated in the following way: 75, 78, 95, 117, 124 and 130 in group 1; 76, 96, 116, 119, 123, 128, 277 in group 2 (Fig. 1a).

Pinot n. clones were associated in the following way: 113, 375, 459, LB4 in group 1; 052, 292, 386 in group 2; 114, 115 in group 3 (Fig. 1b)

Sauvignon b. clones were associated in the following way: 242, 297 in group I; 376, 377 in group 2; R3 in group 3 (Fig. 1c).

All the variables investigated showed any significant difference between groups of *Chardonnay* clones and year of cultivation (Tab. 3a).

Group 3 of *Pinot n.* has the highest S.A.R. values. Maximum and harvest TSS were lower in 1992 and 1993. 1990 and 1993 were the most productive years (Tab. 3b). Significant group year interactions were noticed for S.A.R. 7 and 21 and maximum and harvest TSS. In 1992 group I showed the lowest S.A.R. 7 (Fig. 2). Maximum TSS recorded in 1990 and 1991 for group I were low, whilst were similar to 1992 and 1993 for groups 2 and 3. In 1990 harvest TSS was lower in groups 1 and 2 and higher in group 3. Group 3 of *Sauvignon b.* showed the lowest crop level, and the highest S.A.R. 21, maximum and harvest TSS (Tab. 3c). Years were not significantly different whilst interactions were significant for the same variables of *Pinot n.* In 1991 S.A.R. 7 was high for group I and low for the remaining groups and the differences seemed less apparent for S.A.R. 21. In 1990 maximum and harvest TSS were very high for group 3 (Fig. 3).

Sensorial evaluation of clonal selection

5 Factors represented 62.2% of total variability of data of *Chardonnay* wines (tab. 4a). Factor 1 represented utmostly acid and sapid taste as well as structure and persistence in the mouth and was defined as "body" of the wine. Positively correlated to tropical fruit, floral (lime tree), spicy and tobacco flavours, Factor 2 was defined as "varietal" character. As Factor 3 was compounded by floral (geranium) and grass cut green flavours it was defined as "pungent" character. Factor 4 was positively correlated to sour apple and lemon descriptors and it was defined as "sour fruity" character. Factor 5 was described by bitter descriptors and therefore it was defined as "bitter" character.

5 Factors represented 53.2% of total variability of data of *Pinot n.* wines (Tab. 4b). As Factor 1 was characterized by positive values of berry fruity, jam and earthy descriptors was defined as "fruity" character. As Factor 2 was compounded by acid and bitter descriptors it was considered as "acid-bitter" character. Factor 3 was positively related to sapid and spicy in the mouth and it was defined as "sapid-spicy" character. Factor 4 was characterized by positive values of resinous, phenolic, and alcohol descriptors and was defined as "phenolic" character. Factor 5 was positively characterized by dried fruit, spicy and negatively by yeasty descriptors was defined as "dried fruit-spicy" character.

5 Factors represented 53.1% of total variability of data of *Sauvignon or.* wines (Tab. 4c). At Factor 1 represented fruity descriptors it was defined as "fruity" character. Positively correlated to grass cut green and elder descriptors, Factor 2 was defined as "fresh vegetative" character. Factor 3 positively compounded by lemon and negatively by bitter was defined as "citrus fruity" character. Factor 4 was characterized by fig and green beans descriptors and was defined as "vegetative" character. Factor 5 was defined as "acid" character.

In the case of *Chardonnay* such factors were analysed by ANOVA and differences were significant neither between groups of clones nor for interactions. 1993 showed the highest "sour fruity" character (Tab. 5a). "Sapid-spicy" character of group 2 of *Pinot n.* was significantly lower when compared with the remaining groups, higher in 1991 (Tab. 5b) as well as "Acid-bitter" character. The latter and the "phenolic" characters showed significant interactions. "Acid-bitter" character of group 2 was higher in 1991, whilst "phenolic" of group 1 was higher in 1991 and that one of group 3 higher in 1992 (Fig. 4).

Group 3 of *Sauvignon b.* showed the highest "fruity" and "fresh vegetative" characters, but any difference was noticed for year of cultivation (Tab. 5c). Significant interactions were noticed for "fresh vegetative" and "vegetative" characters. Group 2 showed low values of both characters in 1991 and 1992, and it showed very low values of "fresh vegetative" character in 1991 (Fig. 5).

Discussion

As reported by the same authors (Stefanini *et al.* 1993c) sugar accumulation in berries during ripening time may be successfully modelised through the quadratic model with plateau. Such an approach allows to estimate all the critical variables of maturation as date of veraison and sugar accumulation rate at any moment. It is opinion of the authors (Stefanini *et al.* 1993a and Iacono 1993a) that sugar level at harvest time, yield/vine, and sugar accumulation rate after veraison characterize the single clonal selections from the agronomic and oenological point of views. On the base of the results of collected and estimated data, the clones of each variety resulted grouped in cluster of likeness that allow to simplify the variability in the field.

The groups of *Chardonnay* showed any significant differences in such parameters indicating that the clonal selections used in the present study do not appear very different from the agronomic point of view. All the groups were also similar throughout the years and therefore it is possible to assume that such a variety is adapted to the environment of Trentino. *Chardonnay* wines, evaluated as the groups agronomically characterized, showed any sensorial difference.

S.A.R. discriminated the groups of clones of *Pinot n.*, years appeared to be different particularly for harvest TSS and yield/vine and significant interactions between groups of clones and year demonstrate that in relation to the climatic conditions clones show appreciable differences. Such a behaviour is strongly related to the oenological characteristics of the wines: "sapid and spicy" character was low in group 2 and both "acid-bitter" and "phenolic" characters were interactive with the year.

Similar behaviour was showed by *Sauvignon b.*, the wine of which showed significant differences between groups of clones. Particularly the fruity and vegetative profiles appeared to be affected by the agronomic parameters studied.

The present paper confirms that the agronomic characteristics of clonal selections are related to the oenological ones: TSS at harvest and yield/vine are very important quality control factors, but the way with which the different sugar levels are reached throughout ripening time by the specific clones appears to be critical to define the sensorial properties of wines.

As previously reported (Bogoni *et al.* 1993) it is important to plant in the same vineyard several clones of *Pinot n.* and *Sauvignon b* cultivars with different agronomic characteristics in order to obtain year after year high quality wines. In fact the strong interaction between group of clones and year suggests that it is possible to optimize the high variability of varietal responses. Such a recommendation appears to be less important for *Chardonnay* that shows relatively high stability in the tested environment.

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		Veraison date ^c (days)	S.A.R. 7 (°Brix/day)	S.A.R. 21 (°Brix/day)	Max TSS date ^c (days)	Max TSS (plateau) (°Brix)	Harvest TSS (°Brix)	Yield/vine (Kg)
group of clones	1	207	0.56182	0.40310	272	22.402	20.78	5.09
	2	206	0.54748	0.40071	269	22.021	20.59	4.82
Pr > F ^a		n.s. ⁴	n.s.	n.s.	n.s. ⁴	n.s.	n.s.	n.s.
Year	1990	204	0.44203 b ^b	0.36890 b	296	25.00 a	20.43 b	6.28 a
	1991	211	0.65242 a	0.44145 a	262	22.18 b	21.91 a	3.87 b
	1992	211	0.49758 b	0.36385 b	270	19.71 c	19.13 c	5.39 a
	1993	201	0.62658 a	0.43341 a	253	21.96 b	21.27 ab	4.27 b
Pr > F		n.s.	**	**	n.s.	n.s.	*	**
Interaction								
Pr > F		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

^a: Probability of F values (n.s., *, **, ***: not significant, 95%, 99% ,and 99.9% of probability respectively)

^b: Mean separation by Duncans Multiple Range Test

^c: Days from the 1st January

⁴: Probability value of X²

Tab. 3a: Group of clones and year effects on agronomic variables of *Chardonnay*

		Veraison date ^c (days)	S.A.R. 7 (°Brix/day)	S.A.R. 21 (°Brix/day)	Max TSS date ^c (days)	Max TSS (plateau) (°Brix)	Harvest TSS (°Brix)	Yield/vine (Kg)
group of clones	1	206	0.4965 ab ^b	0.3717 b	272	20.93	20.23	2.17
	2	207	0.4856 b	0.3656 b	273	20.75	19.85	2.40
	3	207	0.5647 a	0.4138 a	267	22.12	21.33	1.78
Pr > F ^a		n.s. ⁴	*	*	n.s. ⁴	n.s.	n.s.	n.s.
Year	1990	211	0.5411	0.3933	271	21.15	21.15 a	2.51 a
	1991	206	0.5144	0.3866	271	21.55	22.59 a	1.44 b
	1992	206	0.4842	0.3586	272	20.34	19.16 b	1.48 ab
	1993	204	0.5251	0.3964	269	22.03	19.00 b	2.63 a
Pr > F		n.s.	n.s.	n.s.	n.s.	n.s.	**	*
Interaction								
Pr > F		n.s.	**	n.s.	n.s.	**	**	n.s.

^a: Probability of F values (n.s., *, **, ***: not significant, 95%, 99% ,and 99.9% of probability respectively)

^b: Mean separation by Duncans Multiple Range Test

^c: Days from the 1st January

⁴: Probability value of X²

Tab. 3b: Group of clones and year effects on agronomic variables of *Pinot noir*

		Veraison date ^c (days)	S.A.R. 7 (°Brix/day)	S.A.R. 21 (°Brix/day)	Max TSS date ^c (days)	Max TSS (plateau) (°Brix)	Harvest TSS (°Brix)	Yield/vine (Kg)
group of clones	1	217	0.5578	0.3942 ab ^b	272	20.60 ab	20.68 ab	3.82 b
	2	212	0.4653	0.3510 b	278	19.98 b	19.61 b	7.56 a
	3	211	0.5760	0.4297 a	275	23.52 a	22.82 a	1.79 c
Pr > F ^a		n.s. ⁴	n.s.	*	n.s. ⁴	*	*	***
Year	1990	215	0.5583	0.3976 a	271	20.79	21.69	4.30
	1991	210	0.5592	0.4197 a	276	23.34	22.19	4.26
	1992	217	0.4615	0.3430 a	282	19.63	20.42	4.74
	1993	212	0.5531	0.4063 a	272	21.72	19.83	4.25
Pr > F		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Interaction								
Pr > F		n.s.	**	n.s.	n.s.	**	**	**

^a: Probability of F values (n.s., *, **, ***: not significant, 95%, 99% ,and 99.9% of probability respectively)

^b: Mean separation by Duncans Multiple Range Test

^c: Days from the 1st January

⁴: Probability value of X²

Tab. 3c: Group of clones and year effects on agronomic variables of *Sauvignon blanc*

Variety	Clone	Source
<i>Chardonnay</i>	B 75	France
	B 76	France
	B 78	France
	B 95	France
	B 96	France
	B 116	France
	B 117	France
	B 119	France
	SMA 123	Italy
	B 124	France
	B 128	France
	SMA 130	Italy
	B 277	France
<i>Pinot n.</i>	113	France
	114	France
	115	France
	292	France
	375	France
	386	France
	459	France
	52	France
	Lb 4	Italy
<i>Sauvignon b.</i>	242	France
	297	France
	376	France
	377	France
	R 3	Italy

Tab. 1: Description of tested clones

Sensory Analysis Group I.S.M.A

NAME _____ DATE _____

Sample code _____

DESCRIPTORS

	min.	med.	max.
Aroma			
Tropical fruit
Sour fruit
Floral (lime tree)
Floral (geranium)
Lemon
Spicy (thimus)
Tobacco
Grass cut green
Taste			
Acid
Bitter
Sapid
Structure
Persistence

Tab. 2a: Descriptive card for the evaluation of *Chardonnay*

Sensory Analysis Group I.S.M.A

NAME _____ DATE _____

Sample code _____

DESCRIPTORS

	min.	med.	max.
Aroma			
Berry fruity
Jam
Dried fruit
Resinous
Spicy (cinnamon)
Grass cut green
Phenolic
Earthy
Alcohol
Yeasty
Taste			
Acid
Bitter
Sapid
Spicy in mouth
Structure

Tab. 2b: Descriptive card for the evaluation of *Pinot n.*

Sensory Analysis Group I.S.M.A

NAME _____ DATE _____

Sample code _____

DESCRIPTORS

	min.	med.	max.
Aroma			
Banana
Elder
Rose
Peach
Lemon
Fig
Grass cut green
Green beans
Mushroom
Taste			
Acid
Bitter
Spicy in mouth

Tab. 2c: Descriptive card for the evaluation of *Sauvignon blanc*

Descriptors	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Tropical fruit	0.09625	0.78548	-0.14109	-0.03627	0.02402
Sour fruit	0.10523	0.03547	0.11636	0.87841	-0.08726
Floral (lime tree)	0.08351	0.77804	-0.23812	0.17364	0.12312
Floral (geranium)	0.03185	0.10886	0.68546	0.19714	0.00196
Lemon	0.00339	0.01206	0.37208	0.54466	0.29176
Spicy (thymus)	0.03232	0.56580	0.45962	-0.05906	-0.25444
Tobacco	0.20634	0.52746	0.40660	-0.06400	-0.05778
Grass cut green	0.08495	-0.07248	0.58821	0.17053	0.38787
Acid	0.57621	0.01908	-0.03312	0.17574	0.30556
Bitter	0.13654	0.01491	0.08435	0.00332	0.81314
Sapid	0.68626	0.06956	0.02279	-0.12257	0.33569
Structure	0.82412	0.10816	0.10362	0.01939	-0.12288
Persistence	0.86210	0.15087	0.07432	0.09867	-0.02967

Tab. 4a: Factor Analysis for *Chardonnay*: descriptors according to Noble *et al.* (1987)

Descriptors	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Berry fruity	0.71534	-0.20437	0.07175	0.10334	0.06205
Jam	0.68723	-0.12772	0.22710	0.10347	0.11120
Dried fruit	0.27956	0.03126	-0.01044	-0.10583	0.75673
Resinous	-0.16909	0.02338	0.37775	0.61135	-0.05841
Spicy (cinnamon)	-0.03705	-0.15791	0.35563	0.02610	0.56168
Grass cut green	0.43864	0.28782	0.03299	0.14907	-0.02641
Phenolic	0.02539	0.04178	-0.10038	0.62364	0.34187
Earthy	0.56919	0.28331	-0.09770	-0.20009	-0.09408
Alcohol	0.30891	-0.04865	-0.12481	0.68054	-0.20566
Yeast	0.16128	0.04748	0.26706	-0.11470	-0.52898
Acid	-0.00942	0.85961	0.05217	-0.02927	-0.07115
Bitter	-0.00490	0.84531	0.02322	0.03776	-0.02378
Sapid	0.33426	0.14636	0.57049	0.05307	0.00040
Spicy in mouth	-0.00858	-0.01935	0.80504	-0.01220	-0.03279
Structure	0.30145	0.03350	0.29813	0.34133	0.02069

Tab. 4b: Factor Analysis for *Pinot noir*: descriptors according to Noble *et al.* (1987)

Descriptors	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Banana	0.74808	-0.07718	-0.03807	0.00900	0.08210
Elder	0.03210	0.73957	-0.12597	0.15698	0.09429
Rose	0.58033	0.38712	0.14286	0.13061	-0.37891
Peach	0.75012	-0.09874	0.10096	-0.07183	0.04472
Lemon	0.22968	0.13855	0.81422	0.03844	0.12467
Fig	-0.00952	0.37593	-0.00781	0.66509	-0.28281
Grass cut green	-0.22383	0.76088	0.18393	-0.13300	0.01266
Green beans	0.02628	-0.09444	-0.07307	0.76668	0.23497
Mushroom	-0.29696	-0.29897	-0.05488	0.33712	-0.15455
Acid	0.07636	0.13493	-0.02747	0.03918	0.90958
Bitter	0.11527	0.13863	-0.69118	0.13298	0.16339
Spicy in mouth	0.42872	0.46024	-0.26735	-0.00647	0.02805

Tab. 4c: Factor Analysis for *Sauvignon blanc*: descriptors according to Noble *et al.* (1987)

Treatment		Factor1	Factor2	Factor3	Factor4	Factor5
group of clones	1	-0.003	-0.039	0.036	0.065	-0.055
	2	0.003	0.036	-0.033	-0.059	0.050
Pr > F ^a		n.s.	n.s.	n.s.	n.s.	n.s.
Year	1990	-0.137	-0.089	-0.186	-0.116 b ^b	-0.053
	1991	-0.063	-0.147	0.048	-0.234 b	0.012
	1992	0.003	0.022	0.015	-0.044 b	0.048
	1993	0.186	0.199	0.101	0.397 a	-0.130
Pr > F		n.s.	n.s.	n.s.	***	n.s.
Interaction						
Pr > F		n.s.	n.s.	n.s.	n.s.	n.s.

^a: Probability of F values (n.s., *, **, ***: not significant, 95%, 99% ,and 99.9% of probability respectively)

^b: Mean separation by Duncans Multiple Range Test

Tab. 5a: Group of clones and year effects on sensorial variables of *Chardonnay*

Treatment		Factor1	Factor2	Factor3	Factor4	Factor5
group of clones	1	0.126	-0.032	0.121 a ^b	-0.051	0.016
	2	-0.016	-0.028	-0.301 b	-0.078	-0.060
	3	-0.227	0.107	0.209 a	0.219	0.058
Pr > F ^a		n.s.	n.s.	**	n.s.	n.s.
Year	1990	-0.120	-0.155b ^b	-0.249 b	-0.238	0.115
	1991	0.121	0.395a	0.483 a	0.170	0.195
	1992	-0.003	-0.175b	-0.114 b	0.084	0.003
	1993	0.019	0.070ab	0.018 b	-0.013	-0.229
Pr > F		n.s.	*	**	n.s.	n.s.
Interaction						
Pr > F		n.s.	***	n.s.	**	n.s.

^a: Probability of F values (n.s., *, **, ***: not significant, 95%, 99% ,and 99.9% of probability respectively)

^b: Mean separation by Duncans Multiple Range Test

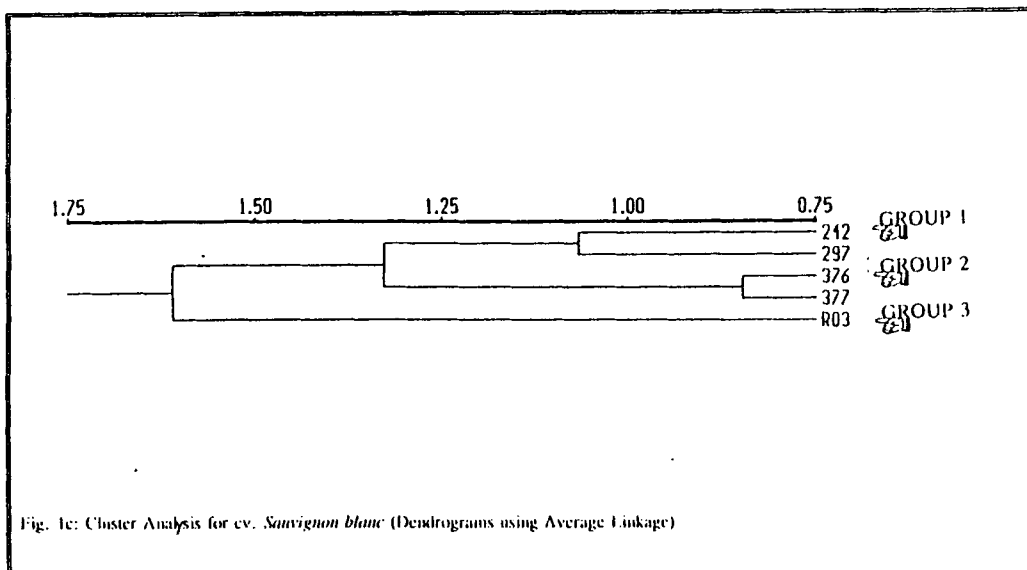
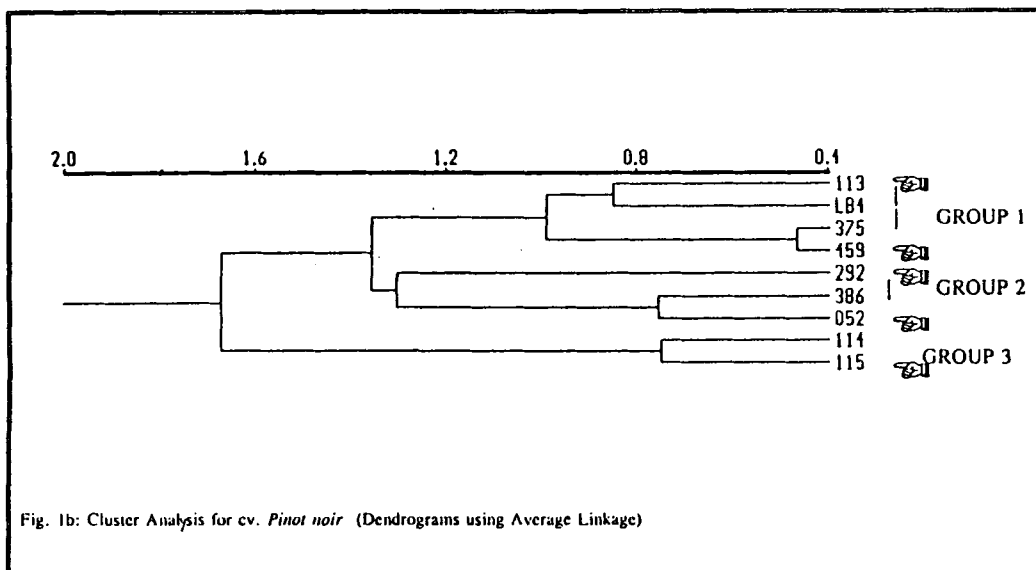
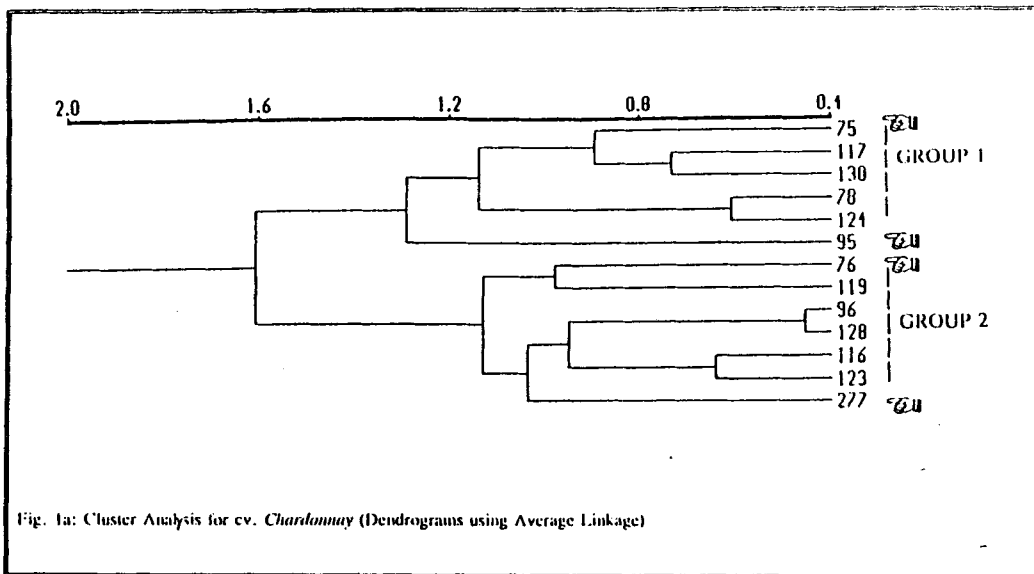
Tab. 5b: Group of clones and year effects on sensorial variables of *Pinot noir*.

Treatment		Factor1	Factor2	Factor3	Factor4	Factor5
group of clones	1	-0.342 b ^b	-0.211 b	0.302	-0.163	-0.030
	2	-0.020 b	0.071 ab	0.123	-0.104	-0.104
	3	1.031 a	0.581 a	-0.312	-0.154	0.319
Pr > F ^a		**	*	n.s.	n.s.	n.s.
Year	1990	0.609	-0.024	0.396	0.074	-0.689
	1991	-0.062	0.129	-0.124	-0.343	0.280
	1992	-0.092	0.218	0.263	-0.085	0.040
	1993	-0.070	-0.149	0.107	-0.081	-0.003
Pr > F		n.s.	n.s.	n.s.	n.s.	n.s.
Interaction						
Pr > F		n.s.	**	n.s.	*	n.s.

^a: Probability of F values (n.s., *, **, ***: not significant, 95%, 99% ,and 99.9% of probability respectively)

^b: Mean separation by Duncans Multiple Range Test

Tab. 5c: Group of clones and year effects on sensorial variables of *Sauvignon blanc*



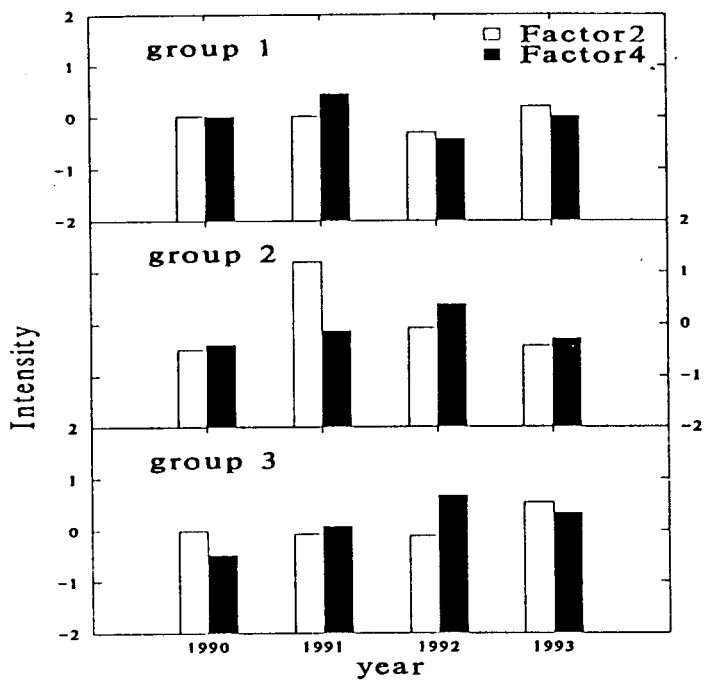


Fig. 4: Significant interactions between year and groups of clones for sensorial variables (*Pinot noir*)

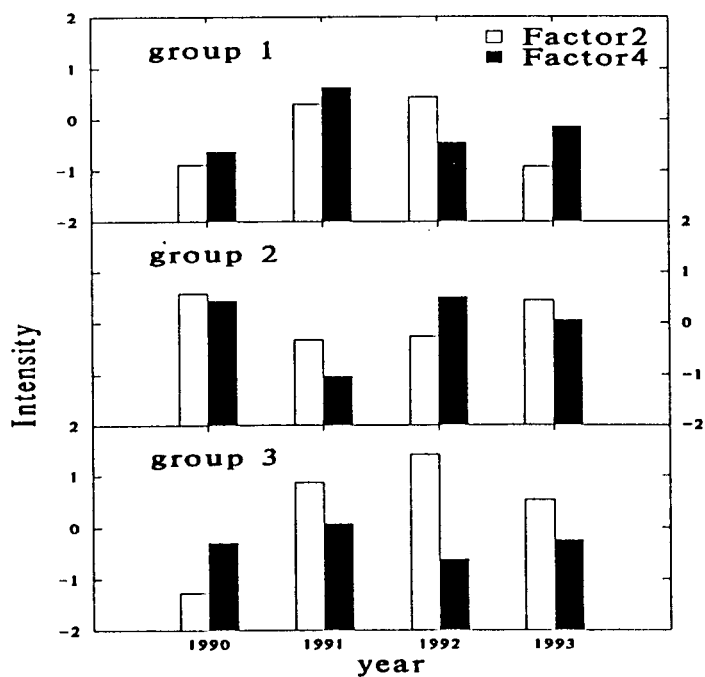


Fig. 5: Significant interactions between year and groups of clones for sensorial variables (*Sauvignon blanc*)

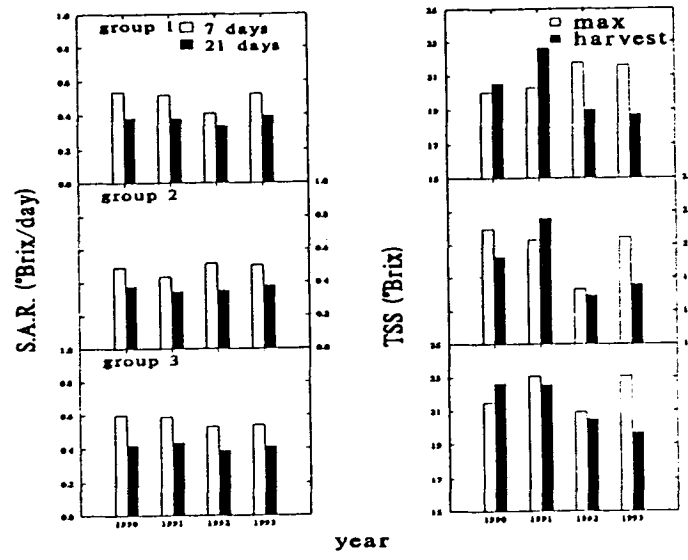


Fig. 2: Significant interactions between year and groups of clones for agronomic variables (*Pinot noir*)

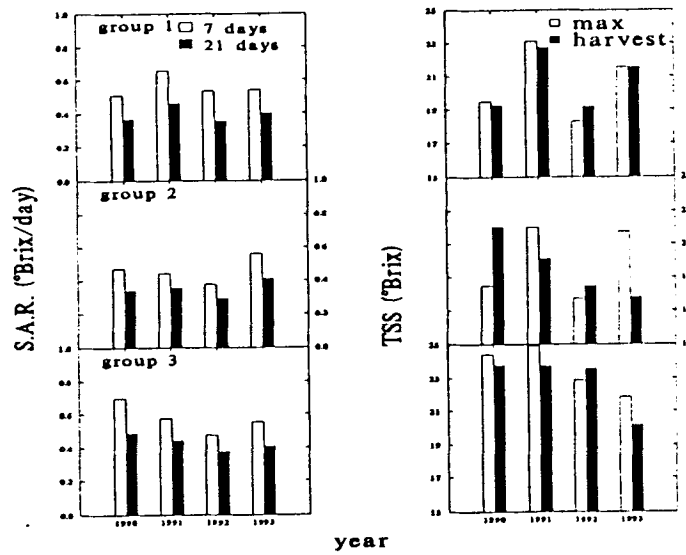


Fig. 3: Significant interactions between year and groups of clones for agronomic variables (*Sauvignon blanc*)

SESSION 5 : CLONAL SELECTION

RECENT PROGRESSES IN THE DETECTION OF VIRUSES AND PHYTOPLASMAS OF THE GRAPEVINE : APPLICATION TO SANITARY SELECTION

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INTRODUCTION

Virus-induced, virus-like diseases and yellows diseases caused by phytoplasmas (formerly called MLOs) constitute one of the major limiting factors to the development, quality and productivity of grapevine. The only ways to control the spread of these diseases are :

- the production of healthy planting material and
- the elimination of inoculum sources like infected plants and natural vectors .

For an efficient sanitary selection the development of reliable, easy-to use and universally available diagnostic methods is thus essential. The initial selection is only valid if it is followed by serious and constant monitoring of the sanitary status of the grapevine material from the primary source through all the steps of multiplication.

A good sanitary status has also become a prerequisite for international movement of grapevine plants and cuttings which go through quarantine. Viruses, phytoplasmas and virus-like diseases are greatly concerned by the quarantine measures applied by most grape-growing countries all over the world. Of course, the lists of quarantine pathogens differ from a country to another. The main goal of quarantine is to avoid the introduction of pathogens into an area where they do not occur. On these premises quarantine should not be lifted because of economical or political backgrounds. The General Assembly of OIV recently voted a resolution recommending to member States not to hinder the international exchange of certified grapevine materials when these have obtained an official recognition.

Concerning the sanitary selection, distinction should be made between major and minor diseases, according to their geographical distribution and economical importance. A group of pathologists of the major grape-growing countries of the European Union have recently agreed upon a scheme for grapevine certification, proposing that minimal sanitary requirements for inclusion of scion cultivars, clones and rootstocks taken into the scheme in the basic or certified categories are freedom from :

1. Grapevine degeneration complex, including grapevine fanleaf nepovirus (GFLV) and other european nepoviruses,
2. Grapevine leafroll complex,
3. Grapevine rugose wood complex (rupestris stem pitting, corky bark, Kober stem grooving, LN33 stem grooving),
4. Grapevine fleck disease (marbrure),
5. Grapevine enation disease,
6. Any of the grapevine closteroviruses,
7. Grapevine diseases caused by phytoplasmas.

Although most of these diseases may be spread naturally by vectors (nematodes, mealybugs, leafhoppers) the major mean of dissemination is through infected propagative material. This is the main reason for developing and maintaining efficient and adequate certification programs.

Besides the detection methods that can be used everywhere without sophisticated technology, namely biological indexing on indicator grapevine varieties and herbaceous hosts, an increasing number of serological and biochemical methods is now available. They have different levels of technical difficulties from the easy-to-use ELISA to the more complicated and quite expensive immune-electronmicroscopy, molecular hybridization and PCR-based techniques.

In this paper, an overview is presented of the state of the art and recent progresses in the field of detection of the different grapevine virus-, virus-like- and yellows-diseases, emphasizing pros and cons of each detection technique.

1. BIOLOGICAL INDEXING

The use of *Vitis* indicators is a compulsory step of grapevine certification programmes, for there are diseases which can be identified only by the reaction of woody differential hosts inoculated by various grafting techniques. These techniques are :

1. Whip or cleft grafting in the field.
2. Chip-bud grafting. This technique is recommended for detection of *rupestris* stem pitting.
3. Machine or bench grafting.
4. Green grafting, whose use is encouraged because of the distinct advantages over other grafting techniques.

These advantages are :

- rapidity, most of the symptoms appearing in a few weeks compared to months or even years with traditional indexing using woody plants;
- possibility of doing indexing all over the year;
- standardization of conditions for testing when green indexing is done in greenhouses and growth chambers, where temperature, light, air humidity can be adapted to an optimized expression of the symptoms .

INOCULATION TO HERBACEOUS HOSTS

Herbaceous indicators detect mechanically transmissible viruses, including those of minor relevance. The use of herbaceous hosts is complementary to other diagnostic procedures. It may be useful for preliminary screening and random testing.

For example, *Chenopodium quinoa* reveals the presence of all nepoviruses after mechanical inoculation with an extract of grapevine tissues. However the symptoms induced in *C. quinoa* do not allow to clearly distinguish between nepoviruses thus to identify the one present in the vine. This identification is not always required for the sanitary selection ; if needed, further identification of the virus can be done by serological methods such as ELISA, using reagents specific to each virus.

It. ELISA

Serological testing is regarded as a complement to, but not as a substitute for, other diagnostic procedures. However, the use of ELISA is recommended for identification of nepoviruses, closteroviruses and grapevine fleck virus (GFkV). For most of the characterized grapevine viruses and phytoplasmas which may concern sanitary selection antisera have been produced.

The optimal sampling strategy for ELISA must answer at least 4 main questions :

- (1) what is the best source of antigens - in other words - what is the best grapevine organ to be tested ?
- (2) in what period of the year can the samples be collected from the vine ?
- (3) what is the minimal number of samples to be collected from a batch of plants or in a field to minimize the risk of failing to detect any viral infection ?
- (4) how many samples can be cropped together for analysis keeping the @ highest probability of a positive response if only a single sample in the batch is infected?

For NEPOVIRUSES AND GFkV, ELISA is the most valuable technique.

However, for large scale and routine analysis (for quarantine purposes or sanitary selection) limits have to be considered in the sampling strategies, the periods of collection and the grapevine species analysed (*V. vinifera* cultivars or rootstock species/hybrids).

The best organs are young leaves which give the highest results in dilution tests, but sanitary control of dormant canes and grafted plants can be made without problems using cortical scrapings or rootlets. Seasonal variations of virus titer has been demonstrated for nepoviruses and GFkV. During the hottest period of the year the detection of these viruses is not possible. Under climatic conditions of Alsace, the best period for collection of leaves was shown to be the end of spring for both FkV and nepoviruses. Interestingly, for nepoviruses, the sensitivity of ELISA was about the same throughout the seasons when rootstock samples were analysed. Because of possible uneven distribution of the virus in a plant, more than one sample (leaves or pieces of canes or rootlets) should be collected from a single vine to minimize the risk of false negative responses in ELISA. Statistical analysis of the risks determines the number and the distribution of samples to be taken from a vine or from a batch of cuttings or grafted plants. For example, to detect a contamination at a level of 1% in a batch of 1,000 plants on the 99% level of significance, 368 plants have to be analysed. This means that, if 2 or 3 leaflets are collected from 368 plants, 736 to 1,104 leaflets need to be

sampled. Fortunately, due to the high sensitivity of ELISA, up to 20 or even more grapevine leaves can be grouped in a single test : this means that for the analysis of the 736 to 1,104 leaves, only 37 to 56 tests are needed.

CLOSTEROLIKE VIRUSES. For these viruses the problem is more complex because of the low number and irregular distribution of particles in infected plants, and the poor sensitivity of serological methods, except for GLRaV III. For these reasons, and because all closterolike viruses associated with leafroll and rugose wood are probably not yet known, the diagnosis of these diseases is best obtained based on the complementary results of ELISA and biological indexing. This means that the presence of closterolike viruses revealed by a positive ELISA response leads to the elimination of the candidate clone in the sanitary selection. In contrast, negative ELISA responses must be double checked by indexing. The best organs for the detection of closterolike viruses are cortical scrapings, petioles and the main veins of old leaves. The best periods for sample collection are the beginning of summer/end of autumn for leaves and petioles and autumn-winter for green and mature bark. No definitive sampling strategy has been established yet for these viruses, but studies are in progress in a way similar to that followed for nepoviruses. Better results are being obtained with the use of monoclonal antibodies raised against trichoviruses (GVA, GVB) especially because of the increase of the specificity with regard to masking reactions caused by plant proteins from grapevine extracts when polyclonal antisera are used.

PHYTOPLASMAS. The detection of phytoplasmas in grapevines has not yet been mastered. Addition of a detergent and 10-fold concentration of the grapevine extract by molecular filtration are necessary. In these conditions, detection of flavescente doree phytoplasma is possible in *Vitis vinifera* cultivars. Positive results can also be obtained, in some cases, with symptomless rootstock varieties grown in the greenhouse, but detection is impossible when the rootstocks are grown in the field. These difficulties are now being circumvented, especially with the help of monoclonal antibodies raised against flavescente doree phytoplasmas recently purified by immunoaffinity.

Relationships have been demonstrated between noir noir phytoplasma and stolbur. Because the purification of noir noir phytoplasmas and raising of specific antibodies were not possible the use of polyclonal and monoclonal antibodies against stolbur phytoplasmas for the detection of bet noir is under investigation.

All the above mentioned infectious agents - viruses and phytoplasmas - can be specifically visualized and identified by immunosorbent electron microscopy (ISEM) but the use of this technique for routine analysis cannot be envisaged because of the cost of the equipment and the technical expertise needed.

III. DETECTION OF DOUBLE-STRANDED RNAs

Double stranded RNA analysis - i.e. the visualization of replicative forms of viral genomes - may be used as an alternative to ELISA for closteroviruses and rugose wood, although it remains an aspecific method and is subject to misinterpretations due to interfering molecules of cryptogamic origin. Today, this method is not routinely used for sanitary selection, but its potentialities need a proper examination. Ds RNA technology could be a tool for a preliminary coarse screening of diseased material.

IV. POLYACRYLAMIDE GEL ELECTROPHORESIS AND MOLECULAR HYBRIDIZATION

Polyacrylamide gel electrophoresis is used for detection and tentative identification of viroids in the grapevine. Six viroids have been described up to now, widely distributed in grapevine varieties all over the world. Four of them are not known to induce disease. The only disease for which a viroidal aetiology has reasonably been established is yellow speckle disease. The causal agents of yellow speckle disease are grapevine yellow speckle viroids I and Z which may have a worldwide distribution : they have been found in vines originating from many different countries of all continents.

Molecular hybridization methods (dot-blot, Northern, Southern) proved to be very sensitive and specific for the identification of grapevine viruses, viroids and phytoplasmas, but they are not well adapted to routine analyses because of the laborious sample preparation and the expensive probe labelling techniques. The use of non radioactively-labeled probes circumvents part of these difficulties.

With the recent acquisition of sequence data of some closterolike viruses (GLRaV III, GVA, GVB) and of phytoplasmas, access to the polymerase chain reaction (PCR)-based techniques is now possible and has already been shown to be valuable because of the very high sensitivity and realibility, and, for phytoplasmas, the possibility of differentiating the agents associated with the different characterized yellows diseases by their similarities with other known plant phytoplasmas. A direct PCR technique is used for the detection of phytoplasmas, followed or not by restriction mapping of PCR products for variability studies. However, a primary immunocapture step is needed for the detection of closteroviruses (and nepoviruses) in

grapevine tissues to circumvent the inhibitory effects of some grapevine compounds (probably tannins and polysaccharides) in the reverse transcription step.

Nevertheless, the use of ELISA plates for the immunocapture step and the possibility to make all further steps in the plate make this technology similar to double antibody sandwich ELISA and works are in progress to develop easy-to-use "one well" PCR-based methods eliminating the gel electrophoresis step for analysis of the PCR products. The main limiting factor is still the high cost of this method in the view of its application to large scale routine diagnosis.

However one interesting goal will be to design broad range PCR primers which could allow detection of all closteroviruses by the identification of consensus genomic sequences specific at the genus level.

DISCUSSION

In the recent past, progress has been made in the detection of viruses and phytoplasmas for the sanitary selection of the grapevine. This progress is the consequence of both new data in the characterization of the pathogens and in the application of recent or improved detection technologies.

For example, the recent purification of the virus responsible for the fleck disease, GFkV, resulted in the development of ELISA for the reliable detection of this virus.

The pros and cons of the different detection methods have to be carefully weighed in order to choose the best adapted method to the goal and to the level of the control.

For the virus-like diseases, the only method still available is grafting onto grapevine indexing varieties, as is the case, for example, for vein mosaic, vein necrosis, summer mottle or enations. Graft indexing also remains mandatory for the detection of leafroll and rugose wood in those clones that are negative in ELISA with the closterolike reagents.

The essential characteristics of the best detection methods adapted to sanitary selection are :

- sensitivity,
- ease to use / possible automation,
- low cost,
- rapidity.

For the time being, ELISA - when reagents are available - is the method the best adapted for routine analysis. ELISA is sensitive, partially automated, rapid (24-48 hrs) and of low cost especially when samples are grouped. Diagnostic kits are now marketed by different companies for the detection of nepoviruses, closterolike viruses and GFkV. But for the routine use of ELISA precautions have to be taken, as we have seen, in the sampling.

The diagnosis of the yellows diseases caused by phytoplasmas such as *flavescence dorée* or *bois noir* by ELISA needs more developments. Difficulties probably come from the low and irregular concentration of the phytoplasmas in the grapevine, especially in rootstock varieties. Molecular techniques based on the use of specific probes or universal primers complementary to the common 16S ribosomal RNA are very promising : adaptations have to be brought to make these techniques simpler and cheaper.

The PCR techniques, particularly the IC-RT-PCR, nowadays have a main disadvantage : the high cost of some reagents such as the polymerase enzymes and the rights of the patents pending. Further developments for large scale use of PCR probably will bring acceptable solutions.

Of course, the advantages and disadvantages of the methods have also to be considered in respect to the amount of samples to be analysed and to the level of risks that is accepted. More time and money have to be spent for the control of the initial nuclear stock plants, which are in low number and for which a bad sanitary status can be disastrous due to the large multiplication of these plants. On the other side, a posteriori survey of large quantities of mother blocks or grafted plants has to be done in a simpler and cheaper way.

Finally, all people concerned by sanitary selection or quarantine - scientists, technicians, plant protection and control agencies, as also politicians - should make efforts towards an harmonization of the lists of pathogens and diseases that have to be taken into account. Efforts should be also made in the elaboration of common and recognized protocols for the detection methods. A group of grapevine virologists from the main grapegrowing countries in the EU are working, with the financial support of the European Commission, in this direction. Permanent research on the characterization of the viruses and phytoplasmas of the grapevine and their interactions is a prerequisite for further improvement and simplification of their detection : consequently this research should be strongly supported.

SESSION 6 : MIXED TOPICS

DISEASE RESISTANT HYBRIDS - A STEP FORWARD TO SUSTAINABLE VITICULTURE

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There is an increasing interest for disease resistant grape varieties in Switzerland. Grape growers who are planting a small piece of their acreage with one or more interspecific hybrids are becoming numerous. This may result from the motivation to do something beneficial for the environment by reducing the routine fungicide spraying, or because they are following a consumer trend towards ecologically sound products. Another motivation for the interest for hybrids is based on criteria of labour economy. Most vineyards in Switzerland are located on more or less steep slopes. The spraying work under these conditions can be very hard and time consuming. The sparing of fungicides opens a perspective of rationalization in an ecological way.

With resistance we generally mean the resistance against fungus diseases. However we have to consider that most other viticultural pests can occur in a hybrid vineyard. We have noticed different susceptibilities to rust mites (*Caleptitrimerus vitis*) and thrips (*Drepanothrips reuteri*) between different hybrids, as it is known with the European varieties (Remond and Basler 1992). The same is with phylloxera (*Viteus vitifolii*) concerning the formation of galls. This pest has not yet occurred in our relatively young hybrid trials. Maybe the use of predators against phylloxera should be considered, as it is a common technique with typhlodromes against spider mites, if the acreage of interspecific hybrids should grow considerably. There are indeed predators in America from where the phylloxera was spread to Europe (Townsend and Millington 1988; Peterson 1951), and it would seem convenient to import these predators from there.

Practical experiences with interspecific hybrids Trials

The first trial was started in 1983 with a series of new hybrids. The building up of trials is going on constantly, and those cultivars that are no longer of interest are removed immediately. At present the following varieties are in production; Seyval blanc, Orion, Phoenix, Bianca, GfGa-64-170-1, GfGa-48-12, Leon Millet, Marechal Foch, De Chaunac, Baco noir and Regent. From a large number of other hybrids there are just a few vines planted for preliminary observation. The trials are located in the lake of Zurich area, where conditions for fungus diseases are very favourable. The average annual rainfall in Wädenswil is about 1350 mm (54 inches). There is no fungicide spraying at all in the trials.

Results

The results obtained to date about fungus diseases refer to the years 1989 to 1993. The infestation of downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*) and gray mold (*Botrytis cinerea*) was rated on the base of a scale of 1 to 9, where: 1 - „healthy" or "very good", 3 - „little infection (tolerable)", 4 - "just tolerable", 5 - "medium", 7 - "bad", 9 - "very bad". The results referring to the hybrids of the most interest at the present time are summarized in table I and 2

Table 1: 1989 to 1993 observations of downy mildew (*Plasmopara viticola*) and estimations of bunch botrytis (in brackets) of the hybrids of most interest at the present time.

Variety	Downy Mildew (Leaf) Median and Extremes	Bunch Botrytis (Estimation)
Bianca	1 - 1,0 - 2	(1,0)
Gf.Ga-48-12*	(2,8)	(2,0)
Seyval blanc	2 - 3,0 - 6	(3,5)
Phoenix	2 - 2,0 - 2,5	7 - 7,0 - 8
Orion	2 - 3,0 - 4	5 - 5,0 - 5
Regent	1 - 2,5 - 3	(1,5)
Léon Millot	2,5 - 3,0 - 4	(3,5)
Marechal Foch	1,5 - 3,0 - 3	(1,5)
De Chaunac	3 - 3,5 - 6	(1,0)

* young vines

Table 2: 1989 to 1993 observations and estimations (in brackets) of oidium (*Uncinula necator*) on the leaves and on the bunches of the hybrids of most interest at the present time.

Variety	Oidium Leaves Median and Extremes	Oidium Bunches (Estimation)
Bianca	1 - 2,0 - 2	(2,3)
Gf.Ga-48-12*	(1,0)	(3,0)
Seyval blanc	1 - 1,0 - 2	(1,0)
Phoenix	(1,7)	1 - 1,0 - 1
Orion	(2,7)	1 - 1,0 - 1
Regent	1 - 2,0 - 3	(1,0)
Léon Millot	1 - 2,5 - 4	(1,0)
Marechal Foch	1 - 1,0 - 2,5	(1,3)
De Chaunac	1 - 1,0 - 3	(1,0)

* young vines

The resistance or the susceptibility to fungus diseases may vary very strongly between locations with different local climatic conditions. Thus, in a site with little infection potential a partly resistant cultivar can be grown easily, whereas this may not be possible at another site. Consequently the level of resistance of the varieties is variable, and there is never a total resistance.

Wines from different varieties were made in small containers such as stainless steel tanks of 100 liters or in balloonflasks of 25 liters. After bottling they were repeatedly tasted blindly and rated by different groups of people, i.e. by specialists on viticulture and also by consumers. We also had the opportunity to present the wines on a public fair for agriculture, food and household at a tasting bar, which gave us the chance to reach almost 800 people (table 4). At these tastings the people had to take part in a hedonic inquiry for gustatory evaluation and decide upon each wine how much they liked it or not. There were 5 levels of rating: „very good“, „good“, „medium“, „bad“ and „very bad“. Out of the ratings, by counting the votes for every level of appreciation, a hedonic grade, expressed in percent, was established, according to the formula:

$$\text{Hedonic grade\%} = \frac{(\text{very good}) + (\text{good}) + (1/2 * \text{medium})}{\text{number of tasters } n} * 100$$

The results of the wine tastings of the vintage 1992 are presented in table 3 and 4. Besides the test wines there were also some traditional vinifera wines placed in the tastings. The number n indicates how many people tasted and rated each wine.

Table 3: Wine tasting results of the 1992 wines; summarized from tastings with various groups of people and indication of the number of tasters per wine. IP, 40° and 0° means immediate pressing, fermented on the skins up to a density of must of 1.040 and 1.000 respectively. *Italic* - commercial wines with traditional vinifera varieties.

Variety	Hedonic grade %	n Tasters
Regent IP (1991)	78,3	76
Gf.Ga-64-170-1	78,3	60
Bianca	77,0	250
Léon Millot 0°	73,9	71
<i>Granoir Lake of Zürich</i>	70,3	64
Léon Millot 40°	69,3	101
Gf.Ga-48-12	68,8	69
Phoenix	67,3	173
<i>Müller-Thurgau Wädenswil</i>	67,0	150
<i>Pinot noir Selection Stäfa</i>	59,0	67
Orion	58,6	210
Seyval blanc	58,4	261
Regent 0°	57,3	186
<i>Chasselas Lake of Geneva</i>	53,3	46
Regent 40°	50,8	181
De Chaunac 40°	49,6	123
Maréchal Foch 0°	48,3	89
Baco noir 40°	47,9	71
De Chaunac 0°	46,8	31
Maréchal Foch 40°	45,2	176
<i>Pinot noir Wädenswil</i>	35,8	60

As the numbers of tasters in table 3 shows, there is a very heterogeneous grouping of results that have been obtained under quite diverse circumstances. With the results obtained at the fair (table 4) the conditions of the tasting were more homogeneous as well as the number of tasters for each wine.

In former wine tastings with the 1990 wines we had the best results with Seyval blanc, Leon Millet, Regent, Orion and Bianca together with Pinot noir, Mullet-Thurgau and Chasselas. With the 1991 wines it was the same with Leon Millot, Regent, De Chaunac, GfGa-64-170-1, Phoenix, Orion and Bianca together with Mullet-Thurgau, Pinot noir and Chasselas (Basler and Wiederkehr 1993). In the interpretation of the wine tastings we do not aim to discriminate statistically the different cultivars; we are just showing tendencies and emphasizing, that the sensoric judgment of some hybrids is within the range of the traditional European varieties. It appears evident that it is possible to make wines with hybrids that are equivalent to those from European varieties.

Table 4: Wine tasting results of the 1992 wines; summarized from the tasting on the fair, with indication of the number of tasters per wine. IP, 40° and 0° means immediate pressing, fermented on the skins up to a density of must of 1.040 and 1.000 respectively. *Italic* = commercial wines with traditional vinifera varieties.

Variety	Hedonic grade %	n Tasters
<i>Fondant Leytron (Chasselas)</i>	75,0	144
Gf.Ga-48-12	74,0	152
Bianca	73,0	152
<i>Müller-Thurgau Wädenswil</i>	72,3	159
Regent IP (1991)	71,3	120
<i>Granoir Lake of Zürich</i>	68,1	113
<i>Pinot noir Selection Stäfa</i>	64,0	107
Léon Millot 0*	61,3	106
Orion	60,9	147
Regent 40*	58,8	136
Phoenix	58,7	155
<i>Pinot noir Wädenswil</i>	58,0	132
Seyval blanc	57,5	154
<i>Domaine de Richaume France</i>	53,9	115
Léon Millot 40*	53,1	112
Maréchal Foch 40*	51,0	103
Maréchal Foch 0*	40,4	114
Regent 0*	39,5	110

Depending on the way of winemaking, the duration of fermentation on the skins or even without fermentation on the skins (IP), there will be quite different types of wine produced with the red hybrids. This has been shown clearly in the tasting results of Regent at the fair (table 4) The variety has been rated differently depending on the way of winemaking.

Discussion and consequences for commercial viticulture

If we accept that resistance to diseases doesn't need to be 100 percent, there are indeed some varieties with a sufficient practical resistance available. The results have been obtained in an area with a high infectious potential. In traditional viticulture one is used to having healthy vines through the whole season; but there is already a satisfactory resistance, when there are no economic losses, i.e. if the bunches remain healthy and a possible attack of the leaves doesn't affect photosynthesis considerably. Out of the experiments to date the following varieties emerge as provisional favourites: Regent, Bianca, GfGa-48-12, Orion, Leon Millet and Seyval blanc. Within a few years some other cultivars may become the favourites, because new varieties enter continuously experimentation thanks to the progress being achieved in grape breeding. With Bianca there will be some more investigations, specially to solve the problem of oxydation in the wine. With all the red hybrids there will be further winemaking experimentation, too; it concerns the optimum maceration time as well as the blending of varieties for more complexity.

As we have experienced in the wine tastings it is possible to make wines equivalent to those from European grapes. Compared with the traditional wines, specially those from our area, we may obtain very different and unusual types of wine on the base of hybrids. A similar historical experience was made when the variety Müller-Thurgau was introduced. It had been bred in 1882. The wine was first judged in a negative sense as unfamiliar (Eggenberger 1982). But it is also a matter of personal taste, whether a type of wine is accepted or not. The chances for marketing wines from interspecific hybrids look rather good in today's social and political context, even if there will not be a majority of consumers to turn to these wines. But it would be wrong not to go this way, because there is not a majority of consumers who appreciate hybrids, as it is the case with Pinot noir or Müller-Thurgau. Looking at the continuous development in grape breeding that produces constantly even better varieties, and based on the progress in winemaking with hybrids, we may be optimistic for the perspectives of the coming years. We consider the hybrids as an alternative or an option in addition to the traditional European varieties; the latter are not to be substituted.

The first interest for interspecific hybrids came from the organic growers because they saw a means to abandon the spraying completely. Thus, the possibility of growing disease resistant varieties will enhance the further development of sustainable viticulture such as integrated production or organic growing. The important advantage is that large quantities of fungicides could be spared. That way viticulture has the chance to become a very ecological crop worldwide. However this requires a little more freedom for the growers for the choice of the variety, as it is the case in America.

To date quite a large number of grapegrowers (up to 70 to 80) have planted a small plot of one or more interspecific hybrids for their own experiment, from 400 m² (1/10 of an acre) up to 2 hectares (5 acres). We welcome this kind of experiment, for it enables us to obtain more quickly a general appreciation of varieties in different areas than by field trials in only one or two locations. Thus the growers have also better possibilities to show and explain the new varieties to their customers and see how they appreciate them.

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PHYSIOLOGICAL ASPECTS OF TIKE-INDUCED CHLOROSIS IN SOME VITIS SPECIES. II. GENOTYPE RESPONSE TO STRESS CONDITIONS.

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Summary: one-year-old cuttings from seven *Vitis* species (*V. amurensis* Rupr., *V. berlandieri* Planch., *V. californica* Benth., *V. cinerea* Engelm., *V. longii* Prince, *V. monticola* Buckl., *V. riparia* Michx.) were grown in pots of a non calcareous and a calcareous soil. Leaves selected at the middle of the second year growing season were assayed to test the iron concentration of the dry matter and the total iron uptake. The most significant findings of the trial were: a) the shoot growth of *V. amurensis*, *V. longii*, *V. monticola* and *V. riparia* was strongly depressed by the calcareous soil; b) *V. berlandieri* and *V. californica* did take up a higher amount of iron when growing on the calcareous soil c) a high ash alkalinity occurred in the chlorotic leaves of *V. riparia* compared to the green ones, under the same iron concentration.

Key words: *Vitis* species, alkalinity, iron.

Introduction

The range of some physiological parameters of ten *Vitis* species growing on calcareous soil has been reported in the first part of this paper (Bavaresco et al., 1994). The ten species behaved according to their known degree of tolerance/susceptibility to lime-induced chlorosis given by the main ampelography treatises, except for *V. champini*, which is considered susceptible by Galet (1988), while it behaved like tolerant. The experiment of this paper deals with seven species out of those ten, comparing the behaviour of each of them growing on two different soils.

Materials and methods

One-year-old grapevine cuttings from seven *Vitis* species (*V. amurensis* Rupr., *V. berlandieri* Planch., *V. californica* Benth., *V. cinerea* Engelm., *V. longii* Prince, *V. monticola* Buckl., *V. riparia* Michx.) were grown in ID I - pots of a non calcareous and a calcareous soil. The non calcareous soil was prepared by mixing one part of peat, one part of soil and two parts of sand, while the calcareous soil was collected from the field and it had the following main characteristics: pH (H₂O) = 8.4; total carbonates = 64 %; active lime = 19 %. The soil was maintained near field capacity by drip irrigation. Every species included 14 plants (7 plants per each soil) trained to two shoots and the pots were placed in the open on a platform with a hail protection net. The shoot growth was checked every 10 days and young leaves (3rd, 4rd, 5rd, 6rd leaf from the shoot tip) were sampled 75 days after bud burst; the leaves were washed for 1 minute in 1 % NaOCl solution, then rinsed for 5 minutes in tap water and analysed as follows.

Chlorophylls: chlorophyll (Chl) a, b and total Chl were extracted from leaf discs by using 80% acetone for 72 hours in the dark, at 4 °C (Torrecillas et al., 1984). The Chl concentrations were determined by reading the absorbencies at 665 nm and 649 nm and calculated using the equations given by STRAIN and SVEC (1966). **Ash alkalinity:** it was assayed by the method of JUNGK (1968). 0.25 g of oven-dried leaves (the remaining blade after the discs) were ground (< 1 mm) and ashed at 556 °C in a muffle-furnace for about 2 hours till the ash got white. After cooling down, the ash was added with 15 ml of 0.1 N HCl and put inside a flask filled up to 100 ml with deionized water. A titration with 0.1 N NaOH followed, by using 0.1 % methyl orange as an indicator.

Mineral elements: mineral element (N, P, K, Ca, Mg, B, Fe, Mn, Cu, Zn) concentrations were assayed after wet destruction of the oven-dried leaves (the remaining blade after the discs). The following methods were used: flame photometry for K and Ca; atomic absorption spectrometry for Mg, Fe, Mn, Zn, and Cu; colorimetry for total N, P and B. **Chlorosis rating:** the scale of POUGET and OTTENWALTER (1978) was utilized, ranking from 0 (no symptoms) to 5 (severe chlorosis, with more than 10 % of the blade with necrosis), with graduations in between. At the end of the growing cycle (162 days after bud burst) the rating was repeated.

The aerial part of the plants was oven-dried, weighed and analysed to test the iron concentration, at the end of the growing cycle. The statistical analysis provided for a two-way-ANOVA with interaction and the means were compared by the LSD test, at a 5 % level.

The data were recorded at the 2nd growth year, when chlorosis symptoms occur much more, as observed in other pot trials (BAVARESCO et al., 1992 and 1993 a).

Results

The shoot growth was depressed by the calcareous soil except for *V. californica* (Fig. 1). The species suffering much more from stress conditions were *V. amurensis*, *V. longii*, *V. monticola* and *V. riparia*. The calcareous soil depressed the chlorophyll leaf concentration of each genotype in a significant way, except for *V. californica* and *V. cinerea* (Tab. 1.). The visual score (Tab. 2) did accord with the chlorophyll concentration. Along the growing cycle (2nd chlorosis rating) the effect of the soil was more evident for the species more susceptible to lime-induced chlorosis (*V. amurensis* and *V. riparia*), while the chlorosis occurrence of *V. berlandieri* and *V. cinerea* (tolerant species) was not affected in a significant way by the soil (Tab. 2).

Ash alkalinity of all the species, save *V. cinerea*, was statistically higher in the calcareous soil than in the other one (Tab. 2) and this parameter did relate in a negative and significant way to Chl concentration (Fig. 2). Iron leaf concentration was affected in a significant way by the soil only in the case of *V. amurensis*, *V. berlandieri* and *V. longii* (Tab. 2), with higher values in the calcareous soil for *V. amurensis* and *V. longii*. The shoot dry matter was depressed by the soil in all the species except for *V. californica* (Tab. 2). The most susceptible species (*V. amurensis* and *V. riparia*) strongly decreased the shoot content of iron when growing on the calcareous soil (Tab. 2), while *V. berlandieri* and *V. californica* did take up more iron in the same stress conditions.

The iron efficiency ratio was affected by the soil for *V. amurensis*, *V. longii*, *V. monticola* and *V. Riparia* (Tab. 2). The soil also affected the mineral element concentration of the leaves, specially for K and Ca (Tab. 3).

Discussion

The responses of the species to stress conditions have been various. The species most susceptible to lime-induced chlorosis, like *V. amurensis* and *V. riparia*, have been very impaired by the calcareous soil; the two species did produce 90 % and 65% less shoot dry matter respectively. The tolerant species, on the other hand, were less impaired by the stress conditions, and they decreased only 35% (*V. berlandieri*) and 40 % (*V. cinerea*) the shoot dry matter. Interesting was the behaviour of *V. californica*, a medium tolerant species, which increased 5 % the shoot dry matter when growing on the calcareous soil.

Another parameter emphasizing the different response of the genotypes to stress conditions was the chlorosis occurrence, checked by the chlorophyll analysis and the visual score. *V. riparia* and *V. amurensis* did show their susceptibility by getting very yellow, specially at the end of the growing cycle. On the other hand *V. berlandieri* and *V. cinerea* did not show chlorosis symptoms when growing on the calcareous soil. *V. riparia* behaved chlorotic also when cultured in vitro on a high bicarbonate substrate (BAVARESCO A parameter emphasizing very much the different response of the genotypes to stress conditions was the shoot iron content or, in other words, the capability of the plant to take up iron from the soil. *V. berlandieri* showed its tolerance features by taking up 46 % more iron when growing on calcareous soil, while *V. cinerea* did take up 25 % less iron. *V. riparia* and *V. amurensis* did show their susceptibility by taking up 88% and 98% less iron respectively. The iron content of the shoots was therefore more related with the chlorosis occurrence than the leaf iron concentration.

The iron efficiency ratio of the shoots is not the classical nutrient efficiency ratio, which is generally defined as total plant biomass produced per unit nutrient absorbed, but the amount of harvestable production per unit of iron absorbed (GOURLEY et al., 1994). The index was not related to the tolerance/susceptibility to chlorosis, and therefore it is not a useful tool to screen genotypes for iron efficiency. It seems to be in grapevine species, in fact, two ways of tolerance to lime-induced chlorosis, one with a high iron efficiency ratio (*V. champini*) and another one with a low value of that index (*V. berlandieri*)e this is due to the different mechanisms of tolerance (BAVARESCO et al., 1994).

The results of the present trial disagree with those of a previous experiment (BAVARESCO, 1990) in which three rootstocks were studied, and the most susceptible to chlorosis (101-14) had the lowest iron efficiency ratio; in that case, anyway, the growth conditions were different from the ones of this trial.

Ash alkalinity seems to be responsible for different chlorophyll concentrations under the same iron level, as already observed in a pot experiment with grafted plants (BAVARESCO et al., 1993 a). *V. riparia*, in fact, had the same iron concentration in both the soils, but different chlorophyll and alkalinity levels. The plants showing chlorosis symptoms had a higher ash alkalinity than the green ones. The ash alkalinity is related to the apoplast pH of the leaf and increasing pH of the leaf apoplast results in the precipitation of Fe (III) oxide hydrate (MENGEL and GEURTZEN, 1988; MENGEL et al., 1994).

This latter occurrence would decrease the uptake of iron from the leaf apoplast to the mesophyll cells, resulting in decreased chlorophyll synthesis and chlorosis, while at the same time the iron concentration of the leaf does not change.

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Tab. 1. Range of some physiological parameters depending on the genotype and the soil

	<i>V. amurensis</i>		<i>V. berlandieri</i>		<i>V. californica</i>		<i>V. cinerea</i>		<i>V. longii</i>		<i>V. monticola</i>		<i>V. riparia</i>		LSD
	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	
Tot. Chl. mg/100 g DW	239	193	403	291	318	287	402	375	369	324	425	223	438	239	40.4
Ash Alkalinity meq/100 g	97	127	94	121	78	100	74	79	83	98	89	106	69	104	7.0
Fe (leaf) ppm	59	95	71	58	59	58	77	77	57	74	67	62	55	59	9.2
Shoot DM g/plant	35	3	125	81	74	78	125	74	127	44	160	81	114	42	26.2
Shoot Fe mg/plant	12	0.2	13	19	14	20	20	15	19	2	32	3	25	3	5.9
Fe efficiency ratio g DM/mg Fe	29	16.9	9.3	4.4	5.4	4.2	6.4	4.9	7.0	21.9	5.0	29.8	4.6	14.5	5.4

n.c.: non calcareous soil c.: calcareous soil

Tab. 2. Chlorosis rating of the different genotypes depending on the soil

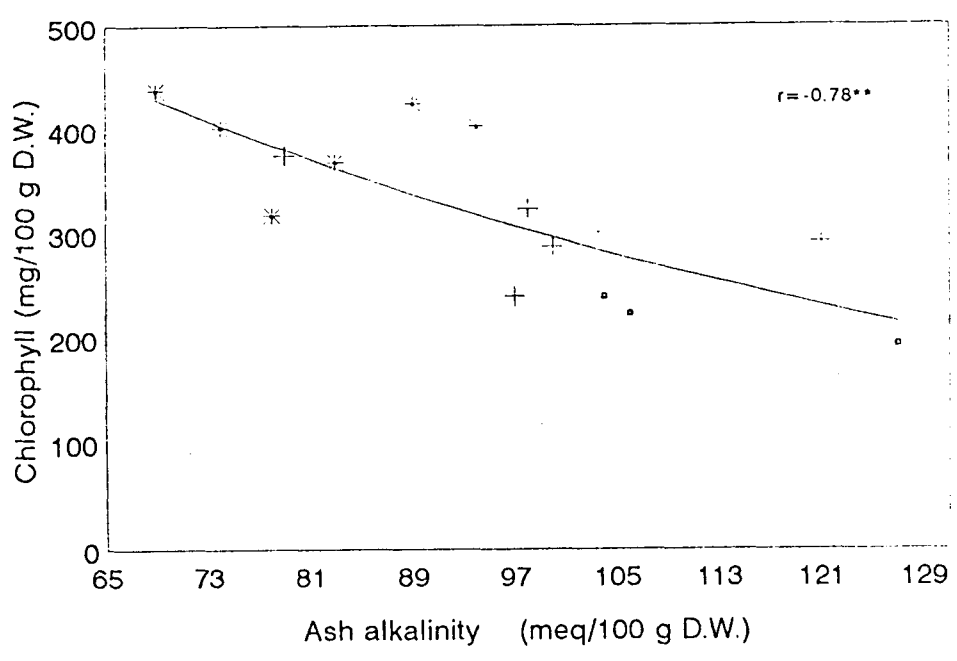
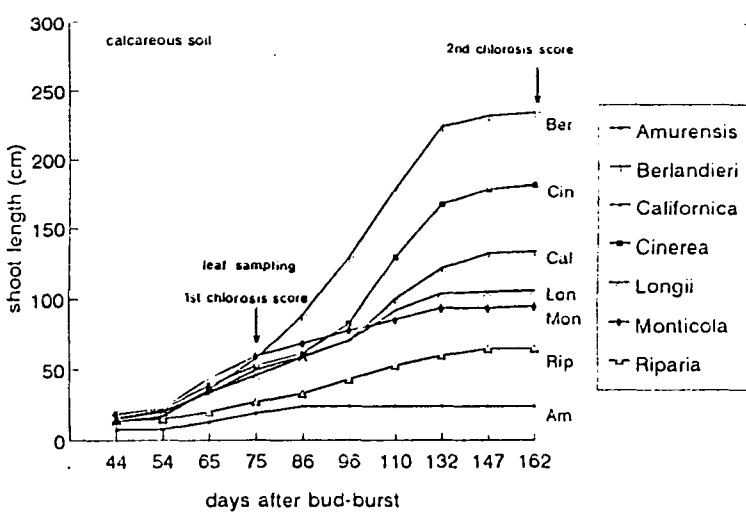
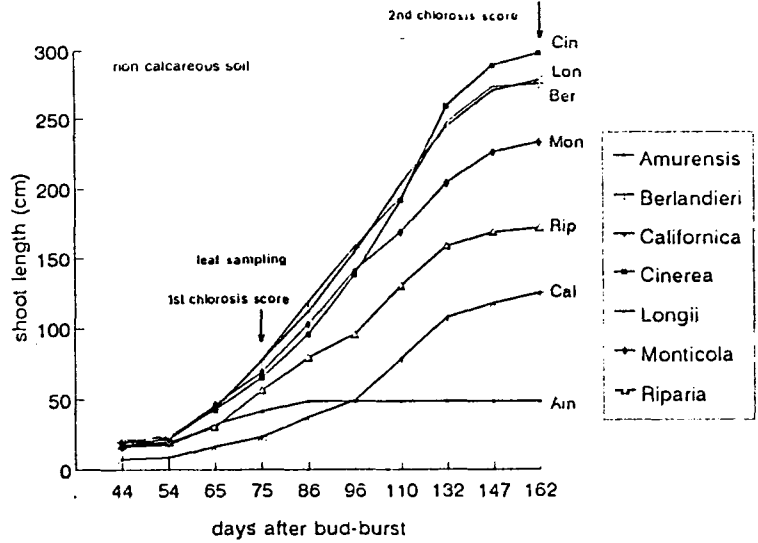
	<i>V. amurensis</i>		<i>V. berlandieri</i>		<i>V. californica</i>		<i>V. cinerea</i>		<i>V. longii</i>		<i>V. monticola</i>		<i>V. riparia</i>		LSD
	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	
1st chlorosis rating*	0.8	3.0	0.0	0.8	0.0	1.4	0.2	0.8	0.2	1.4	0.0	1.6	0.0	2.2	0.77
2nd chlorosis rating*	0.8	4.7	0.0	0.7	0.0	1.1	0.0	0.0	0.0	2.1	0.0	0.9	0.0	4.1	0.80
Mean*	0.8	4.0	0.0	0.8	0.0	1.2	0.1	0.4	0.1	1.8	0.0	1.2	0.0	3.2	0.63

*: 0: none; 5: severe n.c.: non calcareous soil c.: calcareous soil

Tab. 3. Leaf concentration of mineral elements depending on the genotype and the soil

	<i>V. amurensis</i>		<i>V. berlandieri</i>		<i>V. californica</i>		<i>V. cinerea</i>		<i>V. longii</i>		<i>V. monticola</i>		<i>V. riparia</i>		LSD
	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	n.c.	c.	
N%	2.79	2.64	2.51	2.25	2.41	2.72	3.03	2.54	2.77	2.91	3.02	3.27	3.03	4.04	0.31
P%	0.30	0.27	0.27	0.31	0.23	0.31	0.27	0.23	0.30	0.29	0.31	0.34	0.33	0.42	0.05
K%	1.09	1.41	0.88	1.71	0.87	1.59	0.89	1.18	0.90	1.66	0.90	1.63	0.87	1.33	0.17
Ca%	0.87	1.23	0.91	1.32	0.79	1.35	0.61	1.18	0.68	1.36	0.97	1.17	0.81	1.32	0.27
Mg%	0.29	0.38	0.40	0.35	0.38	0.35	0.27	0.38	0.27	0.42	0.29	0.35	0.34	0.33	0.09
Mn ppm	17	47	27	28	18	22	31	53	22	33	20	31	20	26	2.1
Cu ppm	11	10	11	11	8	9	10	9	11	11	11	11	11	10	1.1
Zn ppm	29	29	23	24	23	25	23	28	24	26	27	26	29	28	4.6
B ppm	14	24	16	15	14	18	8	12	11	14	17	16	15	15	5.4
K/Ca	1.25	1.27	0.96	1.30	1.10	1.21	1.46	0.99	1.33	1.24	0.95	1.39	1.09	1.01	0.34
P/Fe	50.8	28.6	37.4	55.2	39.5	53.9	35.6	29.8	53.9	39.0	44.4	53.8	59.5	70.9	12.2

n.c.: non calcareous soil c.: calcareous soil



• Chlorotic leaves + Green leaves * Dark green leaves

SESSION 6 : MIXED TOPICS

LES PRINCIPAUX GROUPES ECOGEOGRAPHIQUES DANS L'ENCEPAGEMENT FRANCAIS

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GENERALITES

Element de la flore spontanee dans son habitat naturel depuis le Neolithique. la vigne sauvage, a laquelle GMELIN 1806 a donne le nom de *Vitis silvestris* Gmel., soumise a la fois aux influences ecogeographiques et a la selection effectuee par l'homme depuis plusieurs millenaires, demeure bien l'ancetre de nos vignes cultivees *Vitis vinifera* L.

L'individualisation des cultivars ou cepages s'est effectuee dans diverses regions a partir de formes sauvages distinctes et, liee aux mouvements des peuples, des civilisations, des religions, elle a conduit a la formation locale d'unites possedant, des caracteres anatomiques et des proprietes physiologiques voisines. Apparus en conditions ecogeographiques identiques, ces groupes possedent une aire determinee d'extension et ont ete obtenus par selection artificielle. Il est logique avec NEGRUL 1946 de les nommer Proles (fig. 1).

A l'interieur de chacune des trois proles reconnues et caractrisees par cet auteur se presentent d'autres unites plus ou moins homogenes et proches de leur origine, notamment semis naturels apres autofecondation ou hybridation intraspecifique. Ce sont la les sortotypes NEGRUL op. cit. ou groupes ecologico-geographiques LEVADOUX 1948.

Formes de cultivars proches les uns des autres, ces sortotypes possedent des caracteres anatomiques voisins qui permettent d'etablir leur parente. Cette methode s'acquiert par la frequentation constante des collections et des vignobles dans lesquels s'effectue le releve objectif des caracteres communs majeurs presentes par les cultivars. Les descriptions, souvent subjectives quoique codifiees (OIV 1983), peuvent etre completees par des criteres ampelometriques plus objectifs et parfois discriminatifs (BISSON 1985).

Chez la vigne multipliee vegetativement les cultivars qui repondent aux besoins de la pratique viticole mais qui n'ont aucune signification botanique, sont constitues, selon le leur degre d'anciennete et d'evolution par un clone ou le plus frequemment par un melange de clones.

Enfin, un cultivar donne et ses mutations gemmaires ne differant que par un seul caractere ou un nombre restreint de caracteres forment un sortogroupe Negr., unite qu'ODART 1874 avait deja nommee "tribu". Le Chasselas et le Pinot avec leurs variations de couleur de la baie fournissent de bons exemples du sortogroupe.

Telle se presente achevement l'espece *Vitis vinifera* L. et ses differentes unites taxonomiques resumees dans le tableau 1 et sur lesquelles se base la classification des cultivars et leur amelioration genetique et clonale.

APPLICATION A L'ASSORTIMENT VARIETAL FRANCAIS

Une nation viticole relativement ancienne comme la France, s'est constituee progressivement a partir du fonds plus ou moins evolue des lambrusques autochtones un assortiment de cultivars adapte au milieu, aux besoins culturels, technologiques et economiques. Les vignes sauvages signalees en France dans le passe par differents auteurs, et en maints endroits, ont ete recensees par PLAXCHON 1887 avant leur disparition quasi complete sous l'influence des grands parasites americains, *Phylloxera vastatrix*, *Uncinula necator*, *Plasmopara viticola*, etc. (fig. 2).

La creation d'une viticulture moderne post-phylloxerique, la rationalisation de la production des raisins, des vins et des eaux de vie, les progres scientifiques, la selection varietale et clonale ont des moyens de communication, conduit a une reduction et a une simplification de l'assortiment varietal. De 2000 peut-etre au milieu du XVIIIe siecle, sans tenir compte des synonymies nombreuses et embrouillees, apres de serieuses etudes ampelographiques et Ahomastiques, le recensement necessite par le cadastre viticole vers 1960 a montre que les cultivars restes en exploitation atteignaient a peine 400 aujourd'hui, y compris les cepages d'origine etrangere.

Avant que la situation ne se simplifie encore davantage, un tableau synthetique et taxonomique des cultivars francais toujours presents le vignoble quelle que soit leur importance, doit etre dresse.

DEFINITION DU SORTOTYPE

C'est LEVADOUX op. cit. qui, sans connaitre alors les travaux de NEGRUL, devait, en France, fonder la theorie des groupes de cepages ecologico-geographiques suscitant la constitution d'une ecole de pensee et d'action. Dans un travail s'inspirant du systeme LEVADOUX op. cit., BISSON 1989 rappelle l'historique de cette conception et de ses principales applications. Le sortotype ou ecogroupe se definit par un certain

nombre de caracteres anatomiques, a partir d'un cultivar de reference choisi pour son importance ou anciennete culturale et son role dans l'evolution du

groupe ou dans son onomastique.

A) Les principaux caracteres distinctifs d'un sortotype

1) Le bourgeonnement principal (forme, villosite, coloration)

2) Le rameau herbage (relief, coloration)

3) La feuille (forme generale, iaille, decoupage denture marginale, mouvement, couleur, relief du limbe).

Bien definis, ces caracteres suffisent a circonscrire un sortotype. La grappe, la baie, le sarment, observables sur une periode plus courte que la feuille et les caracteres phenologiques ou technologiques peuvent offrir des criteres secondaires.

B) Les differents sortotypes LEVADOUX op. cit. a deja distingue des groupes tres homogenes formes de cultivars proches et constituant des ensembles bien caracterises qu'il a nommes "groupes fermes" et d'autres unites plus larges ou se retrouvent des cepages voisins mais exteriorisant des parentes plus laches definis comme "groupes ouverts". Ces derniers presentent des cultivars pouvant assurer des maillons de passage entre differents groupes (voisinage geographique ou cohabitation sur une zone).

C) Aire geographique d'origine et d'extension d'un sortotype Les cultivars d'un ecogroupe issus de lambrusques autochtones occuperent d'abord le milieu naturel d'origine et d'adaptation. Une zone d'extension et de dissemination variable en etendue ou en eloignement du depart s'est creee par la suite. conditionnee par les exigences ecologiques du sortotype ou les besoins technologiques locaux. Il demeure probable que l'aire d'extension englobe souvent le lieu d'origine ou qu'il en reste proche.

D) Stabilité et perennite du sortotype

Les lois biologiques parfois inflechies par l'homme ont entraine l'evolution des ecogroupes (semis, mutations, selections) sans que l'existence et la nature fondamentales de ceux recenses aujourd'hui ne soient encore tres eloignees de leurs origines. Ce sont les regles taxonomiques etablies par l'homme qui n'assurent pas un caractere definitif aux classifications. Par exemple, le Tannat classe primitivement dans les MANSIENS par LEVADOUX op. cit. a ete plus logiquement ramene vers les COTS par ARTOZOUL et BAUDEL 1954. De meme, le Sauvignon a l'origine range parmi les CARMENETS, a ete rattache recemment aux MESSILES Ces quelques modifications, plus frequentes dans les "groupes ouverts" ne remettent pas en cause le systeme fondamental.

LES SORTOTYPES FRANCAIS ACTUELS

Aujourd'hui, les sortotypes francais atteignent a peine la vingtaine. Les effectifs des cultivars encore exploites chez chacun, les importances culturelles individuelles, leur interet technologique different largement. De meme que le nombre des cepages exploites, celui des ecogroupes tend a se restreindre d'ou l'urgence de leur etude. La figure 3 donne la situation des sortotypes nationaux avec leurs zones globales d'extension geographique. Quelques uns presentent encore une grande importance culturale ou oenologique. Certains possedent un caractere historique ou taxonomique particulier. D'autres, en voie de disparition culturale, n'offrent plus qu'une valeur genetique conservatoire.

1) Les NOIRIENS (Nigrales Lev.) avec le Pinot noir pour type, occupent en France la plus large zone d'extension. Ils reunissent des cultivars aussi repandus que le Chardonnay, le Gamay et le Meunier qui fournissent des vins mondialement reputes tels les Bourgogne, Champagne et Beaujolais. Ils comptent aussi de nombreux cepages en voie de disparition (Brumeau d'Auvergne et Genouillet du Centre, par exemple).

2) Les SERINES type Syrah occupent la zone viticole rhodanienne Nord et Centre. La Mondeuse, la Roussanne, La Marsanne le Viognier, entr'autres, appartiennent a ce sortotype et produisent de grands vins rouges ou blancs (Cote Rotie, Hermitage Condrieu, Chateau-Grillet, etc..).

3) Les CARMENETS type Cabernet franc forment le sortotype d'elite du Sud-Ouest. Les cepages archaiques que sont le Petit Verdau, la Carmenere, la Here sont proches du Merlau et du Cabernet-Sauvignon generateurs des grands Bordeaux rouges (Medoc, Saint-Emilion, Pomerol, etc..), d'appellations excentrees (Bergerac, Cotes de Buzet, Irouleguy rouges, etc...) ainsi que Touraine et Anjou (Bourgueil, Chinon, Saumur-Champigny, etc..).

4) Les COTS (Cadurcae Bis.) avec le Cot pour type comptent parmi l'assortiment varietal du Sud-Ouest. La Negrette le Tannat, la Merille, etc..., appartiennent a ce sortotype dont les vins de Cahors, le Madiran, le Fronton, etc..., ont tire leur originalite et leur reputation pres de proches parents qui fournissaient des vins de table (Grapput, Valdiguié Pejac, etc..).

5) Les MANSIENS avec notamment le Petit et le Gros Mansenc qui figurent parmi les cepages nobles des appellations pyreneennes (Jurancon. Pacherenc du Vic Bilh, etc..) constituent un groupe typique. Par contre, les Courbuts nettement differents, sont a rattacher aux CARMENETS.

6) Les FOLLES (Folloides Lag.) avec la Folle blanche, la Folle noire, les Jurancon blanc et noir, l'Ondenc, etc..., contribuerent jadis a la production de vins de grande consommation. De nos jours, sauf pour le Gros plant du Pays nantais, leur role est bien reduit. Les membres de ce groupe disparaissent ou contribuent a l'elaboration des grandes eaux de vie (Cognac, Armagnac) encore que discretement en raison de la sensibilité de la Folle greffee au Botrytis cinerea.

7) Les MESSILES (Miscellae Bis.) type Chenin blanc dominant dans la vallee de la Loire. Les Mesliers le Sauvignon, l'Arbois l'Aunis, constituent les principaux cepages de ce sortotype. Le Chenin blanc permet

d'obtenir de grands vins blancs divers (secs, moelleux, liquoreux, effervescents : Vouvray, Anjou, Cremant de Loire, etc.). Le Sauvignon fournit des secs reputes (Blanc-fume de Pouilly, Sancerre, Quincy, etc.).

8) Les GOUAIS (Gubiae Lev.) representes par le Gouais type avec l'Enfarine, le Samoireau, le Saint-Pierre dore, etc... occuperent de nombreux vignobles en France ou leur forte production et, la grande acidite de leurs vins les faisaient apprecier. Actuellement, seuls la Muscadelle et l'Aligote maintiennent quelques positions.

9) Les GRAS avec la Graisse ou Plant de Graisse pour type et qui regroupent diverses Chalosse fournissent l'exemple d'un sortotype recherche autrefois comme le precedent pour sa fertilité et l'acidite de ses raisins dans le Sud-Ouest et en voie d'extinction complete en culture.

10) Les LANGUEDOCIENS forment un ecogeogroupe compose de cultivars autochtones (Aspiran, Piquepouls, Cinsaut, etc...) presents et exploites avant l'introduction des cepages espagnols (Aramon, Grenache, Carignan, etc...) ou italien (Ugni blanc).

11) Les PROVENCAUX dans une zone tres anciennement convertie a la viticulture par les Pheniciens, les Grecs et les Romains presentent le cas d'un fonds indigene spontane ou postcultural articule probablement autour de cultivars semblables a la Panse de Provence et qui possedaient trois fins : la cuve, la table et le sechage.

12) Les ALPINS ne sont sans doute pas vraiment individualises et pourraient avec le Persan, l'Etraire, etc... ne constituer qu'un seul groupe rhodanien joints aux SERINES dont la forme archaïque serait le Mondeuse a cote d'un second sortotype DURIF.

13) LES RHENANS cultivés en France appartiennent a plusieurs groupes d'origine germanique ou d'Europe centrale. Chacun des trois cepages principaux : Riesling, Sylvaner, Gewurztraminer appartient a des sortotypes differents. Seul le dernier, avec les Savagnins, le Trousseau, le Cifiquien, etc... devrait sans doute permettre d'individualiser un groupe COMTOIS plus francais. Tels sont avec 11, 12 et 13 les types de problemes resolus ou a resoudre encore au niveau de la caracterisation et de la classification des sortotypes ou ecogeogroupes francais.

CONCLUSIONS

Le systematique taxonomique des principaux sortotypes francais permet

- 1) de conforter la theorie des sortotypes ou ecogeogroupes
- 2) d'apporter des precisions eventuelles sur l'origine et l'evolution des cultivars autochtones
- 3) de fournir d'interessants elements a la genetique classique dans le choix des geneiteurs pour l'hybridation ou pour le genie genetique
- 4) d'etablir ou de confirmer une classification botanique servant d'hypothese a des travaux bases sur des composés biochimiques precis ou sur des procedés physiques de pointe.

Cette communication se presente enfin comme le sommaire d'un travail plus complet qui devrait faire l'objet d'une publication ulterieure.

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SUMMARY

The natural french vine varieties assortment has been much reduced during the years because of many factors, principally the post-phyllloxeric reconstitution, the rationalisation of the production and the varietal and clonal selections.

Before a still more important reduction is to be made it is necessary to give a classification of french autochton cultivars based on ecogeographic groups or sortotypes.

These works keep classic genetic interest in possible crossings : less inbreeding, more heterosis. Besides they enable a better approach of modern classifications based on biochemistry or atomic physics' (pigments, enzymes, tracers and so on) so as to confirm or infirm botanic results.

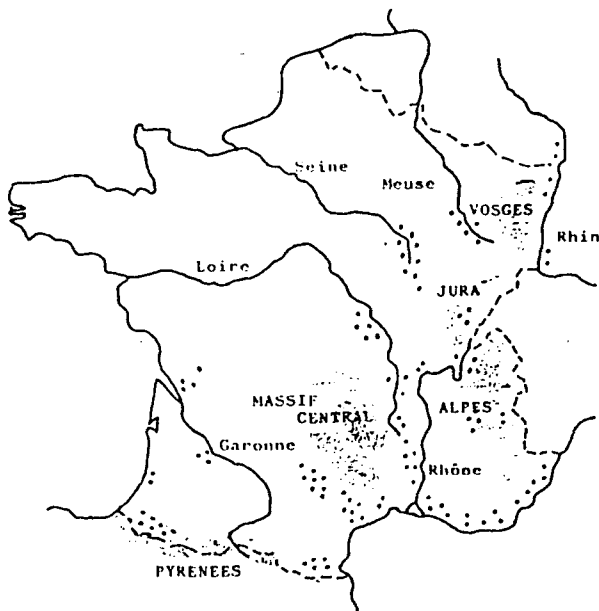
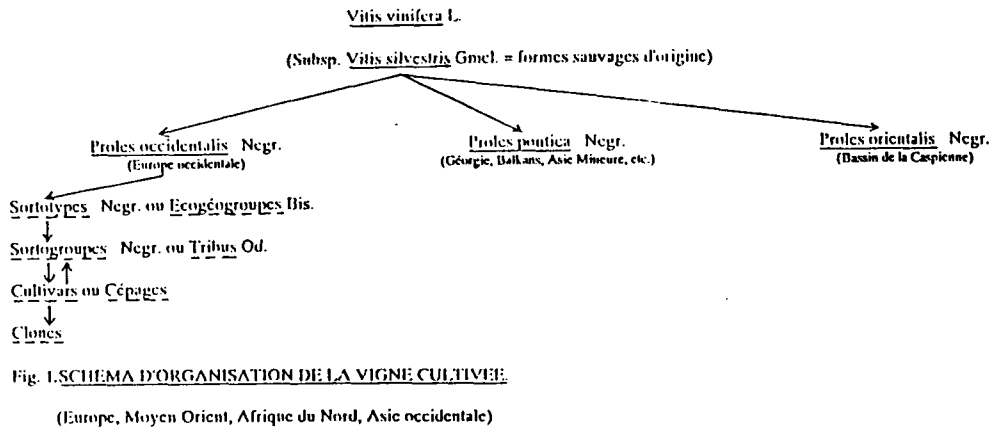


Fig. 2. LES VIGNES SAUVAGES EN FRANCE D'APRES PLANCHON 1967.



Fig. 3 - Zones d'origine ou d'extension des principaux groupes ampelographiques français.

SESSION 6 : MIXED TOPICS

BREEDING OF NEW, RESISTANT "POLYVITIS TRIHYBRID" GRAPE VARIETIES IN EGER

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INTRODUCTION

Following the Second World War the Hungarian Research Institute for Viticulture and Enology has carried out the reorganisation of the national research network. Within this program a Research Station for Viticulture was established in Eger with the task of grape breeding. One of the contracted research tasks of the Station was to produce new grape varieties that are suitable to replace direct producer grape varieties and are resistant but still produce European quality grapes (2,3). The monographic surveys made at that time showed that on 40 to 45 thousand hectares in Hungary direct producer vines were grown. This significantly large area had a serious effect on the vine industry which was trying to orient itself towards the international wine market. Development of resistant grape varieties by cross breeding was started in our institute in 1948. French hybrids contained American lines were selected as basic material. Two clones - EGER I and EGER 2 - were selected from tree pollinated *Serve Villard 12286* and *Serve Villard 12375*, respectively. Consequently, the inheritable resistance was given in these clones. They were used as one of the parents and *Muscat Ottonel*, *Medoc noir*, *Bouvier*, *Csabagyongye*, *Kadarka*, etc. - are derived from Europe and give good quality - were other parent. The first result of this work was a new variety named *Zalagyongye* (Part of Zala), which was given a general, state-wide recognition in 1978. Further achievements of this work are varieties as *Bianca*, *Medina* and others (1-9).

The experience gained in growing these interspecific hybrids in vineyards has urged us to increase production reliability by increasing resistance mainly in the field of winter hardiness (5, 7).

MATERIALS AND METHODS

We contacted the Grape and Wine Institute of Novochockassk, near Rostov in the Soviet Union, in 1970 in order to continue the research work under a joint program. The Institute in Novochockassk was mainly doing crosses with *Vitis amurensis* of Asian origin, therefore, we selected those varieties for crossing partners that had the explicitly frost resistant *Vitis amurensis* in their combination (2, 3, 10).

From Eger the new resistant varieties and hybrids were selected, because these already combined the hereditary base of American and European grape varieties.

The geographical location of the two towns is almost the same since both located close to the 48° latitude. Thus the time of flowering is almost the same.

The distance between the two institutes is about 1000 km. Therefore, the pollen needed for the cross was collected in Eger early in the morning and was sent by plane in disintegrators from Ferihegy Airport in Budapest. The date of shipments was agreed beforehand and we received the exchange pollen in the same way.

Seeds obtained from about 60 different cross combinations were germinated using identical procedures, the seedlings were grown and selected after reaching the stage of full bearing.

RESULTS AND DISCUSSION

Four "polyvitis trihybrid" variety candidates were selected from the huge seedling population originating from the above mentioned cross combinations (10). The crossing formulas of the four selected trihybrids are shown in Table 1. Considering the viticultural features (Table 2) of hybrids, it can be pointed out, that all varieties have excellent resistance against downy mildew and powdery mildew and varieties originating from *Zalagyongye* x *Kazachka* combinations have worse resistance against *Botrytis* than the two other varieties. The variety *NOVO EGER H* has excellent frost resistance, the others are of medium resistance. The bud burst is usually at the end of April and generally the flowering starts in the second week of June. All varieties are of early ripening; it means in the middle of September. The bunch size ranges from little to big. The berries are mainly of middle size, yellow coloured and juicy, having pleasant and spicy taste.

The main characteristics of hybrids

NOVO EGER I is a white wine variety. It is of early ripening and of medium cropping potential. It has medium growing capacity, good frost resistance and medium big bunches. The berries are medium big, yellow coloured and pleasant to taste. Its wine is fresh and of reductive character, it can be qualified as quality wine.

NOVO EGER II is a white wine variety It is of early ripening and of medium cropping potential. It has medium growing capacity, very good frost resistance and little-medium bunches The berries are little, rounded, yellow coloured and pleasant to taste Its wine is of varietal flavour, harmonic, it can be qualified as quality wine

NOVO EGER III is a white wine variety It is of early ripening and of medium cropping potential. It has medium growing capacity, medium frost resistance, little bunches The berries are medium big , yellow coloured, of thick skin and pleasant to taste Its wine has reductive character , it can be qualified as table wine

NOVO EGER IV is a white wine variety It is of early ripening and of good cropping potential. It has good growing capacity, medium frost resistance and big bunches The berries are little and yellow coloured Its wine has reductive character, it can be qualified as table wine

The enological data are summarised in Table 3 Must of hybrids harvested on the 9th and 21st of September have about 22-24 Brix-grade and a total acidity of 6-8 g/l The must of hybrid harvested earliest has the highest acidity and the lowest sugar content Naturally, the wine of this hybrid has the lowest alcohol content , and the rest of varieties have about 13-14% v/v The hybrids from Zalagyongye x Kazachka combination are of high sugar-free extract and have pleasant acidity The other two varieties have lower values in both characteristics

In 1993 and 1994 some of these varieties were tasted on the regular wine-tasting of our institute where the well-known Hungarian enologists participated Table 4 summarises the results of wine tasting in 1993 The NOVO EGER I hybrid has as good result as Chardonnay, Pinot blanc and Sauvignon blanc with different bud loading and harvesting time In 1994 a blind tasting was performed from interspecific hybrids and Vitis vinifera varieties Table 5 shows the results of this tasting It was a big surprise that Zalagyongye and the trihybrids from Zalagyongye x Kazachka combination proved to be as valuable and tasty as Pinot gray, Rhein riesling and Leanyka clon This results show that as good wine can be produced from interspecific hybrids as from Vitis vinifera varieties We think there is a serious prejudice against interspecific hybrids

On the basis of above mentioned data the hybrid NOVO EGER I was picked up from these varieties as the most valuable hybrid and was named " Viktor" as a variety candidate (3) Table 6 shows the crossing formula of Viktor

The field trials showed that regarding the morphological features the characteristics of both varieties could be observed in the hybrid "Victor" We contributed to the fact that the two varieties combined well and united the characteristics of the two parents It shows good resistance to diseases Phylloxera forms galls on the leaves and this is a phenomenon indicating the American hereditary base Good winter hardiness was also indicated by the morphological features, and the wood pith ratio (2/1) also points to this fact Sensory evaluations showed that its sugar to acid ratio is good When harvested in the second part of September the must has 20 Brix grade and a total acidity of 8 g/l Its wine is pleasant, harmonious with a moderately aromatic flavour

SUMMARY

On the basis of world-wide information the assumption can be made that in the next century production will definitely be controlled by environment protection regulations The use of pesticides will be restricted Moreover, there will be prohibited chemicals, just like there are already very strict regulations for the "bio" products Grapes treated with prohibited chemicals and the wines made from them could well be batted front the market This situation will do a further selection on the grape varieties remaining in production The conclusion from this is that the road of the future is the more widespread use and international recognition of resistant and environment friendly grape varieties In accordance with the expectation of the past few years damages made to the environment must be reduced to minimum In viticulture this could be guaranteed through resistance provided on genetic basis.

The Research Station for Viticulture and Enology in Eger has enriched its several-decade old grape breeding activity, with yet again a new valuable achievement In fact we can announce the birth of several new grape varieties, polyvitis trihybrids The genetic composition of these hybrids may open new avenues for the production of additional interspecific grape varieties

Based on their favourable cultivation results known so far these hybrids, especially Victor may count on international interest and may enlarge the range of varieties of both the Hungarian and international "eco-bio viticulture".

ACKNOWLEDGMENT

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No 804-7 Zalagyöngye x Kazachka
NOVO EGER I

No 804-9 Zalagyöngye x Kazachka
NOVO EGER II

No 814-1 (Muscat Ottonel x Eger 2)
NOVO EGER III x Stepniak

No 815-1 [Eger 2 x (Medoc noir x Csabagyöngye)]
NOVO EGER IV x Fioletovyi Rannii

Table 1.: The crossing formulas of the four selected trihybrids

	Zalagyöngye x Kazachka No 804-7 NOVO EGER I	Zalagyöngye x Kazachka No 804-9 NOVO EGER II	(Muscat Ottonel x Eger 2) x Szepmik No 814-1 NOVO EGER III	{Eger 2 x (Medoc noir x Csabagyöngye)} x Fioletovyi Rannii No 815-1 NOVO EGER IV
Downy mildew resistance	excellent	excellent	excellent	excellent
Powdery mildew resistance	excellent	excellent	excellent	excellent
Botrytis resistance	medium	medium	excellent	excellent
Frost resistance	medium	excellent	medium	medium
Mean time of bud burst	28. 04.	29. 04.	01. 05	24. 04.
Mean time of start of flowering	12. 06.	14. 06.	14. 06.	08. 06.
Vigour	medium	medium	medium	strong
Period of maturity	early	early	early	early
Bunch	medium	little-medium	little	big
Berry size	medium	little	medium	little
Berry colour	yellow	yellow	yellow	yellow
Berry flesh	rich in juice, soft	poor in juice	rich in juice	rich in juice, soft
Berry flavour	pleasant, aromatic	pleasant, aromatic	pleasant, weak	pleasant

Table 2.: Viticultural features of the polyvitis trihybrids

	Zalagyöngye x Kazachka No 804-7 NOVO EGER I	Zalagyöngye x Kazachka No 804-9 NOVO EGER II	(Muscat Ottonel x Eger 2) x Szepmik No 814-1 NOVO EGER III	{Eger 2 x (Medoc noir x Csabagyöngye)} x Fioletovyi Rannii No 815-1 NOVO EGER IV
Harvest time	21. 09. 1993	01. 09. 1993	09. 09. 1993	09. 09. 1993
Crop quantity t/ha	5.0	3.4	5.9	5.7
Must				
Brix°	24.1	18.8	21.8	21.9
Total acidity g/l	7.7	9.2	6.1	6.4
Wine				
Alcohol v/v %	14.18	10.83	12.97	12.90
Sugar g/l	8.5	11.9	3.6	6.7
Sugar-free extract g/l	24.02	20.62	16.18	17.76
Total acidity g/l	6.0	7.3	4.6	5.2

Table 3.: Harvest data and analytical features of must and wine made from the polyvitis trihybrids

Gained position	Wine variety	Mean value
1	Chardonnay III/1	18.38
2	Bianca	17.71
3	Chardonnay III/3	17.62
4	Chardonnay III/2	17.29
5	Rhein riesling T.68	17.22
6	Sauvignon bl. III/1	17.06
7	Pinot bl. III/1	17.02
8	NOVO EGER I	16.90
9	Zenit	16.89
10	Zalagyöngye (Pearl of Zala)	16.86
11	Auxerois	16.79
12	Chardonnay II/1	16.75
13	Sauvignon bl. III/2	16.69
14	Pinot bl. III/2	16.68
15	Pinot bl. II/3	16.66
16	NOVO EGER II	16.63
17	Sauvignon bl. II/1	16.59
18	Kerner	16.40
19	Sauvignon bl. III/3	16.35
20	Pinot bl. III/3	16.34

Table 4.: Results of wine tasting in Research Station for Viticulture and Enology of University of Horticulture and Food Industry Eger, May 15, 1993.
Roman numbers mean different harvest times, Arabic numbers mean different bud loading.

Gained position	Wine variety	Mean value
1	Zalagyöngye	17.09
2	NOVO EGER I	16.99
3	NOVO EGER II	16.97
4	Red tramini Fr.	16.94
5	Rhein riesling	16.92
6	Bianca	16.90
7	Gyöngyüzling	16.87
8	Leányka E-100 clone	16.81
9	NOVO EGER IV	16.59
10	Gocsei zamatos	16.53
11	EB-6	16.49
12	Pinot grey	16.41

Table 5.: Results of wine tasting in Research Station for Viticulture and Enology of University of Horticulture and Food Industry Eger, May 10, 1994.

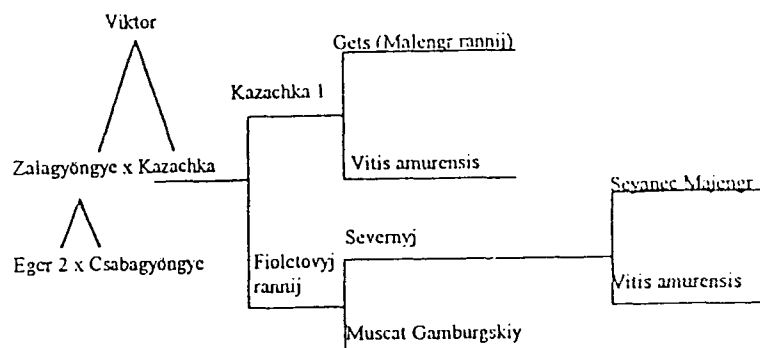


Table 6 . The crossing formula of Viktor

BIOCHEMICAL. STUDIES ON RESISTANCE OF GRAPEVINES (VITIS SPP.) TO DOWNY MILDEW (PLASMOPARA VITICOLA)

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SUMMARY

Host-parasite interactions between grapevines (*Vitis* spp.) and downy mildew (*Plasmopara viticola* (B. et C.) Bert. et de T.) were studied by histochemical analysis. Three kinds of behavior, susceptible (*Vitis vinifera* cv. Grenache), intermediate (*V. rupestris* cv. du Lot), and resistant (*V. rotundifolia* cv. Carlos) were chosen for this study. After inoculation, in resistant cv. Carlos, flavonoid compounds were detected at 2 days. In *rupestris* cv. du Lot, resveratrol and peroxidase activity were detected at 5 days, flavonoids were induced at 8 days, and lignin was formed at 15 days. In susceptible cv. Grenache, only a small amount of yellow autofluorescence was observed 8 days after inoculation. These data suggest that the rapidity of flavonoid formation plays an important role in the resistance of *V. rotundifolia* to *P. viticola*. The formation of resveratrol, flavonoids and lignin in *rupestris* cv. du Lot probably restricts the development of this pathogen.

INTRODUCTION

Downy mildew caused by *Plasmopara viticola* (Berk et Curt.) Berlese et de Toni is an important disease threatening many of the world's viticultural areas. Resistance to the pathogen is found in *Muskadina* spp. as well as in a number of *Vitis* spp (particularly native American species) that cross readily with *Vitis vinifera* L., and these have all been used as sources of resistance in grape breeding programs of many countries (Doazan, 1980).

In *Vitis* spp., synthesis of stilbene derivatives can be induced by UV irradiation, various chemicals and inoculation with *Botrytis cinerea* Per. or *P. viticola* (Langcake, 1981; Langcake and Pryce, 1976). Biochemical work carried out with *B. cinerea* and *P. viticola* suggested the importance of stilbene in resistance, but less information is available concerning histochemical studies on the interaction between *Vitis* spp. and *P. viticola*.

The objective of the present work was to gather information on the relationship between grapevines and *P. viticola*, with the aid of histochemical observation. The results give an overview of induced defence reactions found in *Vitis* species with different susceptibility to this fungus, and the involvement of resveratrol, flavonoids, lignin, and peroxidase activity in resistance.

MATERIALS AND METHODS

Plant material. *Vitis rotundifolia* cv. Carlos (resistant), *V. rupestris* cv. du Lot (intermediate resistant), and *V. vinifera* cv. Grenache (susceptible) were chosen for this study.

Histochemistry and observation. Discs (1 cm in diameter) were removed from the leaves and mounted on microscope slides with the abaxial surface uppermost. Sections (39 µm thickness) of test leaves were cut with a freezing - stage microtome. After staining with the different reagents, the discs or sections were mounted in the reagents or in glycerine : water (15 : 85, v:v) and examined using a light microscope (Nikon Optiphot) with two filter sets: a UV filter set with 365 nm excitation and a 400 nm barrier filter, and a blue filter set with 420 nm excitation and a 515 to 560 nm barrier filter.

The flavonoid compounds were detected using Neu's reagent (Neu, 1956; Dai et al., 1994a). The results were confirmed with Wilson's reagent (Hariri, 1991).

Two tests for lignin were employed: phloroglucinol-HCl (Gabon, 1984) and Mirande reagent (Deysson, 1954). Peroxidase (EC 1.11.1.7) activity in fresh sections was localized with 3,3',5,5'-tetramethylbenzidine (TMB, Sigma) using the method previously described (Imberty, 1984). Autofluorescence was also monitored under UV light.

RESULTS

Histochemistry.

A few stomatal cells with yellow autofluorescence were detected 8 days after inoculation in lesions in the susceptible cv. Grenache. In *rupestris* cv. du Lot, a blue autofluorescence was seen in the diffuse necrotic areas 5 days after inoculation. This blue autofluorescent compound was extracted and identified as resveratrol by

comparison of the UV spectrum and HPLC retention time of a commercial sample of resveratrol with those of the extracts.

After treatment with either Neu's or Wilson's reagents, the guard cells and the cells around the necrotic stomata, of cv. Carlos, 2 days after inoculation, emitted a yellow fluorescence after excitation at 365 nm, and a lemon-yellow fluorescence after excitation at 420 nm. In rupestris cv. du Lot, the cell walls between the healthy tissue and the necrotic tissue emitted a yellowish fluorescence under UV, and a yellow fluorescence under blue light. These results demonstrate that the flavonoid compounds were induced following infection by *P. viticola* in both the resistant cv. Carlos and in rupestris cv. du Lot. Staining with Mirande reagent and phloroglucinol-HCl for lignin was negative in both cv. Grenache and cv. Carlos. Positive stainings for lignin with Mirande reagent and phloroglucinol were observed in the tissue around the necrotic tissue in the intermediate resistant cultivar 15 days after inoculation.

Peroxidase activity

The cell walls around the necrotic areas reacted with TBM in rupestris cv. du Lot 5 days after inoculation, indicating peroxidase activity. However no reaction with TBM was found in cultivars Carlos or Grenache.

DISCUSSION

Flavonoids were induced only by infection with *P. viticola* in resistant cv. Carlos and in intermediate resistant cultivar. Flavonoids represent one component of the resistance phenomenon, as has been shown for other pathogens such as fungi (Friend, 1981) or parasitic plants such as *Viscum album* (Hariri et al., 1991).

In *V. rupestris* cv. du Lot, from 5 days, peroxidase activity was detected in tissue surrounding the necrotic areas, where accumulation of resveratrol and the later accumulation of flavonoids and lignin also occurred. It is possible that the diffuse necrosis formed in *V. rupestris* cv. du Lot in response to *P. viticola* may be derived by the action of peroxidase on resveratrol and flavonoids in a process of lignification. As peroxidases are involved in lignin biosynthesis, the increased peroxidase activity following pathogen inoculation may intensify the formation of lignin (Vance, 1980). By inhibiting pathogen growth and enzyme activity, peroxidase-generated free radicals may provide additional time for the accumulation of stable end-products, such as lignin-like materials, that can probably induce host cell death by interrupting nutrient flow into and from cells, prevent intercellular hyphal growth and suppress sporulation (Ride, 1983).

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SESSION 6 : MIXED TOPICS

GRAPE BREEDING IN THE TROPICS

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Introduction

Pierce's Disease (PD) is a bacterial disease that damages the xylem of grape causing it to become non-conductive. The plant eventually dies of water stress, the process of which is marked by various stages of degeneracy and remittance, showing symptoms such as leaf scald, dry fruit, stunting, and chlorosis over a period of two to five years in *vinifera*. The casual organism was named *Xylella fastidiosa* (Wells et al, 1987), it is considered fastidious because it can be cultivated artificially only on a narrow range of media (mavis et al, 1978). In the wild, on the contrary, it can feast on a vast variety of hosts where it is found strictly in the xylem (Raju et al.1980; Jimenez-Mora 1982).

Plasmopara viticola is widespread in the tropics, but unlike PD, it can be controlled chemically.

Background

Data gathered in Montezuma, Costa Rica (Fig. 1) (Jimenez and Ingalls 1990) from various hybrids and backcrosses of *Vitis caribaea* with *vinifera* seemed to indicate an extraordinary high proportion of resistant plants when compared to the work of Mortensen in Florida (Mortensen 1968). He hypothesized a 3 dominant gene model with all three needed for resistance. Knowing that PD symptoms are visible more commonly on plants that are under stress, we decided to consider only those plants that are under the stress of cropping (in the tropics many varieties do not bloom due to the short photo period) and we used a parameter which we called sustainability to follow the progress of selection. Sustainability was defined as the number of plants in a cross that are producing the current year divided by the total number of plants that have ever produced in that cross. Sustainability plotted against years for a family of plants derived from the backcross (*caribaea* x Tokay) x M. Hamburg, exemplifies this concept (Fig. 2). It has a maximum at four years as the plants come into production followed by an exponential decay as PD kills the acceptable progeny. Here we see that instead of reaching a level of 1/8 predicted by Mortensen, at seven years less than fifty percent of the plants have died. We also found a resistant plant, 56c1, that did not transmit resistance to its progeny thus violating the dominance hypothesis of Mortensen.

We offered two alternate hypothesis to explain this:

1. Mortensen's three dominant genes have an additive effect which is not observed in Florida because the disease is much more virulent in that climate.
2. *Vitis caribaea* has a single gene resistance.

At the time we didn't have enough data to decide between these alternatives.

Results and Discussion of Pierce's Disease Resistance Breeding

After five more years of additional observations in Costa Rica, the first hypothesis of dominant resistance genes with an additive effect was winning. In its 12th year 56c1, an apparently resistant plant, died; the absence of a complete set of resistance genes would explain why the selection could not transmit resistance. The extended curves for sustainability of the test crosses now descend much closer to the level predicted by Mortensen, but even after 12 years, selection is far from complete. (see fig. 3) The indeterminate nature of the proportion of dead or weak plants is indicative of the need for the stress due to cropping to obtain selection; after plants become infertile, they go on living with PD for years. We will probably have to wait for unpredictable extremes of climate to predispose the plants with 2 genes for resistance to an attack of PD. The sustainability curves are zigzag because some plants produce in alternate years. To smooth this out and see the long term rate of degeneracy of the offspring of a cross, we have chosen a parameter which is the number of plants that have not stopped producing divided by the total number that have ever produced. This new parameter when plotted against the age of the plants in years results in a graph (fig.4) that can be divided into three regions: the first region is flat and represent the incubation period which is from 2 to 3 years in *vinifera* and 4 to 5 years in the back crosses. The second region follows an exponential decay which has a constant ratio to the number of plants left as we mentioned in the earlier paper (Jimenez and Ingalls 1990). The third region starting near the 9th year has a catastrophic rate mechanism in some of the families. In these the rate increases as the plants die off.

Unfortunately we see very little correlation between the sustainability in the parents and that in the offspring. This can be seen on the (fig. 3) where families with dead or weak parents are in the left column of

graphs or in the next chart (Fig. 4) where weak parent in some cases produce can be seen to produce better families than productive parent.

Results and Discussion of *Plasmopara viticola* Resistance Research

In the tropics, where one would expect hybrid varieties to be used extensively, vinifera varieties are still the most common (Possingham et al. 1990). This has been justified by saying that fungal disease pressure is so great under tropical conditions that any tolerance gained from hybridization is insufficient to control the diseases and therefore we might as well stay with *vinifera*. This is a challenge to everything we are doing.

Vitis caribaea is immune to *Plasmopara viticola*. Some F1 plants have almost the same resistance as *caribaea*, but in the backcrosses the fungal attack is so devastating that it becomes very disheartening to count leafspots when very soon the whole plant becomes defoliated.

We decided on the more practical method of leaving a whole field of unsprayed and observing the outcome for a year (1993).

Field Observations - Heavy rains began in late May after most of the crop had been harvested, instantly animals (especially crabs) and insects (killer bees) quickly destroyed any fruit that was unpicked.

Plasmopara began to appear in June accompanied by heavy rains. By the end of the month, less than 1/4 of the leaves were infected and none of the leaves had more than 1/8 of their area affected, the old leaves were still intact, new shoots were growing, and spontaneous flowers stimulated by the rain after the intense dryness were blooming. The general appearance of the vineyard was a lush dark green.

In July (heavy rains) over 1/2 of the leaves were affected, the spot covered more than 1/4 but less than 1/2 of the leaf area. Half the old leaves had fallen, but the new leaves were large and compensated for this. There were some straggling young bunches of grapes (which would go unused). In general the vegetation looked healthy with a little thinning. The overall color had become a lighter green, due to the development of new shoots. It was observed that some plants withstood the disease better than others.

By August (heavy rains), all except 4 to 8 of the youngest leaves on each shoot were affected, over 1/2 of the leaf area was damaged. Old leaves were extremely rare. The general impression was that of destruction. The vegetation was very thin and yellow. There was still a little difference between good and poor plants.

In September (heavy rains), all the leaves except the newest ones on secondary shoots were gone. These immediately got sick and fell off. It was no longer possible to choose between good and poor plants.

In October (very rainy) and November (little rain), the plants were pruned which was very easy since there were few leaves to get in the way.

In December (almost no rain; the dry season having begun) the plants budded out very strongly and bloomed. At this time, we had a good chance to select for resistance. With most of the plants, the pruning had broken the reproductive cycle of the fungus, and the plants went into the dry season totally free of it. Some had a few lesions on the leaves, while in others the flowers were destroyed by *Plasmopara*.

In January, which was intensely dry, the *Plasmopara* died on all the plants, but the plants that had lost their flowers would not produce.

February and March were also dry and in March and April we got a good crop on those plants that did not lose their flowers, at least as good as the previous year when we had sprayed every week during the rainy months.

Discussion - Grape is tolerant of being pruned either once or twice a year, but in a damp climate like Costa Rica, there is nothing to be gained by pruning wine grapes twice a year. Any grapes that might be produced in the rainy season are of extremely bad quality. Trimming back after taking the crop can be useful to control growth and keep it harmonious, but this should be done sparingly so as not to stimulate too much new growth that would promote disease. With a once a year pruning schedule, the defoliation due to mildew was beneficial in improving bud break.

Hydrogen cyanamide was not needed. Pruning was also easier. Once a practical method of growing a crop in a particular climate has been worked out, this can be combined with a method to manage diseases under the prevailing conditions. Genetics can then be used to develop varieties more suitable to this system. It is unnecessary to look for a plant that has absolute resistance or no spots on the leaves ever. In our case, we need enough resistance to prevent the fungus from reestablishing itself in the short time between pruning and the intense dry heat of summer. Also, we need to look for a plant that does not dissipate all its resources by constantly making new leaves during a fungal attack but tends to wait it out. These two conditions can be easily satisfied by simply selecting plants that produce fruit under the no spray conditions.

Future Research

We are interested in other wild plants in the grape family besides *V. caribaea* that could be a source of disease resistance. There are dozens of species of *Cissus* in Costa Rica. Most have compound leaves, but the simple leaf varieties look morphologically very much like grape. The flowers and fruit however, are very different. The flowers being hermaphrodite and having 4 petals, and the fruit growing in flat topped corymbose

cymes instead of panicles. The other genus in the grape family is *Ampelocissus*. Plants in this genus differ from grape in that their bark does not shed in strips and the petals spread from the top during flowering and are not connected in the form of a hat as in grape. These are very small details, however compared to the many ways they are similar: The leaves, the five petals and five anthers, and the bunches of fruit in the form of panicles.

Ampelocissus costaricensis Lundell (Lundell 1937ii) grows over large trees in the deepest and rainiest jungles on the Atlantic coast of Costa Rica. It has a very thin tightly adhering greenish bark that gives an appearance of a giant annual plant. It carries huge metallic bronze colored bunches of fruit that can weigh 10 Kg. The round berries which weigh approximately 10G, are not poisonous (personal experience) but have an objectionable parathion like odor and are not eaten by any insect or animal, and for this reason are thought poisonous by the Indians who hold them in awe. The fruit texture is crunchy, the skin is very thick and tough. Their reproductive system is interesting: In spite of being hermaphrodite, the flowers can not self pollinate. When the flower first opens, it is male with well developed anthers. At this time the pistil is very short and non-functional. As soon as the anthers fall off, the pistil begins to grow longer and becomes receptive about a week after the anthers finished shedding their pollen. Until we had these plants identified at the Missouri Botanical Garden, we thought them to be grape and a most likely candidate for hybridized with *vinifera*. After all, they would be recognized intermediately by anyone passing by as being a giant grape.

We found the pollen sheds with difficulty. It has a waxy texture, perhaps to protect it from the constant rains. We had to dry the flowers in the sun and beat them against a sheet of glass and then repeat the process several times over a period of hours to get enough pollen out. We took the pollen to the Pacific coast, where it is not indigenous, to pollinate some female *caribaea* x *vinifera* hybrids in Montezuma. Various female varieties were used, only two had any fruit set. The seeds of this fruit were very small and did not germinate. The next year (1994) we tried again, we had drier weather, and the pollen shed easier. When the females in Montezuma were pollinated with this pollen, the same two female plants set fruit. Only two seeds out of 78 germinated, but the two seedlings obtained died in the two leaf stage. We had many bagged but unpollinated flowers as controls, none of the controls set fruit.

In order to see if a reciprocal cross would be more successful we took *A. costaricensis* seeds to the Pacific coast. They germinated without difficulty and grew with extraordinary vigor in small pots. These plants had huge tops and small roots as compared to the *caribaea* Pacific coast plants which have small tops and large roots. When set out they grew well until the dry weather came, at which time, most of them started to die back, even though we watered them well. The herbaceous green bark was susceptible to sun scald and most of them eventually died, possibly because of this damage to the bark. Probably for this same reason *Ampelocissus* reacted very negatively to pruning and to the sudden entrance of sun to the branches, not at all like *caribaea* which, as *vinifera*, reacts to pruning by blooming. We have now tried planting them in the shade. None have bloomed yet, they may need to grow over lots of space before they can bloom.

Conclusions

Selection for Pierce's disease resistance is very inefficient in the tropics, however, by the same token perhaps we could get along with only the 2 gene resistance. After all, our partially resistant plants often live longer than the average seven to ten year life span for grapes in the tropics (Possingham 1990). The best method at this time is to release a mixture of several similar varieties, pooled as green or red wine grapes, and let the final selection happen in the vineyards. A mixture of plants might also withstand fungal attacks better.

It is possible to produce good wine grapes in the tropics without spraying them for downy mildew, if we choose the right climatic zone, prune them at the correct time of year, and use a variety with a some tolerance to disease. If we wish to change any of these parameters, we will have to spray to compensate for the changes we make. *Vitis caribaea* is a very good source of resistance. It adds no foreign taste to *vinifera* except high acidity which is very useful to preserve the wine under tropical conditions, it contributes no weakness to the attachment of the berry to the bunch, and it can maintain good textural qualities when crossed with table grapes. Crosses with the other genera might lead to some interesting exotic fruit in the future.

We wish to thank Prof. Jorge Gomez Laurito, Herbario de Universidad de Costa Rica, who in association with the Missouri Botanical Garden, identified *A. costaricensis*.

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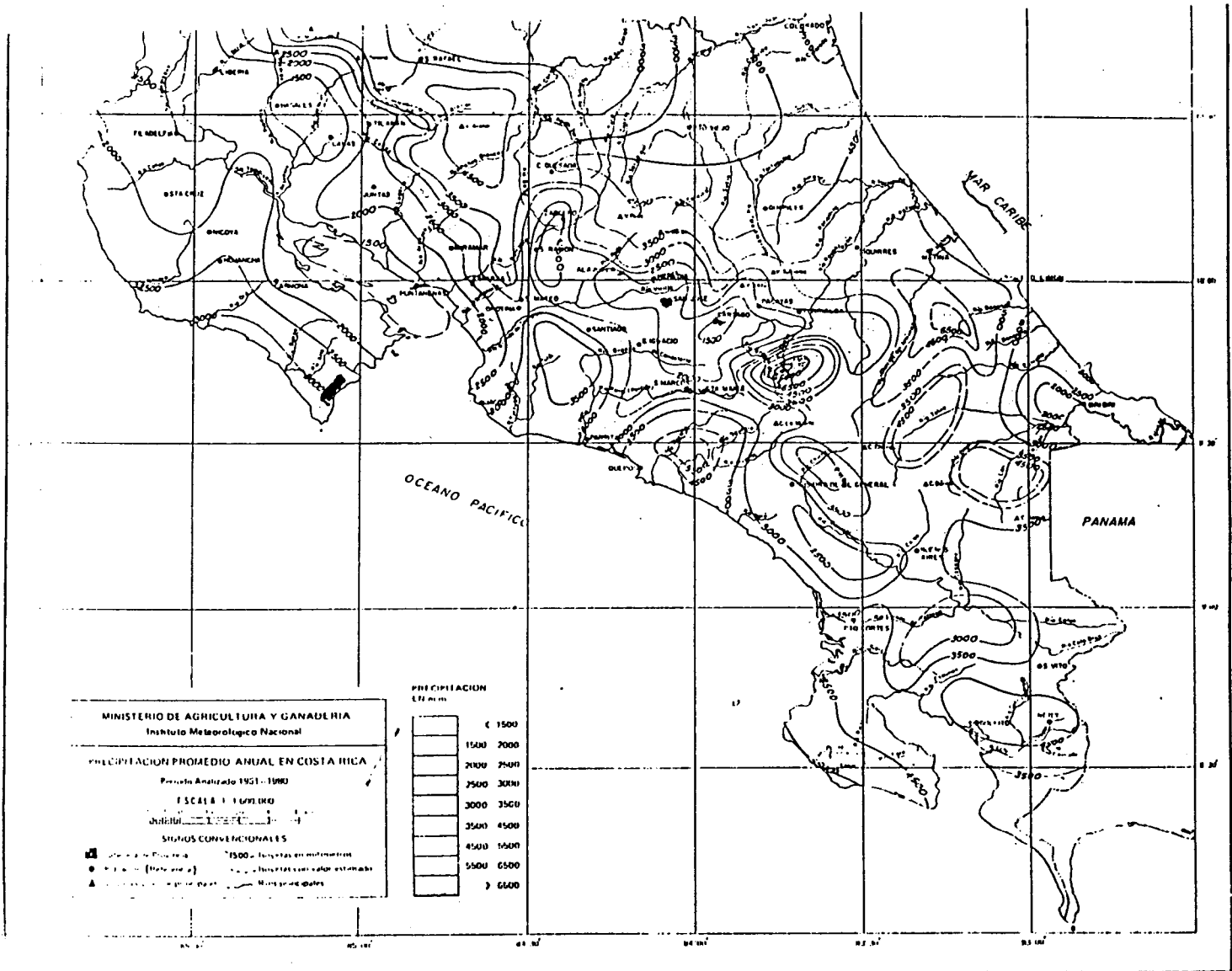
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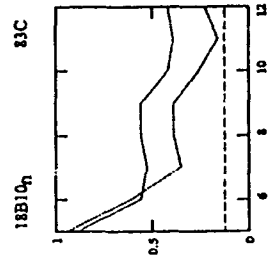
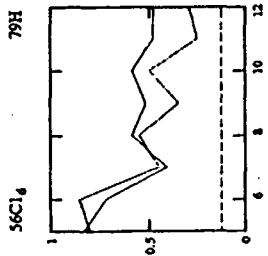
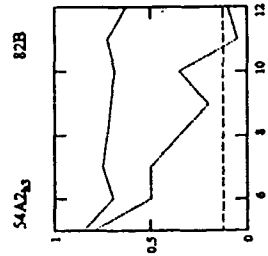
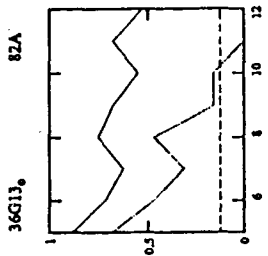
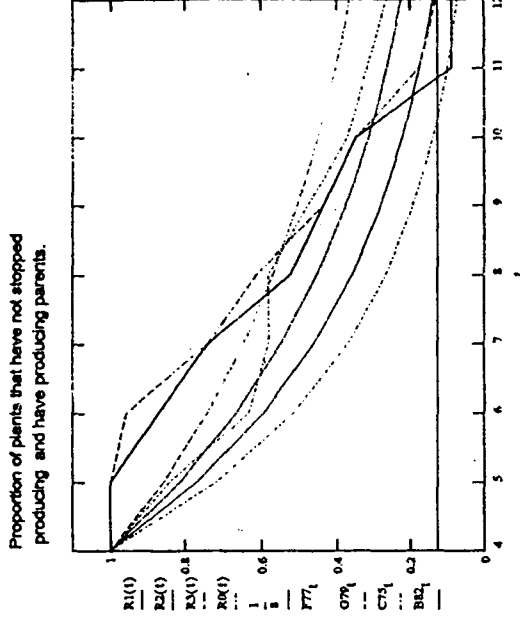
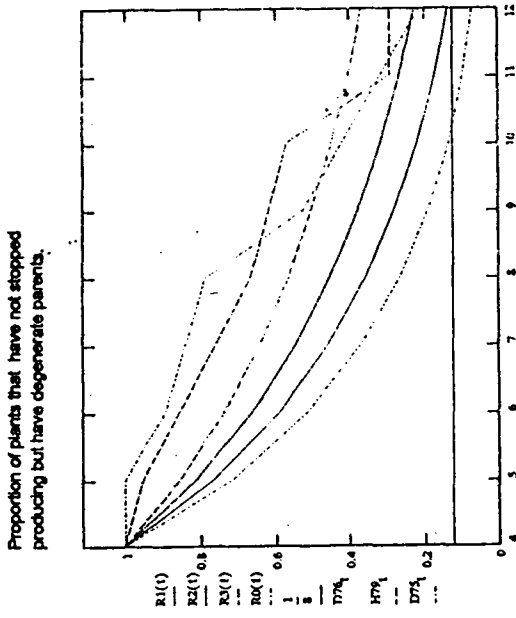
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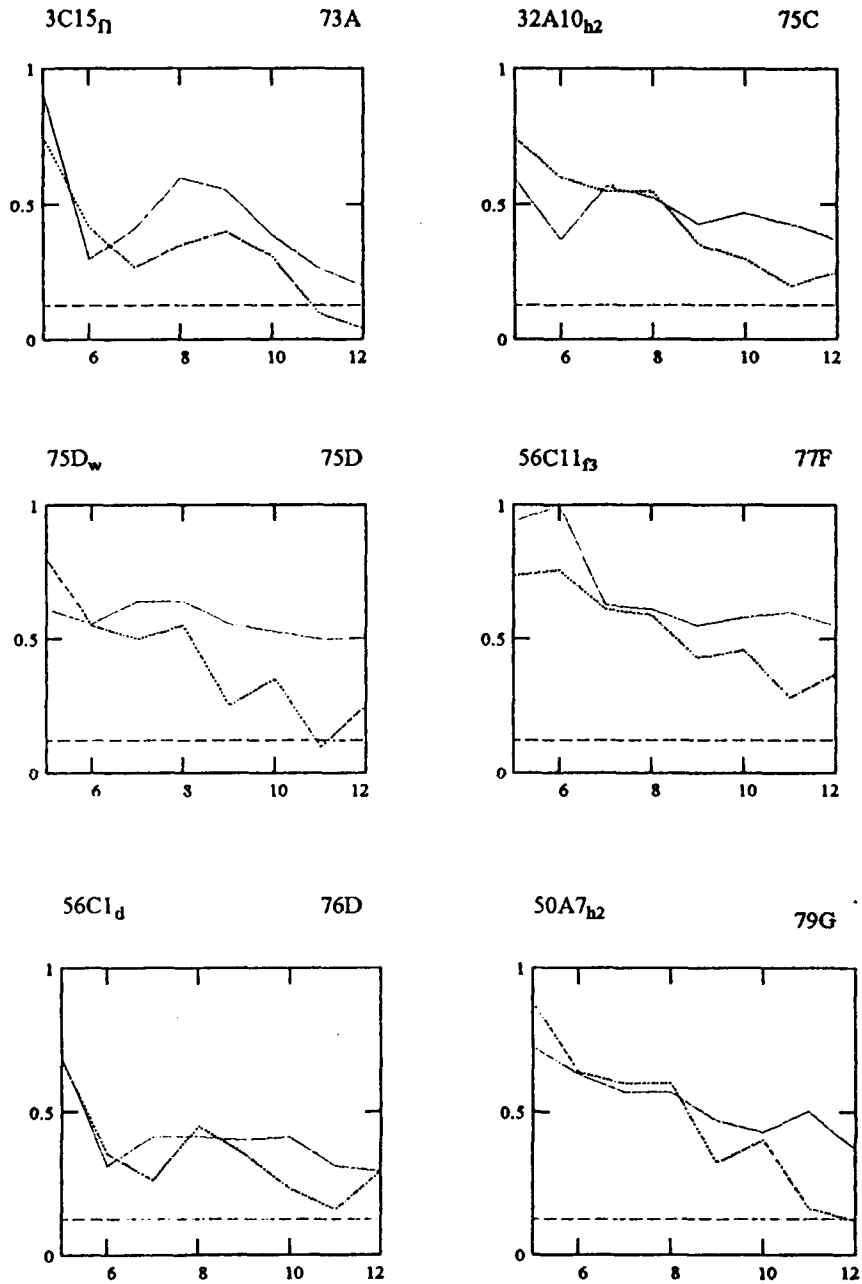
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Top left :resistant parent.
 Top right :cross.
 Subscript :condition of parent.
 W :weak.
 d :dead.
 O :fruitless.
 h,f :sex.
 dotted :sustainability.
 solid :proportion healthy plants.
 dashed :1/8 level (Mortensen).
 time :4 to 12 years

Progress of selection for Pierce's disease in Montazuma, Costa Rica 1982-1994



SESSION 6 : MIXED TOPICS

BREEDING FOR COMPLETE RESISTANCE AGAINST PHYLLOXERA ON THE BASIS OF VITIS CINEREA ARNOLD

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Phylloxera problems in Europe

The introduction of Phylloxera into Europe in the second half of the last century, finally resulted in the use of rootstocks in viticulture. Since then *Vitis vinifera* has only been used as scion grafted on to selections or hybrids of American *Vitis* species in order to prevent them from being destroyed by the Phylloxera. Therefore the resistance of a rootstock against the insect is the most important factor in deciding its suitability. All rootstocks used until today commercially are not completely resistant but only tolerant to Phylloxera. At least theoretically under certain circumstances tolerance might not be sufficient to prevent any damages (2, 4, 5, 10)

For example in the German vine growing region Rheingau, the density of the Phylloxera population increased in the last few years resulting in Phylloxera damage's on grafted vines.

This is caused by various reasons :

1. Climatic conditions during the past years. Since 1988 Germany had mild winter temperatures and warm spring weather. Thereby Phylloxera was able to reach higher numbers of generations. With every new generation the population density might grow in an exponential way. (11, 12)

2. The warm and dry conditions during late summer and autumn favoured the winged form, whose following generations are responsible for the sexual form, migration over longer distant and the leaf galling in the following season.

3. Another problem are an increasing number of abandoned vineyards due to economical reasons. Usually only the scion is removed and the root left untouched. Therefore buds of the berlandieri x riparia rootstocks are bursting, resulting in rootstock leaves, well suited to Phylloxera. The insect is then able to develop a complete life cycle. From these places it easily spreads to the neighbourhood (8)

4. Layering in older vineyards is an other reason for increased Phylloxera problems and a sign for the decreasing Phylloxera awareness of vine growers. Some of them are not able to identify Phylloxera damage or leaf galls.

Phylloxera inspection has continuously been done in the Rheingau region. The acreage of Phylloxera infested vineyards increased from 20 ha in 1970 to 150 ha in 1993. Replantations done in these vineyards with common rootstocks have been of no success (8) (Fig.: 1)

The introduction of *Vitis cinerea* in rootstock breeding

In 1934 Carl Borner detected the complete resistance of the *Vitis cinerea* type Arnold against Phylloxera and introduced this species into his rootstock breeding program. Research since then has shown that the resistance mechanism of *Vitis cinerea* Arnold is a hypersensitive reaction. The Phylloxera attacked cells become necrotic immediately and are cut off by a suberin layer. Preventing any feeding of the insect and any secondary infection. This reaction is not restricted to the roots, but also active on the leaves (4, 7; 9)

Some of this seedlings, produced by Borner's breeding work, were followed up by Helmut Becker and eventually resulted in the release and classification of the variety Borner.

Breeding for Phylloxera resistance

Since then the variety Borner has demonstrated its superior resistance in field and glasshouse trials, even under high Phylloxera pressure (Tab 2). Its affinity to range of scion varieties also proved to be satisfactory. Of course no rootstock can be suitable for all soil types and climatic conditions, therefore this resistance mechanism now has to be combined with other viticultural characters.

For this breeding program early screening for Phylloxera resistance is essential to be able to use high population numbers initially. To develop a fast and easy screening method the variety Borner was used as male parent and crossed in 1992 with the varieties Binova, Berlandieri Resseguler and different clones of Kober 125 AA. The 1813 seeds harvested resulted in 400 seedlings (Tab 1)

Tab.:1 Number of seeds and grown seedlings of various population

The potted seedlings were grown together with 46 standard varieties and clones in an insect proofed greenhouse. As the reaction of the standard varieties against Phylloxera is known, they were used as controls .

On June 3, 1993 we infected the leaves of every 5th plant in the greenhouse with Phylloxera. Leaf galls were collected from a local rootstock mother block. After two weeks the first galls appeared on young leaves. After six weeks Phylloxera had spread all over in the greenhouse. At this time (July 13, 1993) we assessed the leaf galls for the first time. Plants were ranked on a scale from 1 to 9

- 1 = no galls
- 3 = galling without egg production
- 5 = small galls
- 7 = gall production
- 9 = extreme gall production

A second assessment was conducted on August 3, 1993 and a third one on September 14, 1993.

On that day root reaction was measured too. The scaling of the roots proved to be much more difficult. We only observed nodosities but no tuberosities. The frequency of nodosities and egg production was therefore used to rank plant reaction.

Relationship between leaf and root reactions

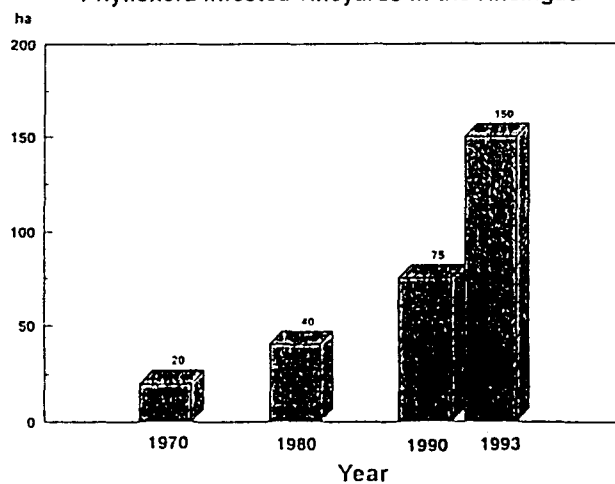
On most standard varieties no interaction exists between leaf and root reaction. Therefore screening Phylloxera resistance has to be based on both leaf and root observations . On standard rootstock varieties a high gall production on the leaf was observed. This was expected and a sign for the high pest pressure in the greenhouse.

The hybrid populations tested showed a different reaction (Tab 3). In all three populations identical results were found. Most of the hybrids, which showed complete resistance on leaves (necrotic reactions), showed no nodosities on their roots .In 54% of the 125BB x Borner, 75% of the Berlandieri x Borner and 36% of the Binova X Borner hybrids no egg production was found both in leaf galls and on nodosities. This reaction is different from the reaction reported in other rootstock varieties. This indicates that a different type of resistance appears to exist in *Vitis cinerea* Arnold.


An other interesting fact was the high proportion (30%) of hybrids with complete resistance on roots, which points to the high heretability of this mechanism in Borner as parent. It also appears that the mechanism is only controlled by a small number of genes (possibly only one).

This rises the question of the stability of this mechanism, as mono-genetic resistances e.g. in wheat breeding often proved to be short lived. In case of the Phylloxera resistance in *Vitis cinerea* Arnold off-springs we have to consider that the mechanism is of general physiological nature as a matter of fact only a very fast wound reaction, which can also be observed after nematode attack or mechanical damage. It therefore appears to be highly unlikely that Phylloxera will be able to overcome such a barrier

Fig.: 1 Phylloxera infested vineyards in the Rheingau



Tab.: 1 Number of seeds and grown seedlings
of various population

	parentage	Number of vines · vines in %		
		seeds	of seeds	of seeds
Gm 9225	Binova x Börner	940	146	15.5
Gm 9226	125AA x Börner	175	41	23.4
Gm 9227	125AA x Börner	165	43	26.0
Gm 9228	125AA x Börner	180	57	31.6
Gm 9229	125AA x Börner	135	54	40.0
Gm 9242	125AA x Börner	58	12	20.6
Gm 9230	Berl. Fess. x Börner	160	47	29.3

Tab.: 2 Phylloxera susceptibility of rootstocks

rootstock varieties	cross	leaf	root
5C Kl. 6 - 13 Gm	Berlandieri x Riparia	9	3
SO4 Kl. 47 Gm	Berlandieri x Riparia	9	3
125AA Kl. 3 Gm	Berlandieri x Riparia	9	3
5BB Kl. 13 -3 Gm	Berlandieri x Riparia	9	3
8B Kl. 361 - 3 Gm	Berlandieri x Riparia	9	3
3309 Kl. 18 Gm	Riparia x Rupestris	9	3
26G Kl. 27-7 Gm	Trollinger x Riparia	9	7
Börner	Riparia x Cinerea	3	1
Na 5153-579	Riparia x Cinerea	3	1
Na 5153-117	Riparia x Cinerea	9	3

Source: Beschreibende Sortenkarte 1992, Herausgeber: Bundesministerium, Hannover, Verlag Alfred Strohle, Frankfurt

Tab.: 3 Reaction of the used hybrid populations

Phylloxera susceptibility on leaves and roots of
Gm 9230 Berl. Fess. x Börner hybrids

leaf	1	3	5	7	9	tot.
1	19	1	0	0	0	20
3	8	7	7	0	0	22
5	4	1	0	0	0	5
7	0	0	0	0	0	0
9	0	0	0	0	0	0
tot.	31	9	7	0	0	47

Phylloxera susceptibility on leaves and roots
of Gm 9225 Binova x Börner hybrids

leaf	1	3	5	7	9	tot.
1	18	11	2	0	0	31
3	12	12	11	3	3	41
5	7	13	3	2	5	30
7	9	6	7	3	2	27
9	3	5	4	2	3	17
tot.	49	47	27	10	13	146

Phylloxera susceptibility on leaves and roots
of all 125AA x Börner hybrids

leaf	1	3	5	7	9	tot.
1	38	22	7	0	0	67
3	20	31	11	9	2	73
5	11	17	4	0	0	32
7	4	9	2	5	2	22
9	0	2	4	1	6	13
tot.	73	81	28	15	10	207

Conclusion

Complete resistance on leaves and roots against Phylloxera can be found in some *Vitis cinerea* hybrids. The variety Borner is a cross between *Vitis riparia* and *Vitis cinerea* Arnold. It is the first classified rootstock with complete Phylloxera resistance and can be used successfully as cross breeding partner. The resistance mechanism appears to be transmitted to the progeny in a high percentage. Therefore it appears to be easy to achieve Phylloxera resistance by utilising *Vitis cinerea* genes.

Due to the complete Phylloxera resistance and its high heritability *Vitis cinerea* Arnold or its descendants should be more considered in rootstock breeding programs.

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**VI МЕЖДУНАРОДНЫЙ СИМПОЗИУМ
ПО СЕЛЕКЦИИ ВИНОГРАДА.**

ДОКЛАДЫ.

Ялта, 4 - 10 сентября 1994 г.

Освещаются результаты исследований более 100 ученых из Австралии, США., Турции, Франции, Швейцарии, ЮАР и стран СНГ по таким направлениям, как зародышевая плазма; культура *in vitro*; генный анализ; перенос генов, выбор при помощи маркеров; клоновая селекция и др.

Организатор - Институт винограда и вина "Магарач".

Подготовка к печати доктора биологических наук
Л.П.Трошина
Перевод на английский язык с русского Л.Е. Гельгар
Компьютерный набор и печать РА "ARTmachine"

