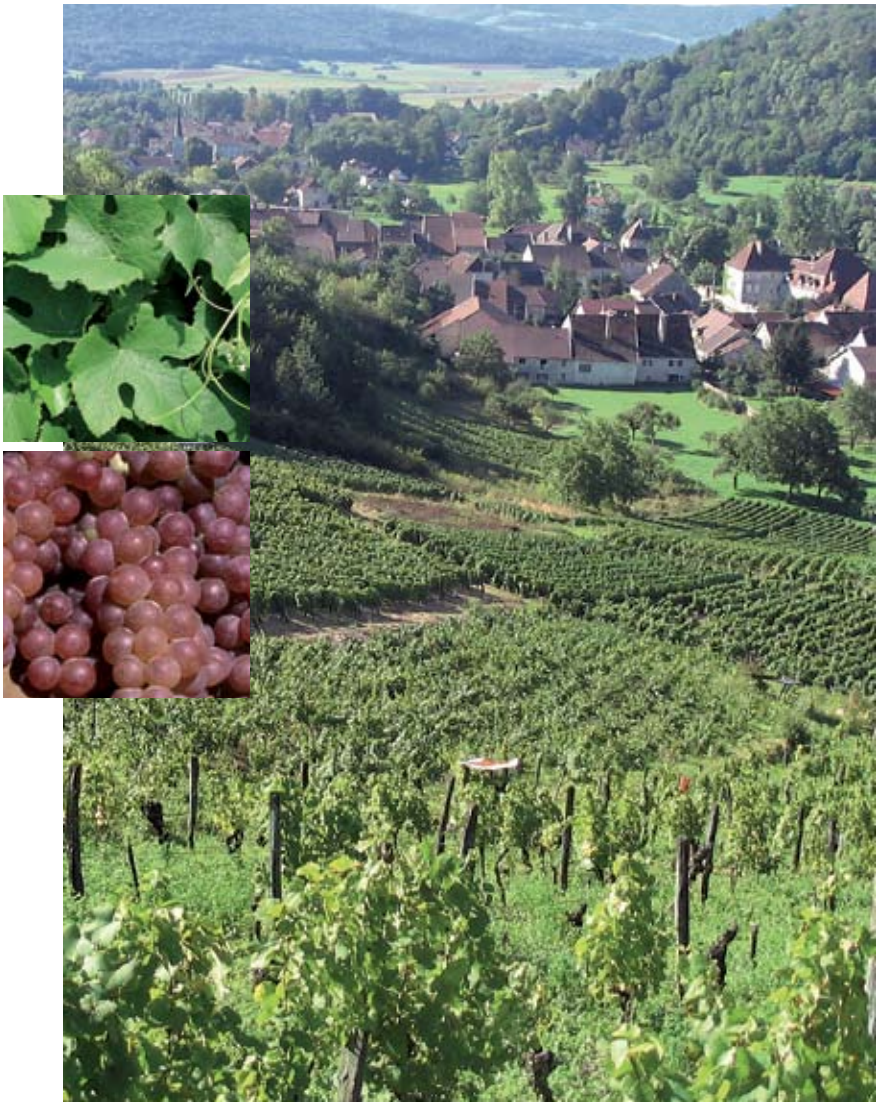
European Cooperative Programme for Plant Genetic Resources

ECP/GR

Report of a Working Group on *Vitis*

First Meeting, 12-14 June 2003, Palić, Serbia and Montenegro

E. Maul, J.E. Eiras Dias, H. Kaserer, T. Lacombe, J.M. Ortiz, A. Schneider, L. Maggioni and E. Lipman, *compilers*



Bioversity International

IPGRI and INIBAP operate under the name Bioversity International

Supported by the CGIAR

European Cooperative Programme for Plant Genetic Resources

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Bioversity International is an independent international scientific organization that seeks to improve the well-being of present and future generations of people by enhancing conservation and the deployment of agricultural biodiversity on farms and in forests. It is one of 15 centres supported by the Consultative Group on International Agricultural Research (CGIAR), an association of public and private members who support efforts to mobilize cutting-edge science to reduce hunger and poverty, improve human nutrition and health, and protect the environment. Bioversity has its headquarters in Maccarese, near Rome, Italy, with offices in more than 20 other countries worldwide. The Institute operates through four programmes: Diversity for Livelihoods, Understanding and Managing Biodiversity, Global Partnerships, and Commodities for Livelihoods.

The international status of Bioversity is conferred under an Establishment Agreement which, by January 2008, had been signed by the Governments of Algeria, Australia, Belgium, Benin, Bolivia, Brazil, Burkina Faso, Cameroon, Chile, China, Congo, Costa Rica, Côte d'Ivoire, Cyprus, Czech Republic, Denmark, Ecuador, Egypt, Ethiopia, Ghana, Greece, Guinea, Hungary, India, Indonesia, Iran, Israel, Italy, Jordan, Kenya, Malaysia, Mali, Mauritania, Morocco, Norway, Oman, Pakistan, Panama, Peru, Poland, Portugal, Romania, Russia, Senegal, Slovakia, Sudan, Switzerland, Syria, Tunisia, Turkey, Uganda and Ukraine.

Financial support for Bioversity's research is provided by more than 150 donors, including governments, private foundations and international organizations. For details of donors and research activities please see Bioversity's Annual Reports, which are available in printed form on request from bioversity-publications@cgiar.org or from Bioversity's Web site (www.bioversityinternational.org).

The European Cooperative Programme for Plant Genetic Resources (ECPGR) is a collaborative programme among most European countries aimed at facilitating the long-term conservation and the increased utilization of plant genetic resources in Europe. The Programme, which is entirely financed by the member countries, is overseen by a Steering Committee composed of National Coordinators nominated by the participating countries and a number of relevant international bodies. Bioversity International provides the Coordinating Secretariat. The Programme operates through nine networks in which activities are carried out through a number of permanent working groups or through ad hoc actions. The ECPGR networks deal with either groups of crops (cereals; forages; fruit; oil and protein crops; sugar, starch and fibre crops; vegetables) or general themes related to plant genetic resources (documentation and information; *in situ* and on-farm conservation; inter-regional cooperation). Members of the working groups and other scientists from participating countries carry out an agreed workplan with their own resources as inputs in kind to the Programme.

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Vineyard in Port-Lesney (Jura, France, 2006) and leaves of cultivar 'Herbemont' (Ardèche, France, 2004), courtesy of © Thierry Lacombe, INRA-Montpellier, France.
Bunch of 'Grisa rossa' grown in Piedmont and in other European areas, an ancient, now neglected cultivar known in the past as the "marvellous grape". Courtesy of © Anna Schneider, CNR–Unit of Grugliasco, Italy.

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Bioversity International
Via dei Tre Denari, 472/a
00057 Maccarese
Rome, Italy

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FOREWORD

Dear reader,

This book is being published five years after the event that it reports, namely the First Meeting of the Working Group on *Vitis*.

This delay has been due to the heavy demand by the ECPGR Networks for high quality meeting reports during the past five years, while staff time resources for this purpose at the ECPGR Secretariat have not been increased to match these needs.

The "Discussion and Recommendations" chapter was published on-line in 2003 and has been used by the Working Group as a reference document for action ever since then.

Inevitably, this section must largely reflect the ideas current at a past point in time and it therefore maintains an historical record for the Working Group. Although many things have changed since 2003 (for example, the name of the country where the meeting was held), we consider that this book contains much relevant and valuable information. Many of the papers have been revised and updated recently.

ECPGR Secretariat

PART I. DISCUSSION AND RECOMMENDATIONS

Introduction

Opening of the meeting

On behalf of the Ministry of Agriculture and Water Management of the Republic of Serbia, Ivana Dulić Marcović welcomed all the participants to the first meeting of the *Vitis* Working Group of the European Cooperative Programme for Crop Genetic Resources Networks (ECP/GR[1]). She explained that she was director of the Federal Department for plant and animal genetic resources. After the formation of the Serbia and Montenegro Union, this department became a constituent part of the Ministry of Agriculture and Water Management of the Republic of Serbia. She expressed her hope that this change would ensure the commitment of the government to continue supporting genetic resources in agriculture and that the current budget for genetic resources within the agriculture budget would rapidly rise. She said that she used to work with plants, and now, after two years spent working with people, she really appreciated her previous work and was looking forward to returning to it. Working with plants is considered to be a gift from God, but she believed that working with *Vitis* was a real privilege. Another privilege was the opportunity to share the results with people who can understand and appreciate them. She thought that these three coming days would be about this. She encouraged the Group to enjoy meeting each other and to try to make the best of this opportunity. She finally wished everybody a successful meeting and an enjoyable stay at Palić.

Lorenzo Maggioni, ECP/GR Coordinator, welcomed all participants to the first meeting of the Working Group on *Vitis,* including representatives from 14 member countries and observers from 5 additional countries. He expressed his pleasure in being able to convene a meeting in Serbia and Montenegro, 15 years after the last ECP/GR meeting was held in this part of Europe. He then asked Erika Dettweiler to chair the meeting and she kindly accepted, in agreement with the Group.

All the participants briefly introduced themselves and their field of activity.

General briefing on ECP/GR

The ECP/GR Coordinator summarized the objectives and structure of the programme, explaining that the establishment of the *Vitis* Working Group had been accepted in October 2001 by the ECP/GR Steering Committee. This was in response to a request made by E. Dettweiler[2], coordinator of the EU-funded project GENRES 081, "European Network for Grapevine Genetic Resources Conservation and Characterization". This arrangement will enable the network established within the EU project to renew its collaboration and to extend it to other countries outside the European Union.

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[1] Following the decision of the 10th meeting of the ECPGR Steering Committee in September 2006, the name of the Programme was simplified to "European Cooperative Programme for Plant Genetic Resources" and the acronym was also modified to "ECPGR", removing the traditional slash of "ECP/GR".

[2] See "Proposal for the acceptance of a Vitis Working Group within ECP/GR" (Appendix I, pp. 171-173).

He explained that the members of the Working Group (WG) are expected to ensure effective links between ECP/GR and the respective stakeholders at the national level and WG members and enable other scientists from participating countries to carry out an agreed workplan with their own resources as inputs in kind to the Programme. The Chair and Vice-Chair should ensure that both attending and corresponding members are involved in the planning and implementation of crop-specific workplans. Action can be facilitated with focused meetings of a restricted number of WG members and resource persons.

He then said that the Web page recently prepared by the Secretariat for the *Vitis* WG was planned to become, in the near future, a more active vehicle for distribution of up-to-date information on the Group's activity. Comments and contributions to improve the use and effectiveness of this tool would be welcome.

While Phase VI of ECP/GR will be coming to an end in 2003, a task force of the Steering Committee has produced a draft document containing a set of recommendations for the next phase. This document was circulated to all the WG Chairs for comments in advance of the end-of-phase Steering Committee meeting, planned to be held in Turkey in October 2003. According to this draft, the programme should increasingly focus its activity towards five specific areas: 1) documentation; 2) molecular markers and genomics; 3) task-sharing; 4) characterization and evaluation; and 5) *in situ* and on-farm conservation. The Working Groups would remain the operational units, but only those with more urgent and clearly identified priorities and measurable targets would receive approval for funding of meetings and other actions.[3]

A brief account was given of current international events, with mention of the International Treaty on Plant Genetic Resources for Food and Agriculture, which is establishing a Multilateral System (MLS) for facilitated exchange of plant genetic resources and for the sharing of the derived benefits. This MLS will however initially be limited to a list of crops that does not include *Vitis*.

The FAO and CGIAR initiative to establish a Trust Fund (Global Crop Diversity Trust) for the conservation in perpetuity of the most important plant genetic resources (PGR) collections in the world was also mentioned.

Regarding opportunities for funding PGR activities in Europe, a new EC Regulation is expected to be launched for a Community programme on the conservation, characterization, collection and utilization of genetic resources in agriculture and repealing Regulation (EC) No 1467/94. The first call for proposals is expected for the end of 2003 or early 2004.[4]

Book of abstracts

A book of abstracts of the presentations given during the meeting was distributed to all the participants before the meeting and uploaded on the Web until publication of the final report. This book included sections on the status of national collections, the status of the European *Vitis* Database (EVDB) and its descriptors, the problems of identification of grapevine varieties and reports of recent surveys on *Vitis* genetic resources.

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[3] The structure of the Programme has been re-defined by the ECP/GR Steering Committee during its end-of-phase-VI meeting held 22-25 October 2003, Izmir, Turkey (see http://www.ecpgr.cgiar.org/Introduction/AboutECPGR.htm).

[4] Regulation 870/04 was published in 2004 and two calls for proposals were launched in 2005 and 2006, respectively.

National collections

A brief account of the national collections was presented by the representatives of Albania (A. Çakalli), Armenia (S. Gasparyan), Austria (H. Kaserer), Croatia (E. Maletić), Cyprus (S. Savvides), the Czech Republic (O. Jandurová), France (T. Lacombe), Georgia (D. Maghradze), Germany (E. Dettweiler), Italy (M. Gardiman), Macedonia F.Y.R. (K. Beleski), Malta (R. Caruana), Moldova (G. Savin), Portugal (J.E. Eiras Dias), the Russian Federation (A. Smurygin), Serbia and Montenegro (P. Cindrić), Spain (J. Ortiz) and Ukraine (S. Goryslavets).

Additional information was received before the meeting from Azerbaijan (M. Musayev, unable to attend), and after the meeting from Bulgaria (P. Abracheva) and Romania (M. Stoian).

All available papers and abstracts are included in Part II of this report (pp. 47-105).

Documentation

The EPGRIS project and the new Multi-crop Passport Descriptors (MCPDs)

Lorenzo Maggioni presented the progress of the EU-funded project EPGRIS for the establishment of a European Plant Genetic Resources Infra-Structure.[5] This 3-year project (2000-2003) was developed within the ECP/GR Documentation and Information Network and was approved for funding within the Fifth Framework Programme of the European Union. The objective is to establish a European Internet Search Catalogue (EURISCO) with passport information of plant genetic resources maintained *ex situ* in Europe. Before the end of 2003, the first version of EURISCO is expected to be launched on-line and to contain a combination of data available from the existing national inventories and from the existing Central Crop Databases (CCDBs). EURISCO is expected to gradually develop and become the most complete and reliable source of passport data in Europe. The catalogue will host an important minimum set of passport data, frequently and automatically updated from the national inventories. These data will be based on the revised version of the FAO/IPGRI *Multi-crop Passport Descriptor List* (MCPDv2) finalized in December 2001.[6] National focal points, already designated in all European countries, will be responsible for data sources, data quality and accuracy, data availability and provision of data in the EURISCO–MCPD format. The central node receiving the data at IPGRI[7] will be responsible for checking data compatibility with the catalogue, providing feedback to national partners, importing data into EURISCO and developing and maintaining the front end.

The launching of the first version of EURISCO is expected to take place at the occasion of the final meeting of the EPGRIS project, which is planned for September 2003 in Prague, Czech Republic, jointly with a meeting of the ECP/GR Documentation and Information Network.[8] On this occasion, all European National

[5] See http://www.ecpgr.cgiar.org/epgris/index.htm

[6] http://www.ipgri.cgiar.org/publications/pubfile.asp?ID_PUB=124

[7] With effect from 1 December 2006, IPGRI and INIBAP operate under the name "Bioversity International", Bioversity for short. This new name echoes their new strategy, which focuses on improving people's lives through biodiversity research.

[8] EURISCO was launched officially at the Final Conference of the EPGRIS Project, 11-13 September 2003, Prague, Czech Republic. The catalogue is available at http://eurisco.ecpgr.org/

Inventory focal persons and Central Crop Database managers, including the EVDB manager, will have the chance to discuss the future relationship between EURISCO and the CCDBs. A document distributed in April 2002 to the ECCDB managers by the EPGRIS project suggested a way forward in this relationship, i.e.:

1. CCDBs to harmonize their structure with EURISCO (the Centre for Genetic Resources, the Netherlands is undertaking this harmonization);
2. CCDBs to continue gathering data until EURISCO becomes the preferred source of passport data;
3. Once EURISCO becomes operational, consider retrieving data from EURISCO.

Three possible scenarios are also expected to exist at any point in time, depending on the specific crop, i.e. that: 1) EURISCO contain less data than CCDB; 2) EURISCO contain more data than CCDB; and 3) EURISCO contain different data than CCDB. A transition phase lasting 2-3 years is considered likely before EURISCO and CCDBs are harmonized. The role of the CCDBs and their managers will also be on the agenda of the Prague meeting. It is foreseen that this role will increasingly focus on helping to improve data quality, tracing duplicates, gaps, Most Original Samples, gathering characterization/evaluation data, analyzing information (geographical information system (GIS), etc.), providing users with data in various formats, helping to define core collections, safety-duplication and collecting needs, etc.

The European Vitis Database (EVDB)

Presentations on the results of the GENRES 081 project, the status of the database and progress on the harmonization of descriptors were made by Erika Dettweiler and Anne Schneider (see full papers in Part II, pp. 13-46).

Enlargement of the EVDB

The Group noted that the EVDB, as a tool for international germplasm management, would benefit from the addition of passport data of grapevine collections from countries not yet included in the database (in particular from eastern Europe) and of regional and departmental collections from countries that have already included their main collection data in the database. The objective would be to obtain an almost complete inventory of the genetic resources maintained in germplasm collections in Europe.

It was also noted that the reliability of the database is guaranteed by continuous updating of the existing data.

Recommendation

The Group agreed on the opportunity to update the existing data and to complete the EVDB with the inclusion of missing data.

It was clarified that it would also be appropriate to continue including in the EVDB accession data related to hybrids, rootstocks and wild species, including Vitis vinifera *subsp.* sylvestris *conserved in European collections.*

Management of the EVDB by BAZ, Geilweilerhof and ZADI/IBV, Bonn

Erika Dettweiler, EVDB manager, informed the Group that the Centre for Agricultural Information and Documentation/Institute for Biological Diversity (ZADI/IBV), had agreed on continuing its support for the development of the EVDB and specifically:

1. to include further grapevine collection passport and characterization data, such as from countries which were not involved in the GENRES 081 project (Albania, Armenia, Azerbaijan, Cyprus, Georgia, Macedonia F.Y.R., Malta, Russian Federation, Serbia and Montenegro and Ukraine);
2. to extract from the database subsets of passport data to be updated by the GENRES 081 project partners and then re-incorporate them into the database; and
3. to add additional primary and secondary descriptor data and photographs to the database.

Passport data

The Group considered it important to adapt the passport data used in the EVDB to the standards adopted by the EURISCO catalogue, which are based on the FAO/IPGRI *Multi-crop Passport Descriptors*.

In this way, the EVDB would become compatible with the EURISCO catalogue and would be able to draw updated passport data directly from the on-line catalogue in the near future, as soon as EURISCO becomes fully operational. Additionally, it was acknowledged that it would be easier to obtain data from new data donors if requests were to conform with the increasingly accepted international standards for multicrop passport data rather than with a different format.

Recommendation

The Group agreed to adopt the EURISCO descriptors 1–33 (i.e. the extended list of the 28 FAO/IPGRI Multi-crop Passport Descriptors + 5 specific EURISCO descriptors) for the new version of the EVDB.

Additionally, the following descriptors would be part of the EVDB passport list:[9]

A. Variety name

B. Berry colour

C. Country of origin of the variety

D. Year of crossing

Characterization and evaluation data

Regarding the addition of primary and secondary characterization data, no further description of grapevine varieties is envisaged at the moment. UPOV, IPGRI (now Bioversity) and OIV descriptors are being reconsidered for further harmonization and it is planned to have a new harmonized list available in 2004-2005. Further characterization of grapevine varieties is therefore not expected to take place before then.[10]

Rules for notation

Experience in the compilation of the EVDB indicated divergences among the different contributors in the way notations were scored.

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[9] Descriptors C and D were agreed upon further to the meeting.

[10] The achieved harmonization results were presented as the final version for the "2nd edition of the OIV Descriptor list for grapevine varieties and Vitis species" at the OIV expert group "Genetic resources and vine selection" in March 2007.

Recommendation

In order to avoid extra work for data set harmonization, the Group recommended that each data contributor should give proper attention to the specific rules that are defined for descriptor recording.

The full list of agreed passport descriptors with instructions and examples, followed by a summary of the recording rules, is available from the Web page of the *Vitis* WG (http://www.ecpgr.cgiar.org/workgroups/vitis/vitis.htm). This document will be referred to as the "EVDB agreed format".

Workplan

- **Update of the EVDB**
  - **By the end of October 2003**, the EVDB manager, E. Dettweiler, with the help of ZADI/IBV, will send to each institution the respective data subset, after extraction from the EVDB.
  - As soon as possible, but not later than the **end of 2004**, each partner will: a) harmonize its data according to the EVDB agreed format; b) update the data subset received; and c) send the updated file back to the EVDB manager. The harmonization will require, inter alia, the replacement of institution and country names with appropriate FAO and ISO codes.
  - All updated data files will be included by ZADI/IBV into the EVDB, shortly after receipt from the partners.
- **Enlargement of the EVDB**
  - Working Group members and representatives from observer countries will provide the available passport data related to collections that are not yet included in the EVDB. These data will be sent to the EVDB manager in the EVDB agreed format **by the end of 2003**. Should an extension of this deadline be required, WG members and observers will inform the WG Chair and EVDB manager.
  - A specific commitment to send the available data to the EVDB manager **by the end of 2003** was taken during the meeting by the representatives of Albania, Armenia, Croatia, Cyprus, Georgia, Malta, Macedonia F.Y.R., Moldova, Russian Federation, Serbia and Montenegro and Ukraine.
  - ZADI/IBV will incorporate into the EVDB all the data files received.
  - Working Group members and observers will make sure that each collection curator intending to add his/her data to the EVDB would provide the following general information to the EVDB manager for inclusion on the Web page (**by the end of 2003**):
    1. Name of the institute or organization holding the collection
    2. Full address, with the name of the curator or responsible person, mailing address, telephone, fax, email and Web site address
    3. Total number of accessions in the collection. Specify the number of autochthonous and traditional cultivars (table and wine) and the number of introduced cultivars (table and wine), rootstocks and wild species
    4. Number of plants per accession
    5. Training system
    6. Plantation density
    7. Geographical location: longitude, latitude and altitude
    8. Type of soil

9. Climate: mean annual rainfall and temperature
10. Most commonly used rootstocks

- **On-line updating of the EVDB**
  With the aim of allowing data donors to make minor updates directly on-line, at the end of 2003 the EVDB manager will discuss with ZADI the possibility of distributing a password for access authorization, to be implemented in 2004.

Microsatellite markers database

Presentations on the problem of correct identification of grapevine varieties and the development of a simple sequence repeat (SSR) marker database were made by E. Dettweiler, A. Jung and J. Ortiz (see full papers in Part II, pp. 109-148).

The Group noted that to detect the existing synonymies, homonymies or misnamings in grapevine collections and viticulture, ampelographic characterization has to be completed with data on microsatellites (SSR markers) analysis. Following the standardization work carried out by the GENRES 081 project, this analysis turned out to be a suitable and reliable tool for grapevine variety identification.

Therefore, the *Vitis* Working Group members decided to immediately start the establishment of an SSR marker database as part of the EVDB.

Workplan

- As soon as possible, the results of GENRES 081 for the 50 analyzed varieties will be made publicly available, as well as the descriptors of the SSR markers listed below (Action: E. Dettweiler, project coordinator).
- In the medium to long term, the SSR marker database will become part of the EVDB. This will include all the available marker data. The possibility of searching for varieties corresponding to a specific data profile will be an additional feature of the database. (Action: E. Dettweiler, EVDB manager).
- The Working Group members agree to transfer all available SSR marker data to the EVDB manager for inclusion into the EVDB. They will also inform the EVDB manager of every upcoming publication referring to the six SSR markers concerned.

In order to facilitate the rapid establishment of a comprehensive *Vitis* SSR marker database, the WG on *Vitis* decided to recommend the following practice to researchers working with *Vitis* SSR marker analysis:

Recommendation to researchers working with *Vitis* SSR marker analysis

1. *A comprehensive SSR marker database will be of benefit to the whole grapevine community. Therefore, it is highly recommended to include in all SSR marker research at least six microsatellite loci which would allow immediate comparison with the variety identification data obtained by the GENRES 081 project. These loci are: VVS2, VVMD5, VVMD7, VVMD27, ssrVrZAG62 and ssrVrZAG79.*
2. *It is recommended to use the reference cultivars according to the six corresponding OIV descriptors to achieve comparability and for the expression of allelic sizes in the coded format, e.g. MU1, or CS1, etc.*
3. *Data sent to the SSR marker database should be provided in the EVDB agreed format. Allelic sizes can be provided as well.*

4. *SSR marker data sent to the database manager should be accompanied by the following passport information: Institution code (of the institute holding the accession being analyzed), Accession number, Accession name, Variety name (if identified), Name and Internet address of the institution providing the SSR marker data and reference to a published article, if appropriate.*
5. *In order to verify whether the variety is true to type, whenever possible microsatellite data should be accompanied by ampelographic descriptor data plus photographic documentation.*

Conservation of genetic diversity within varieties

The European Catalogue of grapevine varieties

H. Kaserer reported that the European Commission is giving high priority to the establishment of a European Catalogue of grapevine varieties and that discussion should start in autumn 2003. The aim is to create an Internet database including all varieties officially accepted for sale of propagating material. The responsible EC Officer, Mr Bruno Foletto, has been informed about the results of the GENRES 081 *Vitis* project (improved ampelographic and new SSR descriptors) and about the ongoing discussion for harmonization between GENRES/OIV/IPGRI and UPOV descriptors.

In the updated Council Directive 68/193/EEC (marketing of vegetative propagating material of vine) it was possible to introduce the wording "taking into consideration the biodiversity" in Art. 3 (5)a concerning possible elimination of standard material of a variety.[11] Therefore it will be necessary to be able to document intra-varietal genetic diversity in order to have good arguments to oppose possible requests for elimination of standard material in the near future.

Clones and variety preservation

The ECP/GR WG on *Vitis* stressed the great importance of the diversity of grapevine cultivars for future generations and agreed to promote public awareness on the value of inter- and intra-varietal grapevine diversity.

It was reiterated that without this diversity, viticulture and oenology would be endangered and the consequence of genetic erosion would be a uniform viticulture, which would be susceptible to any kind of biotic or abiotic stress. For this reason, the building blocks (i.e. genetic diversity) for breeding and the development of new products – wines, varieties and clones – has to be preserved.

The Group expressed deep concern for the ongoing serious genetic erosion of the grapevine variability and clonal diversity. The causes of this erosion can be listed as follows:

- Increased international trade
- Predominance of a small number of varieties in several countries

[11] Council Directive 2002/11/EC of 14 February 2002 amending Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine and repealing Directive 74/649/EEC (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002L0011:EN:NOT).

- Predominance of a few clones of each single variety
- Replacement of old vineyards by replanting with modern cultivars
- A fall in the area of land devoted to viticulture, especially in those sites particularly rich in biodiversity
- Restrictive laws not allowing the use of traditional varieties for planting and marketing.

The Group appreciated the example of France, where collecting, characterization, evaluation and maintenance of clones have an almost 30-year-old tradition. The complex system established in that country guarantees the maintenance of maximum intra-varietal diversity. Even though already 15 000 to 20 000 clones of 88 varieties have been gathered and are preserved, collecting in old vineyards (representing still 5% of the grapevine growing area) still continues. The clonal preservation in France is a joint undertaking of the National Technical Association for Viticultural Improvement (Etablissement National Technique pour l'Amélioration de la Viticulture, ENTAV), the National Institute for Agricultural Research (Institut National de la Recherche Agronomique, INRA), departmental authorities and professional associations (see the contribution of T. Lacombe, pp. 157-163).

Considering the critical situation in some wine-growing countries and to promote public interest, the *Vitis* WG stated that extra support is needed for safeguarding the remaining grapevine diversity and wished to recommend that each country should take responsibility for the preservation of its own biodiversity and promote cooperation with other countries in this action.

Recommendations

- *Each country should maintain its own traditional varieties in national or regional ampelographic collections and should also protect* Vitis vinifera *subsp.* sylvestris *in situ.*
- *Each country should strive to preserve its clonal variability as far as possible. This involves identification of old vineyards, seeking and collecting of clones representing the widest intra-varietal variability. According to the French experience, depending on specific cultivar variability and history, up to 500 clones per variety are necessary for the establishment of clonal collections.*

Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region

On behalf of G. Tamai, the IPGRI consultant based at the University of Milano, Italy, L. Maggioni presented the progress of a project funded by the government of Luxembourg and implemented by IPGRI in collaboration with the University of Milano for the conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region. The project aims to identify, characterize and collect the rich grapevine genetic diversity in this area. The first step was to invite the six countries involved (Armenia, Azerbaijan, Georgia, Moldova, Russian Federation and Ukraine) to provide information about the status of grapevine genetic resources by means of a questionnaire.

As a result of this survey, it was noted that serious genetic erosion is occurring due to the poor sanitary condition of some collections, massive introduction of international

cultivars replacing autochthonous ones and lack of information on confirmation of varietal identity. The project is expected to help to identify the unknown varieties and to ensure the long-term conservation of local varieties. The establishment of national collections and local duplication and evaluation sites is envisaged. Capacity-building initiatives will also follow, with fellowships and exchange visits between the different institutions. Specific training will focus on general ampelography, including management of collections and molecular genetics.

Progress in the preparation of a new central grapevine field genebank collection at Vashlidjvari (Georgia) was reported by D. Maghradze (see also paper by N. Chkhartishvili in Part II, pp. 152-154).

The first project meeting, involving all the partners, was said to be planned to take place in the Caucasus in the autumn of 2003.

Discussion

A question was asked about the relationship between the IPGRI project and the ongoing activity of F. Lefort and collaborators for the development of a germplasm database of Ukrainian, Moldovan and Russian *Vitis vinifera* cultivars using microsatellite markers (see full paper in Part II, pp. 150-151). It was agreed that it would be useful to increase coordination between these initiatives and an appropriate opportunity might occur at the first project meeting.

A second question was whether the Caucasus project would be open to wider European collaboration. L. Maggioni replied that any opportunity to extend the collaboration to other institutions would be welcome and that interested people could contact the person responsible for the Caucasus project (Dr Jozef Turok, Director Regional Office for Europe, email: j.turok@cgiar.org).

Conclusion

The section *Discussion and Recommendations* of the report was presented to the participants and was approved with minor modifications.

Jesús Ortiz and Edi Maletić were selected by the Group as respectively Chair and Vice-Chair.

Closing remarks

J. Ortiz thanked the Group members, Erika Dettweiler, the local organizers and IPGRI for their commitment dedicated to the success of this meeting. He was pleased to have seen a wide representation of European countries at this meeting, which could be the start of a new age of collaboration on grapevine genetic resources in Europe. One of the challenges of this Group would be to be able to define how many grapevine varieties exist in Europe. The very large number often suggested – in the range of several thousands – lacks solid data to confirm a specific figure.

The Group agreed that it would be very important to hold a second meeting in 2-3 years' time, i.e. towards the end of 2005 or early 2006.

PART II. PRESENTATIONS AND PAPERS

GENRES 081 – a basis for the preservation and utilization of *Vitis* genetic resources[12]

***Erika Maul*[1] *and Patrice This*[2]**
[1] *Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany*
[2] *Institut National de la Recherche Agronomique (INRA) - UMR 1097 Diversité et Génome des Plantes Cultivées (DGPC), Equipe Génétique Vigne, Montpellier, France*

Introduction

EU Council Regulation No. 1467/94 of 20 June 1994 aimed at the coordination of the conservation, characterization, collection and sustainable utilization of genetic resources in agriculture. The intention was to bring together the work undertaken in the Member States and to enable it to benefit the needs of the Community, in line with the Common Agricultural Policy and the Convention on Biological Diversity.

The EU project GENRES CT96 No. 81 "European Network for Grapevine Genetic Resources Conservation and Characterization" ran from 1 March 1997 to 28 February 2002. Because more time was needed to finish the project work, a prolongation until 30 September 2002 was approved by the European Commission. The objectives of the project were: (1) the establishment of a European *Vitis* Database, (2) the selection of appropriate primary and secondary descriptors for (3) morphological description and the evaluation of agronomic traits, mainly of old and long-neglected grapevine varieties and (4) the utilization of simple sequence repeat (SSR) marker analysis for variety identification.

Nineteen partners from 14 countries participated: Austria, **Bulgaria**, **Croatia**, **Czech Republic**, France, Germany, Greece, **Hungary**, Italy, **Moldova**, Portugal, **Slovenia**, Spain and **Switzerland** (Table 1). Countries in bold are those for which Bioversity (formerly known as IPGRI, International Plant Genetic Resources Institute), provided funds to help them to take part in the project.

Material, methods, results and conclusion

The European *Vitis* Database

The European *Vitis* Database, established within the scope of the project, is the inventory of the grapevine accessions that exist in 13 European wine-growing countries. To make the inventory, the project partners agreed on a common format for the passport descriptors for the European *Vitis* Database, while also following the FAO/IPGRI *Multi-crop Passport Descriptor* guidelines (IPGRI *et al.* 1997). Grapevine collection lists were gathered from 18 partners and data on the existing ca. 27 000 accessions were compiled. The number of accessions held in the project partners' grapevine collections is given in Table 1.

[12] Updated 2008

Table 1. Partners of the EU project GENRES 081 and number of accessions in their grapevine collections (figures from 1998-99)

Partner	Country	Institute	No. of accessions
1	Germany	Institut für Rebenzüchtung Geilweilerhof 76833 Siebeldingen	2582
2	France	UFR de Viticulture, Centre ENSA.M/INRA 34060 Montpellier	7179
3	Austria	Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau, 3400 Klosterneuburg	411
4	Spain	Junta de Andalucia, Consejeria de Agricultura y Pesca 11480 Jerez de la Frontera	1452
5	Spain	Departamento de Biologia Vegetal Universidad Politecnica de Madrid 28040 Madrid	2573
6	Greece	Research Center of Makedonia and Thraki Greek Gene Bank 57001 Thermi Thessaloniki	259
7	Greece	NAGREF Vine Institute, 14123 Lykovrissi	791
8	Portugal	Estaçáo Vitivinicola Nacional, 2560 Dois Portos	645
9	Italy	Istituto Sperimentale per la Viticoltura 31058 Susegana	2223
10	Italy	Centro Miglioramento Genetico e Biologia della Vite 10095 Grugliasco (TO)	404
11	Italy	Istituto Agrario di San Michele all'Adige 38010 San Michele all'Adige	1564
12	Italy	Università degli Studi di Udine 33100 Udine	349
13	Switzerland	Station Fédérale de Recherches Agronomiques de Changins, 1009 Pully	367
14	Hungary	FM Szölészeti és Borászati Kutató Intézet Allomása 7634 Pécs	1096
15	Bulgaria	Institute of Viticulture and Oenology, 5800 Pleven	1676
16	Czech Republic	Research Station for Viticulture, 26718 Karlstein 9	719
17	Croatia	University of Zagreb, Faculty of Agriculture 1000 Zagreb	(-)*
18	Moldova	Institut National de la Vigne et du Vin 2019 Kishinev	2574
19	Slovenia	Biotechnical Faculty, University of Ljubljana 1000 Ljubljana	209
Total number of grapevine varieties in the project partners' grapevine collections			**27074**

* Collection is going to be established

Each of the 27 000 accessions was characterized by 12 passport descriptors such as accession name, holding institute, accession number and pedigree. The on-line searchable database (http://www.genres.de/eccdb/vitis) (Fig. 1) was conceived by the Centre for Agricultural Documentation and Information/Institute for Genetic Resources (ZADI/IGR) in Bonn, Germany.

Incorrect variety designations have an impact on research, grapevine breeding and the rationalization of collections. Errors in naming are propagated worldwide through material exchange. It is known that about 95% of the accessions in the world grapevine collections may be true-to-type (Dettweiler 1992). The errors, due to homonyms, synonyms, different spelling (e.g. 'Bahran Chirei', 'Bahian Shirei', 'Baian Schirei', 'Baianshyra', 'Bayan Shirei', etc.) and about 5% misnamed accessions impede the estimation of the real number of different accessions existing in the 18 grapevine collections. Hence the checking of the trueness-to-type of accessions is indispensable.

Fig. 1. Internet layout of the European *Vitis* Database (http://www.genres.de/eccdb/vitis).

Concerning the preservation of grapevine genetic resources, because of the above-mentioned problems of misnaming, within the scope of the GENRES 081 project little knowledge could be gathered about the most highly endangered accessions. Highly endangered accessions are those which risk being lost, since they occur only once or twice worldwide. Therefore in future high emphasis has to be laid on the use of efficient methods for grapevine identity assessment in grapevine collections, e.g. by using DNA marker-based techniques.

Primary and secondary descriptors

Since the first compilation and utilization of the descriptor lists of the International Organisation of Vine and Wine (OIV) (OIV 1983, 1st edition), the International Union for the Protection of New Varieties of Plants (UPOV) (UPOV 1977, 1st edition) and the International Board for Plant Genetic Resources (IBPGR) (IBPGR 1983, 1st edition) this was the first time that the numerous ampelographers participating in GENRES 081 could meet again to discuss descriptors for variety characterization, to record descriptor data on a common set of reference varieties and to compare the findings.

Within the reference varieties, some were described by all project partners during 2 to 4 years ('Chardonnay', 'Cabernet Sauvignon', 'Merlot', 'Pinot noir' and 'Trebbiano Toscano'), and some for 2 years ('Barbera', 'Cabernet franc', 'Chasselas blanc', 'Gewürztraminer', 'Muscat à petits grains blancs', 'Sauvignon blanc', 'Semillon' and 'Primitivo').

For primary descriptor recording during the first and second workshops of GENRES 081, 33 ampelographic, 21 ampelometric and 14 secondary descriptors of the OIV Descriptor List (OIV 1983) were selected and slightly modified, e.g. for the time of observation, expression stages or reference varieties. Some newly created descriptors were added.

For each descriptor, the notations and measurements recorded by the partners were compared during the third and fourth workshops. In case of differences, the suitability of the descriptors (easy to record, objective, minimal sensitivity to modification) was discussed and appropriate changes were made where advisable. The results are listed in Table 2.

Table 2. Modification of OIV descriptors: final results after 4 years' comparison of notations of a common set of varieties

Modifications	Primary descriptors		Secondary descriptors (14)
	Ampelographic (33)	Ampelometric (21)	
Unchanged	8	19	1
Wording of the descriptor	4		1
Levels of expression, addition of values	5		5
Example varieties: addition / elimination	18	2	8
Definition (observation time, explanations, etc.)	18	2	9
Pictures	8		2
New descriptors	3		6

Partner 8 carried out a statistical analysis on the primary descriptor data of the 5 reference varieties described during 4 years. Even though the descriptions were made in different environments and by 13 different institutes (Partners 1-12 and Partner 16, see Table 1), discriminate analysis showed a good assignment of varieties to their groups. All chosen descriptors are reliable and hence suited for variety description.

The primary and secondary descriptors of the GENRES 081 project were published as the "Primary Descriptor List for Grapevine Cultivars and Species (*Vitis* L.)" (Anonymous 2002a) and the "Secondary Descriptor List for Grapevine Cultivars and Species (*Vitis* L.)" (Anonymous 2002b).

- ***Primary Descriptor Priority List***

A list with useful descriptors for a quick characterization of varieties discovered *in situ* was suggested by Partner 17. The GENRES 081 project partners agreed on the compilation of a "Primary Descriptor Priority List" comprising 14 primary descriptors (Table 3). They discriminate well between varieties and are easy to score.

Table 3. The 14 descriptors of the *Vitis* Priority List

OIV code N°	Bioversity N° (ex-IPGRI N°)	Descriptor
OIV 001	6.1.1	Young shoot: opening of the shoot tip
OIV 004	6.1.3	Young shoot: density of prostrate hairs on the shoot tip
OIV 016	6.1.14	Shoot: number of consecutive tendrils
OIV 051	6.1.16	Young leaf: colour of upper side of blade (4th leaf)
OIV 067	6.1.22	Mature leaf: shape of blade
OIV 068	6.1.23	Mature leaf: number of lobes
OIV 070	6.1.24	Mature leaf: area of anthocyanin coloration of the main veins on the upper side of the blade
OIV 076	6.1.27	Mature leaf: shape of teeth
OIV 079	6.1.30	Mature leaf: degree of opening / overlapping of petiole sinus
OIV 081-2	6.1.32	Mature leaf: petiole sinus base limited by vein
OIV 084	6.1.35	Mature leaf: density of prostrate hairs between main veins on lower side of blade
OIV 087	6.1.38	Mature leaf: density of erect hairs on main veins on lower side of blade
OIV 223	6.2.6	Berry: shape
OIV 225	6.2.8	Berry: colour of skin

Bioversity, UPOV and OIV have hitherto worked with differing descriptor lists. The partners within GENRES 081 agreed that an approach should be initiated to bring the descriptors of these three lists closer together, with the objective of achieving the greatest correspondence between descriptors. In March 2002 the OIV expert group "Vine selection" invited representatives from Bioversity and UPOV. Both organizations indicated their interest in working on descriptor harmonization. After several working meetings, nearly 80% of the 50 descriptors common to all three descriptor lists were brought to match completely, even though Bioversity, UPOV and OIV pursue different purposes and each depends on its own internal regulations.

The harmonized results achieved (Table 3) will be presented as the final version for the "2nd edition of the OIV Descriptor List for Grape Varieties and *Vitis* species" at the OIV expert group "Genetic resources and vine selection" in March 2007.[13]

Description of old and endangered varieties

The scientific discovery that the high quality varieties 'Chardonnay' and 'Syrah' both descend from parents which are rare and have been abandoned by viticulture has demonstrated again the necessity for grapevine genetic resources characterization, identification and preservation for today and for future requirements. The very old, indigenous but abandoned variety 'Heunisch weiss', widespread in Middle Europe in the Middle Ages and even before, was discovered to be the direct (as parent) or indirect

[13] The new OIV descriptor list will be available from the OIV Web site (http://www.oiv.int/).

ancestor of at least 76 varieties sharing one allele of each of 14 SSR markers with 'Heunisch weiss' (Boursiquot *et al.* 2002). 'Heunisch weiss' in combination with 'Pinot' is the parent of at least 16 varieties, among them the famous variety 'Chardonnay' (Bowers *et al.* 2000).

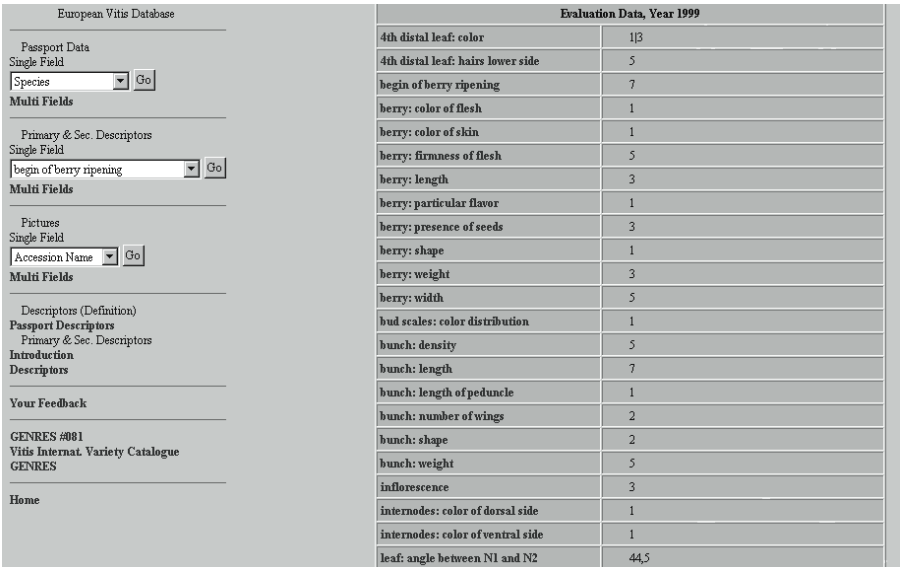
Within the GENRES 081 project, description focused mainly on indigenous and long-neglected varieties. Within the scope of the project, for the first time 54 primary descriptors of 802 varieties and 14 secondary descriptors of more than 432 varieties were recorded according to a common code and with two repetitions per cultivar.

The ampelographic descriptors were recorded in the field collection at three observation times: at flowering time (11 descriptors), from berry set to veraison[14] (12 descriptors), and at maturity (8 descriptors). For the leaf measurement (19 descriptors), 10 mature, healthy leaves above the sixth node and within the medium third of the shoot were collected, pressed and dried. Most of the partners used a digitizer tablet and the leaf measurement program developed by Partner 3. The density of the prostrate and erect hairs on the lower side of the leaf was evaluated by using a binocular microscope. Recording of berry length and width was carried out in the laboratory.

The secondary descriptors were recorded in the field collection at four observation times: at bud burst (1 descriptor), at flowering time (3 descriptors), at veraison (1 descriptor) and at maturity (9 descriptors).

All the descriptor data gathered during the 5 project years were made available via the Internet (http://www.dainet.de/eccdb/vitis) by the ZADI/IGR (Fig. 2).

On the basis of the collected data an identification procedure can be developed in the next few years.



Evaluation Data, Year 1999	
4th distal leaf: color	1|3
4th distal leaf: hairs lower side	5
begin of berry ripening	7
berry: color of flesh	1
berry: color of skin	1
berry: firmness of flesh	5
berry: length	3
berry: particular flavor	1
berry: presence of seeds	3
berry: shape	1
berry: weight	3
berry: width	5
bud scales: color distribution	1
bunch: density	5
bunch: length	7
bunch: length of peduncle	1
bunch: number of wings	2
bunch: shape	2
bunch: weight	5
inflorescence	3
internodes: color of dorsal side	1
internodes: color of ventral side	1
leaf: angle between N1 and N2	44,5

Fig. 2. Primary and secondary descriptor data of the Spanish cultivar 'Alarije dorada' in the European *Vitis* Database.

[14] Veraison: first colour change, beginning of ripening in grapes.

- ***Photos of shoot tips, leaves and clusters***

To illustrate the rare and endangered grapevine varieties described in the scope of GENRES 081, photographs of different anatomical parts of the plant (shoot tips, leaves and clusters) were taken. Pictures are a useful supplement for variety distinction and identification and can be helpful for winegrowers and breeders who are interested in cluster and berry shape and size.

About 1700 photographs of about 500 varieties were added by the ZADI/IGR to the corresponding accessions of the European *Vitis* Database (example in Fig. 3).

Fig. 3. Cluster of the French cultivar 'Aouillat' in the European *Vitis* Database.

Microsatellite (SSR marker) analysis for grapevine variety differentiation and identification

Because microsatellites turned out to be extremely efficient and useful for grapevine variety differentiation and identification, the partners of the GENRES 081 project agreed that the project is an excellent platform to utilize SSR markers which have already been developed for the implementation of a universally accessible SSR marker database for variety identification purposes.

The most informative markers (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79) were selected. Three circular tests were carried out, where 8 to 11 partners worked with identical DNA which was distributed either through shipment of DNA or through shipment of woody cuttings of the varieties. The polymerase chain reaction (PCR) protocol was not standardized owing to the many factors affecting PCR procedure, such as the different methods for allele length assessment

(manual sequencing or capillary- or gel-based automatic sequencing) and because of the different equipment already existing in the partners' laboratories.

The first round of analysis with five varieties revealed several discrepancies. But an important observation was that in most cases the differences in size between the two alleles of a variety and between the partners were conserved. Therefore in the second round of analysis, with 16 varieties, length standards commonly used were replaced by defined reference varieties with known allele lengths as the standards.

Except for minor discrepancies owing to (1) new alleles rather far from the reference alleles, (2) alleles situated close together, which could be interpreted as stutter bands, and (3) homozygous varieties, the shiftings in the size of the markers remained largely constant between partners and varieties. Subsequently for all alleles found, varieties of international importance were chosen as reference (example) varieties. In comparison with the reference varieties, the allele sizes of all the other varieties were coded. After this procedure the results of the different laboratories were largely identical.

The existence of additional alleles mainly present in American *Vitis* species and rootstocks, announced by recent scientific publications, led to the third round of analysis with another 36 varieties (16 *Vitis vinifera* and 19 rootstocks). The results of the third round of analysis have shown that the choice of the additional varieties was justified in the context where:

1. The number of alleles has been increased. Thirteen to 23 alleles per marker have been found.
2. The extension of the markers has been enlarged with a scale ranging from 26 to 46 base pairs difference between the shortest and the longest allele.

Most of the possible alleles seem to have been found. According to the results of the third round of analysis, 31 reference varieties are necessary to represent the 101 alleles which were found.

Descriptors were developed for the six SSR markers (available at http://www.genres.de/CF/eccdb/vitis/_cfm/markers.cfm). The descriptor layout was conceived according to the OIV "Descriptor List for Grapevine Varieties and *Vitis* species" (OIV 1983). For each existing allele, reference (example) varieties were chosen. The variety names were codified.

A detailed description and the results of SSR marker analysis within GENRES 081 were published by This *et al.* (2004).

Conclusion: benefits of GENRES 081

The project has set several new and internationally agreed standards such as the utilization of SSR markers as an additional tool for variety differentiation/identification. These achievements will result in more efficient and sustainable handling of *Vitis* genetic resources.

1. **Documentation:** Inventory of grapevine varieties existing in 13 European countries. Recording of the 27 000 accessions of the 18 GENRES 081 partners' grapevine collections in the European *Vitis* Database.
2. **Characterization:** Improvement of descriptor definition, which will enhance objectivity in descriptor recording.

3. **Description:** Common use of the improved descriptors for the characterization of old and endangered varieties, which were poorly described in the past.
4. **Differentiation/identification:** Besides ampelography, SSR markers proved to be suited for variety distinction and identification by using the alleles of grapevine varieties as length standards. The results of SSR marker analysis are independent of the equipment or the method applied.
5. **Preservation/utilization:** (a) Safeguarding, description and evaluation of rare old grapevine varieties was considerably stimulated. (b) Owing to the varieties' different geographic (climatic) origin, the descriptions carried out cover a wide range of grapevine diversity. (c) Some of the old and long-neglected varieties described by the project partners can now be reconsidered and can be utilized as varieties of special value for consumers or can be involved in grapevine breeding.
6. **Acceptance of the ECP/GR *Vitis* Working Group in October 2001:** On the basis of the GENRES 081 achievements a follow-up will comprise:
   - Involvement of partners from eastern European countries (Albania, Armenia, Macedonia FYR, etc.)
   - Ongoing characterization and evaluation of endangered varieties
   - Utilization of SSR marker data for grapevine identification
   - Work on the problems of synonymy/homonymy (trueness-to-type) and misnaming, by using SSR marker data and ampelography to sort out grapevine collections
   - On-line work in the European *Vitis* Database through partner-specific passwords.

The new European Project on Grapevine of the EU Council Regulation No 870/2004 (GrapeGen06, Management and Conservation of Grapevine Genetic Resources; http://www.montpellier.inra.fr/grapegen06/) lasting for four years (January 2007-December 2010) and comprising 25 partners from 17 countries will continue, enhance and broaden the activities started within the scope of GENRES 081 (Bacilieri 2007).

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Documentation

Status of the European Vitis *Database*[15]

Erika Maul
Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany

Introduction

Cost-efficient and rational breeding programmes require structured information to be made available about the genotypes to be used as parents. In other words, the varieties within germplasm collections are more useful for breeders if corresponding information on their properties is available.

For this reason the European *Vitis* Database (http://www.genres.de/eccdb/vitis/) was created: it is a further *Vitis* database in addition to the *Vitis* International Variety Catalogue (VIVC) (http://www.vivc.bafz.de/index.php; updated in 2007).

These two databases are information platforms for research, breeding and viticulture by providing grapevine variety-specific data and they support (1) the maintenance of genetic resources, (2) the differentiation and identification of grapevine varieties and (3) the availability and exchange of germplasm.

The establishment of two *Vitis* databases

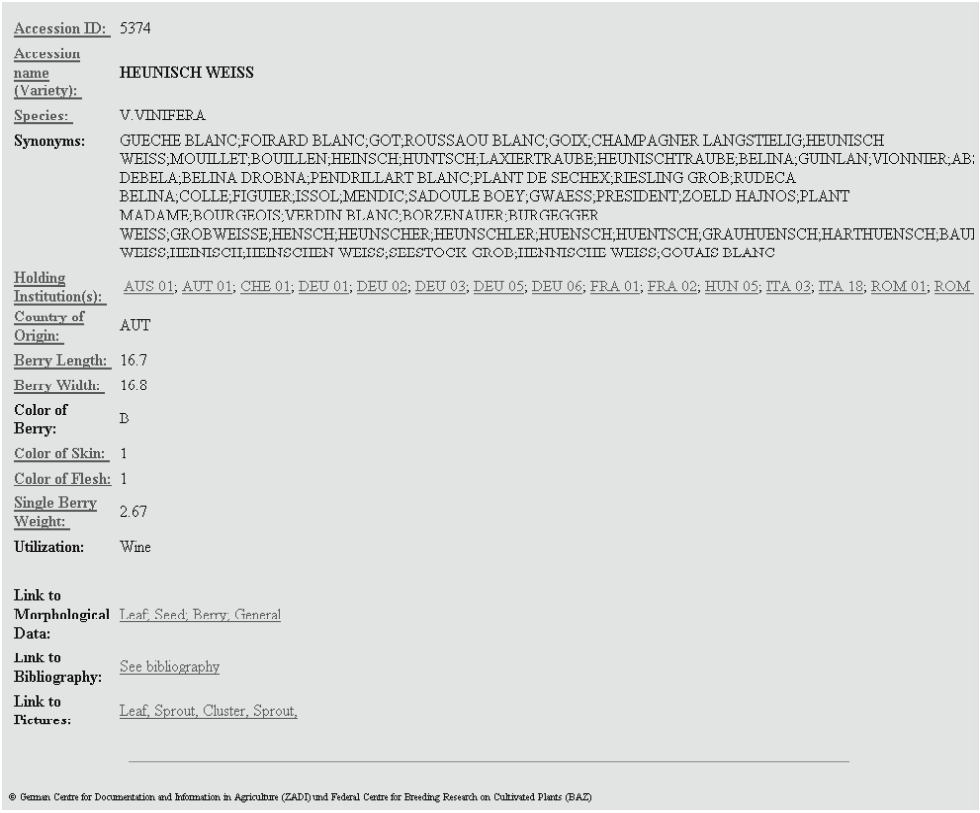
- ***The* Vitis *International Variety Catalogue (VIVC)***

In the early 1970s, experts worldwide realized that plant genetic diversity was endangered by the progress of development and by destructive environmental incidents. *Vitis* species were not excluded from this phenomenon. In 1982, far-seeing experts on grapevine breeding and at the International Board for Plant Genetic Resources (IBPGR) pointed out (1) the urgency of germplasm collection because of the loss of wild forms and old indigenous varieties of *Vitis* and the need for maintenance of Vitaceae, *Vitis* species and cultivars and clones in repositories and (2) the need for international cooperation for their characterization and evaluation and the free exchange of genetic material (OIV General Assembly Resolution No 2/82) (Dettweiler 1990).

The VIVC was the result of international efforts in the field of plant genetic resources conservation. In 1983, with initial support from the International Plant Genetic Resources Institute (IPGRI, now Bioversity International) and the International Organisation of Vine and Wine (Office International de la Vigne et du Vin, OIV), the Institute for Grapevine Breeding, Geilweilerhof, started to compile the inventory of *Vitis* species, varieties and genotypes existing in grapevine collections worldwide. The resulting database has been accessible via the Internet since 1996. It provides an inventory of the currently existing grapevine genetic resources which are also documented by passport descriptors, such as accession name, species and synonyms, parentage and breeder (Fig. 1).

[15] Updated 2007

The records in the VIVC are based on information found in the literature (Fig. 1, "Link to Bibliography") and on varieties maintained in grapevine field collections (Fig. 1, "Holding Institution(s)"). More than 400 books with ampelographic descriptions of varieties and more than 750 publications on grapevine varieties were studied thoroughly and the grapevine varieties of about 120 grapevine collections were assigned to previously determined prime names. Today more than 18 500 prime names of *Vitis* species, cultivars and genotypes are registered.



Accession ID:	5374
Accession name (Variety):	HEUNISCH WEISS
Species:	V.VINIFERA
Synonyms:	GUECHE BLANC;FOIRARD BLANC;GOT;ROUSSAOU BLANC;GOIX;CHAMPAGNER LANGSTIELIG;HEUNISCH WEISS;MOUILLET;BOUILLEN;HEINSCH;HUNTSCH;LAXIERTRAUBE;HEUNISCHTRAUBE;BELINA;GUINLAN;VIONNIER;AB; DEBELA;BELINA DROBNA;PENDRILLART BLANC;PLANT DE SECHEX;RIESLING GROB;RUDECA BELINA;COLLE;FIGUIER;ISSOL;MENDIC;SADOULE BOEY;GWAESS;PRESIDENT;ZOELD HAJNOS;PLANT MADAME;BOURGEOIS;VERDIN BLANC;BORZENAUER;BURGEGGER WEISS;GROBWEISSE;HENSCH;HEUNSCHER;HEUNSCHLER;HUENSCH;HUENTSCH;GRAUHUENSCH;HARTHUENSCH;BAUI WEISS;HEINISCH;HEINSCHEN WEISS;SEESTOCK GROB;HENNISCHE WEISS;GOUAIS BLANC
Holding Institution(s):	AUS 01; AUT 01; CHE 01; DEU 01; DEU 02; DEU 03; DEU 05; DEU 06; FRA 01; FRA 02; HUN 05; ITA 03; ITA 18; ROM 01; ROM
Country of Origin:	AUT
Berry Length:	16.7
Berry Width:	16.8
Color of Berry:	B
Color of Skin:	1
Color of Flesh:	1
Single Berry Weight:	2.67
Utilization:	Wine
Link to Morphological Data:	Leaf, Seed, Berry, General
Link to Bibliography:	See bibliography
Link to Pictures:	Leaf, Sprout, Cluster, Sprout,

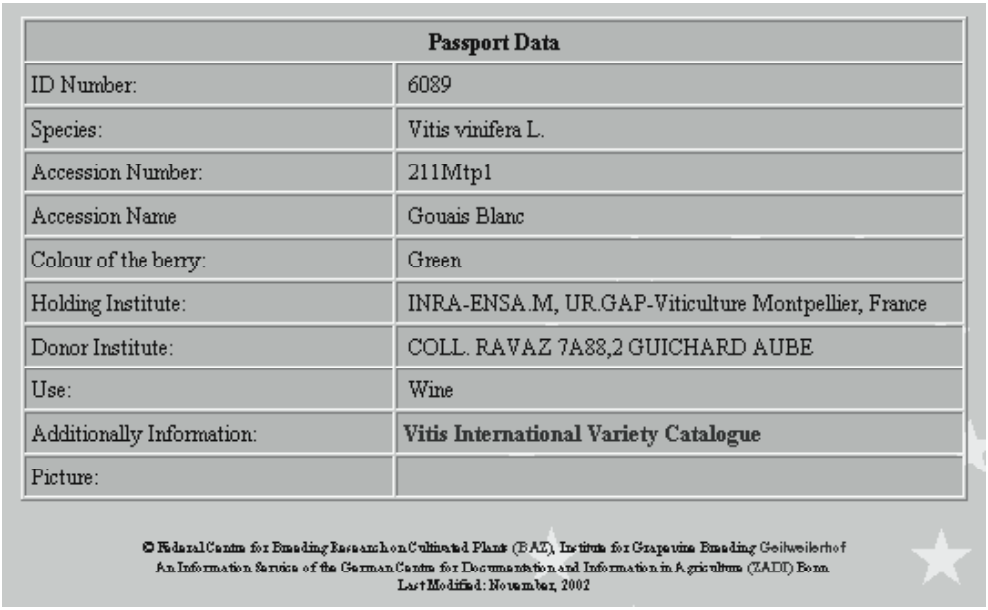
Fig. 1. *Vitis* International Variety Catalogue: documentation on 'Heunisch weiss', an immensely old but abandoned variety, widespread in the Middle Ages and even before. Molecular analysis revealed the importance of this vine as a breeder of new varieties (Boursiquot 2004).

- ***The European* Vitis *Database***

The European *Vitis* Database, established within the EU project GENRES CT96 081 "European Network for Grapevine Genetic Resources Conservation and Characterization", which ran from 1 March 1997 to 30 September 2002, is an accession-linked database, which is not the case for the VIVC. Each accession is identified by its accession number (Fig. 2). This is indispensable owing to the high number of misnamed, synonymous or homonymous grapevine varieties, which amount to about 5 to 10% in the worldwide grapevine collections (Dettweiler 1992).

Thus, every record, whether primary or secondary descriptor, picture or SSR marker, will be assigned to the corresponding accession from which the information was obtained.

During the first meeting of the GENRES 081 project partners in July 1997, the participants agreed on a common database format for the passport and the descriptor data, which comprised the following fields: yes/no field (remark: yes was given for cultivars with verified identity, no for cultivars with uncertain identity), official name of the cultivar, berry colour (B=blanc, G=gris, RG=rouge, RS=rose, N=noir), accession number in the collection, name in the collection, country of origin, source of the material, date of entry into the collection, *Vitis* species, parentage, breeder, use (W=wine, T=table, R=raisin, RS=rootstock), remarks (e.g. observed synonyms), plus 53 descriptor fields.



Passport Data	
ID Number:	6089
Species:	Vitis vinifera L.
Accession Number:	211Mtp1
Accession Name	Gouais Blanc
Colour of the berry:	Green
Holding Institute:	INRA-ENSA.M, UR.GAP-Viticulture Montpellier, France
Donor Institute:	COLL. RAVAZ 7A88,2 GUICHARD AUBE
Use:	Wine
Additionally Information:	**Vitis International Variety Catalogue**
Picture:	

Fig. 2. European *Vitis* Database: 'Gouais blanc', a synonym to 'Heunisch weiss', with its accession number and the link to the *Vitis* International Variety Catalogue.

Both databases are linked if the trueness-to-type of varieties in the European *Vitis* Database is recorded (Fig. 2, option "Additional Information": "Vitis International Variety Catalogue").

The GENRES 081 partners sent their data to the coordinator. The coordinator assembled the information in a single file and sent it to the Centre of Agricultural Documentation and Information/Information Centre for Biological Diversity (ZADI/IBV) in Bonn, Germany, so as to make the European *Vitis* Database available via the Internet.

Prerequisites for smooth-running database management

In spite of the agreement on a common format and standardized descriptor field names, numerous problems can occur and additional work is generated if the

agreed format and terms are not respected. Database updating and expansion require strict adherence to the previously established rules. Switching the order of descriptor fields, for example, will result in confusing and illogical information. There can be considerable consequences, e.g. if the key field, which is the accession's code number, should be modified over time and if the change is not communicated for recording in the European *Vitis* Database. If an accession's code number, registered in the database, does not match up with the accession's code number for the descriptor data and pictures, they cannot be correctly assigned. The same happens if an accession's code number is present twice. Concerning the primary and secondary descriptors, the indications given within the definitions need to be followed to ensure the comparability of the data from different sources. This concerns for example the units for length. If the berry length is to be recorded in mm, then any accessions where the data were recorded in cm will not appear in the right order. Or if for ampelometric descriptors numeric data are requested, notations (scores) cannot be accepted, as this will result in a mix up of measured data and notations in the database and thus impede database search functions.

Background and details of the GENRES 081 project can be found on the European *Vitis* Database homepage by choosing "Genres #081", where the objectives, project partners and workshop summaries are available.

The European *Vitis* Database currently comprises:

- passport descriptors of 27 074 accessions;
- primary descriptor data of 802 accessions of rare old indigenous grapevine varieties;
- secondary descriptor data of 432 accessions of rare old indigenous grapevine varieties and varieties of valuable germplasm for breeding; and
- 2200 pictures illustrating different parts of the vine, useful for grapevine variety recognition of 450 accessions.

The future objective is the addition of SSR marker data for differentiation and identification purposes.

In October-November 2002 the European *Vitis* Database was redesigned by the Centre for Agricultural Documentation and Information/Institute for Genetic Resources (ZADI/IGR), offering multiple options in the search of *Vitis*-specific information. Beside the search for a single field, the "Multi Fields" option enables the combination of several fields for a search directed to specific objectives.

The two examples below – one for passport data and another for primary and secondary descriptor data search – will demonstrate the menu's flow.

Part I. Passport descriptor data

- ***Example for passport data: search for a specific pedigree via "Multi Fields"***

The objective would be to find a grapevine variety with 'Cabernet Sauvignon' as ancestor, black berry colour, utilized as a wine grape and available in Montpellier, France, as indicated in Fig. 3. These four fields have to be connected by the "and" option (see ninth row of the table, "Field Connector").

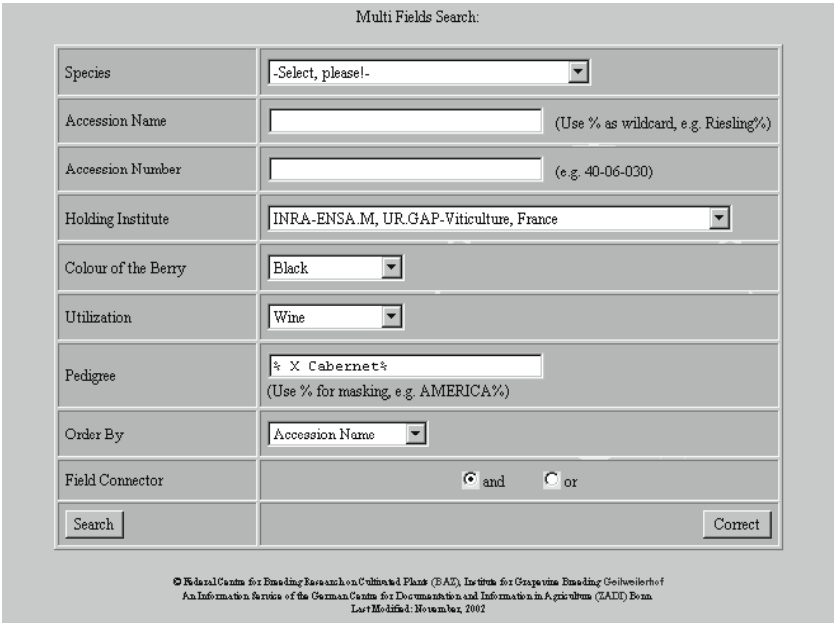
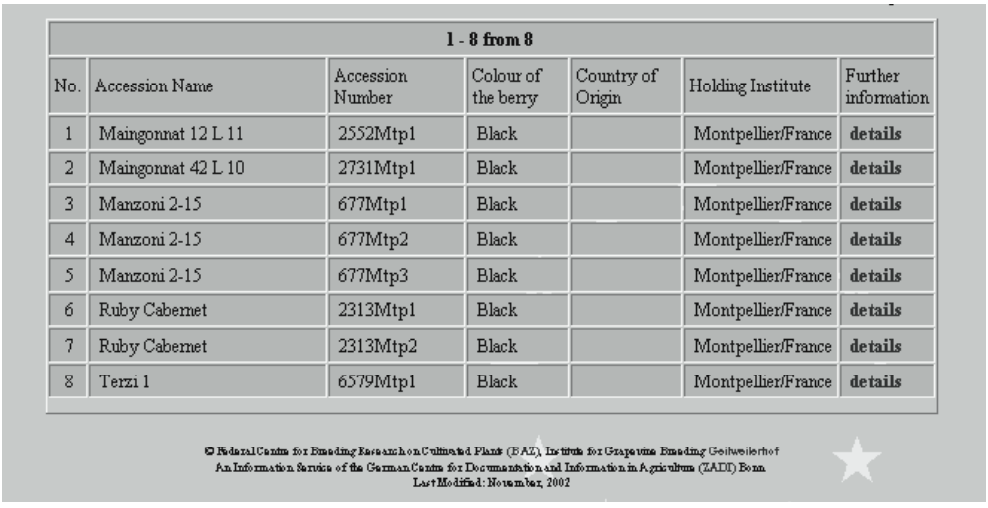


Fig. 3. Passport data: pedigree search via "Multi Fields". Choice of options.

At the end of the search a table will be generated, displaying the accessions fulfilling the requested conditions. At Montpellier 8 accessions correspond (Fig. 4).



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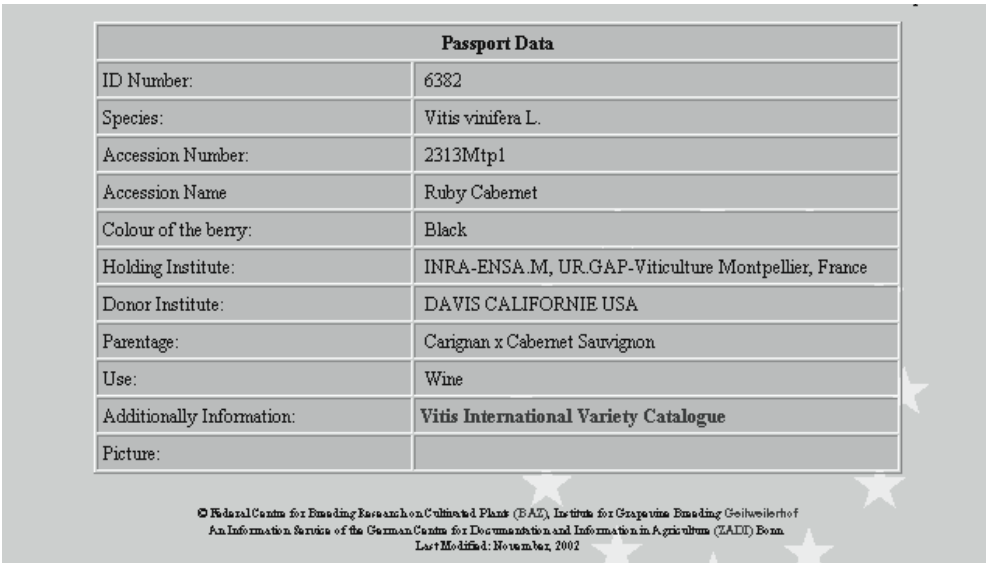
No.	Accession Name	Accession Number	Colour of the berry	Country of Origin	Holding Institute	Further information
1	Maingonnat 12 L 11	2552Mtp1	Black		Montpellier/France	**details**
2	Maingonnat 42 L 10	2731Mtp1	Black		Montpellier/France	**details**
3	Manzoni 2-15	677Mtp1	Black		Montpellier/France	**details**
4	Manzoni 2-15	677Mtp2	Black		Montpellier/France	**details**
5	Manzoni 2-15	677Mtp3	Black		Montpellier/France	**details**
6	Ruby Cabernet	2313Mtp1	Black		Montpellier/France	**details**
7	Ruby Cabernet	2313Mtp2	Black		Montpellier/France	**details**
8	Terzi 1	6579Mtp1	Black		Montpellier/France	**details**

Fig. 4. Passport data: pedigree search via "Multi Fields". Table with accessions corresponding to the request.

More information about the accessions is to be found if "details" is clicked. Passport data (Fig. 5), and, if present, primary and secondary descriptor data and pictures are available.



Passport Data	
ID Number:	6382
Species:	Vitis vinifera L.
Accession Number:	2313Mtp1
Accession Name	Ruby Cabernet
Colour of the berry:	Black
Holding Institute:	INRA-ENSA.M, UR.GAP-Viticulture Montpellier, France
Donor Institute:	DAVIS CALIFORNIE USA
Parentage:	Carignan x Cabernet Sauvignon
Use:	Wine
Additionally Information:	**Vitis International Variety Catalogue**
Picture:	

Fig. 5. Passport data of the accession 'Ruby Cabernet' in the European *Vitis* Database.

The option "*Vitis* International Variety Catalogue" offers additional information. If the accession in the given grapevine collection was checked and considered to be true-to-type, then the relevant accession is linked to the VIVC. By clicking this option some redundant but also some complementary data can be obtained (Fig. 6). In the case of 'Ruby Cabernet' additional specifications are synonyms, further holding institutions, resistance data to *Botrytis* and anthracnose, and bibliography.

Accession ID:	**10313**
Accession name (Variety):	**RUBY CABERNET**
Species:	V.VINIFERA
Pedigree:	CABERNET SAUVIGNON X CARIGNAN
Synonyms:	CALIFORNIA 234 F 2;RUBI KABERNE
Holding Institution(s):	ARG 01; AUS 01; BGR 01; BRA 01; CAN 01; CHN 01; CZE 01; ESP 01; ESP 02; FRA 01; FRA 02; HUN 03; ISR 01; ITA 01; ITA 07; ITA 27; MEX 02; NZL 01; SVK 01; USA 01; USA 06; YUG 01; YUG 02; YUG 03; ZAF 01
Country of Origin:	USA
Color of Berry:	N
Utilization:	Wine;Table
Botrytis C:	7
Anthracnose LC:	7
Link to Morphological Data:	Leaf
Link to Bibliography:	See bibliography

Fig. 6. Passport data of the accession 'Ruby Cabernet' in the VIVC.

Part II. Characterization and evaluation data

- ***Example: primary descriptor data: search via "Multi Fields"***

In the European *Vitis* Database the exact definition of characteristics is available under "Primary & Secondary Descriptors". Besides the 54 primary and the 16 secondary descriptors, the forewords to the editions are also accessible, tracing back the reasons for modifications carried out within the scope of GENRES 081.

Again an example will illustrate the steps necessary in selecting a grapevine accession with particular desired attributes. The objective could be to find varieties with a late time of bud burst to avoid spring frost damage, or to find varieties with an early bud burst which would benefit from a longer vegetative period in warmer climates.

The first step would be to examine the descriptor itself to be aware of the recording and the notation adopted for the characteristic. Fig. 7 shows the descriptor "Time of bud burst" with the code numbers OIV 301, UPOV 1 and Bioversity (ex-IPGRI) 7.1.1. Supposing the aim is to search for "very early" bud burst varieties, then the varieties should be those recorded with the notation "1".

Carattere: Caractère: Merkmal: Characteristic: Carácter:	Epoca del germogliamento Époque du bourgeonnement Beginn des Knospenaustriebs Time of bud burst Epoca de la brotación	Codes N^{os} OIV 301 UPOV 1 Bioversity 7.1.1.

Livelli di espressione / Notation / Bonitierung / Notes / Notación:

1	3	5	7	9
molto precoce	precoce	media	tardiva	molto tardiva
très précoce	précoce	moyenne	tardive	très tardive
sehr früh	früh	mittel	spät	sehr spät
very early	early	medium	late	very late
muy precoz	precoz	media	tardia	muy tardia

Varietà di riferimento / Exemples de variétés / Beispielssorten / Example varieties / Ejemplos de variedades:

1	3	5	7	9
V.amurensis V.romanetii	Chardonnay B	Cabernet Sauvignon N	Mourvèdre N Trebbiano Toscano B	Airén B

Indicazioni / Définitions / Definitionen / Definitions / Indicaciones:

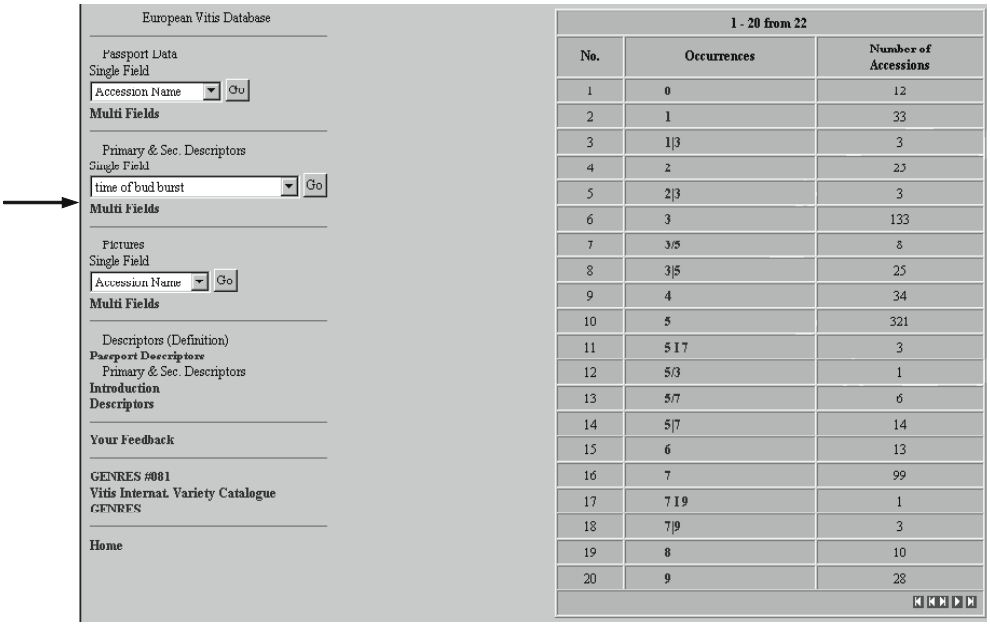
I: Osservazione da effettuare quando il 50% delle gemme si trova allo stadio di punta verde (stadio C di Baggiolini).
F: Observation à faire quand 50% des bourgeons se trouvent au stade pointe verte (stade C de Baggiolini).
D: Feststellung wenn bei 50% der Knospen die grüne Spitze deutlich sichtbar ist (Stadium C nach Baggiolini).
E: Observation when 50% of the buds are in green - tip stage (stage C of Baggiolini).
S: Observación a realizar cuando el 50% de las yemas se encuentran en el estado de punta verde (estado C de Baggiolini).

Gemma: Stadio punta verde
Bourgeon: Stade pointe verde
Knospe: Stadium grüne Spitze
Bud: Green - tip stage

Fig. 7. OIV descriptor 301: "Time of bud burst".

The desired information can be obtained by searching under "Primary & Secondary Descriptors", "Single Field", where the descriptor scroll is available. By clicking "time of bud burst" and "Go" a table is generated, listing the number of accessions recorded for the desired expression stages (Fig. 8). From the roughly 760 accessions described, 33 have been evaluated as having a very early time of bud burst (notation 1).

Expression stages can be mixtures of different notations when the expression appears to be in-between two consecutive notations. Some notations occur twice or three times (Fig. 8), because either of two kinds of slashes used or the presence or absence of spacing between the numbers.



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No.	Occurrences	Number of Accessions
1	0	12
2	1	33
3	1|3	3
4	2	23
5	2|3	3
6	3	133
7	3/5	8
8	3|5	25
9	4	34
10	5	321
11	5 1 7	3
12	5/3	1
13	5/7	6
14	5|7	14
15	6	13
16	7	99
17	7 1 9	1
18	7|9	3
19	8	10
20	9	28

Fig. 8. Secondary descriptor data.

By clicking on "1" in the second row of column "Occurrences", a list with the 33 accessions is generated. Passport data and primary and secondary descriptor data of the listed accessions are accessible by clicking "details" under "Further information". Fig. 9 shows the data recorded for accession 'Albillo' at the Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA), Madrid, Spain.

Pictures of different parts of the plant, suitable for variety differentiation and identification and for breeders' information are also available (see the berries of the accession 'Albillo' in Fig. 10).

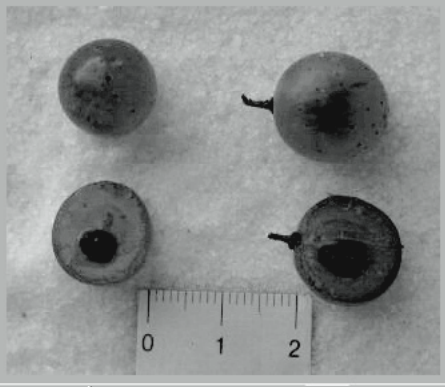
The interest in *Vitis*-related information is high. The frequency of use of the European *Vitis* database for the first quarter 2003 was provided by ZADI/IBV (Table 1).

Passport Data	
ID Number:	13982
Species:	Vitis vinifera L.
Accession Number:	BGVCAM1104
Accession Name	Albillo
Colour of the berry:	Green
Holding Institute:	Instituto Madrileño de Investigación Agraria y Alimentaria (I.M.I.A.), Spain
Country of Origin:	SPAIN
Use:	Table, Wine
Edition Information:	**Vitis International Variety Catalogue**
Picture:	**bayas / berries; racimos / bunches; sumidad / shoot tip; hojas / leaves;**
Evaluation Data, Year 1999	
4th distal leaf: color	1
4th distal leaf: hairs lower side	7
berry: color of flesh	1
berry: color of skin	1
berry: length	14,6
berry: particular flavor	1
berry: presence of seeds	3
berry: shape	2
berry: weight	1,6
berry: width	13,8
bud scales: color distribution	1

Fig. 9. Passport and evaluation data, year 1999 of the accession 'Albillo', recorded at IMIA.

Picture Data

If photos may be reproduced you are kindly requested to quote the source of the data.



Accession Name	Albillo
Accession Number:	BGVCAM1104
Part of the Plant:	bayas / berries
Origin of the Picture:	Instituto Madrileño de Investigación Agraria y Alimentaria (I.M.I.A.), Spain
Passport Data	**details**

Fig. 10. Berries of the accession 'Albillo', photographs taken by IMIA.

Table 1. Web site statistics of the European *Vitis* Database, January to April 2003

Frequency of use	2003			
	January	February	March	April
Homepage	838	712	563	540
Search for pictures	938	974	1250	865
Search for passport and descriptor data	1545	1286	1165	862

Conclusion

The European *Vitis* Database was established by the GENRES 081 partners, thanks to the support of the European Commission. The database was made available via Internet, thanks to the ZADI/IBV. Various search options are offered.

The database structure enables the addition of:

- passport data from further grapevine collections (= grapevine germplasm repositories);
- grapevine varieties descriptor data;
- photographs of shoot tips, leaves, clusters and berries; and
- SSR marker data.

The individual accessibility of the European *Vitis* database with the possibility of on-line modification by each partner is envisaged, as well as the download option in Excel format.

It should be kept in mind that for smooth database management and to ensure the reliability of the data, certain specific and necessary rules must be followed.

References

Boursiquot, J.M., T. Lacombe, J. Bowers and C.P. Meredith. 2004. Le Gouais, un cépage clé du patrimoine viticole européen [Gouais, a key vine of the European wine heritage]. Bulletin OIV 77(875-876):5-19. (in French).

Dettweiler, E. 1990. Genetic resources - Gene banks. Vitis 29:57-59.

Dettweiler, E. 1992. The grapevine herbarium as an aid to grapevine identification - First results. Vitis 31:117-120, Newsletter 4.

GENRES 081 descriptors for Vitis *and the Priority Descriptor List*

Anna Schneider
Institute of Plant Virology, National Research Council – Unit of Grugliasco (Turin), Italy

Introduction

The EU-funded project GENRES 081 (European Network for Grapevine Genetic Resources Conservation and Characterization, http://www.genres.de/vitis/vitis.htm) was mainly focused on scion cultivars, with special regard for local, rare, endangered or questionable (as regards identity) grape varieties. The aim of this programme was genotype identification, characterization and evaluation.

The GENRES 081 team developed a list of descriptors specifically for these purposes. This list was derived from the original list formulated by the International Organisation of Vine and Wine (OIV 1983, 1st edition) and incorporated the work carried out by experts on *Vitis* (ampelographers) before and during the 5 years of the programme (1997-2002). Guidelines for descriptor development were devised for accuracy, ease and speed of use, and accessibility for less experienced staff. All the project partners, representing most of the European countries involved in viticulture, agreed to use such descriptors.

In the GENRES list, new descriptors were introduced, other descriptors partly modified, and five languages were provided (Italian, French, German, English, and Spanish) instead of the previous four.

The current list includes 103 descriptors:

a. 28 passport descriptors: general information on access to the sample;
b. 54 primary descriptors: the vine's morphological traits used for identification and characterization of grapevines;
c. 21 secondary descriptors: related to vine physiology, aimed to the evaluation and exploitation of grapevine germplasm.

In addition, six further descriptors based on molecular marker techniques were developed within GENRES 081.

Passport descriptors

The whole list comprises 28 descriptors, originating from the FAO/IPGRI *Multi-crop Passport Descriptors* (MCPDs); this list was designed for recording detailed information on every single accession, such as its origin, the location of the collection, classification, etc. Beside accession name and number, the most relevant passport descriptors in GENRES 081 were considered to be: species, holding institute, country of origin, collection/acquisition source, acquisition date, common crop name (utilization), ancestral data (i.e. pedigree). In addition to the MCPDs, trueness-to-type, berry colour and variety name (after identification) were specifically adopted for *Vitis* within GENRES 081.

Primary descriptors

Depending on the organ to be observed on the vine, 11 primary descriptors are related to the young shoot, 10 to bunch and berry, and as many as 33 to the mature leaf, considered in *Vitis* as the most significant useful organ for identification.

Most of the observations are carried out on leaves, between fruit set and veraison (the time when the grape colour changes can first be seen), when many healthy and mature leaves are available; however, suitable leaves last until grape maturity or longer.

In the GENRES 081 list, particular effort was dedicated to descriptors and the specification of their stages of expression, improving definitions and drawings. For each stage of expression, further common and appropriate example (reference) cultivars were selected.

Descriptor development proceeded step by step. The 16 project partners described the same 5 reference cultivars in the 16 different collection fields, using both old and improved descriptor sets. The improved descriptors often reduced the average ranges of scores assigned by the participants to the same variety. By way of example, Fig. 1 illustrates the considerable improvement of the scores assigned by participants after modification of the drawings (from 4 to 2 differential points) using descriptor 079 (mature leaf: degree of petiole sinus opening).

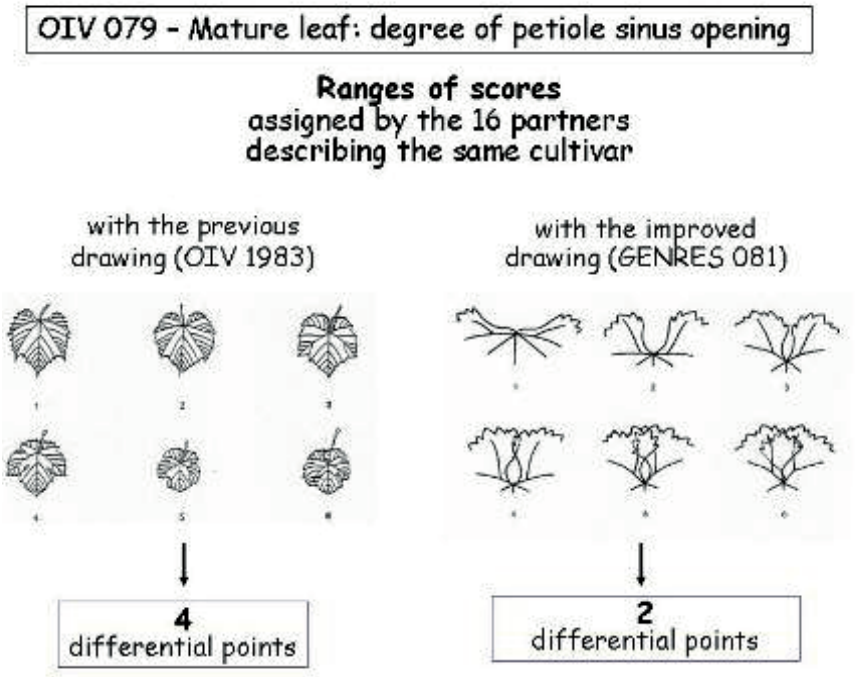


Fig. 1. The improvement of descriptor drawings reduced the ranges of scoring applied by the 16 GENRES 081 partners describing the same cultivar.

As to recording rules, GENRES 081 allows the use of more than one stage of expression for one single descriptor if:

- two or more expression stages are observed in the sample being described (phenotype variability);

- the appropriate notation is between the two closest expression stages; by way of example, for a "short elliptic berry" as in 'Cabernet Sauvignon', the appropriate notation will be 2 and 3, i.e. between round (2) and elliptic (3).

Primary descriptors grouped by the methodology used are:
- ampelographic, i.e. based on observations/notations: 33 descriptors;
- ampelometric: 21 descriptors, 2 of which refer to berry size and 19 are based on the measurement of parameters describing leaf blade morphology.

Leaf biometry proved in fact to have a significant impact on ampelography, decreasing description subjectivity and providing parametric data suitable for specific statistical tests. The mature leaf is suitable for semi-automatic data recording, i.e. the measuring of significant parameters on leaf samples by means of a digitizer or scanned leaf images. In order to provide a quick and accurate tool for recording leaf biometrical data, specific software was developed within GENRES 081. This software performs leaf measurement using a digitizer, and manages and stores the resulting data (Blahous *et al*. 2000).

Secondary descriptors

Although highly affected by environment and season, secondary descriptors refer to the agronomic performance of the material. They are thus significant for the evaluation and the screening of new and old (neglected) varieties of unknown value. Secondary descriptors express vine phenology (bud burst and starting of veraison), cultural traits (basal fertility, yield, vigour), and grape juice composition (sugar, acidity, pH). GENRES 081 put particular emphasis on the evaluation of tolerance/sensitivity to fungal diseases. During the programme, several workshops were held focusing on laboratory techniques to assess fungus resistance, with special regard to *Oidium*, *Plasmopara viticola*, *Botrytis* and *Eutypa lata*. Such workshops provided a forum for discussion, training and learning. New descriptors for screening of material according to the tolerance to powdery and downy mildew, grey rot and *Eutypa* dieback were then developed. Depending on the fungal agent, these screening methods are based on the infection of leaf disks, leaf blades or woody cuttings with measured pathogen quantities, and on the rating of symptoms shown by the test material compared with reference varieties. Detailed and accurate descriptions of testing techniques (laboratory materials and laboratory conditions, statistical design, etc.) are given in every descriptor.

Vitis Priority List

Fourteen primary, non-biometric descriptors were selected to be among the most relevant and most discriminant, thus establishing a *Vitis* Priority List (Table 1). The Priority Descriptor List is meant for the preliminary (often *in situ*) description of materials. Guidelines for the selection of descriptors forming the Priority List are based on their high discriminant value and their ease of scoring (usually in one single survey, through observations in the field by less experienced ampelographers). The *Vitis* Priority List does not include descriptors requiring measurement or laboratory work.

Table 1. The 14 descriptors of the *Vitis* Priority List

OIV code N°	Bioversity N° (ex-IPGRI N°)	Descriptor
OIV 001	6.1.1	Young shoot: opening of the shoot tip
OIV 004	6.1.3	Young shoot: density of prostrate hairs on the shoot tip
OIV 016	6.1.14	Shoot: number of consecutive tendrils
OIV 051	6.1.16	Young leaf: colour of upper side of blade (4th leaf)
OIV 067	6.1.22	Mature leaf: shape of blade
OIV 068	6.1.23	Mature leaf: number of lobes
OIV 070	6.1.24	Mature leaf: area of anthocyanin coloration of the main veins on the upper side of the blade
OIV 076	6.1.27	Mature leaf: shape of teeth
OIV 079	6.1.30	Mature leaf: degree of opening / overlapping of petiole sinus
OIV 081-2	6.1.32	Mature leaf: petiole sinus base limited by vein
OIV 084	6.1.35	Mature leaf: density of prostrate hairs between main veins on lower side of blade
OIV 087	6.1.38	Mature leaf: density of erect hairs on main veins on lower side of blade
OIV 223	6.2.6	Berry: shape
OIV 225	6.2.8	Berry: colour of skin

Conclusions and further remarks

Over 800 scion cultivars, mainly neglected or questionable, have been described and characterized by the GENRES 081 project. This work forms the first nucleus of the European *Vitis* Database available on the Web site (http://www.genres.de/eccdb/vitis/). On the same Web site, each complete descriptor of the list (including drawings, specifications and example varieties) is available in pdf format by clicking on "Descriptors (definition)", selecting "Passport descriptors" or "Primary & Sec. Descriptors", and clicking on "details" for every single descriptor.

GENRES 081 descriptors were mainly designed for *Vitis* scion cultivars, by far the most numerous grape varieties; a further progressive development of the list could be envisaged in order to introduce more specific descriptors for the characterization of other genotypes, such as *V. vinifera* subsp. *silvestris* or rootstock cultivars. As to descriptors' improvement, the addition of further example varieties for every stage of expression will extend descriptor use and acceptance by numerous countries. A next step in the development of primary descriptors could be the use of photographic images of vine organs and vine traits, especially when they describe colours and hues (i.e. definition of berry skin colour).

The GENRES 081 list being an improved version of the OIV one (introducing new descriptors and/or modifying the existing ones), it is recommended that the new edition of the OIV "Descriptor list for grapevine varieties and *Vitis* species"

be integrated with the results from the GENRES 081 project, which would then be officially recognized.[16]

The OIV descriptor list from which GENRES 081 worked was designed and developed in cooperation with other institutions involved in plant germplasm conservation and description, such as Bioversity (formerly known as IPGRI, International Plant Genetic Resources Institute) and the International Union for the Protection of Plant New Varieties (UPOV). Bioversity, UPOV and OIV descriptor lists, although still somewhat different from each other, are closely related: most of the descriptors are cross-referenced in the three lists (as shown by the code numbers of each descriptor at the top right corner). A further effort in the harmonization of descriptor definitions and stages of expression from these three lists is envisaged and will provide a suitable tool, accepted worldwide, for grapevine variety description and identification. In conclusion, it is worth stressing that *Vitis* germplasm identification and evaluation is nowadays a fundamental instrument for the advancement of viticulture legislative frameworks, the improvement of viticulture and oenology products, the development of research on *Vitis* and the conservation of *Vitis* genetic resources.

References

Blahous, D., H. Kaserer and L. Alzinger. 2000. Leaf measurement. Version 1.3.16. Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau, Vienna, Austria.

OIV. 1983. Descriptor list for grapevine varieties and *Vitis* species. Office International de la Vigne et du Vin, Paris.

[16] The *2nd edition of the OIV Descriptor List for Grape Varieties and Vitis species* will be available from the OIV Web site (http://www.oiv.int/).

Harmonization of international descriptors for Vitis[17]

Erika Maul
Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtug Geilweilerhof, Siebeldingen, Germany

Introduction

For a long time (since about the end of the 18th century in Germany), grapevine varieties have been characterized very individually. The chosen characteristics and the method of evaluation varied according to the person or ampelographer involved, and in more recent times they varied according to the aims that were being pursued. Some of these aims are the description of new varieties for their protection (International Union for the Protection of New Varieties of Plants, UPOV), the development of internationally recognized tools to describe and record passport, management, characterization and evaluation data of crops and facilitate data exchange, storage and retrieval (International Plant Genetic Resources Institute (IPGRI), now Bioversity International) and the description of accessions for differentiation and identification purposes and for the evaluation of their breeding aptitudes (International Organisation of Vine and Wine, OIV).

With the ultimate aim of standardizing grapevine variety description, in 1979 experts of all three organizations made proposals on the number and type of characteristics and their definition. In the subsequent years the three organizations worked on descriptor harmonization. The outcomes were the "Descriptors for Grape" (IBPGR 1983), the "Descriptor list for grapevine varieties and *Vitis* species" (OIV 1983) and the "Guidelines for the conduct of tests for distinctness, homogeneity and stability" (UPOV 1985).

Over the years the usage of the three lists revealed that improvements of descriptors are still necessary, in particular to achieve more objectivity and thus to obtain a better comparability of data recorded by different observers and at different locations.

IPGRI and UPOV worked on the descriptors and discussed the modifications on the occasion of an UPOV Subgroup meeting of the Technical Working Party on grape held at Conegliano, Italy in 1996. A revision of the OIV descriptor list took place in November 1996 at Geilweilerhof, Germany. In the framework of the EU project GENRES 081 (http://www.genres.de/eccdb/vitis), running from 1997 to 2002, 60 OIV descriptors were used for grapevine variety description. Most of them were adopted from the OIV descriptor list (1983). Owing to experience gained over four years of descriptor recording and due to the variation which occurred between the notations of the GENRES 081 partners, descriptors were modified when necessary.

The second edition of the "Descriptors for Grapevine *(Vitis* spp.)" was published in 1997 (IPGRI *et al.* 1997). Some descriptor modifications which had been made during the first GENRES 081 workshop in 1997 were already included. In 1999 UPOV published the revised "Guidelines for the conduct of tests for distinctness, homogeneity and stability". The descriptors used within GENRES 081 were published in the "Primary

[17] Updated 2007

Descriptor List for Grapevine Cultivars and Species (*Vitis* L.)" (Anonymous 2002a) and the "Secondary Descriptor List for Grapevine Cultivars and Species (*Vitis* L.)" (Anonymous 2002b).

At the final meeting of the GENRES 081 project partners in Conegliano in September 2001, the workshop participants agreed that OIV should be asked to request a working group of representatives of all the organizations to discuss continuing the harmonization of the different lists.

During the 34th session (March 2002, Paris) of the OIV expert group "Vine selection", the invited delegates of IPGRI and UPOV, together with the OIV expert group "Vine selection" decided to review the three existing lists aiming at the harmonization of descriptors. The harmonization of most of the descriptors was achieved in 2002 and 2003. A final discussion by the ampelography experts of the three organizations took place in October 2003. Minor improvements, the addition of several drawings, final translations and a review of the English language text were carried out in 2004 and 2005. A last meeting of UPOV and OIV representatives took place in November 2006.

Material, methods and results

The number and the classification of characteristics differ in the four descriptor lists (Table 1). Bioversity lists 96 descriptors (biochemical, molecular markers, etc. are not counted since they are not specified), UPOV 50, OIV 115 plus 17 ampelometric and 2 isoenzyme descriptors, and GENRES 081 51 plus 21 ampelometric descriptors.

Table 1. Contents of the descriptor lists

Institution	Descriptors	Total no. of descriptors
Bioversity	Characterization (vegetative – 42, inflorescence and fruit – 16) Evaluation (plant descriptors – 21, abiotic stress susceptibility – 6, biotic stress susceptibility – 11, biochemical markers – not specified, molecular markers – not specified, cytological characters – 5, identified genes)	96 (101)
UPOV	Characterization (41) and evaluation (9) descriptors Order according to phenological development stages	50
OIV	Plant descriptors (characterization – 71, evaluation – 14) Phenology (6), growth (4), abiotic resistance (3), biotic resistance (8), yield (6), rootstock (3), ampelometry (17) and isoenzymes (2)	134
GENRES 081	Primary descriptors (33) Secondary descriptors (18) Ampelometric descriptors (21)	72

Work was carried out on 41 descriptors of GENRES 081, 16 OIV descriptors and all 50 UPOV descriptors. In total 58 descriptors were considered. The work covered the wording of the descriptors, notations (scores), example (reference) varieties, definitions and drawings. Some examples will demonstrate the differences e.g. in descriptor recording and vocabulary and the kind of modifications carried out. They will be presented from the OIV descriptors' perspective. Incompatible opinions resulted in the maintenance of two different descriptors.

- ***Example 1***

Characteristic: "Young shoot: intensity of anthocyanin coloration on prostrate hairs of the tip" with code numbers OIV 003, UPOV 5 and Bioversity 6.1.2 (Fig. 1).

Characteristic: Young shoot: intensity of anthocyanin coloration on prostrate hairs of the tip	Codes N os OIV 003 UPOV 5 Bioversity 6.1.2.

Livelli di espressione / Notation / Bonitierung / Notes / Notación:

1	3	5	7	9
absent or very weak	weak	medium	strong	very strong

Varietà di riferimento / Exemples de variétés / Beispielssorten / Example varieties / Ejemplos de variedades:

1	3	5	7	9
Furmint B Garganega B	Riesling B	Müller -Thurgau B Barbera N	Aleatico N Cabernet Sauvignon N	*V. aestivalis*

Indicazioni / Définitions / Definitionen / Definitions / Indicaciones:

E: Observation during flowering. Shoot tip: scope above the first unfolded leaf. The leaves of closed and half open shoot tips (OIV 001) have to be unfolded to record the corresponding part of the tip. Mean value of 10 shoot tips.

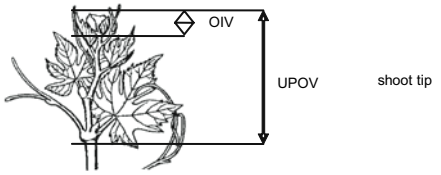


Fig. 1. OIV descriptor 003.

For UPOV the shoot tip comprises the tip with the first two unfolded leaves. For OIV, the shoot tip is defined as the tip above the first unfolded leaf.

Initially UPOV recorded the distribution and intensity of the anthocyanin coloration of the shoot tip (leaf tissue and prostrate hairs), while the OIV descriptor registers the intensity of the anthocyanin coloration of the prostrate hairs of the shoot tip only. Since usually the coloration of the prostrate hairs is observed, UPOV agrees to adapt to OIV definition.

The observation time also differs. UPOV records the characteristic from "inflorescences visible" to "flowering time". OIV does it during flowering.

To adapt to the UPOV definition, the indication "The leaves of closed and half open shoot tips have to be unfolded to record the corresponding part of the tip" was added to the OIV descriptor.

Conclusion: harmonization was achieved.

- ***Example 2***

Characteristic: "Shoot: colour of the dorsal side of internodes" with code numbers OIV 007, UPOV 11 and Bioversity 6.1.6.

Three expression stages exist in both lists: (1) green (OIV) – completely green (UPOV), (2) green with red stripes (OIV and UPOV) and (3) red (OIV) – completely red (UPOV).

The three expression stages were conserved. Both lists will use green as notation 1, red as notation 3. With regard to notation 2 the text "green with red stripes" will be replaced by green and red, characterizing internodes where both colours are expressed.

Conclusion: complete coincidence was achieved.

- ***Example 3***

Characteristic: "Bunch: length (peduncle excluded)" with code numbers OIV 202, and Bioversity 7.1.5 (Fig. 2).

Characteristic: Bunch: length (peduncle excluded)	Codes N^{os} OIV 202 Bioversity 7.1.5.

Livelli di espressione / Notation / Bonitierung / Notes / Notación:

1	3	5	7	9
very short	short	medium	long	very long
ca. 8 cm	ca. 12 cm	ca. 16 cm	ca. 20 cm	ca. 24 cm or longer

Varietà di riferimento / Exemples de variétés / Beispielssorten / Example varieties / Ejemplos de variedades:

1	3	5	7	9
		Müller-Thurgau B	Trebbiano Toscano B	Nehelescol B

Indicazioni / Définitions / Definitionen / Definitions / Indicaciones:

E: Observation at maturity. Mean value of the largest bunches of 10 shoots.
To be measured: height from the uppermost to the lowest berry of the primary bunch.
Secondary bunches (inserted on the knot of the bunch peduncle, see Code OIV 206) will not be considered).

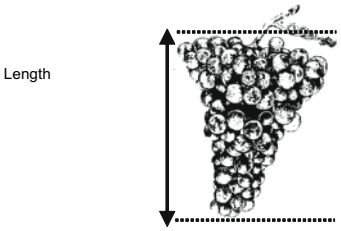


Fig. 2. OIV descriptor 202.

The main difference is that UPOV descriptor 36 records the bunch size, whereas Bioversity and OIV record the bunch length.

In the OIV descriptor at "notation" the ranges have been replaced by the average value, e.g. at notation 2: 12 cm instead of 10-14 cm. The reason for this change was that in case UPOV would wish to use this descriptor, expression stages 2, 4, 6 and 8 would be needed.

The definition was clarified by defining the bunch length and by excluding secondary bunches from measurements. The change from "mean value of all bunches" to "mean value of the largest bunches" should improve coincidence and avoid too high a variation.

Conclusion: both UPOV and OIV descriptors are distinct. No assignment of code numbers exists.

- ***Example 4***

Characteristic: "Berry: colour of skin" with the code numbers OIV 225, UPOV 41 and Bioversity 6.2.8 and the expression stages: green-yellow, rose, red, gray, dark red-violet and blue-black.

In contrast to OIV, UPOV notes the colour of skin without bloom which leads to a different perception of the colour. UPOV has suggested that more expression stages would be useful for variety distinction, e.g. green, yellow, orange brown, rose-yellow, green-red, etc.

Conclusion: harmonization has not been achieved.

- ***Example 5***

Characteristic: "Mature leaf: length petiole sinus to upper lateral leaf sinus" with the code numbers OIV 605, UPOV 24 and Bioversity 6.1.34 (Fig. 3).

Characteristic: Mature leaf: length petiole sinus to upper lateral leaf sinus	Codes N os OIV 605 UPOV 24 Bioversity 6.1.34

Livelli di espressione / Notation / Bonitierung / Notes / Notación:

1	3	5	7	9
very short	short	medium	long	very long
≤ 30 mm	50 mm	70 mm	90 mm	≥ 110 mm

Varietà di riferimento / Exemples de variétés / Beispielssorten / Example varieties / Ejemplos de variedades:

1	3	5	7	9

Indicazioni / Définitions / Definitionen / Definitions / Indicaciones:

E: To be measured on 10 lea ves: distance petiole sinus to upper lateral leaf sinus on both halves of the leaf.

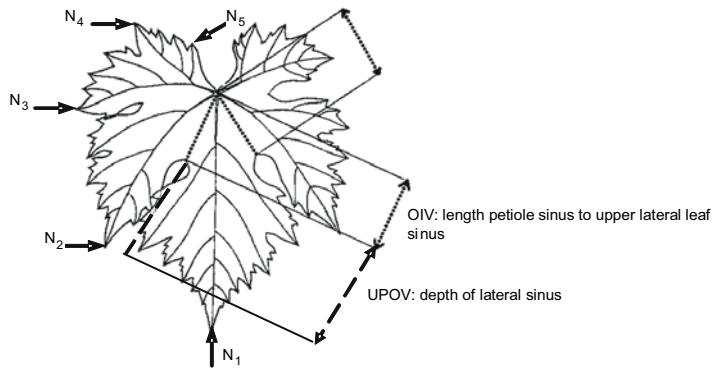


Fig. 3. OIV descriptor 605.

For OIV this is an ampelometric descriptor, for UPOV and Bioversity an ampelographic descriptor. In addition the correspondence with UPOV and Bioversity is inverse, because both institutions are describing the "depth of upper lateral sinuses" whereas OIV is measuring the distance between the petiole sinus and the upper leaf sinus.

OIV will keep descriptor OIV 605 and create a new descriptor identical to UPOV 24 and Bioversity 6.1.34, describing the "depth of upper lateral sinuses" as "absent or very shallow", "shallow", "medium", "deep" and "very deep".

Conclusion: complete coincidence was achieved.

Final result

With regard to the 50 common UPOV, Bioversity and OIV descriptors: 41 descriptors are identical, differing only slightly in vocabulary; 4 descriptors were considered as being different even though they describe similar characteristics and 5 descriptors could not be harmonized for the time being.

Final conclusion

The intention of descriptor harmonization by UPOV, Bioversity and OIV was to create a common language in grapevine description, which will lead to conformity and thus to comparable inputs in databases. It will enhance the use and the utility of descriptor data.

Nearly 80% of the 50 descriptors were brought to match completely, even though these three institutions pursue different purposes and depend upon their own internal regulations.

The results now achieved have been presented as the final version for the "2nd edition of the OIV Descriptor List for Grape Varieties and *Vitis* species" at the OIV expert group "Genetic resources and vine selection" in March 2007.[18] The expert group decided that the complete document will be presented to the OIV General Assembly in June 2007 for recognition.

With regard to the description of mainly neglected indigenous varieties to be carried out in the scope of the new European Project GrapeGen06, Management and Conservation of Grapevine Genetic Resources (http://www.montpellier.inra.fr/grapegen06) of the EU Council Regulation No 870/2004, the OIV expert group "Genetic resources and vine selection" agreed that the new version of the OIV descriptor list would be applied.

Acknowledgements

OIV expresses its sincere thanks to all contributors. OIV honours their willingness to offer their knowledge, expertise and time for the "2nd edition of the OIV Descriptor List for Grape Varieties and *Vitis* species", acknowledged by the OIV General Assembly in June 2007.

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National reports

Status of Vitis *germplasm in Albania*

***Adriatik Çakalli*[1], *Hipokrat Fiku*[2], *Endrit Kullaj*[3] *and Frida Çarka*[4]**
[1] *Albanian Genebank, National Seed and Seedling Institute, Tirana, Albania*
[2] *Fruit Trees Institute, Vlorë, Albania*
[3] *Department of Horticulture, Faculty of Agriculture and Environment, Agricultural University of Tirana, Tirana, Albania*
[4] *Fruit Trees Institute, Tirana Experimental Station, Albania*

Introduction

Albania, a typical Mediterranean country, enjoys soil and climatic conditions that are ideal for the development of viticulture. Many cultivars have developed here, adapting to the agroecological conditions through continuous natural and human selection. They are of high value in terms of yields and quality and therefore compete with many introduced cultivars (Sotiri *et al.* 1996). This rich diversity of grapevine cultivars and biotypes is spread out widely across the country (up to 1000 m altitude) and many of the cultivars are very well adapted to local conditions (Fig. 1).

From this large number of cultivars, those with inferior qualities have disappeared (or are gradually disappearing) because of the severe genetic erosion during the transitional period (1990–2000), while others, with superior qualities, are increasing owing to their high economic value to many farmers.

Vitis germplasm

Within the wide range of native grapevine cultivars available in Albania, geographically distributed in all the wine-producing areas of the country, we will mention only those which are the most frequent in terms of their distribution and which received special attention as essential genetic resources because they are very valuable for hybridization and breeding programmes.

The predominant native grape cultivars in Albania are 'Shesh i Bardhë' and 'Shesh i Zi', which constitute 60% of the plantings throughout the country, except in the cold eastern and northeastern areas, where their cultivation is limited because of their late maturation and consequently low sugar content.

Since the 1990s the preference of the Albanian farmer has been for the above-mentioned cultivars (at least in areas I and II, Fig. 1) originating in the area of Shesh in Tirana.

- 'Shesh' cultivars are very well adapted to Albanian soil and climate conditions, giving high annual yields. They are the most abundant cultivars.
- They are very flexible to all types of cultivation, due to the production potential of all the shoots.
- Until recent years, 'Sheshs' have been used for both table and winemaking owing to their fine gustatory qualities, which result from their very well balanced acid/sugar ratios (Sotiri *et al.* 1973).
- They are relatively more resistant to fungal diseases than sensitive cultivars such as 'Italia', 'Afuzali', etc.

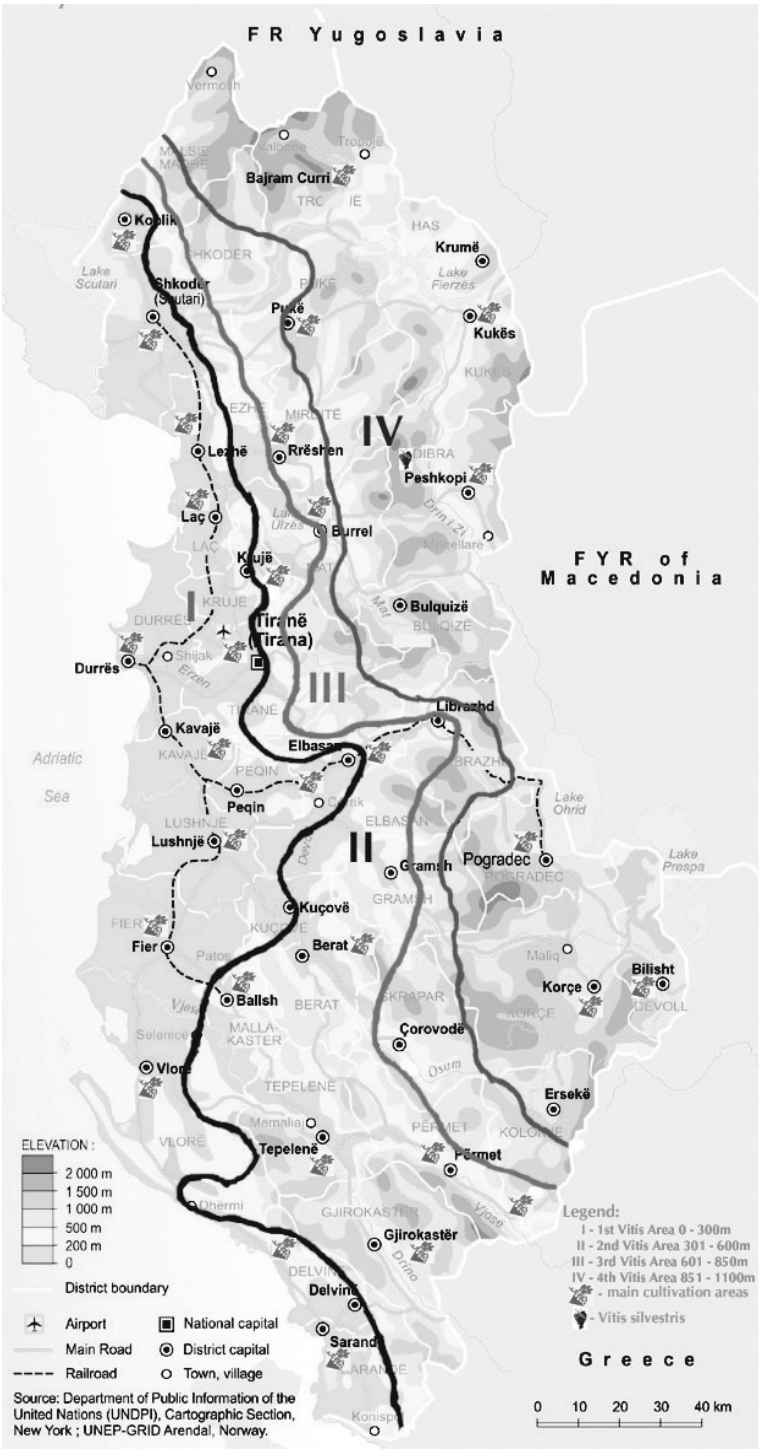

Fig. 1. Phytoclimatic areas of viticulture in Albania and locations of *Vitis sylvestris*.

However, in spite of the undeniable preferences of our farmers and the advantages just listed, we should stress that, regarding competitiveness with other well-known wine grapes such as 'Merlot', 'Cabernet–Sauvignon', 'Riesling' and 'Pinot Nero', which are appreciated and required all over the world, the wine produced by the 'Shesh' cultivars is inferior with respect to the quality of colour and bouquet (especially in cases of high yields or when cultivated in deep and cold soils). This is reflected in the trading difficulties faced by the wines produced from these grapes. Since almost the majority of areas are cultivated with the 'Shesh' cultivars, it is now becoming necessary to plant other cultivars, in order to produce better-quality wines. In the near future, when we can foresee that competition in the wine market will be harder, the 'Shesh' cultivars should be sold mainly for domestic use and in limited quantities for trading purposes, either for fresh consumption or in winemaking.

Cultivar 'Vlosh', which can now be found mainly in southern Albania, used to represent a considerable proportion of the plantings and was very popular with our consumers, as it was well known for producing good quality wines and valued for the fine flavour of the grapes. Interest in this cultivar has now fallen. In part this was due to discrimination against the wine in terms of its colour and bouquet, and also because of the change in the type of cultivation from traditional to intensive cropping, aiming at higher yields. This cultivar is not suited to high and cold areas since it is classified as a late cultivar. During the 1980s-1990s it has undergone breeding by both clonal selection and sexual hybridization. The grapes kept well under cold storage, and were also appreciated for fresh grape consumption.

Cultivar 'Kallmet' is grown mainly in northern Albania and in a few locations of central Albania. It is a remarkable cultivar, especially for winemaking. Wines produced from this cultivar have been awarded medals in various competitions.

Other cultivars such as 'Serin i Zi', 'Serin i Bardhë' and 'Debinat', which are early-maturing, are found mainly in cold areas such as Korça, Ersekë, Përmet, Skrapar, Leksovik, etc. They are very well adapted to those particular ecosystems, especially when grown on pergolas and are being considered for the development of organic viticulture.

The same can be said of cultivars 'Kotekë e Bardhë', 'Kotekë e Zezë', 'Rrush Dhelpre', etc.

Because of the preference for foreign cultivars, these Albanian cultivars are becoming less appreciated and the percentage of native cultivars within the general assortment of varieties is decreasing.

In a time of severe genetic erosion, it is imperative to explore, collect, study and grow all the viticultural germplasm at the Fruit Science Institute of Vlorë, and to exploit all the genetic material for hybridization work. We should also mention the potential use of *Vitis sylvestris*, which enjoys an old tradition of cultivation in Albania and has also been reported to be still in existence by the latest explorations by specialists.

The Albanian national grapevine collection

Vitis collections in Albania can be found both in research institutes and at private farms. The national grapevine collection is held at the Fruit Science Institute, Vlorë, responsible for the collection and maintenance of fruit trees in Albania. This collection contains 61 grape cultivars (Table 1).

Table 1. The *Vitis* collection available at the Fruit Science Institute, Vlorë

Native cultivars	Type	Foreign cultivars	Type
Table grape		**Table grape**	
Durrsaku i bardhë	white	Afuzali	White
Qelibar i hershëm	white	Regina dei vigneti	White
I bardhi cipë fortë	white	Italia	White
Dimjat	white	Shasla skuta	White
I bardhë kokërkëndezi	white	Perla Ksaba	white
Pulëz	white	Stambollesh	white
Muzhaku	white	Moskat D'ada	black
Gomaresh	white	Alfons Lavale	black
Celepyzum	white	Sidheritis	red
Meresnik	white	Kardinal	pink
Serin e bardhë	white	Tarif rozë	pink
Jediveren	red	Shaslla violet	pink
I rrumbullakët i vonët	red	Moskat rozë	pink
Dimerak	pink	**Wine grape**	
Dimerakes	red	Fitore	black
Korrithi	red	Kaberne savinjon	black
Tajgë e Liut	pink	Malvasia	white
Wine grape		Aligote	white
Rrush Zhepove	pink	Barbera	black
Rrush i Hodos	pink	**Raisins**	
Debin Leskoviku	white	Sulltanina e Bardhë	white
Shesh i bardhë	white		
Debin Përmeti	white		
I bardhi cipëhollë	white		
Kotekë e bardhë	white		
Sinanbel	white		
Rrush Bureli	white		
Sinanbel no.2	white		
Tajgë e zezë	black		
Rozë	red		
Rrush kishe no.2	pink		
Verë breshkëza	pink		
Tajgë rozë	pink		
Shesh i zi	black		
Vlosh	black		
Rrush vere	black		
Krakie	black		
Debinë e zezë	black		
Kosinjot	black		
Kolek e zezë	black		
Rrush vere me supe	black		
Kozarka	black		

In recent years, scientific institutes and the Albanian genebank collaborated in the genetic enrichment of this collection. Another collection was established in Sarandë, containing exclusively native cultivars.

Despite these efforts, much work is still needed for the collection and maintenance of *Vitis* genetic resources in Albania. Attempts must be made to reduce the impact of negative factors such as:

- urban migration leading to reduced use of native cultivars,
- unfair competition with planting material of other uncertified cultivars entering the country at lower prices,
- lack of awareness at farmers' level regarding the benefits of cultivating native cultivars,
- lack of adequate funds for the maintenance of existing collections or the establishment of new ones,
- difficulties in finding incentives for the farmers to maintain the indigenous cultivars, etc.

Efforts for the future

More attention to such crops in preservation and utilization programmes is necessary. Conservation of native varieties should be immediate, and it needs to be followed and sustained by on-farm conservation and activities in related areas:

- Maintenance of the existing collections
- Intensification of collecting missions for relevant native varieties of grapevine and for *Vitis sylvestris*
- Establishment of new grapevine collections in the appropriate areas of cultivation
- Creation of a network among institutions holding *Vitis* collections
- Investments to solve the constraints due to lack of funds.

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Vitis *collections in Armenia*

Samvel Gasparyan
International Academy of Viticulture and Winemaking, Armenian National Branch, Yerevan, Armenia

Introduction

Armenia is an ancient centre of grape-growing and grape cultivation. According to archaeological data, viticulture and winemaking were developed in Armenia in the 7th century BC. The main features of the ecological conditions in Armenia are dry and hot summers, which require 75% of the vineyards to be irrigated. Cold and frosty winters are frequent, with a minimum temperature sometimes reaching -30°C and below. As a consequence, more than 85% of the vineyards require earthing up (protection of the vines' roots and the lower stems under a layer of earth in the winter). Different systems of grapevine formation are used according to the local soil and climate conditions: most vineyards (75%) use systems of training on a 4-wired vertical trellis and by a stemless fan-shaped system; 10% are old and on a hedge system; more than 10% are on a middle-stemmed (50-60 cm) fan system; high-stemmed vineyards constitute only 1%.

Most vineyards are found on stony lands.

There are separate viticulture zones on the Armenia-Ararat plateau (foothills) and in the northeastern region, Vayots Dzor and Zangezour. Production grapes (over 65%) are centralized on the Ararat Plateau.

Self-rooted vines account for 90% of Armenian vineyards. The remaining 10% are grafted and resistant to phylloxera and are located in the northeastern zones of the country.

The Armenian ampelographic collections

The largest ampelographic collection of Armenia was that of the Armenian Scientific Research Institute of Viticulture, Winemaking and Fruit growing, which comprised over 800 indigenous and foreign varieties. In 1993 as a result of land privatization, most varieties disappeared forever.

At present there are three ampelographic collections in Armenia:

- Botanical Garden of the Academy of Science of Armenia: approximately 45 varieties
- Scientific Centre of Farming (Husbandry) and Agrichemistry: over 30 varieties
- Scientific Research Institute of Viticulture, Wine making and Fruit Growing: 65 varieties.

Most varieties present in the first two collections are also being grown in the third one.

Table 1 provides the list of grape varieties in the ampelographic collection of the Scientific Research Institute of Viticulture, Winemaking and Fruit Growing (Nalbandyan Experimental Station).

The Nalbandyan ampelographic collection is self-rooted and irrigable. The soil is stony, poor in humus; the climate is strictly continental. The planting scheme is 2.5 x 1.5 m, the form is generally fan-shaped and stemless.

It is impossible to define the precise age of the ampelographic collection, as the vines have been planted gradually. The average age is 30 years.

Table 1. Grape varieties of the ampelographic collection of the Armenian Scientific Research Institute of Viticulture, Winemaking and Fruit Growing

Wine grape varieties		**Table varieties**	
Black grapes	**White grapes**	**White grapes**	**With coloured pulp**
1. Tigrani	1. Azateni	1. Sasun	1. Aygezard
2. Muscat TSKHA	2. Megrabouyr	2. Merdzavani Vaghahas	2. Arevshat
3. Saperavi	3. Muscat Armenian	3. Hayastan	3. Metsamor
4. Nalbandyani	4. Muscat Dessert	4. Parvana	4. Tatev
5. Charentsi	5. Voskehat	5. Muscat Ayvazyani	5. Kapoutan
6. Haghtanak	6. Bourmounk	6. Yerevan Muscat	6. Zartonk
7. Hadisi	7. Arazi	7. Uzbek Muscat	7. Berkarat
8. Nerkeni	8. Ginu Vaghahas	8. Anahit	8. Taroni
9. Armavir	9. Aparatsin	9. Masis	9. Geghard
10. Artashati Karmir	10. Urartu	10. Shahumyani	10. Kishmish Black
11. Dimatskoun	11. Rkatsiteli	11. Tokun	11. Rizamat
12. Arpa	12. Chilar	12. Meghru Vaghahas	12. Armenia
13. Nerkarat	13. Garan Damak	13. Deghin Yerevani	13. Yerevani Vardagouyn
14. Merdzavani	14. Berkanoush	14. Itsaptouk	
15. Ashtaraki	15. Kangoun	15. Kishmish Khishrau	
16. Karmreni		16. Anoushik	
17. Karmrahyout		17. Arevvar	
Universal varieties			
White grapes	**Black grapes**		
1. Mskhati	1. Areni		
2. Muscat Susanna			

At present, in connection with the land privatization processes, the expenses for vineyard cultivation are increasing, the bulk purchase system is absent and the viticultural sector is in a difficult situation: the growth of planting material is being reduced to a critical level, the purity of varieties is being lost, and the old vineyards are being eradicated at an increasing rate. During the vineyard eradication process, many varieties which are not widely spread, as well as unknown local grape varieties may completely disappear. There is no doubt that there are valuable forms among old varieties and clones of local grapes. It is urgent to study grape varieties in the old vineyards and the ancient grape-growing regions of Armenia and to establish ampelographic collections, to identify the local types and forms which have been preserved throughout history and also the diversity of wild grapes.

The areas of wild-growing grapes are now very much reduced. The distribution and the morphological diversity of wild vines in Armenia have not been sufficiently studied. The study of the wild vines is not only of theoretical interest, but also has great practical significance.

A detailed ampelographic study of the Armenian vineyards should be undertaken, with special attention to old varieties. The study of such varieties and their comparison with the wild-growing vines may provide valuable answers to the question of the origin of the cultivated Armenian grape. The collected material will enable us to improve the present collection but will also help to preserve the rich diversity of Armenian grape varieties and clones for the next generation.

Grapevine genetic resources in Azerbaijan

Mirza K. Musayev
Genetic Resources Institute, National Academy of Sciences of the Azerbaijan Republic, Baku, Azerbaijan

There are three types of grapes in Azerbaijan, of which two are cultivated. Researches carried out by A.M. Negrul from 1940 to 1946 led to the conclusion that grape cultivation in Azerbaijan has arisen independently based on the use of local wild grapes, which are now found in woods down the Kura river and its deltas, in valleys of the lower part of the Alazan river and on the foothills of the Great and Small Caucasus. Wild grapes are also found in Tugay woods.

After independence, a State Commission for Plant Genetic Resources was created and a National Programme established. The Commission is organized in eight working groups, including one on fruit, subtropical cultures and grapes. Institutes involved in the collecting, study, preservation and use of grapes in Azerbaijan are the Genetic Resources Institute of the National Academy of Sciences, the Institute of Viniculture and Winemaking, branches of these institutes and the Azerbaijan Agricultural Academy.

Many grape varieties (for wine and table) are cultivated in the country, and are characterized by specific gustatory and technical characteristics. Many varieties are cultivated mainly on foothills, low hills and lowlands. Out of a total of 643 local forms, breeding lines and foreign introductions, 512 varieties, including 250 local types, have been collected and planted in a field genebank; 76 have been lost, and 55 are on the verge of disappearance. More than 100 samples of wild grapes have been collected. The available germplasm is being studied and evaluated with the collaboration of breeders, geneticists, physiologists, phytopathologists, entomologists, biochemists and technologists.

Under the provisions of the National Programme a National Information Programme was established and an inventory of *Vitis* genetic resources carried out. A database is under development and documentation is ongoing.

Grapevine genetic resources in Bulgaria

Penka Abracheva and Iliyan Simeonov
Institute of Viticulture and Enology, Pleven, Bulgaria

Work on the identification of grapevine varieties in the Republic of Bulgaria began in the past century with the foundation of the Experimental Station of Viticulture and Enology in the town of Pleven in 1902. The studies began with the description of wild-growing grapevine species and concerned questions related to the evolutionary process and origin of local varieties. The collection of local and introduced varieties began during that period and as a result in 1925 an ampelographic collection including specimens under 300 variety names was created at the Institute of Viticulture and Enology, Pleven. The work on the ampelographic description of the local and introduced varieties collected is linked to the names of Prof. P. Viala from France and the Bulgarian researchers P. Sirakov (1904), N. Kirmidche (1927) and Prof. N. Nedelchev (1938).

During the 1950s the ampelographic description of the local and introduced varieties was started, using the methodology of M. Lazarevskii (1936) and Baranov *et al.* (1946). It resulted in the "Bulgarian Ampelography" in five volumes, of which only the first one was published (Katerov *et al.* 1990).

As of May 2003 the ampelographic collection of the Institute included about 2000 named varieties.

The Institute of Viticulture and Enology, Pleven participated in the GENRES 081 project "European Network for Grapevine Genetic Resources Conservation and Characterization". A total of 30 varieties (10 local, 8 introduced and 12 newly bred) were described according to the GENRES descriptors (Anonymous 2002a, 2002b). During this work some differences were found with the methodology of Lazarevskii regarding some secondary characters (flower, seed, shoot, agrobiological and technological characterization, etc.) and ampelographic descriptors.

The study carried out in the framework of GENRES in 1999-2002 did not show up significant differences in the process of identification of the variety names. We believe that this work must continue in order to develop a standard methodology for the definition and recording of characters, which will eventually provide the possibility for quicker and simpler identification of grapevine species and varieties.

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Activities of the Vitis *genebank in Croatia*

Edi Maletić, Jasminka Karoglan Kontić and Ivan Pejić
Faculty of Agriculture, University of Zagreb, Croatia

Introduction

- ***Some facts about viticulture in Croatia***

Croatia has diverse and favourable conditions for vine-growing. There are two clearly distinct climatic regions – the "continental" region with features of a continental-type climate (middle European), and the "coastal" region with a pronounced influence of the Adriatic Sea (Mediterranean-type climate). Croatian viticulture has a very long tradition dating back to a few centuries BC, when grapevines were probably introduced by the ancient Greeks or Phoenicians to the Adriatic coast. At the end of the 19th and beginning of the 20th centuries, Croatia was a significant European vine-growing country. At that time there were about 200 000 ha under vineyards, with 90 000 ha in the Dalmatia region alone. Currently, there are only 59 000 hectares of vineyards according to statistical data. However, viticulture remains a vital part of the national economy, involving directly or indirectly about 15% of the country's inhabitants. Hence, it has a capacity to contribute much more to the gross national income than it actually does today.

- ***Some facts about Vitis vinifera germplasm in Croatia***

Leading ampelographers of the "golden days" of Croatian viticulture, Goethe (end of 19th century) and Turkovic (middle of 20th century) were witnesses of the greatest numbers of different grapevine cultivars. According to Jelaska and Briza (1967), during that time more than 400 cultivars were grown, with more than 200 cultivars in Dalmatia alone (Bulić 1949). Beside the favourable climatic conditions, a chequered past and good connections with other countries where grapes were grown had a strong influence on the cultivar numbers. It is probable that some of them had been developed in this area, while the others were introduced a long time ago. Unfortunately, many cultivars were lost at the beginning of the last century in vineyard destruction caused by new fungal diseases and pests (e.g. *Plasmopara, Uncinula* and *Phylloxera*) as well as through modern production demands for high yields and the introduction of globally popular cultivars (e.g. 'Chardonnay', 'Riesling', 'Cabernet Sauvignon', 'Merlot', etc.). Since that time, acreages have constantly been declining, leading to drastic erosion of the native cultivars. Today, the official variety list consists of only 70 native cultivars out of 150 listed. Very likely many of the valuable indigenous cultivars have become extinct, while today many of the surviving ones are very neglected and subject to continuing eradication. Even cultivars that were very important only 50 years ago can today only rarely be found.

- ***Ampelographic researches and genebank activities in the past***

The oldest ampelographic studies of Croatian genotypes were published in the mid-19th century by Trummer (1841), Stražimir (1876) and Goethe (1887). S. Bulic completed

the first book of ampelography and documented almost 200 grapevine cultivars grown in Dalmatia during the period 1887-1925 (first published in 1949). Valuable indigenous cultivar descriptions can be found also in Jelaska (1954), Mirošević (1986), Maleš (1987), Maletić (1993), etc. In some cases, Croatian genotypes have been considered to be the ancestors or relatives of famous cultivars (Maleš 1993; Meredith 1996).

During the 1980s, biodiversity preservation of endangered wild and cultivated plant and animal species, as well as their indigenous cultivars and breeds, started to be taken seriously as an important international task. Modern industrial-based agriculture has greatly contributed to the reduction in the number of cultivars in use and it has led to some cultivars becoming extinct. Croatian scientists and vine-growers during the period of the former Republic of Yugoslavia initiated a project for the preservation of grape genetic resources called "GenBank Vitis sp." The process of inventorying and collecting indigenous cultivars was interrupted during the recent war at the beginning of the 1990s. Regrettably, war destruction did not spare the GenBank plantation in the vicinity of the city of Zadar, and most of the collected material was lost forever. Realizing the importance of the previous attempt at cultivar preservation, a new project on ampelographic and genetic identification of endangered native grapevine cultivars was launched in 1998 at the Faculty of Agriculture, University of Zagreb. Today, we run several projects supported from national and international sources, focused on the preservation, evaluation and revitalization of indigenous grapevine cultivars. The ultimate goal of these projects is to find all the remaining native varieties which are not yet collected, to make thorough ampelographic and genetic analyses of them, and to replant them into one or two *ex situ* collections. In 1998, Croatia became an associated partner of the GENRES 081 project "European Network for Grapevine Genetic Resources Conservation and Characterization". Since that time, we have followed the project's harmonized methods of measurement and description for cultivar identification, and we have made contributions towards establishing a common European network for the preservation of genetic resources of *Vitis vinifera.*

Current research and activities in the field of *Vitis vinifera* germplasm

• *Materials and methods*

The list of Croatian cultivars is provided in Table 1, with indications on studies carried out as described below.

Based upon existing ampelographic literature and previous experience of Croatian professional and scientific institutions dealing with viticulture, we began searching vine-growing regions with the aim of finding, marking and sampling all remaining indigenous cultivars. We described cultivars in the field and obtained samples for ampelometric measurements as well as for DNA analysis. We described every genotype according to the OIV descriptor methods and according to the Primary, Secondary and Ampelometric Descriptor lists accepted and agreed upon among the GENRES 081 project partners (Anonymous 2002a, 2002b). Observations and measurements were repeated for several years and, where possible, at several locations. All the collected data together with photos of the characteristic phases (shoot tip, mature leaf and cluster) will be documented in an electronic database. Using both the data and an assessment by several experts, we ascertained the identity of every genotype.

Table 1. List of Croatian native grapevine cultivars and research carried out (names of endangered cultivars in bold)

Prime name	Berry colour[1]	Ampelographic description (OIV descriptors)	Number of SSR loci analyzed	Photographs taken	Grafted in collection
Babica crna	N	x	25	x	x
Babica plosnata crna	N			x	x
Babić	N	x	16	x	x
Bak crni	N			x	x
Balbut bijeli	B				
Barjanka	B		25	x	
Bašćan	N	x		x	
Belina bakarska	B	x	16	x	
Bena	B				
Beretinjok bijeli	B		16	x	x
Bilan bijeli	B	x		x	x
Biloliska bijela	B			x	
Bjeloruža	B			x	
Bjeljak bijeli	B			x	
Blatina	N			x	
Blatinka bijela	B			x	
Bljuzgavac	N			x	x
Bodul	N				
Bogdanuša	B		7	x	x
Brajda bijela	B		2	x	
Brajda velika	N			x	
Brajdica bijela	B	x		x	x
Bratkovina bijela	B	x	25	x	x
Bratkovina crvena	Rs		25	x	
Cetinka	B		25	x	x
Cibib	B		2	x	
Cipar	Rs	x	16	x	x
Crljenak kaštelanski	N	x	25	x	x
Crljenak viški	N		16	x	x
Crnka	N			x	
Čihovac	N			x	x
Ćoruša	B			x	
Debit	B		16	x	x
Dišeća ranina	B		2	x	x
Divjaka	B			x	
Dobričić	N	x	25	x	x
Dobrogostina	B			x	
Dolcin	B		2	x	x
Drnekuša mala	N		16	x	x

[1] B (Blanc) = green or yellow skin; G (Gris) = gray skin; N (Noir) = black or blue skin; Rs (Rose) = rose skin.

* Original name not known – named according to the place where it was found: Stradun in Dubrovnik

Table 1 (cont.). List of Croatian native grapevine cultivars and research carried out (names of endangered cultivars in bold)

Prime name	Berry colour[1]	Ampelographic description (OIV descriptors)	Number of SSR loci analyzed	Photographs taken	Grafted in collection
Drnekuša velika	N			x	x
Dugovrst	B			x	x
Filipić	B	x		x	
Frmentun	B				x
Galac crni (Gavran ?)	N	x	16	x	x
Garganja	B		2	x	x
Gegić	B	x	16	x	x
Glavanjuša	N		2	x	x
Glavinuša	N	x	16	x	x
Grgićevica	B			x	
Grk	B		25	x	x
Gustopupica	N	x	16	x	x
Gustopupica ninska	Rs			x	x
Hrvatica	N		7	x	
Kadarun	N			x	
Kamenina	N	x		x	
Kleščec	B		2	x	
Kraljevina	Rs		7	x	x
Krstićevica	B		16	x	
Kuč	B	x	16	x	x
Kujundžuša	B			x	x
Kurtelaška	B		16	x	
Lasina	N		7	x	x
Lasina vrgorska crna	N			x	
Lelekuša	N				x
Ljutun crni	N	x	16	x	x
Malvasija dubrovačka	B		16	x	x
Malvasija župska	B			x	
Malvazija istarska	B		7	x	x
Maraština	B		25	x	x
Marinkovića grozje	B				x
Medna	B				
Mekuja	B			x	x
Mijajuša	N			x	x
Mladenka	B	x	2	2	x
Mlinčevac	N			x	
Moslavac	B		7	x	x
Muškat momjanski	B			x	
Muškat ruža	N		7	x	

[1] B (Blanc) = green or yellow skin; G (Gris) = gray skin; N (Noir) = black or blue skin; Rs (Rose) = rose skin.

* Original name not known – named according to the place where it was found: Stradun in Dubrovnik

Table 1 (cont.). List of Croatian native grapevine cultivars and research carried out (names of endangered cultivars in bold)

Prime name	Berry colour[1]	Ampelographic description (OIV descriptors)	Number of SSR loci analyzed	Photographs taken	Grafted in collection
Muškatel	B	x		x	x
Ninčuša	N	x	16	x	x
Očenaš	B	x		x	
Ošljevina	N	x		x	
Pagadebit bijeli	B		2	x	
Palagružanka bijela	B				x
Palaruša	B		16	x	x
Pavicić (Soić ?)	N		16	x	x
Petovka	B	x		x	x
Plavac krčki	Rs	x		x	
Plavac mali crni	N	x	25	x	x
Plavac mali sivi	Gr		20	x	x
Plavac omiški	Rs			x	
Plavac runjavac	N	x	26	x	
Plavac sobotovac crni	N			x	
Plavčina	N	x		x	
Plavec žuti	B			x	x
Plavina	N		25	x	x
Podbil	B			x	
Pošip bijeli	B	x	25	x	x
Pošip crni	N		7	x	x
Pošipica	B			x	
Prč	B		2	x	x
Prošip crni	N			x	
Pršljivka	B				x
Ranfol	B		7	x	x
Rogoznička	N	x		x	
Rožeta	N	x		x	
Rudežuša	N			x	x
Rukatak bijeli	B			x	
Rušljin crni	N	x		x	
Sansigot	N	x		x	
Silbijanac	B	x		x	x
Siložder crni	N				x
Slakarinac	N		2	x	
Stara brančevka	N			x	x
“Stradunska”*	B		2	x	
Surina	Rs			x	
Suščan	N			x	

[1] B (Blanc) = green or yellow skin; G (Gris) = gray skin; N (Noir) = black or blue skin; Rs (Rose) = rose skin.

* Original name not known – named according to the place where it was found: Stradun in Dubrovnik

Table 1 (cont.). List of Croatian native grapevine cultivars and research carried out (names of endangered cultivars in bold)

Prime name	Berry colour[1]	Ampelographic description (OIV descriptors)	Number of SSR loci analyzed	Photographs taken	Grafted in collection
Sušić	N	x		x	
Svjetljak bijeli	B			x	
Svrdlovina crna	N			x	
Šarica trišnjavica	N		2	x	x
Šemperinka crna	N			x	x
Šipelj	B			x	x
Škrlet	B	x	7	x	x
Šljiva	N			x	
Teran	N		7	x	x
Topol	B	x		x	x
Trnjak	N				
Trojišćina	Rs			x	
Vela pergola	B		7	x	
Viška crna	N			x	
Vlaška bijela	B	x	2	x	
Volarovo	B	x		x	
Vranac	N		19	x	x
Vrbić	B	x		x	
Vugava	B		25	x	
Vugava crvena omiška	Rs				
Zadarka	N		16	x	x
Zelenika bukovačka	B				
Zelenka šoltanska b.	B			x	
Zlatarica blatska	B	x	25	x	
Zlatarica vrgorska	B			x	x
Žilavka	B		7	x	
Žlahtina	B	x	7	x	
Žumić	B	x		x	

[1] B (Blanc) = green or yellow skin; G (Gris) = gray skin; N (Noir) = black or blue skin; Rs (Rose) = rose skin.

* Original name not known – named according to the place where it was found: Stradun in Dubrovnik

We obtained cuttings of all analyzed cultivars in the field and grafted them in the collection of Croatian indigenous cultivars situated at the Experimental Station "Jazbina" of the Faculty of Agriculture, University of Zagreb. From the chosen vines with their identity determined we obtained leaf samples for DNA extraction. DNA extracts were performed according to the classic protocol of Doyle and Doyle (1990) or the protocol outlined in the Qiagen DNeasy Plant Mini Handbook (Qiagen, Valencia, CA, USA).

For genetic identification of the grapevine cultivars we used SSR markers according to the protocol of Bowers *et al.* (1996) and ran them on the synthetic gels Spredex™ EL400 (Elchrom Scientific, Switzerland), and they were subsequently dyed with SYBR Gold (Molecular Probes, The Netherlands).

We used six microsatellites (loci VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79), accepted by the GENRES 081 project partners as the core set for identification of grapevine cultivars. As a standard for allele determination, we used globally well-known cultivars and existing *Vitis* databases from the University of California (UC Davis), USA and the Center for Applied Genetics (Zentrum für Angewandte Genetik, ZAG), Vienna, Austria.

- ***Present results and plan for future***

Until now we have been thoroughly exploring the Croatian vine-growing regions in order to locate all the remaining native cultivars, paying special attention to the Dalmatian region. In Dalmatia, viticulture and winemaking are based mainly on native cultivars, which make up more than 85% of all cultivars growing in the region. Croatia's continental region mainly grows introduced cultivars, originating from Western Europe ('Welsch Riesling', 'Riesling', 'Sauvignon blanc', 'Chardonnay', 'Cabernet Sauvignon', 'Traminer', etc.), and there are only a few cultivars considered indigenous. The situation in Dalmatia was caused by the isolation and remoteness of the region from the rest of Croatia. The geographic separation has had a profound impact on viticulture, and as a consequence, the cultivars developed there remain locally specific and differ from the rest of those in Europe. Our work shows that it is still possible to find large numbers of native cultivars in Croatia, many more than we expected when initiating this study. However, many of the cultivars have no economic importance, and some are endangered, with only a few stocks left in old mixed vineyards. By exploring and working in these neglected vineyards, we provide what may be a last chance for saving a valuable natural heritage which would otherwise be lost; and this work needs to be done with a sense of its urgency.

Every identified cultivar which we considered to be native has been assigned a unique number. In order to preserve them, they were planted in a collection in 2001 at the Experimental Station "Jazbina", which serves for viticulture and oenology research at the Faculty of Agriculture in Zagreb. We planted rootstocks at the collection site (Berlandieri x Riparia SO4) and each year a certain number of genotypes are green-grafted. Every genotype is represented in the collection with six vines, which originated from previously documented and ampelographically determined mother stocks. In order to avoid possible mistakes, as well as unforeseen variables within the cultivar, cuttings are collected from single vines whenever possible. Despite the ever-present issues of damage caused by low winter temperatures and drought, to date we have managed to collect and graft more than 70 cultivars in the collection. We are hoping to establish all the indigenous cultivars in our collection at the end of this project, and we plan to establish another one or two collections in coastal regions where it will be possible to carry out other research programmes, such as economic evaluations. Upon completion of the project, it is our intention to achieve the preservation of the existing genetic diversity of grapevines in Croatia; this is of the utmost importance, especially for endangered genotypes.

Thus far, more than 40 cultivars have been described using OIV descriptors (OIV 1983), and we have taken photographs of about 130 cultivars – shoot tip, mature leaf and cluster.

Furthermore, genetic analyses of 57 cultivars have been completed. Comparisons of genetic profiles of the most analyzed cultivars with data from two big *Vitis*

databases (UC Davis and ZAG) proves their genetic uniqueness and their status as indigenous cultivars. In some cases their Croatian origins were further supported by the parentage analysis results. Specifically, in the population of analyzed cultivars we have been able to find parents of the two most economically important cultivars from the Primorska Hrvatska region, 'Pošip' and 'Plavac mali' (Pejić *et al.* 2000; Piljac *et al.* 2002).

DNA markers have been useful in determining cultivars with the same genotype and different names (synonyms), and in one case we also solved a supposed homonymy. For some Istrian cultivars, which were considered to be indigenous ('Teran bijeli' and 'Muškat ruža porečki'), we found they had the same profile as some foreign cultivars. In the case of 'Hrvatica' and 'Croatina', which it was suggested might be the same cultivar, genetic analysis has shown that they are in fact two distinct cultivars (Maletić *et al.* 1999).

All ampelographic and genetic data of the analyzed cultivars, together with their photos, will be placed into an Internet-accessible electronic database.

Conclusions

Despite the difficulties and unfavourable conditions for Croatian viticultural development in the last 100 years, it is still possible to find large numbers of native cultivars. Some of them are economically important and play a vital role in the Croatian wine business, while many others are endangered and are exposed to the threat of extinction. Our activities in the last few years have focused on ampelographic and genetic evaluation, as well as collecting the remaining Croatian native cultivars. In this way, *Vitis* germplasm in Croatia will be preserved from further erosion. As a result of these research efforts, the exact number of cultivars and an official cultivar list, together with their relationships with other world-famous cultivars, will be established. We have observed a high potential for quality in some of the neglected cultivars, and we hope that these findings will support their economic revitalization and involvement in wine production. Furthermore, the increasing demand for original and indigenous products may also enhance the potential for native Croatian cultivars.

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Viticulture and clonal selection in Cyprus

Savvas Savvides
Fruit Trees and Viticulture Section, Agricultural Research Institute, Nicosia, Cyprus

Vine is one of the major cultivated crops in Cyprus and wine and grape production is one of the most important agro-industries in Cyprus. Vine-growing has been an important activity of the rural population of Cyprus since ancient times, both from an economic and a social point of view. The island's climate and soil favour the production of good quality grapes and give a distinctive character to the wine.

Wine grapes are grown on an area of about 19 000 ha covering about 13% of the total agricultural land. They are cultivated mainly under rainfed conditions on hilly and semi-hilly areas and they exploit land on which no other crop could achieve acceptable economic results. The range of wine-grape varieties cultivated is largely dominated by local varieties, mainly by 'Mavro' and 'Xynisteri'.

In 1987 the Plant Protection Section of the Agricultural Research Institute, with the main objective to provide Cypriot growers with healthy propagating material of local and other traditional grapevine varieties grown in Cyprus, implemented a phytosanitary clonal selection programme. Selection of healthy clones was based both on phytotechnological characteristics such as trueness-to-type, plant vigour, productivity and grape quality, and on the results of visual, biological and serological phytosanitary controls. So far 286 clones representing 15 traditional varieties have been processed though the programme. Of these, only 30 clones (less than 10%), representing 10 of the 15 varieties under sanitary evaluation, were found free of major virus and virus-like diseases and were finally selected. These 10 varieties, now available in a virus-free state are 'Mavro', 'Aspro' or 'Xynisteri', 'Malaga', 'Lefkada', 'Opthalmo', 'Maratheftiko', 'Moschato', 'Promara', 'Spourtiko' and 'Morokanella'.

Suitable genetic sources of frost hardiness, earliness in maturation and sugar accumulation in the Czech national grapevine collection

Olga Mercedes Jandurová and Ricardo Augusto Casal
Crop Research Institute (CRI), Research Station of Viticulture Karlštejn, Czech Republic

Introduction

The economic success of grape production under European continental conditions depends on the adaptation of the vine variety to rapid and wide temperature fluctuations during winter and on the long endodormancy period, which protect vines from spring frost damage. These traits influence the stability of grape yield in regions with continental climates and therefore all genetic resources bearing the above-mentioned characteristics are considered as valuable material for breeding programmes.

Material and methods

The evaluation of the time of bud burst, time of grape maturation and sugar content in must[19] from mature grapes was made in the years 1999–2000 in 34 accessions. Phenological data were recorded according to OIV descriptors 301 and 303 (OIV 1983). Sugar content (kg sugar/hl must) was measured according to the CNM scale used in the Czech Republic and in Slovakia.

Results

According to the length of the vegetative period, the evaluated accessions were divided into four groups.

The first, earliest group with a vegetative period from 102 to 105 days comprises four varieties (Fig. 1). 'Perlaut' had the shortest vegetative period. 'Anna Maria' is a very early-bursting Italian variety. The average time of bud burst in this variety was 16 April, which is too early for Czech vineyard regions, where spring frost damage endangers this early-bursting genotype. The varieties 'Zenit' and 'Perlaut' showed higher sugar accumulation (19.9% and 16.75%). Their time of bud burst at the end of April (28 April) seems to be optimal for Czech vineyard regions. The remaining fourth variety 'Topas' belongs to table grapes and therefore the sugar concentration in mature grapes is not comparable to that of the must varieties.

The second group of early-maturing genotypes also includes one promising variety, 'Muskat moravsky' with appropriate time of bud burst and high content of sugar in must (Fig. 2).

The other accessions evaluated belong to the medium- and late-maturing varieties (Figs 3 and 4). The longest vegetative period was observed in 'Ryzlink vlašsky', followed by 'Muskat hamburský', 'Hedvabne zelené', 'Elvín', ´Ryzlink aromatický´,

[19] Must: juice of freshly pressed grapes ready for fermentation.

'Ryzlink červený', 'Chardonnay' and 'Veltlínské zelené'. Although some of these are traditional varieties, commercially grown in the Czech Republic ('Chardonnay', 'Ryzlink vlašský'), their yield and sugar concentration in the must could be lower in seasons with cold and rainy autumn weather.

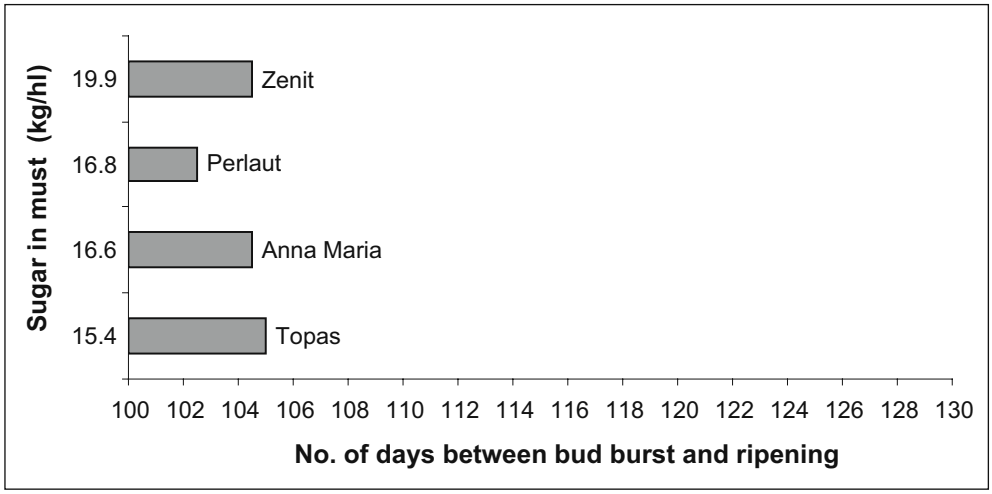

Fig. 1. Amount of sugar in the earliest varieties (102-105 days) compared to the number of days between bud burst and ripening, average of years 1999 and 2000.

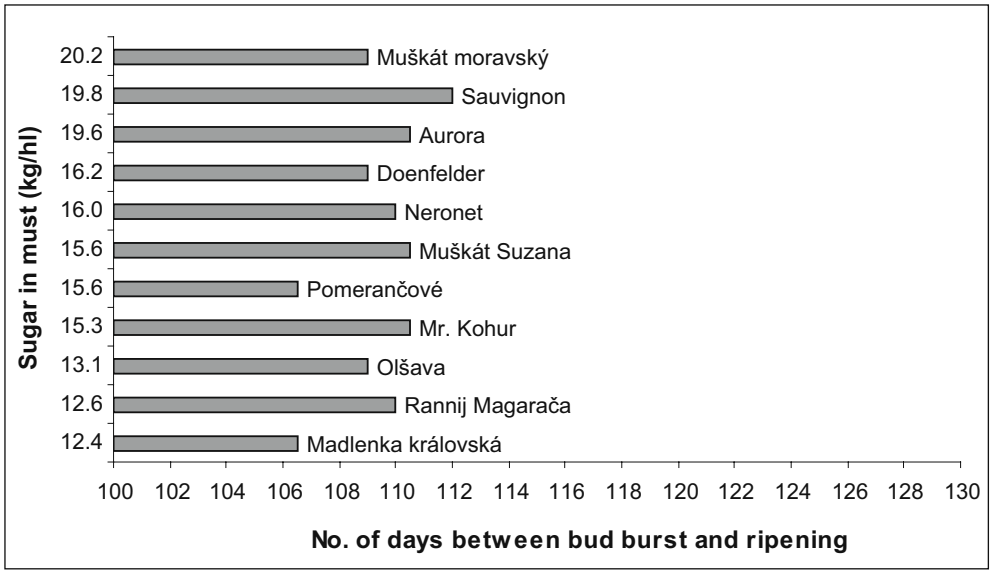

Fig. 2. Amount of sugar in early varieties (106-112 days) compared to the number of days between bud burst and ripening, average of years 1999 and 2000.

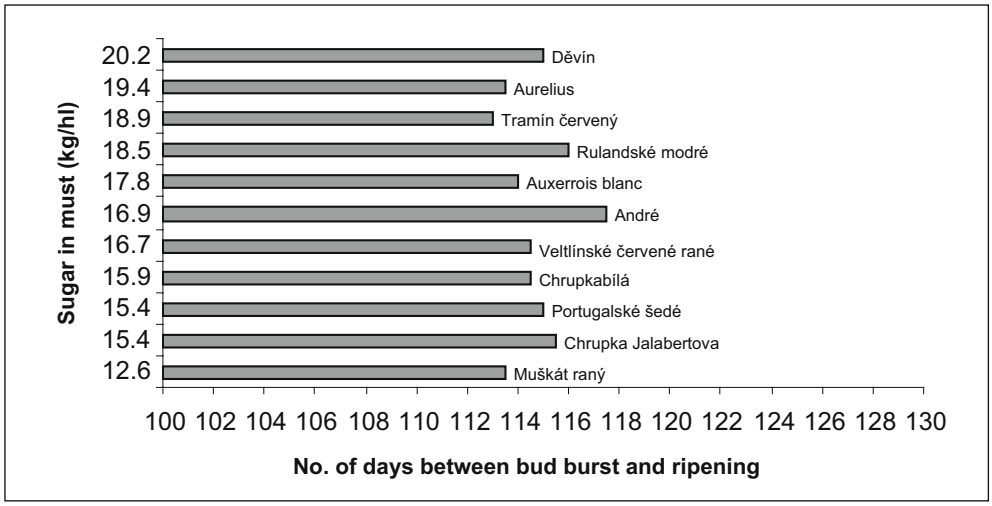

Fig. 3. Amount of sugar in medium-late maturing varieties (113-119 days) compared to the number of days between bud burst and ripening, average of years 1999 and 2000.

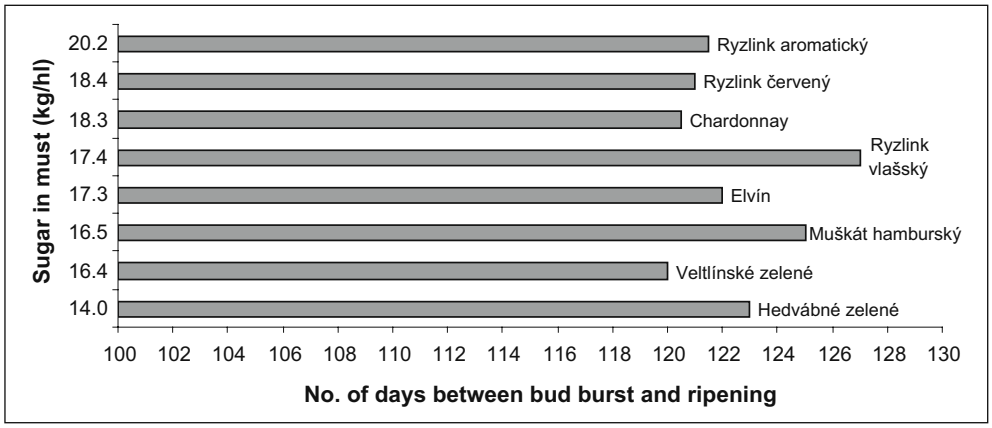

Fig. 4. Amount of sugar in late-maturing varieties (120-127 days) compared to the number of days between bud burst and ripening, average of years 1999 and 2000.

Conclusion

The comparison of earliness in bud burst, grape maturation and sugar accumulation in must detected valuable genetic resources in the Czech National Grapevine collection. The high concentration of sugar in must is not always positively correlated to a long vegetative period. Among early-maturing genotypes with a short vegetative period, there are also valuable varieties with bud burst at the end of April. These varieties represent promising material for further breeding.

Reference

OIV. 1983. Descriptor list for grapevine varieties and *Vitis* species. Office International de la Vigne et du Vin, Paris.

Status of the French Vitis *National Collection*

Thierry Lacombe
Institut National de la Recherche Agronomique (INRA) - UMR 1097 Diversité et Génome des Plantes Cultivées (DGPC), Equipe Génétique Vigne, Montpellier, France

France maintains several different types of grapevine genetic resources (Tables 1 and 2). The curators of *Vitis* genetic resources in France are quite diverse but there is no fully private partner:

- Institut National de la Recherche Agronomique (INRA – National Institute for Agricultural Research)
- Etablissement National Technique pour l'Amélioration de la Viticulture (ENTAV – National Technical Association for Viticultural Improvement)
- Professional partners in the regions
  - Chambres d'Agriculture (Agricultural Chambers)
  - Comités interprofessionels (Interprofessional Committees)
  - Associations techniques (Technical Associations)
  - Syndicats viticoles (Winegrowers' Unions)
- Others : about 10 heritage, tourist-orientated and/or educational collections
- Organizations related to this subject (Office National Interprofessionnel des Vins (Onivins – Interprofessional Wine Organisation), Institut National des Appellations d'Origine (INAO – National Institute for the Labels of Origin), etc.)

Table 1. Grapevine genetic resources maintained in France and holders of the collections

Type of material	Holder
Wild species	INRA
American and Asian *Vitis sp*.	INRA Vassal and Bordeaux
Vitis vinifera subsp. *silvestris*	INRA and *in situ* preservation
Rootstocks	INRA and ENTAV
Interspecific hybrids	INRA
V. vinifera cultivars	
Foreign and table cultivars	INRA Vassal (and Bordeaux)
Old French cultivars	INRA Vassal (and Bordeaux) and ENTAV
Clones (national level)	ENTAV (and INRA Bordeaux, Colmar, Angers)
Clones (regional level)	30 professional partners

Table 2. Number of *Vitis* genetic resources preserved in France

Wild species	35 sp.
American and Asian *Vitis sp*.	400 accessions
Vitis vinifera subsp. *silvestris*	300 individuals *in situ* + 110 accessions
Rootstocks	500 cv.
Authorized	30 cv. (about 800 accessions)
Others	500 cv. (about 600 accessions)
Interspecific hybrids	1000 cv. (1400 accessions)
V. vinifera cultivars	3000 cv. (26 000 accessions)
Foreign and table cultivars	2600 cv. (about 5000 accessions)
Old French cultivars	400 cv. (about 2000 accessions)
Clones (national level)	4000 accessions (for 228 cv.)
Clones (regional level)	about 15 000 accessions (for 88 cv.)

A total of about 45 organizations are involved in grapevine genetic resource conservation, and some are found in each area of production.

Today we are moving towards the development of a national network of collections under the framework of the Bureau des Ressources Génétiques (BRG) which supports this strategy for all species (plants, animals, microorganisms). A general agreement called the "Charte" (Charter) is formulated for each crop. In this context, the term "national collection" is understood as a sub-sample of the whole network collection. In the "grapevine charter" not only certified clones are concerned but the whole of the vine genetic resources. Every voluntary curator who maintains *Vitis* genetic resources, and every organization involved can join the network. The first objective of this new network is to group, manage and share information on *Vitis* genetic resources in France. Therefore the first achievement is to be a national database as a tool for inventory. The other main objectives are diversity analysis and conservation methodologies. The coordination of this network is jointly supported by INRA-Montpellier and ENTAV.

Status of the Vitis *collections in Georgia*

David Maghradze
Research Institute of Horticulture, Viticulture and Oenology (IHVO), Tbilisi, Georgia

The history of grapevine collections in Georgia starts in the 1890s, when the first grapevine collection was established in Sakara. In the 1930s the first state collection was planted in Telavi to conserve 255 Georgian local varieties. Further collections were located in Dighomi (3000 varieties, including 420 local and more than 30 wild and semi-wild forms), Sakara (200 varieties) and Gudauta (312 varieties). As well as these, indigenous varieties were also protected in what appeared to be the first centres or ethno-geographic groups of origin: in Samegrelo (Zugdidi) - 48 varieties, Adjara (Keda) - 42, Kartly (Skra and Galavani) - 39 and 22 varieties respectively.

Today grapevine collections in Georgia are located in Dighomi, Mukhrani, Telavi and Skra: all are field collections. A total of 929 accessions are conserved, including local, introduced and breeding varieties, clones, rootstocks, wild and semi-wild forms of grapevine. Among them 701 are "original" and 248 are local Georgian varieties (Table 1).

Table 1. *Vitis* collections in Georgia

Location	Total no. of accessions	Old local varieties	Clones	Wild and semi-wild forms	Total area (ha)	Date of planting
Dighomi	573	193	-	5+5	9.0	1967-68
Mukhrani	155	155	-	-	1.0	1986-87
Telavi	226	116	10	-	1.2	1987
Skra	75	12	-	-	2.0	1975
Total	**929**	**476**	**10**	**10**	**13.2**	**-**
"Original"	701	248	10	10	-	

There are 228 safety-duplicated accessions.

Each accession contains 5 to 25 plants.

Collections in Dighomi and Mukhrani belong to the State Agrarian University (curated by the Department of Viticulture).

Collections in Telavi and Skra belong to the Georgian Scientific Research Institute of Horticulture, Viticulture and Oenology (IHVO) (curated by the Department of Grapevine and Fruits Crops Germplasm Research, Genetics and Breeding and the Experimental Stations of Skra and Telavi).

The State Agrarian University is under the aegis of the Ministry of Education and the IHVO is under the aegis of the Academy of Agricultural Sciences of Georgia.

According to the Law of Georgia on "Vine and Wine" (1998) the State is responsible for grapevine genetic resources protection in our country. Therefore collections were and still are funded from the state budget. However, as a result of economic problems during the transition period in Georgia, during the last 12-15 years the funding of collections has been very low, which makes their protection and related research difficult. This situation leads to losses and reduces the number of conserved accessions and does not provide any possibility for the enrichment of our collections.

It is therefore necessary to find alternative sources of funding, which, together with the state budget, should build a strong base for grapevine germplasm conservation in Georgia. Collaboration with the international organizations working for the protection of biodiversity is necessary.

One example of this type of collaboration is illustrated by our relationship with IPGRI (now Bioversity International). In the framework of the project on "Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region", planting of new collections of Georgian local varieties was started. One collection was planted in Italy and the second will be planted in Georgia this year [2003], where approximately 240 varieties will be collected.

Varieties in our collections have been described for ampelometric, economic and technological characters over many years. The results of these studies have been published in scientific works and in the ampelographies in Georgian and Russian languages (Ramishvili 1948; Tabidze 1954; Frolov-Bagreev 1946-1956; Ketskhoveli *et al.* 1960; Negrul 1963-1970).

Research based on molecular techniques has not yet started in Georgia.

The documentation of passport or ampelographic descriptors has not been completed in electronic format for grapevine accessions located in the collections of Georgia. An electronic list of varieties protected in our collections is available, but there is no national *Vitis* database in Georgia and therefore the information is not included in the European *Vitis* Database.

Indigenous varieties are severely threatened. Over the centuries 524 local grapevine varieties were obtained through traditional selection (Ketskhoveli *et al.* 1960), from which only 248 remain as of today. A number of other varieties, according to our data, are in collections abroad, mainly in the republics of the Former Soviet Union. Many varieties are under threat or have already disappeared.

The wild grapevine of the Caucasus was a typical plant in our country but after the invasion of phylloxera and fungal diseases in the 19th century the number of plants decreased sharply. However in Georgia the typical wild *Vitis vinifera* subsp. *silvestris* Gmel. ("Usurvazi", "Krikina", "Tkis vazi" in Georgian) was found, described and protected.

Activities planned for the very near future are as follows:

- Complete passport data for entry into the European *Vitis* Database
- Start recording plant descriptors
- Plant a new collection of local varieties (in 2003)
- Prepare and publish a new bilingual ampelography in Georgian/English.

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Negrul, A.M., editor. 1963-1970. Ampelografiia SSSR [Ampelography of the USSR]. Vols. 7-10. Pischepromizdat, Moscow. (in Russian).

Ramishvili, M. 1948. [Grapevine varieties of Guria, Samegrelo and Adjara]. Technika da shroma, Tbilisi. 321pp. (in Georgian).

Tabidze, D. 1954. [Grapevine varieties of Kakheti]. Technika da shroma, Tbilisi. 425pp. (in Georgian).

Maintenance of grapevine genetic resources in Germany

Erika Maul and Reinhard Töpfer
Bundesanstalt für Züchtungsforschung an Kulturpflanzen, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen, Germany

As in most European countries, large private grapevine collections maintaining several hundred cultivars existed in Germany during the 18th century and lasted until the early 20th century. Unfortunately none of them survived and much precious material was lost.

Despite that fact, nowadays six governmental institutions maintain about 5500 accessions in *ex situ* field collections as shown in Table 1.

Table 1. German institutes preserving grapevine germplasm

Collections	WIEWS Institute code*	No. of accessions
Dienstleitungszentrum Ländlicher Raum Rheinland-Pfalz, Neustadt	DEU363	294
Bayerische Landesanstalt für Weinbau und Gartenbau, Veitshöchheim	DEU457	219
Staatliche Lehr- und Versuchsanstalt für Wein- und Obstbau, Weinsberg	DEU456	848
Forschungsanstalt Geisenheim	DEU454	ca. 900
Staatliches Weinbauinstitut Freiburg	DEU455	ca. 300
Institut für Rebenzüchtung Geilweilerhof	DEU098	2927
Total		**ca. 5500**

* WIEWS: World Information and Early Warning System
database of institute codes available at http://apps3.fao.org/wiews/institute_query.htm

Beside simple maintenance, the grapevine material also serves several purposes such as breeding of fungus-resistant cultivars (DEU455, DEU456 and DEU098), phylloxera- and lime-resistant rootstocks (DEU454), characterization and evaluation of breeding features, as well as providing the basis for various research activities.

The national grapevine collection at the Institute for Grapevine Breeding Geilweilerhof

The distribution of the 2927 cultivars of the national *ex situ* grapevine collection according to the type of material is as follows:

- Old and indigenous cultivars, existing in Germany in former times (200)
- German newly-bred cultivars and breeding lines (100)
- Cultivars of national and international importance (500)
- Fungus-resistant cultivars and selections, mainly from French breeders (1600)
- *Vitis vinifera* subsp. *sylvestris* (30)
- *Vitis* species of American and Asian origins (50).

Table 2 shows the classification of these cultivars according to their area of origin, species and utilization.

Table 2. Classification of the cultivars maintained in the Institute for Grapevine Breeding Geilweilerhof according to their area of origin, species and utilization

	No. of cultivars
Area of origin	
Western Europe	1597
Eastern Europe (Bulgaria, Romania, Czech Republic, Slovenia, Moldova, Russian Federation, Tajikistan, Turkmenistan, Turkey, Ukraine, Uzbekistan)	223
Near East (Syria, Lebanon, Israel, Jordan)	5
Middle East (Iran, Iraq, Afghanistan)	18
East Asia (China, Japan, India)	33
Caucasus (Georgia, Azerbaijan, Armenia, Dagestan)	20
North Africa (Morocco, Algeria, Tunisia, Egypt)	5
South Africa	2
North America (Canada, USA)	294
South America (Argentina, Brazil)	8
Australia	2
Without indication of origin	316
***Vitis* species**	
Vitis vinifera subsp. *vinifera*	1019
Vitis vinifera subsp. *sylvestris*	33
Vitis riparia	42
Vitis rupestris	11
Vitis labrusca	9
Other *Vitis* species	46
Interspecific crossings	1285
Without indication of species	78
Utilization	
Wine grapes	1258
Table grapes	230
Rootstocks	79
Multiple use: wine and table grape	222
Multiple use: table grape and raisin	12
Multiple use: wine grape and rootstock	20
Other multiple uses	10
Without indication of uses	692

Future activities focusing on the preservation of valuable *Vitis* germplasm in Germany

Recent activities carried out for the preservation and maintenance of germplasm comprise the collection of diverse clones of recommended cultivars by governmental clone breeders in vineyards planted before 1950. The greatest focus is upon plantations which have not yet undergone clonal selection. An inventory of old vineyards planted with outstanding clones and of old neglected cultivars will be established by the end of 2009 and plant material from endangered plantations will be rescued.

Status of Vitis *collections in Italy*[20]

Angelo Costacurta, Mirella Giust, Roberto Carraro and Massimo Gardiman
Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Centro di Ricerca per la Viticoltura (CRA-VIT), Conegliano, Italy

Since the 1980s the importance of genetic resources preservation has been increasingly recognized at national level. In the past this work has been carried out by public or private institutions autonomously, often without financial means.

For this reason in Italy some public projects, aimed at coordinating the recovery, collection and characterization of *Vitis* species, grapevines and clones of *Vitis vinifera*, and hybrids were implemented by involving stakeholder institutions.

The first project was financed by the National Research Council (Consiglio Nazionale delle Ricerche, CNR) and coordinated by the former Experimental Institute for Viticulture (Istituto Sperimentale per la Viticoltura, ISV), now Agricultural Research Council – Research Centre for Viticulture (Consiglio per la Ricerca e la Sperimentazione in Agricoltura – Centro di Ricerca per la Viticoltura, CRA-VIT). This project, entitled "Difesa delle risorse genetiche delle specie legnose da frutto" (Protection of fruit tree genetic resources) (CNR 1988) included 12 Italian regional partners, and aimed at:

- the recovery of underutilized, highly threatened indigenous varieties,
- the collection and conservation of vines in field repositories, and
- the characterization of collected biotypes.

More than 400 grapevine varieties were discovered and all of them are now being maintained in the main collections of the CRA-VIT located in Spresiano and Tormancina; the regional varieties discovered were included in the regional partners' repositories.

Characterization was then carried out using not only morphological analyses, as in previous research programmes (Giust 1991; Costacurta *et al*. 1992, 1996), but also biochemical and molecular markers (isozymes and DNA analyses) as in the projects "Marcatori molecolari in frutticoltura" (Molecular markers in fruit-growing) and "Mappe genomiche" (Genomic maps).

Starting from 1994, various Italian institutions were involved in two European projects: AIR 1728, "Comparative study and analyses on grapevine vegetative material", and GENRES CT96 081 (Peterlunger *et al.* 1998). The final objectives of these researches were to study and try out new common methods for the reliable description and characterization of vines (This *et al.* 2004), to catalogue all the major existing European collections and to create an on-line database of passport data for all the accessions analyzed (see http://www.genres.de/vitis).

Through these research programmes, inventories of international and Italian grapevines were made (CNR 1994; Sartori *et al.* 2001). Moreover, international ampelographic, phyllometric and molecular descriptor lists have been produced (see the primary and secondary descriptor lists of the GENRES 081 project (Anonymous 2002a, 2002b) and the *2nd edition of the OIV Descriptor List for Grape Varieties and Vitis species*, available from the OIV Web site (http://www.oiv.int/).

[20] Updated 2008

The CRA-VIT is currently involved in two research projects aimed at the protection and enhanced utilization of biodiversity:

1. *Plant Genetic Resources*, a 3-year programme financed by the Italian Ministry of Agriculture, with the following purposes for its grapevine collections:
   - Maintenance, entry and updating of collection accessions
   - Updating of accession data and international descriptor passport data (EURISCO)
   - Characterization of accessions
   - Identification of indigenous varieties, clarification of synonyms and homonyms
   - Recovery and enhanced utilization of indigenous grapes.
2. *Biodiversity*, an annual inter-regional research programme to carry out the inventories of Italian collections and their accessions, supported by the European Union.

These actions of recovery and protection of grapevine germplasm have resulted in the collection and conservation of about 25 000 accessions. The last inventory of Italian collections managed by private and/or public institutions is reported in Table 1.

Table 1. The Italian *Vitis* collections

Region / Province	Institution	Public (P) or Private (PR)	Site	No. of accessions	
				Per institute	Total per Region / Province
Friuli Venezia Giulia	Provincial Administration of Pordenone	P	Spilimbergo (PN)	81	**1137**
	"Vivai Cooperativi Rauscedo"	PR	Rauscedo (PN)	400	
	Farm "Nimis Giovanni"	PR	Nimis (UD)	20	
	University of Udine	P	S. Osvaldo (UD)	138	
	"Ersagricola"	P	Beano di Codroipo (UD)	52	
	"Ersa–Centro Pilota"	P	Gorizia	46	
	Winery of Cormons	PR	Cormons (GO)	400	
Veneto	CRA-VIT	P	Susegana (TV)	3541	**4213**
			Spresiano (TV)		
	Agricultural Institute "G.B. Cerletti"	P	Conegliano (TV)	45	
	Farm "Case Bianche "	PR	Susegana (TV)	16	
	Farm "Ruggeri"	PR	Valdobbiadene (TV)	16	
	Farm "Dal Betto"	PR	Boccon Di Vo' (PD)	16	
	Farm "Da' Lustra"	PR	Faedo (PD)	62	
	Farm "Bedin"	PR	Brendola (VI)	33	
	Winery of Soave	P	Soave (VR)	41	
	Provincial Administration and University of Verona	P	S. Floriano (VR)	24	
	"Veneto Agricoltura"	P	Ceregnano (RO)	368	
			Porto Tolle (RO)	51	

Table 1 (cont.). The Italian *Vitis* collections

Region / Province	Institution	Public (P) or Private (PR)	Site	No. of accessions	
				Per institute	Total per Region / Province
Prov. Bolzano	Research Centre for Agriculture and Forestry Laimburg	P	Vadena-Ora (BZ)	46	**46**
Prov. Trento	Agricultural Institute "S. Michele all'Adige" (ISMAA)	P	Giaroni (TN)	5369	**6525**
			S. Michele all'Adige (TN)		
			Inferno (TN)	1156	
Lombardia	University of Milan	P	Voghera (PV)	5000	**9063**
			Brescia	4000	
	University "Cattolica Sacro Cuore" of Piacenza	P	Torrazza Coste (PV)	63	
Piemonte	CNR–Institute of Plant Virology	P	Grinzane Cavour (TO)	400	**600**
			Canelli (AT)	150	
	Agricultural school "Malva Amaldi"	P	Bibiana (TO)	50	
Valle d'Aosta	Institut Agricole Régional	P	Aosta	21	**21**
Liguria	CNR–Institute of Plant Virology	P	Albenga (SV)	50	**50**
Emilia-Romagna	University "Cattolica Sacro Cuore" of Piacenza	P	Ziano Piacentino (PC)	28	971
	University of Bologna	P	Cadriano (BO)	663	
	Centro Ricerche Produzioni Vegetali (CRPV)	P	Tebano (RA)	280	
Toscana	Research Unit for Viticulture (CRA–VIC)	P	Pratantico (AR)	387	1214
		PR	Massa	42	
		PR	Bibbiena (AR)	79	
		PR	Montevarchi(AR)	68	
	University of Firenze, Department of Horticulture	P	Peccioli (PI)	160	
			Pontremoli (MS)	97	
			Colignola (PI)	11	
	University of Pisa	P	Castelnuovo Berardenga (SI)	260	
			Montalcino (SI)	110	
Marche	Agenzia Servizi Settore Agroalimentare delle Marche (ASSAM)	P	Petritoli (AP)	30	30
Lazio	CRA–VIT	P	Tormancina (RM)	800	858
	Research Unit for Wine Production In Central Italy (CRA–ENC)	P	Velletri (RM)	48	
	University of Tuscia	P	Viterbo	10	
Campania	University of Portici	P	Portici (NA)	30	30
Basilicata	Azienda Agricola Sperimentale Dimostrativa (AASD)	P	Gaudiano (PZ)	33	33

Table 1 (cont.). The Italian *Vitis* collections

Region / Province	Institution	Public (P) or Private (PR)	Site	No. of accessions	
				Per institute	Total per Region / Province
Puglia	Research Unit for Viticulture and Enology in Mediterranean Environment (CRA–UTV)	P	Lamarossa (BA) Turi (BA)	800	1080
	CRSA "Basile-Caramia"	P	Locorotondo (BA)	280	
Sicilia	Vivaio Governativo di viti americane (V.G.V.A.)	P	Palermo	743	827
	Sicily Region – Local Authority of agriculture and forestry	P P	Marsala (PA) Comiso (RG)	42 42	
Sardegna	Consorzio Interprovinciale per la frutticoltura (Interprovincial Fruit Farming Consortium) of Cagliari	P	Villasor (CA)	18	48
	Farm "Sella & Mosca"	PR	Alghero (SS)	30	
Grand total					26746

The former ISV (now CRA-VIT, CRA-VIC, CRA-UTV) collections (Costacurta and Carraro 2005), located in different areas of Italy with various soil and climatic characteristics, are the most important in terms of numbers and typology of accessions (Table 2, Fig. 1).

Table 2. Number and typology of accessions held at the CRA–VIT of Conegliano, CRA-VIC of Arezzo, and CRA-UTV of Turi

Site	Typology	No. of accessions	
		Per type	Total per site
Susegana (TV)	Species	14	2394
	Hybrids	150	
	Rootstock hybrids	240	
	Biotypes of *Vitis vinifera* L.	1990	
Spresiano (TV)	National and international clones of *V. vinifera*	193	1147
	ISV clones on selection	69	
	ISV clones entered in the National Catalogue	115	
	National biotypes of *V. vinifera*	370	
	New table grape hybrids	400	
Arezzo	Rootstock hybrids	47	387
	Biotypes of *V. vinifera* of central Italy	340	
Turi (BA)	Biotypes of *V. vinifera*, rootstocks and hybrids of central-southern Italy		800
Tormancina (Rome)	Grapevines entered in the National Catalogue	360	800
	Biotypes of *V. vinifera*	440	
Grand total			**5528**

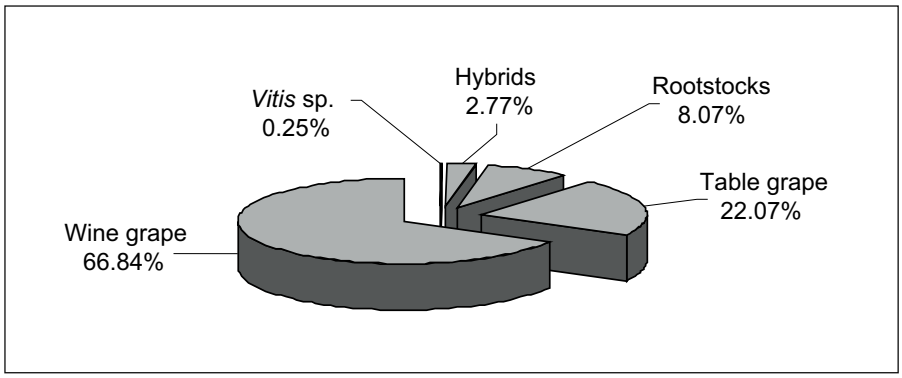

Fig. 1. Typology of the accessions in CRA-VIT, CRA-VIC, and CRA-UTV collections.

Various types of analyses are carried out each year on all the CRA-VIT collections:
- phenology and productivity observations for all the genotypes
- updating of new accessions coming from Italy and abroad, analysis of the phytosanitary status (ELISA test) and of trueness-to-type (morphological and molecular)
- phylogenetic studies
- studies for the clarification of synonyms and homonyms
- studies on intravarietal grapevine variability.

The results obtained so far are highlighting the increasing interest in the protection and recovery of grapevine germplasm, particularly over the past few years. This awareness in the research community has focused actions and efforts with the main aim of reducing genetic erosion.

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Viticulture and grapevine genetic resources in Macedonia (FYR)

Klime Beleski
Institute of Agriculture, Skopje, Macedonia (FYR)

Viticulture in Macedonia (FYR)

The Former Yugoslav Republic of Macedonia, situated in the central part of the Balkan Peninsula, covers an area of 25 713 km^2. It is a predominantly mountainous country, with plateaux and highlands cut by valleys and gorges.

The western and eastern regions of the country have a continental climate. The modified Mediterranean climate has an influence along the valley of the Vardar River all the way to Skopje.

The climate is favourable for grape growing. The main viticulture regions are Pcinsko-osogovski, Povardarski and Pelagonisko-poloski. The Povardarie region has favourable climatic conditions for table grape and for red wine varieties. Varieties for white wine are grown in the Pcinsko-osogovski and Pelagonisko-poloski regions.

The country's total vineyards cover some 28 000 ha (1999), producing annually about 1 000 000 hl wine and 74 000 t of table grapes.

The main grapevine varieties grown in Macedonia (FYR) are:

- for red wine: 'Vranec', 'Prokupec' and 'Gamay'
- for white wine: 'Smederevka', 'Zilavka' and 'Grenashe'
- for table grape: 'Dattier de Beyrouth', 'Cardinal', 'Belo Zimsko', 'Muscat de Hambourg', 'Alphonse Lavallee' and 'Italia'.

The national grapevine collection

The national collection of grape varieties is located at the Institute of Agriculture, Department of Viticulture and Enology, Skopje.

Grape varieties are conserved *ex situ* only by the Department of Viticulture and Enology of the Institute of Agriculture.

The collection has 180 different commercial and local grape varieties and clones, of which 150 are described by internationally accepted descriptors.

In the framework of this collection, the Institute works continually on the introduction, selection, examination and evaluation of varieties and clones. In the future we expect to improve the content of the collection by adding varieties and clones for table grape production, varieties and clones for high quality red and white wine production, seedless varieties with large berries for consumption in a fresh state and also varieties for raisin production.

We expect significant results from the virus-free clones introduced from France and Italy, from the varieties for white and red wine production ('Cabernet Sauvignon', 'Cabernet Franc', 'Merlot', 'Pinot Noir', 'Chardonnay', 'Riesling' and 'Sauvignon') and also from the table grape varieties ('Italia', 'Cardinal' and 'Muscat de Hambourg').

Status of plant genetic resources in Macedonia (FYR)

For the last 10 years, since the independence of the Former Yugoslav Republic of Macedonia, the research community has been striving to reorganize the genebank activities previously coordinated by the headquarters in Belgrade. For various

reasons, mainly lack of consistent support from the government and instability in the region, very little has been done regarding plant genetic resources for food and agriculture.

Three institutes deal with the conservation and evaluation of the working collections:

- Institute of Agriculture in Skopje (Department for Field and Vegetable Crops, Department for Viticulture and Enology, Department for Fruit Orchards) which also includes the Department for Rice in Kochani
- Institute for Southern Crops in Strumica
- Tobacco Institute in Prilep.

As yet there is no legal framework for genebank activities. Starting from 1996 the funding has been provided through a Programme for developmental support in agriculture, by the Ministry of Agriculture, Forestry and Water Economy. This means that the national funding varies every year and can be from US$ 5000 to US$ 50 000 depending on the total budget of the Ministry and the current policy. In 1998 funding was interrupted for one year due to the Kosovo crisis.

A national programme for the protection of biodiversity, including agrobiodiversity, is currently in preparation at the Ministry of Environmental and Physical Planning.

Status of grapevine genetic conservation in Malta

Randall Caruana
Viticulture Unit, National Office for Vitiviniculture and Oleiculture, Ghammieri, Marsa, Malta

In Malta, viticulture is one of the most ancient branches of the agricultural sector. The cultivation of the vine has formed part of Maltese agricultural efforts since immemorial times. There are no documents which trace its origin, but archaeology shows that the culture of the vine dates as far back as the Carthaginians (ca. 300 BC) and continued during the Roman era.

The large number of grape varieties (over 120) that were present in Malta, before phylloxera wiped out many vines, also provides testimony of the Islands' historical links. Their central position in the Mediterranean Sea links the Islands with some of the major grape-growing countries of the world.

In the Maltese Islands there are two indigenous varieties: 'Gellewza' (red) and 'Girgentina' (white). These names actually describe groups of varieties with the respective berry colours. The name 'Gellewza' in Maltese means "hazelnut" and may originally have been used to describe small-berried grapes. The name 'Girgentina' on the other hand is derived from a place in Malta called Girgenti. This could also indicate a former connection with Girgenti in Sicily (modern Agrigento) during the Greek colonization period. In fact one of the varieties found in the 'Girgentina' group has many similarities with the variety 'Ansonica' which was historically an important variety in Agrigento. The area under vines dedicated to the two indigenous variety groups is about 70% of the total area under vines in Malta.

Prof. John Borg (1922) described various table and wine grapes present in Malta at that time. Although he was not a professional ampelographer, he tried to relate the local vine varieties to others known in other countries.

In 1998 a survey was carried out, financed through Project 29 of the IVth Italo-Maltese Financial Protocol, during which a primary selection of more than 150 vines was made in which 20 different cultivars were represented. An *ex situ* collection was subsequently planted out in pots at the Plant Health Department. DNA analysis using 10 microsatellite markers (VVS2, VVS5, VVMD5, VVMD7, VVMD27, VVMD28, ssrVrZAG79, ssrVrZAG47, ssrVrZAG62 and ssrVrZAG64) will be carried out in 2003-2004. Through an EU-financed twinning project experts from Italy will interpret the raw results of the DNA analysis and will train Maltese staff to make ampelographic descriptions and ampelometric measurements.

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Vitis *genetic resources in the Republic of Moldova*[21]

Gheorghe Savin, Andron Popov and Vladimir Cornea
Institutul National pentru Viticultura si Vinificatie (INVV), Chişinău, Republic of Moldova

Viticulture has been carried on for a thousand years or more in our region. Until the end of the 19th century it was mainly based on old indigenous varieties of *Vitis vinifera*. The national collection or assortment mostly consisted of cultivars of wine grapes. The phylloxera invasion caused great damage to viticulture in terms of both the cultivated area and the varietal composition. However the contribution of grapevine genetic resources to the development of viticulture was essential. Since 1832 several ampelographic collections were established for the purposes of disseminating a good quality of different lines of vines, promoting grafted viticulture and creating new cultivars. In particular during the last 60 years the quality of the grapevines available in the Moldovan region progressed from the French-American hybrids to the classical European varieties. Newly created varieties (hybrids of the second and third generations) are well represented due to their quality and relative resistance to pests and diseases.

This evolution was accompanied by some losses, especially of old indigenous varieties. Some of them have definitively disappeared and wine market requirements limit the utilization of those still remaining. This evolution during the last 60 years is reflected in the relationships between the three groups of registered cultivars presented in Table 1. The proportion of old indigenous varieties has decreased in favour of the new creations, while the proportion of classical European varieties remains high.

Table 1. Evolution of the standard *Vitis* varieties in the Republic of Moldova from 1949 to 2005, according to the Registers of cultivars recommended for cultivation in the Republic of Moldova

Years	Total no. of varieties included in the Register	Type of material (%)		
		Introduced varieties, including classical European varieties	Old indigenous varieties	Newly created varieties
1949	43	84	16	-
1964	31	90	10	-
1980	36	82	5	13
1995	53	69	6	25
2001	65	64	5	31
2005	74	62	4	34

This ratio is reflected to some extent in the cultivated areas. The vineyards in the Republic of Moldova include particular vineyards and collective and cooperative farms. The highest level of development in this sector was achieved in the early 1980s, when Moldova held the sixth place after Spain, Italy, France, Portugal and Romania for vineyard area, total harvest and winemaking. Unfortunately this favourable

[21] Updated in 2006

period was followed by a period of decline caused by multiple factors – starting with the political forcible decision concerning the stubbing of wine grape vineyards, followed by economic and social transformations, natural disasters (mainly frosts and droughts), pests and diseases, etc. In order to restore the viticultural sector, a "National Programme for the restoration and development of viticulture and winemaking in the Republic of Moldova in the period 2002-2020" was developed and ratified. According to this Programme, the area under vineyards in the country should reach 100 000-120 000 ha in 2020.

The distribution of each group of varieties over the last 60 years is presented in Fig. 1. The most important areas are under wine varieties and the same trend is foreseen for the future. Concerning the varietal composition, we can note the qualitative evolution of the assortment of cultivars: the share of the French-American hybrids decreased from 65.9% in 1945 to practically zero. Over the same period the share of classical European varieties increased from 21.2% to 71.4%, and this figure is planned to increase up to 80%.

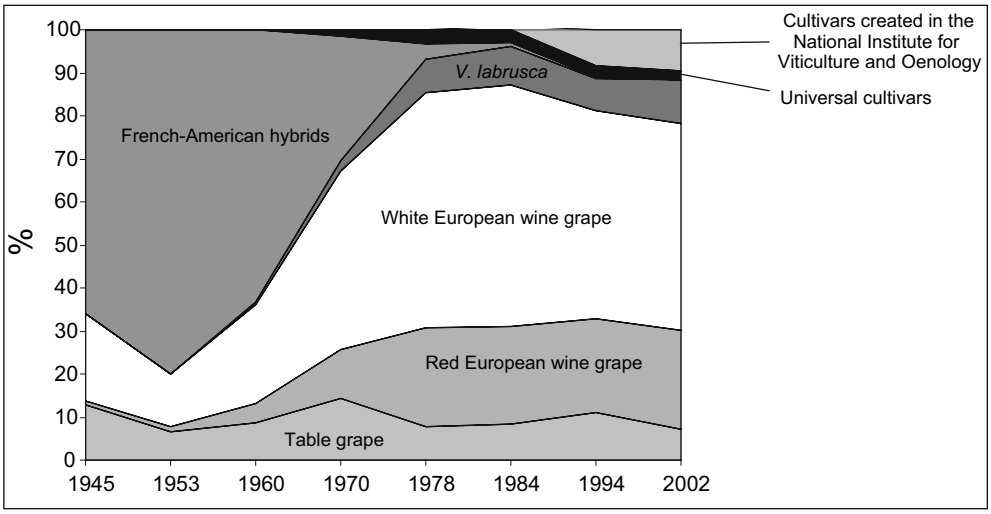

Fig. 1. Evolution of the grape varietal composition in Moldova, 1945-2002.

Universal varieties (for table and wine) were included in the approved assortment since the 1960s and new varieties created by the National Institute for Viticulture and Oenology (Institutul National pentru Viticultura si Vinificatie, INVV) since the 1980s. The table grape varieties with the greatest cultivated areas are 'Chasselas blank', 'Muscat of Hamburg', 'Rein of vine', 'Cardinal', 'Perla of Csaba', the indigenous cultivar 'Coarnă neagră' and the newly bred cultivar 'Moldova'. For table grapes the area of newly created varieties will increase up to 80%.

Among wine grape varieties the most widespread are the classical European varieties 'Cabernet Sauvignon', 'Merlot', 'Aligote', 'Sauvignon', 'Traminer rose', 'Pinot' group, 'Riesling of Rhine' and 'Rkatsiteli'. The share of the old and new indigenous varieties is still low. There are insignificant areas of 'Rara neagra', 'Fetească albă' and

'Fetească neagra'. For the future it is planned to increase the share of classical European varieties up to 80% and to use the new resistant varieties as a "safety belt" in cases of unfavourable years for viticulture due to extreme environmental conditions.

The history of grapevine genetic resources collection and preservation includes the creation of a number of ampelographic collections:

- 1832: establishment of a collection near the locality of Cetatea Alba (Akkerman). From the 330 cultivars included in this collection, 85 were old indigenous cultivars.
- 1849: creation of the collection of Basarabia's Viticulture and Winemaking School in Chişinău on the basis of cultivars received from the Crimea.
- 1910: after the phylloxera invasion, in order to promote the cultivation of high-quality European wine grapes and to change viticulture into a grafted culture, came the creation of the Experiment and Demonstration Station near Chişinău (locality Costiujeni). This station is the precursor of the current INVV. All further important collections were established at this Institute.
- 1956: foundation of the Ampelographic Collection (called "Old Collection") based on the following principles and purposes: representation of traditional and newly created varieties from all viticultural areas; varieties with valuable biological and agrotechnical properties; rootstocks; French-American hybrids; wild forms, etc. By 1980 this collection contained about 2750 genotypes from more than 57 sources (Ivanova 1976). Some of these genetic resources existed only in this collection and served as a basis for the establishment of collections in other viticultural regions.
- 1981: foundation of the "New Collection" based on the "Old Collection" and following the same principles regarding the build-up and composition of the collection, but with other additional strategies according to current demands and to the experience acquired. This collection is situated in Chişinău on the southeastern slopes, on land with a 3-6° of slope. The soil is black earth. Grapevine genetic resources from more than 60 locations of origin worldwide include more than 2500 genotypes, including ca. 78 old and new indigenous varieties, ca. 480 from Western Europe and 344 from Eastern Europe, 270 from Middle Asia, 74 from North America and others (Savin 1980; Savin *et al.* 1995).

  The collection has mostly been increased through introductions. This process was particularly intensive in 1982-1986, at the beginning of the establishment of the New Collection, when the collaboration with viticultural centres worldwide was facilitated by organizational and financial factors. In spite of the difficulties, more than 900 genotypes were introduced in the last 20 years. The main demands for the improvement of the nationally available assortment are to promote the introduction and the creation of early table grape varieties and the renovation of the wine grape collection. Great attention was paid to obtaining varieties resistant to pests, diseases and unfavourable environmental factors (mainly to winter conditions), but with high quality and productivity. The scientists' efforts were oriented towards finding parent material possessing one or more of these properties. As a result important grapevine genetic resources with complex properties, for use in genetic breeding programmes, especially for table grapes, were gathered together in

the Ampelographic Collection. The principal resources with big berries and/or relative disease or pest resistance are from Middle Asia, Ukraine, Russia, Romania and Bulgaria.

On the basis of existing genetic resources a new direction was given to the diversification of the grapevine assortment in the Republic of Moldova: the creation of seedless resistant varieties. Seedless genetic resources with complex properties (early ripening, resistance to unfavourable abiotic and biotic environmental factors) are being collected. Three seedless varieties were created and homologated: 'Apiren alb' (patented), 'Apiren roz' (patented) and 'Apiren negru de Grozesti'. Some forms with remarkable properties (high sugar accumulation, early ripening, resistance) have also been distinguished; they are being tested for their potential for wine, juice, jam or raisin production, for fresh consumption and for medicinal uses.

The limited utilization of the potential of the old indigenous varieties is an omission in the creation of a durable viticulture, because some of them are adapted to unfavourable environmental conditions or resistant to diseases. For example, 'Coarnă neagră', 'Coarnă roşie', 'Fetească albă', 'Fetească neagra', 'Negru de Causeni', 'Băşicată', 'Galbena de Ardeal', 'Codarca' and others are resistant to frost, drought and diseases (Constantinescu 1967). Most of the table grape indigenous varieties are good for long-term storage, which allows diversifying into fresh grape consumption during the winter season. At present attention is focused on the inventory, description and reintroduction of old indigenous varieties. In industrial vineyards there is a tendency to increase the cultivated area of varieties 'Fetească neagra' and 'Fetească regala'. During the unfavourable winter conditions for viticulture of 2005-2006, when frosts seriously affected vineyards, some indigenous varieties performed well and their yield in 2006 was at the level of favourable years. This emphasizes once again the value of a history of millennial selection and the importance of these grapevine resources for local viticulture.

The anthropogenic pressure on the environment threatens the habitats of the wild grapevines which occur in the country. These wild species are important as representatives of the original flora, but also for theoretical studies and breeding purposes. Due to the threat of disappearance *in situ*, *ex situ* conservation of seed and vegetative populations of wild grapevines from the Prut riverbank has been initiated.

Conservation and maintenance of genetic resources are essential. It is not possible to apply adequate phytosanitary and the other necessary treatments due to the lack of human resources, and the spread of viral infections and chronic diseases constitutes a threat to the genotypes. In order to avoid degradation or loss of genetic resources, activities are oriented towards the monitoring of the threatened genotypes, and efforts are also dedicated to the reintroduction of lost resources.

International collaboration contributes significantly to the mobilization, conservation and sustainable use of grapevine genetic resources. This facilitates the exchange of information and of biological material.

The description of grapevine genetic resources is based on descriptor lists published by IBPGR (1983), IPGRI *et al.* (1997) and on the Primary and Secondary Descriptor Lists developed in the framework of the project GENRES 081 "European Network for Grapevine Genetic Resources Conservation and Characterization" (Anonymous 2002a, 2002b).

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Status of the Vitis *national collection in Portugal*

José Eduardo Jorge Eiras-Dias
Estação Vitivinícola Nacional, Instituto Nacional de Investigação Agrária e das Pescas, Dois Portos, Portugal

Portugal has a list of 341 grapevine varieties that can be used to make Portuguese wine. The names of these 341 varieties resulted from an important work on the synonymy and homonymy of the Portuguese varieties and a national name was chosen for each variety. In 1889, Portugal had a list of 1482 names and most of these were still in use before the agreed national names were chosen.

One of the most important strategies to settle this question of cultivar identity was the establishment of the Portuguese National Ampelographic Collection at the National Station of Viticulture and Oenology in 1985, after a national ampelographic project was developed. Working groups in the whole country located all the varieties used in Portugal to make wine and described morphologically the main varieties, using the descriptors of the International Organisation of Vine and Wine (Office International de la Vigne et du Vin, OIV) (OIV 1983). This project resulted in the establishment of ampelographic collections in the main wine-producing regions and the establishment of the Portuguese National Ampelographic Collection.

The institution holding this collection is located in Dois Portos, 40 km north of Lisbon. The local coordinates are 9º11′ West, 39º02′ North and 110 m of altitude. The climate is characterized by rainy winters, and the rain falls predominantly during November, December and January. The summer period is very dry. The average annual temperature is 15.2ºC and the average annual precipitation is 694 mm. The month with the maximum average temperature is August, reaching 21ºC, and the month with the minimum average temperature is January, reaching 10ºC. November has the maximum average rainfall with 95 mm.

The collection area is 2 ha. Vines were grafted on SO4 and there are 724 accessions (691 accessions of *Vitis vinifera*, 24 of rootstocks and 9 *Vitis* species).

Whenever possible each accession came from a single plant (clonal accession).

Nowadays, the national collection aims mainly at preserving the traditional Portuguese varieties, and allows their characterization, identification and the study of synonymy and homonymy. With this aim we continue to develop the morphological and the ampelometric characterization of the accessions, using the descriptors developed by OIV and in the framework of the GENRES 081 project "European Network for Grapevine Genetic Resources Conservation and Characterization" (Anonymous 2002a, 2002b). A working group of four Portuguese institutions carried out the molecular characterization of the 341 varieties used to make wine in Portugal, using the six microsatellites selected by GENRES 081 and accepted by OIV as good molecular markers for *Vitis*.

In addition the collection also aims at:

- Being used as a reference collection for the varieties and rootstocks used in Portugal
- Including the most important varieties used in the world

- Having the varieties used as references for international *Vitis* descriptors (OIV, UPOV or Bioversity)
- Being used for educational purposes.

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Creation of the Russian ampelographic collection

***Alexander S. Smurygin*[1], *Vasilii A. Nosulchak*[1] *and Leonid P. Troshin*[2]**
[1] *Crimean Research Selection Station of the N.I. Vavilov Research Institute of Plant Industry (VIR), Krymsk, Russian Federation*
[2] *Viticulture Department, Kuban State University of Agriculture, Krasnodar, Kuban, Russian Federation*

Russia today needs to establish its own ampelographic collection, since the largest existing collections of the Soviet Union are now located in newly independent states following the collapse of the USSR, and also because of the deterioration of grape germplasm throughout the Community of Independent States (CIS). Three research organizations of the Russian Federation, the N.I. Vavilov Research Institute of Plant Industry (VIR), the North Caucasus Regional Institute for Horticulture and Viticulture (NCRIHV) and the Kuban State Agrarian University (KSAU) are charged with this task.

More than 3600 samples of grapevine germplasm have been introduced into Russia over the last 7 years. Of these, 3150 samples, accounting for 88% of the country's total grapevine germplasm, have been collected through the efforts of VIR and KSAU. This part of the grapevine germplasm is currently under study in greater detail. Contributions to the establishment of the Russian collection of grapevine genetic resources have been received from 25 ampelographic collections in 10 countries worldwide. The most successful introduction has been from the CIS, with 3055 samples (= 94.5% of today's grapevine germplasm of Russia) coming from seven countries of the Community. The largest contributors are the Crimea (1400 samples), Turkmenistan (360), Russia itself (540), Uzbekistan (301) and Moldova (218).

For Russia, representative institutions of 13 geographical units of the country have become part of the collection-holding community. The major contributor is the Far East Experiment Station of VIR (one-third of the Primorski region). Nevertheless, grapevines derived from Povolzh'ie, Bashkortostan, Michurinsk and other regions of amateur grape-growing have not been covered adequately so far.

Another 176 grape varieties and forms have been obtained from the USA, Japan and Germany. These account for only 5.5% of today's grape germplasm of Russia, yet the samples are notable for their diversity. Interspecific hybrids whose parentages include American species account for more than 50% of that contribution. Eighteen seedless and 16 tetraploid forms have been introduced for the first time.

Russia's grapevine germplasm collected until now is highly diverse as regards its specific and genetic composition. The majority of samples (almost 76.7%) belong to *Vitis vinifera* L., including 1471 (57.2%) indigenous grapes and 505 (19.5%) obtained by intraspecific crossing. Varieties obtained by interspecific crossing account for 545 samples and include 40 rootstocks, some 130 varieties having forms of *Vitis amurensis* Rupr. in their parentages and more than 150 samples with hybrids of 'Seyve Villard' as their ancestors. We should also mention the 130 hybrids, 60 clones and more than 100 forms of the species *Vitis* (Tournef.) L. Of special value are the seedless varieties, accounting for more than one-third of this category of the world's grapevine

germplasm. Indigenous varieties of Middle Asia are well covered, with only partial representation of newly bred varieties from the CIS and other countries.

The creation of the All-Russia database of ampelography and viticulture, which contains some databases from research institutes of the CIS, is under way. A Web site (http://www.vitis.ru/) has been established. It contains 65 publications dealing with ampelography, grape genetics and breeding, grapevine growing and winemaking. The site also offers various items about grapes and wine such as painting, poetry, papers open to discussion and promotional material.

Grapevine genetic resources in Serbia and Montenegro

Petar Cindrić[1], Nada Korać[1], Dragoljub Žunić[2], Ljubomir Pejović[3], Vesna Maraš[3] and Saša Matijašević[2]

[1] *Faculty of Agriculture, Novi Sad, Serbia and Montenegro*
[2] *Faculty of Agriculture, Zemun, Serbia and Montenegro*
[3] *Biotechnical Institute, Podgorica, Serbia and Montenegro*

Introduction

Reasons for the maintenance and study of grapevine genetic resources are primarily ethical and historical. They are economic only in the long term, when they assist in achieving success in breeding (Alleweldt and Dettweiler 1992, 1994).

At the time of the establishment of the Bank of Plant Genetic Resources of Yugoslavia (BPGRY) in 1989-1991, an analysis was conducted of the status of genetic resources of the genus *Vitis* on the territory of the Socialist Federal Republic of Yugoslavia (Cindrić *et al.* 1997). It was concluded that the research centres involved had gathered a rich grapevine germplasm collection.

Where grapevines are concerned, the territory of the Balkans does not represent a uniform ecosystem. Large differences exist between the northern parts, prone to the continental climate, and the southern parts, which are under the influence of the Mediterranean climate. Both parts have their indigenous groups of cultivars which have been formed under the effects of specific ecological factors and under the control of the peoples inhabiting these areas. Several grapevine collections, referred to as "ampelographic collections", were established. The best known were those of Radmilovac, Sremski Karlovci, Titograd, Niš, Skopje, Svetozarevo, Split, Vipava, Krško, Nova Gorica, Mostar, Zadar and Zagreb.

Methods

Based on the lists developed by the International Board for Plant Genetic Resources (IBPGR), the International Organisation of Vine and Wine (Office International de la Vigne et du Vin, OIV) and the International Union for the Protection of New Varieties of Plants (Union Internationale pour la Protection des Obtentions Végétales, UPOV), the following descriptors were adopted:

- 32 descriptors for collection passport data;
- 8 descriptors for passport data of accessions: primary name, synonyms, accession origin, location of collecting site, year of entry, accession size, accession status and uses;
- 21 descriptors for characterization and primary evaluation; and
- 57 evaluation descriptors.

We worked principally on the basis of OIV descriptor lists (OIV 1983), which are compatible with those of IBPGR (1983) and UPOV (1985), and we translated them into the Serbian language.

Table 1 provides more details about the characterization and evaluation descriptors.

Table 1. Characterization and evaluation descriptors used in the BPGRY for *Vitis*

	No. of descriptors		
	Characterization and preliminary evaluation	**Evaluation**	**Total**
Morphological characters			
Young shoot	3	0	3
Young leaf	0	3	3
Mature leaf	6	14	20
Woody shoot	1	12	13
Inflorescence, bunch, berry	10	11	21
Tendril	1	1	2
Phenological characters	0	4	4
Biological characters	0	8	8
Agronomic characters	0	4	4
Total	**21**	**57**	**78**

After having defined the descriptors, we started developing a database within the BPGRY information system. This activity was carried out at the Maize Institute in Zemun Polje under the technical supervision of Dr V. Makević.

An accession number, which serves as the sample identifier, is given by a curator when a sample arrives at BPGRY. Once assigned, an accession number cannot be reassigned to another sample even if the former sample is definitively lost.

To resolve the problem of primary names and synonyms, it was necessary to standardize the accessions' names. Cultivars that have been extensively grown for a long time tend to be given several different names. This happened in our country as it did in others. When a cultivar is transferred from one region to another, it frequently acquires a new name. Thus we now have cultivars with several names (synonyms). It also frequently happens that different cultivars bear the same name (homonyms). There is much confusion about the names of some grapevine cultivars both within our country and in international communications. In an attempt to bring some order into this issue, OIV experts decided to assign primary names according to the origin of cultivars or, if the origin is not clear, according to locations where these cultivars are most numerous (Dettweiler 1994). All country members of European Union (EU) are obliged, in mutual communications, to refer first to the primary name and then to mention other synonyms.

OIV, of which our country is a member, also recommended to countries outside the EU to accept the proposed names. A large number of the cultivars from the EU list could be found on the territory of Serbia and Montenegro; however, there were also significant numbers of indigenous and other cultivars in our country which were not on the EU list. It was thus necessary to make an amended list for our country. This list was compiled by a Commission which included Prof. Dr Lazar Avramov, Prof. Dr Petar Cindrić and Prof. Dr Nada Korać.

Based on OIV documentation, technical literature and personal experience, the Commission assigned primary names and most important synonyms to all *Vitis* genetic resources available in the BPGRY. Each primary name is followed by an internationally accepted symbol which designates berry skin colour: B (Blanc) – green or yellow skin; G (Gris) – gray skin; N (Noir) – black or blue skin; Rs (Rose) – rose skin.

Conditions were therefore created in which it became possible to arrange the accessions in the BPGRY alphabetically.

In addition to the compiled descriptor lists, which contain detailed instructions regarding sample size, optimal dates of observations or measurements, and diagrams of plant organs labelling the exact positions to be observed, special worksheets have been prepared for field work.

Results

Ten research centres from all Former Yugoslavian Republics, represented by 18 researchers, took part in the establishment of the BPGRY. The *Vitis* germplasm consisting of 1661 accessions was thus maintained in ten collections (Cindrić *et al.* 1997).

A new appraisal of the status of the grapevine genetic resources in Serbia and Montenegro was made in 2003. The largest collections are held in three research centres, all equipped with the necessary research staff, technical personnel and equipment:

- Radmilovac (Faculty of Agriculture in Zemun): 498 accessions
- Sremski Karlovci (Faculty of Agriculture in Novi Sad): 487 accessions
- Podgorica (Biotechnical Institute in Podgorica): 491 accessions.

The collections in Podgorica and Sremski Karlovci are old, while the collection in Radmilovac is new, most of it having been established in 1994.

The current composition of these three collections according to genetic origin, type of material and uses is given in Tables 2, 3 and 4.

Table 2. Genetic origin of *Vitis* samples in the BPGRY

Species	No. of accessions in the collection			
	Radmilovac	Sremski Karlovci	Podgorica	Total
V. vinifera	418	358	462	1238
Interspecific hybrid	73	128	27	228
V. riparia	4	-	1	5
V. rupestris	3	-	1	4
V. amurensis	-	1	-	1
Total	**498**	**487**	**491**	**1476**

The largest number of samples (84%) belongs to the species *Vitis vinifera*, about 15% of the samples are interspecific hybrids and only a few samples are other species from the genus *Vitis*.

Table 3. Status of *Vitis* samples in the BPGRY

Status of sample	No. of accessions in the collection			
	Radmilovac	Sremski Karlovci	Podgorica	Total
Primitive cultivar	183	157	303	643
Valuable genotype	62	38	13	113
New cultivar	216	244	165	624
Clone	37	48	10	95
Total	**498**	**487**	**491**	**1476**

Most numerous (44%) are primitive cultivars whose pedigree is not known. A large number of accessions (42%) are new cultivars developed by hybridization, i.e. their pedigree is known. The collections also include about 8% of valuable genotypes possessing some important characters and about 6% of clones.

Table 4. Uses of *Vitis* samples in the BPGRY

Use of sample	No. of accessions in the collection			
	Radmilovac	Sremski Karlovci	Podgorica	Total
Wine grape	300	294	296	890
Table grape	148	152	166	466
Raisin	16	15	14	45
Rootstock	34	25	15	74
Not used	0	1	0	1
Total	**498**	**487**	**491**	**1476**

Most cultivars are used for winemaking (60%), about one-third is table grape cultivars, about 3% are seedless cultivars and about 5% are rootstocks. One single wild species has no practical use.

The passport data (8 characters) and the characterization and preliminary evaluation data (21 characters) for the accessions in the collections in Sremski Karlovci and Radmilovac have for the greater part been entered into the BPGRY database. Only the data for the accessions acquired in recent years have not been processed yet.

The results of an evaluation of economically important characters for about 250 cultivars from the collection in Sremski Karlovci are published in a monograph "Grapevine cultivars" (Cindrić *et al.* 2000).

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The Vitis *Germplasm Bank of El Encín (Alcalá de Henares, Madrid, Spain)*

Félix Cabello [1] ***and Jesús María Ortiz*** [2]

[1] *Departamento de Agroalimentación, Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA), Alcalá de Henares, Madrid, Spain*

[2] *Departamento de Biología Vegetal, Universidad Politécnica de Madrid (UPM), Madrid, Spain*

Grapevine varietal collections were initiated in Spain during the second half of the 19th century with the objective of reducing the genetic erosion caused by the phylloxera outbreak and its expansion in the country. The *Vitis* Germplasm Bank of El Encín (Banco de Germoplasma de Vid de la Comunidad de Madrid, BGVCAM) is located at Alcalá de Henares, Madrid, Spain. The starting point of this Bank was the Viticulture and Oenology Station in Haro (Estación de Viticultura y Enología de Haro), located in La Rioja (Spain) and established in 1893 as a varietal grapevine collection. Since 1950 the collection has gradually been moved to its present location.

As of 2003, the BGVCAM includes 2726 accessions, distinguished as follows: 1718 *Vitis vinifera* varieties including wine and table grapes; 848 rootstocks; 71 hybrids; 66 V*itis* spp.; and 23 *Vitis vinifera* subsp. *silvestris*. The objectives are the collecting, conservation, identification and evaluation of the plant material as well as enabling scientific and experimental exchanges. Ampelographic and molecular characterization has been carried out on the accessions included in the collection, detecting the existence of synonyms and solving some cases of misnaming. An "Ampelographic Museum" integrated with a "base collection" was established in 2003, and including 233 accessions, which were fully characterized and documented. A parallel research project is being carried out in order to locate and recover the indigenous grapevines threatened by extinction. The material obtained through this project will also be included in the BGVCAM.

More information on the germplasm bank is available on the Internet (http://www.madrid.org/cs/ - *Museo ampelográfico*).

Conservation and study of grapevine genetic resources in Ukraine

Anatoli M. Avidzba, Misak V. Melkonian, Vladimir A. Volynkin and Alla A. Poluliakh
Institute for Vine and Wine "Magarach", Yalta, Crimea, Ukraine

Conservation and study of grapevine genetic resources remain an important task at the beginning of the 21st century, both for wild and cultivated species and for cultivars. Grapevine is an old cultivated crop which has been studied rather well, and a number of its centres of origin have been identified. The formation of individual species of grapevine is associated with these centres.

Modern Ukraine has primary and secondary centres of origin of grapevine. The former is confirmed by the fact that grapevines belonging to *Vitis vinifera* subsp. *silvestris* are found in the Crimean Mountains and that a group of indigenous varieties of the Crimea has been established within the group of *Vitis vinifera* subsp. *sativa.* Varieties belonging to this group are still cultivated in the Crimea on a commercial scale: they include 'Kokur belyi', 'Kefessia', 'Ekim kara', etc. They produce famous brands of wines such as Solnechnaia dolina, Chernyi doctor, Chernyi polkovnik, etc.

According to data of 2001, farmers in Ukraine cultivated more than 220 grape cultivars on a total area of 105 200 ha. The Register of Plant Varieties of Ukraine contains 113 cultivars officially authorized for commercial cultivation, including 44 of table grape and 69 of wine grape.

Wine grape in Ukraine is cultivated on ca. 80 000 ha, and table grape on ca. 20 000 ha. The principal cultivars and their respective cultivation areas are listed in Table 1.

Table 1. Major grape cultivars and their cultivation areas in Ukraine

Cultivar	Area (ha)	Comment
Wine grape		
Rkatsyteli (W)	23000	local variety of Georgia
Alighote (W)	15000	
Sovin'on zelenyi (W)	5300	
Kaberne Sovin'on (B)	4000	
Sukholymans'kyi bilyi (W)	3500	(Shardone x Plavai)
Fetiaska bila (W)	3200	
Rysling reins'kyi (W)	2500	
Bastardo magarach'skyi (B)	1500	(Bastardo x Saperavi)
Pino chornyi (B)	1400	
Odes'kyi chornyi (B)	1400	(Alikant Bushe x Kaberne Sovin'on)
Kokur bilyi (W)	1100	local variety of Crimea
Merlo (B)	1100	
Shardone (W)	1100	
Table grape		
Moldova (B)	3800	(Guzal kara x SV 12375)
Rannii Magaracha (B)	3500	(Madlen Anzhevin x Kishmish chornyi)
Italia (W)	1600	(Bican x Muskat ghamburz'kyi)
Muskat ghamburz'kyi (B)	1600	
Shasla bila (W)	1400	

Conservation and the further use of indigenous varieties in the commercial grape cultivar assortment of the Crimea and Ukraine are still of importance. It is logical to use them in breeding programmes aimed at obtaining new generations of varieties that would combine in their genome the ability to adapt to abiotic factors and a set of valuable traits such as high-yielding capacity, good quality of fruit and resistance to pathogens. The presence of such a set of traits is necessary to grow new generations of varieties on a commercial scale within the framework of Ukraine's modern viticulture.

Searching for a diversity of traits in various forms of grape requires both a good knowledge of the world's grapevine germplasm and the testing of these new forms under specific ecogeographical conditions, since the formation of the final product of viticulture is determined both by the plant genotype and by the conditions under which the plant grows.

With this in mind, many research institutions of Ukraine located in different ecogeographical zones possess ampelographic collections where a diversity of grapevines are maintained and studied:

- The **Tairov Institute for Viticulture and Oenology** (Odessa) established an ampelographic collection in 1905. A total of 900 cultivars have been studied. The latest collection was re-established in 1988-1990 and contains 470 samples of varieties of which 407 have been identified (contact person: Dr M. Bankovskaia).
- The **Transcarpathian Institute for Agroindustrial Production** (Transcarpathia) began maintaining and studying grapevines in the collection in 1946. In the 1970s, about 400 cultivars were maintained in the collection; this number has decreased to about 200 (contact person: Dr A. Popovich).
- The **Zaporozh'ie Experiment Station** (Zaporozh'ie) has an ampelographic collection containing 85 cultivars (contact person: Mr. V. Laskavy). This is the northernmost zone of commercial viticulture in Ukraine.
- The **Institute for Vine and Wine "Magarach"** (Crimea) holds Ukraine's largest ampelographic collection. It was established in 1828 and maintains 3259 samples of grapevine of which 2400 have been identified. Samples of cultivars are maintained grafted on phylloxera-resistant rootstocks, and each form is represented by five to ten plants, on a total area of 16 ha. The phytosanitary status of the plants is periodically checked by testing for viruses and crown gall. Ampelographic and agrobiological observations are carried out in the field annually on a regular basis (persons in charge: Dr V. Volynkin and Dr A. Poluliakh).

  The collection contains varieties from the whole world (Fig. 1), 26 wild forms, 23 species of the genus *Vitis,* 3 species of the genus *Ampelopsis* and 2 species of the genus *Parthenocissus*.

Studies of varieties belonging to the West-European and Eastern ecogeographical groups has affected Ukraine's nationally grown grapevine assortment. Varieties belonging to both groups are widely cultivated in the country. Besides, the availability of such a wide diversity of grapevine germplasm in terms of geographical origin makes it possible to develop several breeding programmes using varieties belonging

to various ecogeographical groups, species and centres of origin (North America, Europe, Middle Asia and East Asia). This allows the development of new generations of varieties for commercial cultivation in Ukraine and also offers the perspective of obtaining new botanical taxa via multiple hybridization.

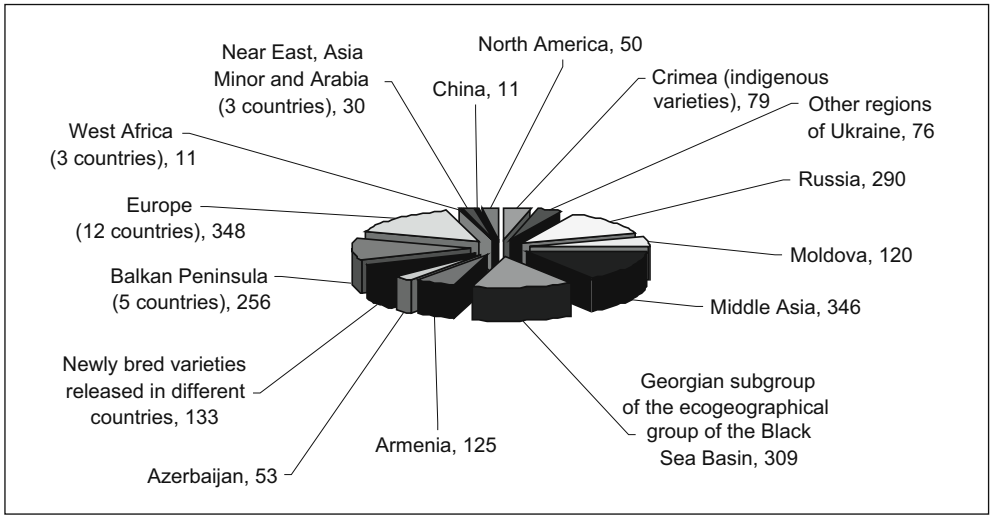

Fig. 1. Number of *Vitis* varieties in the ampelographic collection of the Institute for Vine and Wine "Magarach" (Crimea) according to their geographical origin.

Differentiation and identification of grapevine varieties

Synonymy, homonymy and misnaming are obstacles for an international network on the conservation of Vitis *germplasm in Europe*

Erika Maul
Bundesanstalt für Züchtungsforschung an Kulturpflanzen (BAZ), Institut für Rebenzüchtung Geilweilerhof (IRZ), Siebeldingen, Germany

Introduction

Depending on the specific vine-growing area, viticulture has a tradition estimated at between 2000 to 7000 years (McGovern *et al.* 2000). Through colonization by Phoenicians, Greeks and Romans, the migration of nations, development of trade routes, the foundation of monasteries, etc., grape varieties were brought to new areas where they were not found before. Often on the way to new destinations the varieties changed their names. In addition to this, over the centuries new grapevine varieties were selected for their wine and table grape properties while less-adapted varieties disappeared. More than 18 500 prime names and about 23 000 synonyms are registered in the *Vitis* International Variety Catalogue (VIVC) (http://www.vivc.bafz.de/index.php; updated in 2007).

The names given to grapevine varieties can sometimes be traced back to their geographical origins. They may refer to a location, e.g. to a village from which the variety was imported or where it was found (e.g. 'Dattier de Beyrouth', 'Gamay', 'Chardonnay'), to a region or to a country (e.g. 'Traminer', 'Italia', 'Malvasia di Sardegna'), or may relate to the breeder (e.g. 'Müller-Thurgau', 'Seyval', 'Scheurebe'), to the person who discovered and distributed the variety (e.g. 'Ruländer', 'Ortlieber'), to the cross (e.g. 'Cabernet Sauvignon', 'Rivaner'), or to a special quality attribute (e.g. 'Tempranillo', 'Muscat à petits grains blancs', 'Teinturier', 'Kishmish'), etc. But often the origin of the name remains unknown.

The particular difficulty which we face today is that old grapevine varieties and those which have been widely spread are often known under several local names, which are synonymous to the variety. This is the reason why the same variety occurs under different designations in grapevine collections. The VIVC lists dozens of synonyms for varieties such as the widely grown table grape 'Dattier de Beyrouth' (106), the old prolific variety 'Weisser Heunisch', called 'Gouais' in France (135), 'Pinot noir' (111), 'Pinot gris' (98), 'Palomino Fino' (68), 'Furmint' (61), 'Sangiovese' (48), etc. In addition it can also happen that the same name is used to designate different varieties. In this case the synonymous names are called homonyms.

Owing to a long tradition of plant material exchange between wine growers, botanical gardens, grapevine collections, breeders and researchers, and the high probability of wrong labelling or accession mix-up from the first step (harvesting of cuttings) to the last step (planting in the new location), misnaming is also a real problem, estimated at between 5 and 10% in the worldwide grapevine collections.

Grapevine variety differentiation and identification is indispensable for achieving reliable outputs in research and reliable knowledge of the plant material for breeding purposes, as well as for the exchange of true-to-type material. Efficient management of germplasm conservation, which means at least duplicate conservation in genebanks and the prevention of loss of genetic resources, also depends on the accessions being true-to-type.

Problems to overcome

In the European *Vitis* Database (EVDB) accessions appear in alphabetical order of accession names. A search for synonymous accessions belonging to the same variety is not yet possible. For the utilization of the EVDB as a tool for genetic resources management in a decentralized network of grapevine collections, the problems of synonymy, homonymy and misnaming must be overcome. The objective is a unique designation of varieties, a clear assignment of synonyms and the assessment of trueness-to-type in grapevine collections. The following examples illustrate the current difficulties.

- ***Synonymy***

Example: 'Dattier de Beyrouth'

This table grape from the Orient is known worldwide under numerous synonyms. The more common synonyms and the frequency of their occurrence were looked up in the EVDB. The results show that at least ten different designations of 'Dattier de Beyrouth' occur there. The number of accessions listed in the EVDB for the ten names is shown in brackets after the synonym: 'Dattier de Beyrouth' (17), 'Razaki' (15), 'Regina' (9), 'Afus Ali' (5), 'Actoni Maceron', 'Bolgar', 'Rosaki', 'Rosetti', 'Zeini' (2 each) and 'Hafiz Ali' (1).

Without knowledge about the variety-specific synonyms, the real number of varieties cannot be discovered. This presents a serious problem as the correct identifying of the material is a precondition for germplasm maintenance.

Moreover, several different 'Razaki' types exist in Turkey. Their description and classification have been the focus of a Turkish research group (Samanci and Uslu 1993).

- ***Homonymy***

Example: 'Augusta'

This variety name exists four times in the VIVC. Two are *Vitis labrusca* varieties, the two others are new crosses. Three of them have white berry colour. For the fourth variety the berry colour is unknown. In addition another German variety is called 'Augusta Luise'. Hungary cites an 'Augustana'. A Russian fungus-resistant cross was called 'Augustovskij' (Fig. 1). For the USA the interspecific cross 'Augustina' is mentioned. The French ampelographer Galet mentions 'Augustine blanche' for the region Haute Vienne/France.

Without specific variety indications like *Vitis* species, berry colour or parentage it is difficult to find out which 'Augusta' is present in the collections.

It is worth noting that 'Augusta Luise' has become 'Augusta Suisse' in two Spanish grapevine collections.

1 - 12 from 12

No.	Accession Name	Accession Number	Colour of the berry	Country of Origin	Holding Institute	Further information
1	August Giant	39-37-008	Black	USA	Geilweilerhof/Germany	details
2	Augusta	52-06-030	Green	ROM	Geilweilerhof/Germany	details
3	Augusta	10006	Green	ESP	Jerez de la Frontera/Spain	details
4	Augusta	96	Green		Pleven/Bulgaria	details
5	Augusta	BGVCAM1475	Green	ITA	Alcalade/Spain	details
6	Augusta Luise	52-12-010	Green	DEU	Geilweilerhof/Germany	details
7	Augusta Luise Wü B48-1	BGVCAM1362	Green	DEU	Alcalade/Spain	details
8	Augusta Luisse	9161	Green	ESP	Jerez de la Frontera/Spain	details
9	Augusta Suisse	10007	Green	ESP	Jerez de la Frontera/Spain	details
10	Augustana	Subcoll.3NOVAGORICA-1	Green		Ljubljana/Slovenia	details
11	Auguste Suisse	BGVCAM1756	Green		Alcalade/Spain	details
12	Augustovskij	38-25-027	Green	RUS	Geilweilerhof/Germany	details

Fig. 1. Different varieties carrying the same name (homonyms) in the EVDB.

- ***Similar variety names belonging to the same variety***

For old grapevine varieties especially, which centuries ago were passed to neighbouring villages or other vine-growing areas without documentary evidence, a shifting of consonants and/or vowels in the names was quite common. The 'White Heunisch' for example shows a wide range of similar names: 'Heinish', 'Heinsch', 'Heunsch', 'Heunschler', 'Hinschene', 'Hintsch', 'Huensch', 'Huntsch', etc. Numerous name variations for the variety 'Bayan Shirei' are registered in the EVDB: 'Bahran Chirei', 'Bahian Shirei', 'Baiean Schirei', 'Baianshyra' and 'Ag Shirei'.

If grapevine variety names are in unknown languages, an interpretation of the name itself is not possible and it cannot be decided whether minor variations of the letters still stand for the same variety or if they completely change the meaning and thus the variety. Moreover through the transliteration of Cyrillic letters or of Chinese or Japanese languages, shifting of consonants occurs. In these cases the determination of the true variety is critical.

To avoid different transliterations, in 1983, when the VIVC was established, the International Organisation of Vine and Wine (Office International de la Vigne et du Vin, OIV) decided to use the Chemical Abstracts' recommendations for that purpose.

- ***Similar variety names belonging to different varieties***

Both in the past and today, grape breeding activities create hundreds of new varieties, which have to be named before breeders' rights can be granted and authorized for commercial use. Unfortunately, numerous names which have been given to recently released grapevine varieties are identical. They may even be given the names of old, long-existing varieties. Others differ in a few letters like 'Olimpia' (white berry colour, 'Italia' x 'Thalloczy Lajos'), 'Olimpiada' (parentage unknown – interspecific crossing), 'Olimpiiskii' (black berry colour, 'Sereksiya chernaya' x *Vitis amurensis*), 'Olimpijec' (white berry colour, *Vitis vinifera* x *Vitis amurensis*). A new cross from Japan is called 'Olympia' (4n, red berry colour, 'Kyoho' x 'Kyogei') (Fig. 2).

1 - 11 from 11						
No.	Accession Name	Accession Number	Colour of the berry	Country of Origin	Holding Institute	Further information
1	Olimpia	264	Green	HUN	Vienna/Austria	details
2	Olimpia	2050	Black		Susegana/Italy	details
3	Olimpia	2125			Kishinev/Moldovia	details
4	Olimpia	2168			Kishinev/Moldovia	details
5	Olimpia	707	Green		Pécs/Hungary	details
6	Olimpia	2505			Kishinev/Moldovia	details
7	Olimpia Testvére	708	Green		Pécs/Hungary	details
8	Olimpiada	53-18-027			Geilweilerhof/Germany	details
9	Olimpiiskij	653	Black	UKR	Kishinev/Moldovia	details
10	Olimpija	941	Green		Pleven/Bulgaria	details
11	Olimpijec	67-41-109	Green		Geilweilerhof/Germany	details

Fig. 2. Different varieties carrying similar variety names in the EVDB.

The risk is that small shiftings or the modification of single consonants can affect variety identification.

- ***Prefixes***

Confusion can also be found within variety groups, which seem to be mixed up. Prefixes are often placed in front of names like 'Boal', 'Jaen', 'Kishmish', 'Malvasia', 'Muscat', 'Plant', 'Uva', etc.

- ***Misnaming***

During an international ampelography project, collection holders interested in the differentiation and identification of grapevine varieties described varieties for about 20 characters and sent dried leaf specimens of the cultivars to the Institute for Grapevine Breeding (Institut für Rebenzüchtung, IRZ)-Geilweilerhof (Dettweiler 1991a). Up until 1992, leaf specimens of more than 900 varieties were gathered, of which 350 varieties were represented by more than one accession from different sites. Concerning conformity of plant material and designation, leaf comparison of cultivars of the same name but from different sites revealed that 85% of the 350 different varieties were correctly named. For 5% of the cultivars identity was not obvious and 10% of the cultivars seemed to be misnamed (Dettweiler 1992a).

An ampelographic check of 41 rootstock cultivars at IRZ-Geilweilerhof in 2000 and 2001 has shown that 17% were not true-to-type. A considerably higher percentage of misnomers is estimated for *Vitis* species accessions in European grapevine collections.

Measures to be undertaken for variety identification

- ***Methods: ampelography, isoenzymes, SSR markers, etc.***

The grapevine identity problems explained above came into focus through the danger of grapevine genetic erosion. Besides the compilation of descriptor lists for *Vitis* spp., OIV initiated the organization of international ampelography courses. A multitude of different approaches for grapevine identity assessment

have been presented: ampelometric methods with the advantage of increased objectivity, computerized leaf recognition programs, elliptic Fourier analysis, DNA analysis, the two isoenzyme systems of glucose phosphate isomerase (GPI) and phosphoglucomutase (PGM), phenolic and aromatic compounds of the berries, and others (Dettweiler 1991b). These research papers were limited to a small number of grapevine varieties. They helped to sort out regional and sometimes international confusion, but were not applied on a large scale to whole grapevine collections. Hence the usefulness of the methods was not tested on a larger scale. Often there was no follow-up on grapevine identity programmes or methods as soon as doctoral theses had been completed.

In addition an ampelography course was held aiming at the training of scientists in objective description of grapevines to obtain comparable results. Methodical instructions and practice on field evaluation, leaf- and berry-measuring methods were carried out (Dettweiler 1992b).

The identification procedure of Galet (1988, 1990) offered a practicable ampelographic solution. He classified morphological features of French varieties according to botanical rules, and added leaf drawings and photographs, so that the verification of grapevine variety identity is possible.

New techniques are supporting the proposed undertaking in combination with traditional ampelography. Simple sequence repeat (SSR) marker techniques are a complementary tool (for literature see This *et al.* 2004; the SSR marker database of Grando *et al.* (2002); the Greek *Vitis* Database (http://gvd.biology.uoc.gr/gvd/index.htm), the EVDB and the VIVC with descriptor data and photographs).

The outcome of the project GENRES 081 "European Network for Grapevine Genetic Resources Conservation and Characterization" on SSR markers, aimed at the standardization of marker data, will contribute to the establishment of a universally useable database.

- ***Trueness-to-type assessment in grapevine collections***

The world's largest grapevine collection is located in southern France at the Domaine de Vassal (experimental unit of the Institut National de la Recherche Agronomique, INRA). It is planted in littoral sand, free from *Phylloxera* and nematodes, vectors of the fanleaf virus disease complex. This collection comprises more than 7200 accessions of which 5100 are *Vitis vinifera* from 35 different countries. Since 1876, the year in which the first grapevine collection was founded at Montpellier, many old indigenous varieties have been gathered together. Without their maintenance at Vassal, they would have completely disappeared (Boursiquot 1998). The old Croatian variety 'Dobricic', a parent of 'Plavac Mali', was lost in its own country but was preserved as accession F106 at Vassal.

From the 1950s to about 1990 two worldwide acknowledged ampelographers, Pierre Galet and Paul Truel, worked at the same time at Montpellier and at Vassal. This coincidence brought considerable advantage for the grapevine collections at Montpellier and Vassal concerning their size, description, identification, documentation and maintenance of the intra- and interspecific variability and to a certain degree the intravarietal variability.

The comparison of accessions from different locations, herbarium leaf specimens, descriptions and drawings allowed scientists to (1) bring together identical varieties,

differently named, from different locations (3500 identified varieties, including 2236 of *Vitis vinifera* (Boursiquot 1998)) and (2) differentiate distinct varieties, similarly or identically named.

One of the outcomes is a 3-volume table grape ampelography, illustrating the process (Branas and Truel 1965, 1966). Important morphological traits are described; photographs of the upper and lower sides of the leaves, the bunch, berries and the inflorescence are given, as well as the source of the described material. For the grapevine collections cited, the latter information is of value even today.

Within the scope of GENRES 081, activities on grapevine identity assessment were intensified in the project partners' grapevine collections. At the IRZ-Geilweilerhof for example, 1016 accessions – i.e. about one-third of the grapevine collection – have been checked for trueness-to-type using (1) leaf specimens, obtained from about 60 grapevine collections, (2) descriptions and photographs in ampelographies and (3) SSR markers. Most accessions (923) were true-to-type; 9% of the accessions could not be identified. The non-identified accessions are being maintained. For plant material exchange purposes, the information on trueness-to-type of the accession is specified as "true-to-type" or "identity not verified" to avoid the multiplying of mistakes.

These national or grapevine collection specific efforts have to be combined at international level for the needs of the whole grapevine community. It is only through concerted actions that the aim of grapevine germplasm management by means of the EVDB can be achieved.

- ***Grapevine germplasm management network***

For the aim stated above, several steps are necessary, as follows:

1. For all grapevine varieties a synonymy list needs to be drawn up. This has been initiated and already carried out by OIV for the most important vine varieties in the world. Through this process, 31 countries have contributed with country specific variety designations. The outcome was the *International List of Vine Varieties and Synonyms* (OIV 1996). A list with the remaining grapevine varieties of minor importance and those which are endangered needs to be established. In every winegrowing country complete synonymy lists should be compiled.
2. Connection between the diverse designations in the different countries. Investigations about variety identities not yet examined may be necessary.
3. Incorporation of the synonymy list into the EVDB.
4. Assessment of the identity of national grapevine varieties in grapevine collections. This work is completed or is under way in many countries.
5. Assessment of the identity of foreign grapevine varieties in grapevine collections. Exchanges of descriptor data, DNA, cuttings or other information with the countries of origin may be necessary. This work is also under way in many countries.
6. Organization of *Vitis* germplasm management. Definition of responsibilities at international level.

A reliable grapevine germplasm management network will serve the whole grapevine community. The steps listed above are a long-term enterprise. It will succeed only by cooperation and combination of international experts' efforts. The

new European Project GrapeGen06, Management and Conservation of Grapevine Genetic Resources (http://www.montpellier.inra.fr/grapegen06/accueil.php) of the EU Council Regulation No 870/2004, lasting for four years (January 2007-December 2010) and comprising 24 partners from 17 countries, will push this initiative forward.

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A comparative study of the general utility of SSR markers for grapevine variety characterization and identification: developing a common standard for uniform labelling using reference cultivar-based allele codes

A. Jung[1], P. This[2], P. Boccacci[3], J. Borrego[4], R. Botta[3], L. Costantini[5], M. Crespan[6], G.S. Dangl[7], C. Eisenheld[8], F. Ferreira-Monteiro[9], S. Grando[5], J. Ibáñez[4], T. Lacombe[2], V. Laucou[2], M. Magalhães[10], C.P. Meredith[7], E. Maul[11], N. Milani[6], E. Peterlunger[12], F. Regner[8] and L. Zulini[12]

[1] *Office for Ampelography and Clone selection, Lustadt, Germany*

[2] *INRA - Génétique de la Vigne, UMR Diversité et Génome des Plantes Cultivées, Montpellier, France*

[3]. *Centro di Studio per il Miglioramento Genetico e la Biologia della Vite – CNR, Grugliasco, Italy*

[4] *Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA), Finca El Encin, Alcala de Henares, Madrid, Spain*

[5] *Laboratory of Molecular Genetics, Istituto Agrario di San Michele all'Adige, Trento, Italy*

[6] *Istituto Sperimentale per la Viticoltura, Sezione Ampelografia e Miglioramento Genetico, Susegana (TV), Italy*

[7] *Department of Viticulture and Enology, University of California, Davis, USA*

[8] *HBLA u. BA Klosterneuburg, Langenzersdorf, Austria*

[9] *Instituto Portugues de Viticultura e Enologia (IPVE), Maia, Portugal*

[10] *Instituto de Ciencias Biomedicas de Abel Salazar (ICBAS), Universidade do Porto, Porto, Portugal*

[11] *Bundesanstalt für Züchtungsforschung an Kulturpflanzen (BAZ), Institut für Rebenzüchtung (IRZ) Geilweilerhof, Siebeldingen, Germany*

[12] *University of Udine, Department of Crop Production and Agricultural Technology, Udine, Italy*

Introduction

The genus *Vitis* L. is a moderately diverse genus comprising about 40 species in Asia, about 20 species in North America and a single wild species – *Vitis vinifera* L. subsp. *sylvestris* (Gmelin) – in Europe. Around 7000 varieties of domesticated *Vitis vinifera* L. subsp. *vinifera* are estimated to exist worldwide (http://www.genres.de/vitis), from which less than 400 are of commercial importance (Galet 2000). Therefore, most of the genetic resources of grapevine currently survive only in germplasm collections.

The utilization of grapes for fruit, juice or wine has a long history of about 8000 years (McGovern *et al.* 2000). Owing to the ease of vegetative propagation, grapevine cultivars have been widely exchanged and spread into many areas of the world (Bassermann-Jordan 1923; Dion 1959; Fregoni 1991). Hence, many cultivar names are known, especially for old varieties of wide distribution (Alleweldt and Dettweiler 1992; http://www.genres.de/vitis), but a consensus on variety naming is mostly lacking. Therefore, the clarification of synonymy, homonymy and misnaming is still an important task in the ca. 130 grapevine collections existing worldwide, and the assessment of trueness-to-type of maintained accessions is a basic requirement for the rational management and use of grapevine genetic resources (Dettweiler *et al.* 2000a).

Characterization and identification of grapevine varieties are traditionally based on ampelography (from *"ampelos"*, grapevine and "*graphos*", description) which involves describing and comparing morphological characteristics such as those of shoot tips, leaves, fruit clusters and berries (Viala and Vermorel 1909; Galet 1990, 1991; IPGRI 1997; Anonymous 2002a, 2002b). Currently, this knowledge is restricted to a small number of specialists in the world (Dettweiler 1991). However, expression traits are influenced by the interaction of environmental factors, plant biology and individual life history. Furthermore, juvenile plants are nearly impossible to identify, as they do not show the typical morphological traits of adult plants. Some cultivars which are related by parentage are morphologically very similar and difficult to differentiate by visual comparison (Loureiro *et al*. 1998; Borrego *et al.* 2002; Bowers *et al.* 1999a). On the other hand, intra-varietal clones can considerably differ in phenotype while showing broadly identical morphological "fingerprints" (Franks *et al.* 2002; Riaz *et al.* 2002).

To overcome these limitations, several types of molecular markers have been applied to the differentiation, characterization and identification of grapevines (Reisch 1998; Meredith 2001). Most grapevine varieties were originally bred from single seeds, each characterized by an individual mixture of parental alleles accidentally recombined during the meiosis process. For the characterization of these seedling-born varieties, the polymorphic, stable and robust simple sequence repeat (SSR) markers are the best choice, since the co-dominant allelic profiles at defined microsatellite loci strongly reflect Mendelian laws of allelic inheritance for diploid organisms with a high degree of methodical reproducibility (Sefc *et al.* 2001; Aradhya 2003).

In 1998, within the scope of the project GENRES 081 "European Network for Grapevine Genetic Resources Conservation and Characterization" (Dettweiler *et al*. 2000b), the international partners considered the project as an appropriate platform to: (1) test different modes of microsatellite DNA profiling for general comparability and reproducibility; (2) investigate standardization of methods and definitions of descriptors; and (3) implement a uniform, universally useful SSR marker database for variety identification purposes. At that time, the six most informative markers VVS2, VVMD5, VVMD7, VVMD27, VrZAG62 and VrZAG79 were selected. All participating partners worked on the same plant material. Three circular tests were carried out. Owing to the many different types of laboratory equipment and individually adapted protocols, any standardization of the polymerase chain reaction (PCR) protocols was not considered practicable to implement.

Materials and methods

• *Variety selection*

The laboratories of ten international partners (Table 1) carried out analyses of 50 different grapevine accessions (Table 2). Variety selection and the number of grapevines to be comparatively analyzed reflect the evolution of the microsatellite project within GENRES 081 (This and Dettweiler 2003; http://www.genres.de/vitis/) following three international workshops held in 1998, 1999 and 2001.

First analyses were limited to five regionally important varieties in order to get a preliminary impression of data consistency (Table 2, analysis step 1). This group of varieties was expected to be rather distinct in allelic profiles because of the varieties' different geographical origins.

To reach better coincidence of results in a second step, analyses on the first variety group were repeated, including another ten widespread, well-known grapevine cultivars and one frequently used rootstock variety (Table 2, analysis step 2) in order to reflect a more complete range of existing alleles for each locus.

To complete the allelic ladders for the six microsatellite loci in a third step, 35 additional accessions (34 varieties) including 18 rootstock varieties (Table 2, analysis step 3) were selectively picked out and added. This pre-selection focused on the published database of Sefc *et al.* (2000) and unpublished data of E. Zyprian, IRZ Geilweilerhof and C. Meredith, University of California.

Table 1. Microsatellite research group within GENRES 081, partner numbers according to their listing in the project (http://www.genres.de/vitis/)

GENRES 081 Project partners	Country	Name	Address
Partner 1	Germany	Jung A., Maul E.	BAZ Institut für Rebenzüchtung (IRZ) Geilweilerhof, D-76833 Siebeldingen
Partner 2	France	This P.	INRA - Génétique de la Vigne, UMR Diversité et Génome des Plantes Cultivées, 2, Place P. Viala, F-34060 Montpellier
Partner 3	Austria	Regner F., Eisenheld C.	HBLA u. BA Klosterneuburg, Rehgraben 2, A-2103 Langenzersdorf
Partner 5	Spain	Borrego J., Ibañez J.	Instituto Madrileno de Investigacion Agraria y Alimentaria (IMIA), Finca El Encin, Apdo 127, S-28800 Alcala de Henares (Madrid)
Associated to Partner 8	Portugal	Ferreira Monteiro F.	Instituto Portugues de Viticultura e Enologia (IPVE), Rua Eng. Frederico Ulrich 2650, P-4470-605 Maia
		Magalhaes R.	Instituto de Ciencias Biomedicas de Abel Salazar (ICBAS), Universidade do Porto, Largo Prof. Abel Salazar, 2, P-4099-003 Porto
Partner 9	Italy	Crespan M., Milani N.	Istituto Sperimentale per la Viticoltura Sez. Ampelografia e Miglioramento Genetico, Via Casoni 13/A, I-31058 Susegana (TV)
Partner 10	Italy	Botta R., Boccacci P.	Centro di Studio per il Miglioramento Genetico e la Biologia della Vite – CNR, Via Leonardo da Vinci 44, I-10095 Grugliasco
Partner 11	Italy	Grando M.S., Costantini N.	Laboratory of molecular genetics, Istituto Agrario di San Michele all'Adige, Via Mach 1, I-38010 Trento
Partner 12	Italy	Peterlunger E., Zulini L.	University of Udine, Department of Crop Production and Agricultural Technology, Via delle Scienze 208, I-33100 Udine
External partner	USA	Meredith C., Dangl G.	Department of Viticulture and Enology, University of California, One Shields Avenue, Davis, California 95616 USA

- ***Confirming trueness-to-type of varietal DNA samples***

To prevent possible confusion concerning variety identity (Dettweiler *et al*. 2000a) it was important to guarantee the singularity of source for each varietal DNA sample in examination. For the first and second steps, fresh young leaves of the selected grapevine varieties were centrally collected in one of the partners' grapevine collections (Table 2). DNA samples were prepared by single delegated partners and distributed to all the others. For the third step, late winter cuttings from INRA-Domaine de Vassal were sent to each partner's laboratory for local DNA extraction. DNA samples of all accessions were sent to the external partner because of US quarantine restrictions. This ensured that all ten partners worked on uniform DNA samples extracted from identical plant material.

- ***DNA extraction***

DNA was generally isolated from finely powdered leaf or wood cambium tissues frozen in liquid nitrogen and ground in a mortar. Partners (Table 1) performed different DNA extraction methods using adapted standard protocols or commercial DNA extraction kits as follows: protocols according to Doyle and Doyle (1990) with an additional RNAse A-digestion step (partner 11), Doyle and Doyle (1990) modified by Cipriani and Morgante (1993) (partner 12), Thomas and Scott (1993) (partner 10), Thomas and Scott (1993) without initial step (partner 3), Bowers *et al.* 1996 (external partner), Crespan *et al*. 1999 (partner 9), Ferreira Monteiro *et al.* 2000 (partner 8) or according to the protocol for DNeasy Plant Mini Kit from Qiagen GmbH, Hilden, Germany (partners 1 and 5), partner 2 with addition of 1% Polyvinylpyrrolidone 40 (Sigma-Aldrich, Mi, USA) to AP1-buffer doubling the amount of AE-buffer for elution.

- ***Marker selection***

In 1998, a still manageable number of already detected microsatellite loci were available. At that time, VVS, VVMD and VrZAG markers were the most recognized and had already been introduced to microsatellite analysis of grapevine cultivars (Cipriani *et al.* 1994; Thomas *et al.* 1994; Botta *et al.* 1995; Bowers *et al.* 1996; Regner *et al*. 1996; Sefc *et al*. 1997). Six polymorphic microsatellite loci were mathematically supposed to be the necessary minimum for diploid grapevine variety classification. According to the partners' appraisal, the six microsatellite markers VVS2 (Thomas and Scott 1993), VVMD5, VVMD7 (Bowers *et al.* 1996), VVMD27 (Bowers *et al.* 1999b), VrZAG 62 and VrZAG79 (Sefc *et al.* 1999) were chosen for analysis. Their special qualification for global utilization and general recommendation in grapevine variety characterization still had to be verified.

- ***PCR conditions***

According to partners' preferences for different brands of Taq DNA polymerases and thermal cyclers, various individual strategies for optimization and generalization of PCR conditions were embarked upon. PCR mixes (Table 3) and cycling strategies (Table 4) differed widely. Four partners used a Hot-Start PCR to avoid fluctuating banding patterns. Most partners preferred a 3-step cycling routine, three partners used differing 2-step PCR regimes, and a single partner performed a touch-down PCR. Some partners generalized primer-specific annealing temperatures to reduce the number of cycling programmes.

Table 2. Grapevine cultivar accessions used for microsatellite DNA profiling

Analysis step	Variety name	WIEWS institute code*	True-to-type	Local accession number	Code of reference cultivar
1	Furmint B	DEU098	yes	52-09-034	
1	Merlot noir N	DEU098	yes	52-07-045	ME
1,2	Sultanina Gigas B	DEU363	yes	10|14	SU
1	Touriga nacional N	DEU098	yes	52-10-006	
1	[*Trebbiano Toscano*] B	DEU098	**no**	52-10-019	
2	Barbera N	ITA360	yes	CVT424	BA
2	Cabernet franc N	FRA139	yes	324Mtp39	CF
2	Cabernet Sauvignon N	ITA362	yes	304	CS
2	Chardonnay B	ITA339	yes	-	CH
2	[*Kober5BB*] N	AUT024	**no**	IX-10-75	
2	Merlot noir N	ITA339	yes	-	ME
2	Muscat blanc à petits grains B	FRA139	yes	555Mtp22	MU
2	Pinot noir N	ITA362	yes	1560	PI
2	Silvaner B	AUT024	yes	IV-7-12	SI
2	Traminer rot RG	DEU098	yes	52-03-007	TR
3	Admirable de Courtiller B	FRA139	yes	814Mtp1	
3	Agiorgitiko B	FRA139	yes	1816Mtp2	
3	Alvarelhao N	FRA139	yes	1481Mtp2	AL
3	Carignan noir N	FRA139	yes	18Mtp8	
3	Castel 216-3	FRA139	yes	9017Mtp3	
3	Couderc 1616	FRA139	yes	9039Mtp1	16C
3	Couderc 3309 N	FRA139	yes	9043Mtp4	33C
3	Fercal N	FRA139	yes	9219Mtp2	FE
3	Goethe 9	FRA139	not confirmed	9000Mtp537	GO
3	Hans RG	FRA139	yes	1595Mtp1	
3	Jacquez	FRA139	yes	5000Mtp69	JA

Table 2 (cont.). Grapevine cultivar accessions used for microsatellite DNA profiling

Analysis step	Variety name	WIEWS institute code*	True-to-type	Local accession number	Code of reference cultivar
3	Kober 5 BB N	FRA139	yes	9171Mtp1	
3	Madeleine Royale B	FRA139	yes	653Mtp1	MAR
3	Malègue 44-53	FRA139	yes	9081Mtp3	4MA
3	Mancin N	FRA139	yes	1216Mtp1	MAN
3	Mauzac blanc B	FRA139	yes	443Mtp14	MAU
3	Mavrodaphni N	FRA139	yes	1800Mtp3	
3	Millardet et Grasset 101-14 N	FRA139	yes	9095Mtp1	1MG
3	Millardet et Grasset 420 A	FRA139	yes	9122Mtp3	4MG
3	Mourvèdre N	FRA139	yes	64Mtp2	
3	Muscat of Alexandria B	FRA139	yes	308Mtp2	
3	Paulsen 1103	FRA139	yes	9003Mtp1	
3	Portugieser blau N	FRA139	yes	450Mtp1	PO
3	Richter 110	FRA139	yes	9159Mtp2	11R
3	Richter 99	FRA139	yes	9157Mtp3	99R
3	Romorantin B	FRA139	yes	304Mtp8	RO
3	Rondinella N	FRA139	yes	1295Mtp1	
3	Ruggeri 140	FRA139	yes	9001Mtp1	
3	Salvador N	FRA139	yes	5026Mtp4	SAL
3	Saperavi N	FRA139	wrong samples	1734Mtp2	
3	Schwarzmann	FRA139	yes	9221Mtp1	SCH
3	Teleki 5 C	FRA139	yes, one wrong sample	9179Mtp3	5C
3	Veltliner rot RG	FRA139	yes	284Mtp4	VE
3	Vialla N	FRA139	yes	9005Mtp1	VIA
3	Vital B	FRA139	yes	2103Mtp1	VI

* http://apps3.fao.org/wiews/institute_query.htm

Table 3. PCR mixes

	Partner									
	1	**2**	**3**	**5**	**8 ass.**	**9**	**10**	**11**	**12**	**Ext. Part.**
Reacting Volume (µl)	25	25	20	20	20	25	20	25	10	20
DnA [ng]	20	20	50	50	10	10	50	50-60	20	10
DnA Polymerase [U]	1 HotStart Taq2	0.8 Taq	TAQ Biotherm1	0.5 Biotools DnA Polymerase5	0.5 Taq6	0.5 Taq4	0.5 AmpliTaq Gold3	0.5 AmpliTaq Gold3	0.5 AmpliTaq Gold3	0,5 AmpliTaq Gold3
PCR buffer	10x PCR buffer2	1x PCR buffer2	1x PCR buffer	10 mM Tris-HCl pH 8.3 50mM KCl	10x PCR buffer	10 mM Tris-HCl pH 9 50 mM KCl. 0.01% (w/v) gelatin. 0.1%tritom X –100	10x GeneAmp PCR buffer 3	1x GeneAmp PCR Buffer II3	10 mM Tris-HCl pH 8.3 50mM KCl	10X PCR Gold buffer3
$MgCl_2$[mM]	1.5	2.5	1,5	2.0	1	1.5	1.5	1.5 (2 for VVMD5)	2.5	2.0
Each primer [µM]	0.4 (0.8 for VVMD5)	0.4	0,5	0.2	0.2	0.8	0.5	0.25	0.25	1.0
Forward Primer dye labelling	Unmodified	Unmodified	Fluorescent dye phosphor-amidites	Fluorescent dye phosphor-amidites5	Unmodified	Unmodified	Fluorescent dye phosphor-amidites5	Fluorescent dye phosphor-amidites5	γ33 P-ATP labelled	Unmodified
Each dnTP [µM]	200	200	100	200	200	200	200	200	200	200
Additives		-	Mineral-oil			Mineral oil				Mineral oil

[1] Genxpress, Maria Wörth, Austria [2] Qiagen GmbH, Hilden, Germany [3] Applied Biosystems, Applera Corporation, USA [4] HT Biotechnology, Cambridge, UK
[5] BIOTOOLS Biotechnological & Medical Laboratories, S.A. Madrid, Spain [6] Invitrogen, USA

Table 4. PCR cycle regimes

	Partner									
	1	**2**	**3**	**5**	**8 ass.**	**9**	**10**	**11**	**12**	**Ext. Part.**
Thermal cycler	GeneAmp PCR System 97003	PTC-1002	Hybaid Omnigne1	PTC-1002	Sanyo MIR-D304	PTC-1002	PTC-1002	GeneAmp PCR System 96003, 97003,	GeneAmp PCR System 97003	DNA Thermo-cycler 4803
PCR cycle strategy	3-step	3-step	2-step 3-step for VVMD7 VVMD27	3step	3-step 2-step for VVMD5 VVMD27	2-step	3-step	3-step	Touch down (T.d.)	3-step
Number of cycles	28 x	35 x	40 x	40 x	40 x	10x + 23x	26 x	30 x	9x + 21x VVS,ZAG-13x + 17x VVMD	40 x
Initial denaturation	20 min 95°C	4 min 94°C	2 min 92°C	5 min 95°C	2 min 94°C	5 min 95°C	9 min 95°C	12 min 95°C	10 min 95°C	2 min 92°C
Denaturation	60 sec 94°C	60 sec 94°C	15 sec 90°C	45 sec 94°C	60 sec 92°C	15 sec 94°C (10x) 15sec 89°C (23x)	30 sec 95°C	45 sec 94°C	30 sec 95°C T.d. 45sec 94°C	60 sec 92°C
Annealing VVS2 VVMD5 VVMD7 VVMD27 VrZAG62 VrZAG79	50 sec 56°C VVS2: 52/48°C VrZAG79 50°C	60 sec 56°C	15 sec 50°C	60 sec 50°C	60 sec 52°C VVMD5 VVMD27 56°C	15 sec 50°C	45 sec 52°C	30 sec 51°C	T.d. 30 sec 65 //// 57°C T 45 sec 56°C (21x) VVS,ZAG T.d. 30 sec 65 //// 53°C 45 sec 52°C (17x) VVMD	60 sec VVS2. VVMD7 VrZAG79 : 52°C VVMD5, VVMD27 VrZAG62 : 56°C
Extension	60 sec 72°C	60 sec 72°C	15 sec 72°C (VVMD7, VVMD27)	90 sec 72°C	120 sec 72°C		60 sec 72°C	90 sec 72°C	30 sec 72°C	120 sec 72°C
Final elongation Abrupt cooling	30 min 72°C	6 min 72°C	5 min 72 °C	none	30 min 72°C	>10 min 8°C	45 min 72°C	7 min 72°C	7 min 72°C	7 min 72°C

[1] Hybaid UK [2] MJ Research Inc., MA, USA [3] Applied Biosystems, Applera Corporation, USA [4] SANYO Electric Biomedical Co., Ltd

- ***Fragment separation and size determination***

Manual and automated systems were used for the separation and determination of PCR fragments (Table 5): six partners separated amplification products manually by high voltage electrophoresis on vertical, denaturing polyacrylamide (PAA) sequencing gels (5-6% polyacrylamide, 7-8 M urea). Fragments were visualized by silver-staining procedures founded on standard protocols (according to Sambrook *et al.* 1989, manual of Silver Sequence DNA sequencing System, Promega Corporation, WI, USA or according to Crespan and Milani 2001, following Bassam *et al.* 1991 and Tixier *et al.* 1997 using NaOH instead of $NaCO_3$).

One partner used γ^{33}P-ATP labelled primers detecting PCR fragments on autoradiography film after 1-7 days of exposure to fragment radiation (reaction mix at 37°C, 35 min: 2.5 µM primer f, 0.4-0.52 pmol γ^{33}P-ATP at 2500 Ci/mmol, 70 mM Tris HCL, 5 mM $MgCl_2$, 0.5 mM DTT 0.4 U T4 polynucleotide kinase).

Automated fragment separation was done on single capillary or gel-based electrophoresis systems in ABI Prism Genetic Analysers (Applied Biosystems, Applera Corporation, USA). Adapted amounts of denatured PCR fragments labelled with fluorescent dye phosphoramidites were detected by the system's lasers. The fluorescent marker emissions were analyzed by GeneScan Software 2.1 (Applied Biosystems, Applera Corporation, USA) using internal-lane size standards (ROX or TAMRA) and system's Local Southern Method for automatic size calling of emissive peak positions.

Determination of fragment sizes visually was facilitated by using previously labelled PCR fragments of known cultivars as internal size standards in addition to commercial weight markers. Automatic peak labelling by GeneScan Software needed some additional control by visual inspections of individual peak positions, rounding up or down the decimal variations to reach integer size numbers. Already-labelled peak profiles of reference cultivars were helpful for decision-making. One partner applied the mathematical algorithm of Ghosh *et al.* (1997) (not applicable to VVS2), using the common average value of identical fragments to direct the algorithmic rounding of decimal variations into same integer numbers.

Results

Despite the care taken, some problems occurred concerning variety identity. Comparison of fingerprints with already existing data revealed that the accessions called 'Trebbiano Toscano' and 'Kober 5BB' (in the first step) were misnamed. DNA extracted from winter cuttings of 'Saperavi' did not show the reported allelic profile, indicating an error when collecting fresh plant material (Table 2). The same mistake happened to a 'Teleki5C' sample. As a consequence, all data referring to these confused samples were excluded from the analysis.

- ***Comparison of numerical allele sizes***

Due to the diploid and highly heterozygous character of the *Vitis vinifera* L. genome organization, two distinct alleles can be expected at each polymorphic sequence tagged microsatellite site (STMS) locus. Their numerical sizes are commonly measured in base pairs (bp) as lengths of amplified DNA fragments.

Table 5. Systems used for fragment separation and size calling

	Partner									
	1	**2**	**3**	**5**	**8 ass.**	**9**	**10**	**11**	**12**	**Ext. Part.**
Scoring	Manual	Manual	ABI PRISM 373A1	ABI PRISM 310 1	Manual	Manual	ABI PRISM 377 1	ABI PRISM 310 1	Manual	Manual
Electrophoresis	PAA gel (6%)	PAA gel (6%)	System's PAA gel (6%)	Single capillary POP4	PAA gel (6%)	PAA gel (5%)	System's PAA gel (6%)	Single Capillary POP4	PAA gel (6%)	PAA gel (6%)
Fragment visualization	Silver staining2	Silver staining2	Laser detection, GeneScan1 Computer analysis	Laser detection, GeneScan1 Computer analysis	Silver staining2	Silver staining	Laser detection, GeneScan1 Computer analysis	Laser detection, GeneScan1 Computer analysis	Amersham Hyperfilm MP3	Silver staining2
DNA Weight markers, Internal-Lane size Standards	PBR322 x TaqI x HaeIII, weight marker VIII5, XIII	AFLP DNA-Ladder4 (30-300 bp)	TAMRA 3501	TAMRA 5001	10 bp ladder6	none	ROX-GS 3501	TAMRA 5001	Plasmid PUC18, primer M13 labelled with γ33 P-ATP	none (allele sizes of reference cultivars, determined initially by comparison to a sequencing reaction)
Adjustment of allele sizes	Fragments of selected reference varieties used as primer specific allelic ladders all 6 lanes	Home-made ladder of already identified PCR fragments	Rounding after visual inspection of peak position according to known alleles of reference cultivars	Automated binning of alleles according to algorithm of Gosh *et al.* 1997	Known standard all 10 lanes	Reference cultivars of known allele sizes all 15-20 lanes Scoring was done twice		Visual adjustment of peak size (+/- 0.7 bp) according to known allele sizes of reference cultivars		Reference cultivars of known allele sizes Scoring was done twice

[1] Applied Biosystems, Applera Corporation, USA [2] Promega Corporation, WI, USA [3] Amersham Biosciences [4] Invitrogen, CA, USA
[5] Roche Applied Science, Mannheim, Germany [6] GibcoBRL

Comparing the partners' results for each DNA sample at the six SSR loci (Table 6), there was not a fully satisfactory coincidence with regard to the numerical determination of fragment lengths. Even though some partners' data showed more similarities than others, labelling modes for identical fragments differed by up to 8 bp from each other (VVS2). Depending on the marker, these differences were more or less expressed (e.g. for VVMD7, VVMD27, VrZAG62, VrZAG79 only a switch of 3 bp), but generally data sets could not be compared directly without an additional transformation procedure.

Looking at the relative distances between corresponding fragments, data consistency was better for a majority of partners, but nevertheless it was still rather irregular; deviant shifts in relative distances also occurred, most frequently observed in the samples at VVS2 and VVMD5 (Table 6).

Consequently, the first problem to solve was to harmonize the partners' individually differing labelling modes. The "correct" allele size (n) was defined by a majority of partners with coincident results. Depending on the marker, this major group comprised 3 to 7 partners (not always the same ones).

Two possible methods for better data harmonization were discussed: the first was to label the fragments objectively by their effective number of base pairs counted out by a sequencing procedure. This would result in the definition of true fragment lengths, which all partners had to accept. This solution implied the conversion of 10 individual sizing procedures to one single but objective standard. The second, easier and cheaper possibility was to create a standardized codification system for uniform allele sizing. By using variety-specific PCR fragments as universal length standards for each allele, it was assumed that it should be possible to replace the varying numeric allele sizes by primer-specific variety codes, finally resulting in the broadly completed allelic ladders for each of the six microsatellite loci. This could lead to the creation of a homogenous, multiply-confirmed and generally accepted codification system.

- ***Transforming numerical data into reference cultivar-based allele codes***

To avoid fundamental changes in already established measurement systems, the second option was followed. Variety-specific PCR fragments of different lengths were selectively picked as reference alleles and grouped according to increasing size. By definition, the shortest detected allele at each locus was called "n". All longer fragments were sized n+x, according to their size relative to this smallest allele n. By defining a fixed starting point for relative scaling, all distinct alleles could easily be related to each other by relative distances, thus creating initial allelic ladders for each of the six markers. Detected reference alleles were named according to the varieties they belong to and codified as shorter fragment 1 or longer fragment 2 of a corresponding allele pair. If there were several suitable cultivars, preferably widespread, well-known varieties were selected for the status of reference cultivars, trying to reduce the number of reference cultivars to the minimum by looking at their aptitude for multiple marker representation. In cases of singular detected alleles, only rare or poorly known varieties could be taken as referential candidates. The inclusion of rootstock varieties into the third step of analysis was necessary because of the high number of unique, newly emerged alleles in interspecific hybrids. All those newly detected alleles had to be represented by newly supplemented varieties to be tested for reference qualification, but finally the incorporation of interspecific hybrids resulted in the near completion of the gaps within the allelic ladders for each microsatellite locus.

Table 6. Five variety examples for differences in the determination of numerical allele sizes between partners. The field of a majority of partners with identical results is highlighted in bold; deviant and erratic differences in relative distances between alleles not congruent to regular shifting are emphasized with a grey background.

VVS2	**Data set 1**		**Partners' majority data set**		**Data set 3**		**Data set 4**		**Data set 5**		**Data set 6**		**Data set 7**	
Admirable de Courtiller	130	134	**133**	**137**	133	137	133	137	134	138	135	139	138	144
Agiorgitiko	140	142	**143**	**145**	143	145	**144**	**146**	144	146	145	147	**140**	**148**
Alvarelhao	130	**149**	**133**	**151**	133	151	133	**152**	134	152	-	-	**130**	**134**
Carignan	140	142	**143**	**145**	**145**	145	**144**	**146**	144	146	145	147	134	134
Castel 216-3	134	138	**137**	**141**	137	**142**	137	**142**	138	142	139	143	**134**	**148**
Regular shifts of size (bp)	-3		n		n		n		+1		+2		+5	
Irregular size differences		-2				-2/+1		+1						???
[number of partners]		[2]		[3]		[1]		[1]		[1]		[1]		[1]

VVMD5	**Data set 1**		**Data set 2**		**Data set 3**		**Data set 4**		**Partners' majority data set**		**Data set 6**	
Admirable de Courtiller	223	233	223	233	225	235	226	236	**226**	**236**	228	238
Agiorgitiko	**227**	237	229	237	231	239	232	240	**232**	**240**	234	242
Alvarelhao	219	223	219	223	221	225	222	226	**222**	**226**	224	228
Carignan	223	225	223	225	225	227	226	228	**226**	**228**	228	230
Castel 216-3	233	265	233	**263**	235	267	236	**266**	**236**	**268**	238	270
Regular shifts of size (bp)	-3		-3		-1		n		n		+2	
Irregular size differences		-5		-5				-2				
[number of partners]		[1]		[1]		[2]		[1]		[4]		[1]

VVMD7	**Data set 1**		**Data set 2**		**Partners' majority data set**		**Data set 4**	
Admirable de Courtiller	237	241	238	242	**239**	**243**	240	244
Agiorgitiko	241	247	242	248	**243**	**249**	244	250
Alvarelhao	237	237	238	238	**239**	**239**	240	240
Carignan	237	237	238	238	**239**	**239**	240	240
Castel 216-3	249	259	250	260	**251**	**261**	252	262
Regular shifts of size (bp)	-2		-1		n		+1	
Irregular size differences								
[number of partners]		[1]		[1]		[7]		[1]

Table 6 (cont.). Five variety examples for differences in the determination of numerical allele sizes between partners. The field of a majority of partners with identical results is highlighted in bold; deviant and erratic differences in relative distances between alleles not congruent to regular shifting are emphasized with a grey background.

VVMD27	**Data set 1**		**Data set 2**		**Data set 4**		**Data set 3**		**Data set 5**		**Partners' majority data set**		**Data set 7**		**Data set 8**	
Admirable de Courtiller	183	**191**	183	**191**	184	**194**	184	193	185	**193**	**185**	**194**	185	**195**	186	195
Agiorgitiko	173	183	173	183	174	184	174	184	175	185	**175**	**185**	175	185	176	186
Alvarelhao	183	187	183	187	-	-	184	188	185	189	**185**	**189**	185	189	186	190
Carignan	179	183	179	183	180	184	180	184	181	185	**181**	**185**	181	185	182	186
Castel 216-3	204	207	204	208	206	210	206	210	207	211	**207**	**211**	207	211	208	212
Regular shifts of size (bp)	-2		-2		-1		-1		n		n		n		+1	
Irregular size differences		-3/-4		-3		0				-1				+1		
[number of partners]		[1]		[1]		[1]		[1]		[1]		[3]		[1]		[1]

VrZAG62	**Data set 1**		**Data set 2**		**Partners' majority data set**		**Data set 4**		**Data set 5**	
Admirable de Courtiller	187	193	188	194	**189**	**195**	189	195	190	196
Agiorgitiko	199	201	200	202	**201**	**203**	**203**	203	202	204
Alvarelhao	187	193	188	194	**189**	**195**	189	195	-	-
Carignan	185	187	186	188	**187**	**189**	187	189	188	190
Castel 216-3	189	195	190	196	**191**	**197**	191	197	192	198
Regular shifts of size (bp)	-2		-1		n		n		+1	
Irregular size differences								+2		
[number of partners]		[2]		[3]		[3]		[1]		[1]

VrZAG79	**Data set 1**		**Data set 2**		**Partners' majority data set**		**Data set 4**		**Data set 5**	
Admirable de Courtiller	249	255	250	256	**250**	**256**	251	257	252	258
Agiorgitiko	245	245	246	246	**246**	**246**	247	247	248	248
Alvarelhao	249	257	250	258	**250**	**258**	251	259	252	260
Carignan	249	257	250	258	**250**	**258**	251	259	252	260
Castel 216-3	253	261	254	**260**	**254**	**262**	255	263	256	264
Regular shifts of size (bp)	-1		n		n		+1		+2	
Irregular size differences				-2						
[number of partners]		[1]		[1]		[4]		[3]		[1]

Table 7 shows the results of this codification procedure. The differences in size between the shortest and the longest alleles varied from n+26 bp at VrZAG79 up to n+46 bp at VVMD5 and VrZAG79. The number of detected alleles within the allelic ladders of each locus ranged from a minimum of 13 detected alleles for VrZAG79 to a maximum of 23 alleles for VVS2. For *Vitis vinifera* L. cultivars generally 2-bp shifts from one allele to another occurred except for a single 3-bp-step to the reference allele coded MU2 at VVMD27. Including the rootstock varieties, shifts within the allelic ladders of only +/-1 bp additionally occurred for the two adjacent alleles of GO1 at VVMD27. For VrZAG62 a single 1-bp-step can be mentioned from 1MG1: to 4MA1. Only for VVS2 could the nearly complete allelic range of n to n+34 be represented by at least the allele of one reference cultivar. For the other five microsatellite loci some gaps within the marker-specific allelic ladders could not be covered by suitable true-to-type cultivars. Especially some of the still missing, but hypothetically existing fragments of VrZAG and VVMD loci could not be detected in the scope of this work. Some of these supposed alleles are preliminarily announced to exist in varieties of doubtful identity, therefore no true-to-type accession could be proposed for reference status.

Table 7. Allelic ladders for each of the six markers coded by the alleles of selected reference varieties according to their relative distance to the shortest detected allele n. Shifts of only 1 bp are emphasized with a grey background.

VVS2		VVMD05		VVMD07		VVMD27		VrZAG62		VrZAG79	
n	33C1	n	AL1	n	FE1	n	CS1	n	1MG1	n	RO1
n+2	VIA1	n+4	CF1	n+2	MU1	n+4	MU1	n+1	4MA1	n+2	PI1
n+4	4MG1	n+6	MU1	n+4	VIA1	n+6	CF1	n+6	4MA2	n+6	CH1
n+6	RO1	n+8	MAU1	n+6	JA1	n+8	FE1	n+8	33C1	n+8	CH2
n+8	VE1	n+10	TR1	n+8	CF1	n+10	PI1	n+10	FE1	n+10	CF1
n+10	BA1	n+12	CH1	n+12	TR1	n+11	GO1	n+12	MU1	n+12	SI1
n+12	BA2	n+14	MU2	n+14	33C1	n+12	VIA1	n+14	CH1	n+14	TR2
n+14	CH1	n+16	CH2	n+16	ME2	n+14	CS2	n+16	33C2	n+16	VI2
n+16	CF1	n+18	CF2	n+18	MU2	n+16	ME2	n+18	VE1	n+18	MU2
n+18	16C2	n+22	JA2	n+20	FE2	n+18	4MG1	n+20	CF1	n+20	4MA1
n+20	CH2	n+24	VE2	n+22	SU2	n+19	MU2	n+22	CH2	n+22	CF2
n+22	SU1	n+30	33C1	n+24	PO2	n+20	16C1	n+24	JA2	n+24	4MA2
n+24	CF2	n+34	1MG1	n+26	TR2	n+22	1MG1	n+26	5C1	n+26	99R2
n+26	99R2	n+40	GO1	n+28	33C2	n+26	SAL2	n+28	SCH2		
n+28	SI1	n+42	33C2	n+30	99R2	n+28	5C1	n+30	CF2		
n+30	SI2	n+44	1MG2	n+32	CF2	n+30	4MA1	n+36	5C2		
n+32	MaR2	n+46	11R2	n+34	5C1	n+32	1MG2	n+40	11R2		
n+34	MAN2					n+34	VIA2	n+46	FE2		
n+38	33C2					n+36	16C2				
						n+38	SCH2				
						n+40	4MA2				
						n+42	4MG2				
						n+44	16C2				
19 alleles		17 alleles		17 alleles		23 alleles		18 alleles		13 alleles	

From the evidence, rootstock varieties frequently displayed characteristic allele clusters not congruent to those already detected in European *Vitis vinifera* L. varieties. These alleles might be used to discriminate the wild *Vitis* species from each other, but further investigations into the uniqueness of certain alleles in *Vitis* species are highly recommended.

- ***Comparison of codified data sets***

One intention of the microsatellite project within GENRES 081 was to test the general comparability and reproducibility of microsatellite analysis data produced by different partners under varying local laboratory conditions. If the method of characterizing grapevine varieties by microsatellite fragment analysis is to work universally and independently when using different analysis systems and laboratory equipment, each analysis of identical DNA samples must produce identical allelic profiles.

The standardized codification of numerical data into uniform reference cultivar-based allele codes now allows immediate and direct comparison of all microsatellite data produced by ten partners for all 47 grapevine accessions at the six microsatellite loci.

Comparing coded data (not shown), it can be stated that not all partners succeeded in producing completely identical data sets as should be expected. Especially for the first step, mistakes in determining allele sizes were obviously due to beginner's problems. For the second step, data consistency improved, especially for the five repeated samples from step 1. For the third step, data consistency between most partners was satisfactory, even if it still was not 100% (Table 8).

Table 8. Consistency in partners' data sets: each allele of 44 varieties was compared between each partner

Locus	Data concordance (%)
VVS2	98.8
VVMD5	97.2
VVMD7	98.0
VVDM27	98.6
VrZAG62	96.4
VrZAG79	96.2
Mean value	**97.5**

Excluding missing data from the calculations and only referring to the revised data sets of the second and third steps, 97.5% of the data (4487 alleles out of 4600) were completely identical among the partners. Data consistency was especially high for VVMD7 and VVMD27, which are known to be robust, solid markers. For VVS2, a high coincidence could also be reached eventually by using already known reference alleles for scoring purposes. The coincidence for the VrZAG markers was a little lower since overlapping stutter patterns may have complicated their correct size determination.

Generally all measured fragments (except those of the excluded 'Saperavi') could be verified by absolutely identical data codes by a majority of 7-10 partners, indicating the "correct" allele pairs for each variety at each locus (Table 9). Nevertheless, persistent minority opinions must be mentioned concerning the different interpretation of special fragment patterns. One of these special cases occurred for the allelic ladder of VVS2 whose allele sizes regularly increased by 2-bp steps for the majority of partners. However, a minority group mentioned a displacement of one allele indicated by a single 3-bp shift from n+22 to n+25, thus extending the scaling factors up to the amount of 1 bp. The codification process was not affected by this difference in interpretation as the number and ascending order of the reference alleles did not change (Table 10).

- ***Definition of OIV descriptors for the use of SSR markers***

Based on these multiply-confirmed results (Table 9), new descriptors for the six STMS markers were developed according to the layout of the "OIV Descriptor List for Grapevine Varieties and *Vitis* Species" (UPOV 1977; OIV 1983; IPGRI *et al.* 1997). The results of a distinct majority of partners were accepted as the basis for the general definition of uniform allele codes as recommended references. By definition, each separate reference allele x was related to the smallest detected allele n for each marker, building up an allelic ladder according to its relative distance n+x. If possible, for each reference allele, further example varieties were selected. For the status of reference cultivar, for preference, well-known and widespread varieties were proposed. To reduce the number of reference cultivars to the necessary minimum, the varieties were selected to prevent repeated representation of identical alleles by several references. But to cover the complete range of existing alleles for the six markers, up to 33 reference cultivars were necessary, because of the unique character of some alleles which were only detected in rare, singular varieties.

Fig. 1 shows the proposed OIV descriptor for VrZAG79, English version only.[22]

Discussion

- ***Choice of grapevine varieties***

As already indicated for European *Vitis vinifera* L. cultivars, different allele frequencies are often found in geographically distant areas, reflecting the separate evolutionary centres for family-based variety groups, indicating common descent and parental relationships (Sefc *et al.* 2000; Boursiquot *et al*. 2002; Aradhya 2003). The inclusion of interspecific rootstock varieties turned out to be reasonable, not only because of their general importance in viticulture, but also because of the high number of unique, newly detected alleles, obviously not existing in the single species genepool of pure *Vitis vinifera* L. varieties. Only these additional alleles permitted the construction of broadly completed allelic ladders for each of the six microsatellite loci, while also ensuring that the relative distances between the alleles could be determined. Further investigations on wild *Vitis* species not yet represented in the current study may reveal further new alleles.

[22] Full multilingual version available at http://www.genres.de/CF/eccdb/vitis/_descriptoren/stms_vrzag79.pdf. The five other SSR markers' proposed descriptors are also available at http://www.genres.de/CF/eccdb/vitis/_cfm/markers.cfm

Table 9. Codified data of 46 varieties according to the majority of partners

Variety name	Marker											
	VVS2:1	VVS2:2	VVMD5:1	VVMD5:2	VVMD7:1	VVMD7:2	VVMD27:1	VVMD27:2	VrZAG62:1	VrZAG62:2	VrZAG79:1	VrZAG79:2
Admirable de Courtiller B	BA1	CH1	CF1	MU2	CF1	TR1	PI1	MU2	CH1	CF1	TR2	4MA1
Agiorgitiko B	CH2	SU1	TR1	CF2	TR1	MU2	CS 1	PI1	5C1	SCH2	CF1	CF1
Alvarelhao N	BA1	SI1	AL1	CF1	CF1	CF1	PI1	CS2	CH1	CF1	TR2	CF2
Barbera N	BA1	BA2	CF1	CF1	MU2	SU2	PI1	CS2	VE1	5C1	CH1	CF2
Cabernet franc N	CF1	CF2	CF1	CF2	CF1	CF2	CF1	CS2	CF1	CF2	CF1	CF2
Cabernet Sauvignon N	CF1	SI1	TR1	CF2	CF1	CF1	CS1	CS2	CH1	CF1	CF1	CF1
Carignan noir N	CH2	SU1	CF1	MU1	CF1	CF1	CF1	PI1	MU1	CH1	TR2	CF2
Castel 216-3	CH1	16C2	MU2	11R2	FE2	99R2	1MG2	16C2	33C2	CH2	MU2	99R2
Chardonnay B	CH1	CH2	CH1	CH2	CF1	TR1	CF1	CS2	CH1	CH2	CH1	CH2
Couderc 3309 N	33C1	33C2	33C1	33C2	33C1	33C2	PI1	16C2	33C1	33C2	MU2	4MA1
Couderc 1616	CF1	16C2	33C2	11R2	CF1	FE2	16C1	16C2	33C2	33C2	MU2	4MA1
Fercal N	CH2	CH2	MU2	33C2	FE1	FE2	FE1	CS2	FE1	FE2	CH2	4MA1
Furmint B	BA1	SI2	CF1	CF2	CF1	MU2	MU1	MU2	CH1	CF2	RO1	SI1
Goethe 9	CH1	16C2	GO1	33C2	FE2	5C1	GO1	G02	SCH2	SCH2	4MA2	4MA2
Hans RG	VE1	BA1	CH1	VE2	MU2	SU2	FE1	CS2	VE1	CF2	TR2	TR2
Jacquez N	CF1	CH2	MU1	JA2	JA1	CF1	MU1	CS2	MU1	JA2	SI1	SI1
Kober 5 BB N	16C2	99R2	MU2	1MG2	MU1	5C1	ME2	16C2	5C1	11R2	TR2	CF2
Madeleine Royale B	SI1	MaR2	MU1	MU2	TR1	ME2	CF1	CS2	CH1	CF1	CH2	CF2
Malègue 44-53	CF1	SU1	33C1	33C2	MU1	CF1	4MA1	4MA2	4MA1	4MA2	4MA1	4MA2
Mancin N	CF1	MAN2	TR1	CH2	CF1	CF1	CS1	CS2	CH1	CF1	CF1	TR2
Mauzac blanc B	BA1	SI1	MAU1	TR1	CF1	MU2	PI1	ME2	CH1	5C1	TR2	TR2
Mavrodaphni N	CH2	CH2	CF1	TR1	CF1	CF1	FE1	CS2	MU1	CH1	CH1	CH2
Merlot noir N	CF1	SI1	CF1	MU2	CF1	ME2	CS2	ME2	CF1	CF1	CF2	CF2
Millardet et Grasset 101-14 N	BA1	CH2	1MG1	1MG2	TR1	FE2	1MG1	4MG2	1MG1	33C2	MU2	4MA1

Table 9 (cont.). Codified data of 46 varieties according to the majority of partners

Variety name	Marker											
	VVS2: 1	VVS2: 2	VVMD5: 1	VVMD5: 2	VVMD7: 1	VVMD7: 2	VVMD27: 1	VVMD27: 2	VrZAG62: 1	VrZAG62: 2	VrZAG79: 1	VrZAG79: 2
Millardet et Grasset 420 A	4MG1	CH1	CH2	33C2	FE1	CF2	4MG1	4MG2	33C2	CH2	MU2	MU2
Mourvèdre N	BA1	SI1	CF1	CF2	MU2	MU2	MU1	CS2	CH1	CF2	TR2	4MA2
Muscat of Alexandria B	BA1	99R2	MU1	TR1	MU2	FE2	MU1	MU2	MU1	CF2	CF1	MU2
Muscat à petits grains blancs B	BA1	BA1	MU1	MU2	MU1	MU2	MU1	MU2	MU1	CH2	TR2	MU2
Paulsen 1103	CH1	CF2	MU2	MU2	MU1	TR2	5C1	1MG2	CH2	11R2	TR2	99R2
Pinot noir N	CH1	SI1	MU1	CH2	CF1	TR1	PI1	CS2	CH1	CF1	PI1	CH2
Portugieser blau N	CH2	SI1	CF1	TR1	TR1	PO2	CF1	MU2	CH1	CF2	SI1	CF2
Richter 110	CH1	CH2	CH1	11R2	FE1	TR2	CS2	4MA1	CH2	11R2	CH1	CF2
Richter 99	CH1	99R2	MU2	MU2	FE1	99R2	ME2	1MG2	CH2	5C2	TR2	99R2
Romorantin B	RO1	BA1	CH1	CH2	TR1	MU2	MU1	CS2	CH1	CF2	RO1	CH2
Rondinella N	CH2	SI1	CF1	TR1	CF1	CF1	MU1	CS2	CH1	CF1	CF1	TR2
Ruggeri 140	CH1	CH2	VE2	11R2	FE1	TR2	CS2	4MA1	CH2	11R2	CH1	CF2
Salvador N	BA1	BA1	CF1	33C1	ME2	FE2	CF1	SAL2	CF1	CF1	CH1	CH2
Schwarzmann	CH1	SU1	33C1	1MG2	FE2	5C1	1MG2	SCH2	5C1	SCH2	MU2	4MA1
Silvaner B	SI1	SI2	CF1	TR1	TR1	ME2	CS2	MU2	CH1	CF2	SI1	TR2
Sultanina Gigas B	SU1	SI1	CH1	CH1	CF1	SU2	CF1	MU2	CH1	CH1	CF1	CF2
Teleki 5 C	BA1	CH2	33C1	1MG2	FE1	5C1	5C1	16C2	5C1	5C2	TR2	CF2
Touriga nacional N	CH2	SI1	CF1	MU2	CF1	CF1	CF1	CS2	CH1	CF1	CH2	CH2
Traminer rot RG	SI1	SI1	TR1	CH2	TR1	TR2	CS2	CS2	CH1	CF1	CH2	TR2
Veltliner rot RG	VE1	BA1	CF2	VE2	CF1	SU2	FE1	M U2	VE1	CH2	TR2	TR2
Vialla N	VIA1	BA2	1MG2	1MG2	VIA1	FE2	VIA1	VIA2	SCH2	SCH2	SI1	MU2
Vital B	SU1	SI1	AL1	CF2	CF1	CF1	CF1	MU2	CH1	CH1	CF1	VI2

Table 10. Differences in interpretation of scaling factors between reference alleles of VVS2 with a 3-bp shift n+22 / n+25 as minority vote

Cultivar	Majority		Minority		Reference Codes	
Barbera N	n+10	n+12	n+10	n+12	BA1	BA2
Chardonnay B	n+14	n+20	n+14	n+20	CH1	CH2
Cabernet franc N	n+16	n+24	n+16	n+**25**	CF1	CH1
Cabernet Sauvignon N	n+16	n+28	n+16	n+**29**	CF1	SI2
Touriga national N	n+20	n+28	n+20	n+**29**	CH2	SI1
Sultanina B	n+22	n+28	n+22	n+**29**	SU1	SI2
Traminer RG	n+28	n+28	n+**29**	n+**29**	SI1	SI1
Silvaner B	n+28	n+30	n+**29**	n+**31**	SI1	SI2

Characteristic:	**SSR marker VrZAG79**		Code No OIV
Primer sequence:	VrZAG79a: AGA TTG TGG AGG AGG GAA CAA ACC G VrZAG79b: TGC CCC CAT TTT CAA ACT CCC TTC C		
Relative base pair distance to allele size n	Example varieties Notes (Variety code)		Further example varieties
N	RO 1	Romorantin B: 1	
n + 2	PI 1	Pinot N,G,B: 1	
n + 4			
n + 6	CH 1	Chardonnay B: 1	Barbera N: 1
n + 8	CH 2	Chardonnay B: 2	Pinot N,G,B: 2, Traminer RG: 1,
n + 10	CF 1	Cabernet franc N: 1	Sultanina B: 1
n + 12	SI 1	Silvaner B: 1	
n + 14	TR 2	Traminer Rot RG: 2	Muscat à petits grains blancs B: 1, Silvaner B: 2
n + 16	VI 2	Vital B: 2	
n + 18	MU 2	Muscat à petits grains blancs B: 2	
n + 20	4MA 1	Malegue 44 – 53: 1	Admirable de Courtiller B: 2, Couderc 3309 : 2
n + 22	CF 2	Cabernet franc N: 2	Barbera N: 2
n + 24	4MA 2	Malegue 44 – 53: 2	Mourvedre N: 2
n + 26	99R 2	Richter 99: 2	Paulsen 1103 : 2

Definitions:

E: Approximate size range of alleles: from 235/236 to 261/262 base pairs. Remark that different methods of analyses may result in small deviations. The shortest allele found within Genres081 has been chosen arbitrarily as being "n".
Variety code: CF1 means Cabernet franc N shorter allele, CF2 means Cabernet franc N longer allele, etc. If a new allele is found, e.g. “n – 2”, the corresponding variety code would be "RO – 2“. For allele size codification of cultivars, some example varieties have to be run as standard within the same analysis. There are no restrictions on the method. But (1) the PCR conditions for the example varieties and the varieties to be analysed have to be the same; (2) it is recommended that in a 3-step PCR the final elongation step is at least 30 minutes.

Fig. 1. Proposed OIV descriptor for STMS marker VrZAG79, English version.

Our recommendation is that the most frequent reference alleles can be covered at all six microsatellite loci by using the following 17 grapevine varieties from a total of 33 initially selected reference cultivars: 'Barbera N', 'Cabernet Sauvignon N', 'Cabernet franc N', 'Chardonnay B', 'Merlot N', 'Muscat à petits grains blanc B', 'Pinot noir N', 'Sultanina B', 'Silvaner B', 'Traminer Rot RG' and the rootstock varieties 'Couderc 1616', 'Couderc 3309', 'Millardet et Grasset 101-14', 'Millardet et Grasset 420A', 'Richter 99', 'Richter 110' and 'Teleki 5C'.

- ***Choice of SSR markers***

The six microsatellite markers were found to be suitable for grapevine variety characterization due to their high degree of allelic polymorphism (13-23 alleles per locus) and high discriminatory power. As already introduced and frequently used, these six markers should be recommended in general as the minimal standard marker set for future grapevine variety analyses. This will help to promote the creation of uniform, easily and directly comparable data catalogues which will be valuable and profitable for identification purposes. For rational conservation and evaluation of grapevine genetic resources there is an urgent need to identify unknown or unconfirmed accessions in international grapevine germplasm collections to find duplicates and synonyms.

In all cases the six markers succeeded in differentiating and characterizing the 46 examined grapevine varieties by individual, well distinguishable microsatellite profiles. Only in a very few cases was it reported (Jung unpublished; Boursiquot personal communication) that the allelic profiles produced through the six expressive polymorphic markers needed one or two additional markers to differentiate obviously different varieties which had already been discriminated by ampelographic means. Some closely related, inbred or self-pollinated table grape varieties may need more than six markers to differentiate between them (Crespan *et al*. 1999; Sanchez-Escribano *et al*. 1999).

Therefore, if a grapevine variety is defined as an agriculturally selected and vegetatively propagated descendent from an individual seedling, characterized by the unique recombination of parental alleles during meiosis, then microsatellite profiling will be the method of choice for variety discrimination and characterization. For special questions (e.g. to differentiate intra-varietal clones, berry colour mutants, somatic chimeras or multiple backcrosses), six markers generally will not be sufficient and alternative marker systems such as amplified fragment length polymorphism (AFLP) markers may be more suitable (Cervera *et al.* 1998, 2000). Also, for research on geographic origins or parentage relationships, up to 50 markers may be necessary to obtain results of statistical relevance (Vouillamoz *et al*. 2003).

The number of available microsatellite markers has rapidly increased to more than 400. Not all markers are yet available or useful, but it is undeniable that hundreds of markers can eventually be standardized by a coordinated international research effort. But in order to make best use of the presently available data, and in particular for the aim of direct data comparability, every scientist working with microsatellite markers is called on to accept the six markers as the general minimum standard for uniform grapevine characterization, and furthermore to select only expressive, highly polymorphic markers for the more detailed analyses (Tessier *et al.* 1999). For each additional marker, a fixed standard of cultivar-based reference fragments

should be established, which broadly represent the existing alleles along a scale of relative distances. This would easily enable other working groups to convert their own data into easily comparable data catalogues.

- ***Discrepancies in results***

The complete data of all ten partners are available at http://www.genres.de/eccdb/vitis/. As already stated, some discrepancies between partners' data occurred (Tables 6, 8 and 10) which contradicted certain theoretical expectations. Without going into details, some of these disharmonies between partners' results were recognized as systematic interpretation problems for specific fragment patterns.

1. A systematic problem of interpretation could emerge if corresponding alleles are separated from each other by a distance of only 2 bp. These densely clustered fragments might be interpreted either as homozygous or heterozygous allele pairs, especially if some overlapping stutter was also amplified. During the second step of analyses this problem concerned the alleles of 'Silvaner' at VrZAG79. Here the densely clustered fragment pair was interpreted either as homozygous with a slight stutter (n+12 : n+12; n+14 : n+14) or as heterozygous with nearby standing fragments of 2-bp distance from each other (n+12 : n+14). After reinterpretation, all ten partners agreed about the heterozygous nature of these corresponding alleles. Nevertheless a similar interpretation problem occurred for some other allele pairs (e.g. those in data set 3 of 'Carignan' at VVS2 and in data set 4 of 'Agiorgitiko' at VrZAG62, see Table 6).
2. Due to some gaps in the allelic ladders, data discrepancies between partners occurred if an isolated allele (e.g. the alleles of 'Castel 216-3' at VVMD5, see Table 6) emerged in a still unexplored area of the allelic spectrum, the distance of which could not be directly related to an adjacent reference fragment. Thus exact sizing of distantly grouped fragments led to difficulties of interpretation between the partners, a problem that was finally solved by defining the exact scales between fragments of selected reference cultivars (Table 7).
3. Looking at *Vitis vinifera* L. varieties only, the relative distance between two neighbouring alleles was mostly 2 bp, in accordance with the dinucleotide nature of the repetitive motifs at the 6 microsatellite loci. One exception was a single shift of 3 bp from n+16 to n+19 up the allelic ladder of VVMD27. This shift was not recognized by all partners (see Table 6, data of 'Admirable de Courtiller' at VVMD27). A further shift of 3 bp was reported by a minority of partners at a VVS2 locus that can possibly be explained as an artefact of the automated scoring procedure on Genetic Analysers. Due to the use of simple mathematical algorithms, automated scoring of peak sizes can produce artificial shifts through the effects of automatic rounding up or down. These effects must be visually controlled to manually adjust decimal aberrations into integer numbers which will fit the peak positions of already labelled reference alleles (see Table 6, data sets 1 and 4 of VVS2).
4. Obviously, *V. vinifera* L. as a single species does not represent all existing alleles within the genus *Vitis*. Analyses of hybrids broadly succeeded in filling many gaps between distant reference alleles, but also revealed a resolution problem when discriminating alleles of only 1-bp distance from each other.

There is a potential danger that unconventionally shifted but autonomous alleles may be misinterpreted as electrophoretic casualties or stutter patterns and may be completely ignored. This problem arose for the unconventional alleles of some interspecific hybrids (see Table 7, scaling size n+11 at VVMD27 and n+1 at VrZAG62). The only solution for this high resolution problem is to optimize PCR conditions as skilfully as possible and to control the analysis modes by using varietal reference fragments of defined allele sizes. In general, modern Genetic Analysers have a higher resolution than handmade PAA gels. Low concentrated stutter patterns visualized as low peaks can be more easily differentiated from the higher peaks of truly amplified allele fragments. High resolution is especially important for the detection of somatic chimeras.

5. Some minor data discrepancies could not be explained, obviously due to real mistakes. This only emphasizes the necessity to optimize PCR strategies, visualization techniques and analysis modes so as to prevent unclear fragment patterns. A good strategy might be the use of a hot-start PCR to prevent fluctuation of amplified fragments and to reduce stutter patterns. Some difficulties encountered may have resulted from the trend for generalization of PCR protocols towards uniform annealing temperatures. Obviously optimization and adaptation of working routines to local laboratory conditions still remains an important task. For future work, the utilization of already scored PCR fragments of multiply-confirmed and internationally recommended reference cultivars will facilitate the objective and uniform determination of allelic profiles.

Conclusion

The SSR marker technology has been developed very recently. Investigations into the general comparability of microsatellite profiles carried out within GENRES 081 showed that SSR marker data of different working groups produced under varying laboratory conditions are essentially comparable after codification and are suited for grapevine identification and characterization purposes.

Owing to the enormous efforts and costs of the project, partners did not aim at achieving standardization of laboratory equipment or working routines. Evidently this is not actually necessary, since after data transformation a majority of partners produced highly similar data. The minor data discrepancies can be explained by different point of views on systematic interpretation problems. Errors in allele sizing could mostly be eliminated by selecting distinct fragments of reference varieties as defined standards for uniform coding of allele sizes. In cases of persistent data discrepancies, the predominant scoring of the majority of partners was calculated. By transforming numerical data into reference cultivar-based allele codes, the microsatellite profiles of ten different laboratories became directly comparable and turned out to be broadly identical. Nevertheless, the optimization of working routines individually adapted to each set of local laboratory equipment remains a necessary requirement to be completed for each marker.

The comparative microsatellite analyses on 46 distinct grapevine varieties culminated in the common definition of six new, multiply-confirmed and universally defined OIV descriptors which now enable uniform fragment size labelling.

For future investigations, recommendations are to utilize the variety- and marker-specific PCR fragments presented in this study as well-defined internal size standards in order to replace different modes of numerical fragment labelling by a uniform and multiply-proved coding system. If accepted as an international standard for allele scoring and variety characterization, microsatellite profiles of grapevine accessions become directly comparable, opening an opportunity to create a uniform database of value for general variety identification purposes, while also overcoming the restrictions imposed by regional orientation (Lefort and Roubelakis-Angelakis 2000) or literature-based databases (Grando *et al.* 2002).

For viticulture, grapevine science and the maintenance of *Vitis* genetic resources the assessment of trueness-to-type of collected and conserved grapevine accessions is a substantial precondition. As a matter of course, each DNA sample, used as a reference for genetic analyses, must be taken from identity-confirmed vines, which effectively represent the true type of a variety under consideration (Dettweiler *et al.* 2000a). It is a fact, that the true types of most traditional varieties were historically defined by local ampelographers during the 19th and 20th century (Viala and Vermorel 1909; Galet 2000). They only used morphological characters and local names or synonyms as base of variety description. Over the years, synonymy often got confused. Variety names from distinct regions were wrongly attributed to native varieties and sometimes these mistakes were transliterated unsuspectingly for centuries. As a consequence, the misnaming of historic varieties still occurs frequently. Each recent confirmation and modern redefinition of a historic variety type must first be based on the analyses of ampelographic reference literature. Then, variety characterization can be completed by modern ampelographic descriptions and microsatellite analyses. Genetic profiling and the comparison of genotypes can be very useful for supranational identification purposes and the reconstruction of pedigrees (Sefc *et al.* 1998; Bowers *et al.* 1999; Regner *et al.* 2000a, 2000b; Lefort and Roubelakis-Angelakis 2001). The establishment of a unique, central and uniform European database with multiply-confirmed and well-defined grapevine variety profiles will set new standards and support a better, more rationalized management of world grapevine collections. This will facilitate the efforts for conservation of unique, rare or endangered grapevine varieties (Lopes *et al*. 1999; Boursiquot *et al*. 2002).

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Identification of duplicates in Vitis *germplasm banks by using microsatellites plus ampelography*

***Jesús María Ortiz*[1], *Juan Pedro Martín*[1] *and Félix Cabello*[2]**
[1] *Departamento de Biología Vegetal, Universidad Politécnica de Madrid (UPM), Madrid, Spain*
[2] *Departamento de Agroalimentación, Instituto Madrileño de Investigación Agraria y Alimentaria (IMIA), Alcalá de Henares, Madrid, Spain*

Introduction

A large number of grapevine varieties are mentioned in the literature and exist in the collections of different countries. A widely known fact is the existence of many synonyms which are more or less confirmed by ampelographic or molecular characterization. The International Organisation of Vine and Wine (Office International de la Vigne et du Vin, OIV) published a list in which most of the known synonyms are recorded (OIV 1996). The occurrence of these synonyms has resulted in the existence of many duplicates in grapevine collections located in different countries. During the last decade, a broad study has been carried out in the *Vitis* germplasm bank of "El Encín" (Banco de Germoplasma de Vid de la Comunidad de Madrid, BGVCAM), located at Alcalá de Henares, Madrid (Spain), in order to detect the existence of duplicates, as well as the trueness-to-type of the varieties included in the collection. The number of accessions included in the Bank was 2726 (Cabello *et al.* 2003), with a large number of different varietal names, supposedly including some homonyms as well as synonyms. In order to characterize and document the plant material of the bank, ampelographic descriptors, isozyme systems and molecular markers have been evaluated or analyzed (Ortiz *et al.* 2004). In the present work and as a result of the studies carried out, a standard methodology is presented and recommended for characterization of accessions in *Vitis* germplasm banks in order to detect the synonyms and reduce to a minimum the duplicated accessions.

Material and methods

The plant material for the study consisted of 318 accessions, located at the BGVCAM (Cabello 1995). Each accession consisted of four replications of head-trained plants, without trailing, of around 50 years of age.

- ***DNA extraction and polymerase chain reaction (PCR) amplification***

Leaves from field-grown plants were harvested and stored at -20ºC. DNA was extracted from frozen grapevine leaves using a MasterPure™ Plant Leaf DNA Purification Kit (Epicentre Technologies, Cat. nº MPP92100). Extracted DNA was quantified and a working solution of DNA (10 ng/µl) was made.

A total of six sequence tagged microsatellite site (STMS) loci, fully characterized in earlier studies, were used: VVS2 locus (Thomas and Scott 1993), VVMD5 and VVMD7 loci (Bowers *et al.* 1996) and ssrVrZAG47, ssrVrZAG62 and ssrVrZAG79 loci (Sefc *et al.* 1999). Primer pairs were synthesized (Perkin Elmer Applied Biosystems)

from published sequences. During synthesis, one of the primers of each pair was fluorescently labelled with a Perkin Elmer fluorophore, 6-FAM (blue) TET (green) or Hex (yellow).

Two different multiplexed PCR reactions were carried out: VVS2, VVMD5 and VVMD7 loci (set A), and ssrVrZAG47, ssrVrZAG62 and ssrVrZAG79 loci (set B). Both multiplex PCRs were performed in 20 µl of reaction mixture consisting of 0.2 mM of each of the four dNTP, 2 mM of $MgCl_2$, 1 unit of Tth-DNA polymerase in the buffer provided by the manufacturers of the enzyme (BIOTOOLS, B&M Labs.), 30 ng of template DNA, and different amounts of each primer pair depending on the set: in set A, 0.2 µM of each VVS2 primer, 0.5 µM of each VVMD5 primer and 0.25 µM of each VVMD7 primer; and for set B, 0.5 µM of each primer of both primer pair ssrVrZAG47 and ssrVrZAG79, and 0.1 µM of each ssrVrZAG62 primer. PCR amplifications were carried out in a PTC-100 thermal cycler (MJ Research, Inc.) with heated lid, using an initial cycle of 5 min at 95ºC, followed by 40 cycles of 45 s at 94ºC, 1 min at 50ºC and 1 min 30 s at 72ºC.

- ***Detection of STMS polymorphism***

Amplified products were separated by capillary electrophoresis using an automated DNA sequencer ABI PRISM model 310 (Perkin Elmer Applied Biosystems). Fluorescently labelled fragments were detected and sized using GENESCAN software (PE Applied Biosystems). GENESCAN-350 TAMRA (PE Applied Biosystems) was used as internal standard to assign sizes to DNA fragments.

- ***Ampelographic characterization***

Based on earlier studies carried out in the BGVCAM (Ortiz *et al.* 2004), the following OIV characters were described according to the specifications (Table 1).

- ***Detection of duplicates***

The combined use of microsatellite analysis and ampelographic characterization was applied to obtain evidence of the existing synonyms in order to detect the duplicates in the collection.

Results and discussion

In an earlier work, 621 accessions of the BGVCAM, all corresponding to indigenous Spanish cultivars, were studied by using both ampelographic descriptors and isozyme systems (Ortiz *et al.* 2004). As a consequence, 303 synonyms were detected. The remaining 318 accessions were those included in the present study.

Analysis of the six STMS loci yielded an average of 11 alleles per locus, with slight variation among them (Table 2). Although the average potential number of genotypes for each locus was 67.0, only around 50% of them were obtained with the analyzed accessions. The percentage of homozygous genotypes ranged from 9.1% in VVMD5 to 24.4% in VVMD7, with an average of 16.0%. The STMS results produced a total of 163 different genotypes in the 318 studied accessions (Table 2) (Martín *et al.* 2003).

Table 1. OIV descriptors used for the characterization (OIV 1984)

OIV Code	Plant organ	Description of the character
OIV 001	Young shoot	Tip shape
OIV 002	Young shoot	Distribution of anthocyanin coloration of tip
OIV 004	Young shoot	Density of prostrate hairs of the tip
OIV 007	Shoot	Colour of dorsal side of internodes
OIV 008	Shoot	Colour of ventral side of internodes
OIV 011	Shoot	Density of erect hairs of the nodes
OIV 012	Shoot	Density of erect hairs of the internodes
OIV 016	Tendrils	Distribution on the shoot
OIV 053	Young leaf	Density of prostrate hairs between veins at the lower side of leaf
OIV 067	Mature leaf	Shape of blade
OIV 068	Mature leaf	Number of lobes
OIV 070	Mature leaf	Anthocyanin coloration of the main veins on the upper side of the blade
OIV 081-1	Mature leaf	Presence of teeth in the petiole sinus
OIV 081-2	Mature leaf	Naked petiole sinus
OIV 082	Mature leaf	Shape of upper leaf sinuses
OIV 083-1	Mature leaf	Shape of the base of the upper leaf sinuses
OIV 083-2	Mature leaf	Presence of teeth at the base of the upper leaf sinuses
OIV 091	Mature leaf	Density of erect hairs of petiole
OIV 102	Woody shoot	Surface
OIV 202	Bunch	Length
OIV 203	Bunch	Width
OIV 204	Bunch	Density
OIV 206	Bunch	Length of peduncle
OIV 208	Bunch	Shape
OIV 220	Berry	Length
OIV 223	Berry	Shape
OIV 225	Berry	Colour of skin
OIV 230	Berry	Colour of flesh
OIV 236	Berry	Particular flavour
OIV 241	Berry	Presence of seeds
OIV 244	Berry	Transversal ridges on dorsal side of seed
OIV 503	Berry	Single berry weight

Table 2. Results of the microsatellite analysis

Microsatellite loci	Number of alleles obtained	Potential number of genotypes	Number of genotypes obtained	Obtained vs. potential genotypes (%)	Homozygous percentage
VVS2	13	91	41	45	11.4
VVMD5	10	55	36	65	9.1
VVMD7	12	78	34	44	24.4
ssrVrZAG47	9	45	24	53	14.2
ssrVrZAG62	10	55	25	45	18.2
ssrVrZAG79	12	78	39	50	19.9
Average	**11**	**67**	**33.2**	**50.3**	**16.0**
Total	**66**		**163**		

The ampelographic characterization was carried out with the 32 descriptors listed in Table 1. As a result, 13 varieties, included in 11 groups, each of them with the same microsatellite results, showed differences in one or more OIV characters (Table 3). In most cases, the difference was in the colour of the berry, a frequent mutation in grapevines, not so far detected with these molecular markers. In the case of 'Garnacha Peluda', the morphological difference with 'Garnacha Tinta' was the presence of hairs on the lower side of the leaf in the first variety. 'Palomino' and 'Palomino Fino' differed in the shape of the berry, slightly pointed in the apex in the second versus rounded in the first. Finally, 'Carrasquín' and 'Prieto Picudo Tinto' presented several differences in leaf morphology.

The use of a higher number of microsatellites in order to detect differences has not so far proved useful in the case of mutations with a different colour of berry, as in the case of 'Pinot Blanc', 'Pinot Grey' and 'Pinot Noir' (Sefc *et al.* 1998). Perhaps the analysis of a higher number of loci could detect differences between varieties in other cases, as in 'Carrasquín' vs. 'Prieto Picudo Tinto'.

Table 3. Groups of varieties having the same allelic patterns in the six STMS studied and marked differences in ampelographic observations

Group	Varieties	Berry colour(1)	Other differences
1	Beba	B	
	Calop Rojo	RG	
2	Cariñena Blanca	B	
	Mazuela	N	
3	Jaén Blanco	B	
	Jaén Rosado	RG	
4	Quiebratinajas Rojo	RG	
	Quiebratinajas Negro	N	
5	Temprano Blanco	B	
	Temprano Colorado	RG	
6	Teta de Vaca Blanca	B	
	Teta de Vaca	RG	
7	Moscatel de Grano Gordo	B	
	Moscatel Rosa	RG	
8	Xarello	B	
	Xarello Rosado	RG	
9	Garnacha Blanca	B	Hairless leaves
	Garnacha Gris	G	Hairless leaves
	Garnacha Tinta	N	Hairless leaves
	Garnacha Peluda	N	Hairy leaves
10	Palomino	B	Rounded berry apex
	Palomino Fino	B	Pointed berry apex
11	Carrasquín	N	Several differences in leaf shape
	Prieto Picudo	N	

(1) B = white; RG = pink; N = black; G = grey.

In the varieties studied, microsatellites resolved more than 92% of the identification of varieties. The use of morphological characterization is always needed in order to detect mutations that are very obvious and important from the agronomic viewpoint, but affect a minimum part of the genome and consequently are very difficult to detect with the STMS markers.

As a result of the present work, we strongly recommend the following steps for the characterization, management and documentation of a *Vitis* germplasm bank:

1. Select and analyze a small number of adequate microsatellite loci – VVS2, VVMD5, VVMD7, ssrVrZAG47, ssrVrZAG62 and ssrVrZAG79 in our case – of which a database with previous information should be available (Martín *et al.* 2003; list of Internet databases, see below).
2. Compare the results with the database.
3. Different patterns in microsatellites will correspond to different varieties that can subsequently be characterized by ampelography.
4. Identical patterns in microsatellites will be confirmed or rejected as synonyms based on the ampelographic characterization.
5. A reduced number of ampelographic descriptors, around 32, has been proved useful for the indicated characterization.

List of Internet databases on microsatellites

http://www.neiker.net/BIOVID/	Characterization of Spanish grapevine cultivar diversity using sequence-tagged microsatellite site markers.
http://meteo.iasma.it/genetica/gmc.html	GMC – Grape microsatellite collection A web-backed database of genotypes at SSR loci obtained from IASMA analysis and literature
http://gvd.biology.uoc.gr/gvd/index.htm	The Greek Vitis Database A multimedia web-backed genetic database for germplasm management of Vitis resources in Greece
http://www.boku.ac.at/zag/forsch/	Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars
http://hydra.unine.ch/svmd/index.php?details=87	Swiss Vitis Microsatellite Database

In a further step that is being carried out in the BGVCAM, the detected synonyms of different varieties are being evaluated agronomically and fully characterized in order to elucidate the existence or not of different clones.

As a consequence of the recommended management methodology of a *Vitis* germplasm bank, the number of maintained accessions as well as the costs will probably be reduced.

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Documentation of biodiversity within varieties: genetic differences within the grapevine variety 'Traminer'

Herwig Kaserer and Ferdinand Regner
Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau, Abteilung Rebenzüchtung, Klosterneuburg, Austria

The occurrence of biotypes within old varieties of grapevines is a well-known fact; however differentiation and identification have been very difficult in the past. Within the GENRES project we investigated several clones of the variety 'Traminer' from different sources. We found visible differences in ampelographic characters. An analysis of variance of ampelometric data (leaf measurements) revealed significant differences. Therefore it was decided to check them by DNA analysis. By applying random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers, several genetic differences were revealed within the variety. RAPD polymorphism was used to estimate the heterozygosity. The microsatellite profiles offer the potential for the identification of a single individual. Stable SSR loci usually used for the identification of cultivars are not suited for clonal differentiation. However, SSR loci located on hyper-variable regions provide sufficient polymorphic alleles for identification of clonal material. The supposed variability of a cultivar can be confirmed with these data. Clonal selection will be regarded again as a genetic selection process with some phytopathological aspects and the preservation of clone collections should be accepted as part of the preservation of biodiversity.

On the base of this knowledge, the current European legislation for vegetatively propagated material of vines appears to greatly endanger their biodiversity, due to expensive phytopathological tests requested for certified propagating material and long-term plans for ruling out the standard material as well. According to the update of the Council Directive in 2002[23] it was possible to implement in Art. 3(5) the wording, that ruling out the standard material of a certain variety is only possible if taking into consideration its biodiversity. However it is necessary to document all the estimated and known biotypes for further discussions in Brussels in order to prevent genetic erosion caused by EC seed legislation in the future.

[23] Council Directive 2002/11/EC of 14 February 2002 amending Directive 68/193/EEC on the marketing of material for the vegetative propagation of the vine and repealing Directive 74/649/EEC (http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:32002L0011:EN:NOT)

Development of a genetic database for Ukrainian, Moldovan and Russian germplasm of Vitis vinifera *using microsatellite markers*

***Francois Lefort*[1]*, Valentina Risovannaya*[2]*, Svitlana Goryslavets*[2] *and Leonid P. Troshin*[3]

[1] *Laboratoire de Biotechnologie et Génétique Appliquée, Ecole d'Ingénieurs de Lullier, Haute Ecole Spécialisée de Suisse Occidentale, 150 route de Presinge, 1254 Jussy, Switzerland (email: francois.lefort@hesge.ch)*

[2] *Institute for Vine and Wine "Magarach", 31 Kirov St., Yalta, Crimea, Ukraine*

[3] *Viticulture Department, Kuban State University of Agriculture, 350044 Krasnodar, Russian Federation*

Genetic resources of grapevine found in the territory of the former Soviet Union may account for more than 6000 cultivars and rootstocks. Many of them remain unknown to ampelographers outside the Community of Independent States (CIS countries) since any information on them is scattered in different bibliographic sources and ampelographic collections or else access is hampered by language barriers, since the data available through electronic media are mostly in Russian, Ukrainian or other languages of the former Soviet Union's Republics.

Grape growing of the CIS countries relies on a number of foreign and local cultivars. Although foreign cultivars are believed to be well identified, very little is known about local cultivars of Ukraine, Moldova and Russia, which are important and ancient grape-growing regions. Genetic relationships between local cultivars of these countries and those from the neighbouring regions also need to be highlighted.

Local cultivars from Ukraine are found mostly in Crimea (in the south of Ukraine). In Russia, the highest concentration and distribution of the country's local cultivars and wild forms of grape are associated with the Don region, the North of the Caucasus (Daghestan), the central part of the country and the Far East. It arises from this that genetic relationships between Russian local cultivars and cultivars of the neighbouring regions are also influenced by their relationships inside Russia. The history of cultivated grape distribution in Ukraine, Moldova and Russia is complex, a fact which is related to the very ancient origin of some of these cultivars (indigenous cultivars), the proximity of the putative regions of grape domestication (North Caucasus) and the effects of human migration on local cultivars arising from trade with distant territories. As concerns the origin and time of introduction into cultivation of some ancient indigenous cultivars of grape, only hypotheses may be proposed and confronted, or eventually related, to historical, botanical and genetic data. Mostly, their modern names cannot be considered as reliable indications of their origins. Crimea for instance has seen passing through it so many nations, which settled on the same territory at different times, that many names descending orally from one generation to another have been lost, distorted or replaced by new ones. The present research focuses on studies and conservation of grape genetic resources of Ukraine, Moldova and Russia.

For the identification of cultivars and management of available information, modern information technologies are widely used at the international level. Combination of information technology with information and molecular genetic

data was recently used to offer public databases accessible through the Internet (Lefort and Roubelakis-Angelakis 2001; Russanov *et al.* 2003).

Our multimedia web-backed database for Ukrainian, Moldovan and Russian germplasm of *Vitis* has been developed on the same plan as the Greek *Vitis* Database. Ukrainian and Moldovan cultivars included in the present study are maintained in the ampelographic collection of the Institute for Vine and Wine "Magarach" in Yalta, Crimea. Russian cultivars are maintained in the new ampelographic collection of Russia located in Krasnodar within the framework of the Kuban University of Agriculture. The cultivars were selected as being potentially the most ancient cultivars cultivated in these regions.

The database contains the following basic components: an information database (names, synonyms, history, known pedigrees, cultivar characteristics, etc.); an ampelographic database (images of young shoots, mature leaves and clusters); and a nuclear microsatellite profile database (genetic identity database).

Microsatellite profiling is widely used today in studies of polymorphism of the grape genome.

The technique used genotyping at specific loci called microsatellites.

In the present research, genetic profiling of cultivars has been performed by using nine nuclear microsatellite loci characterized previously by other European researchers: VVS2, ssrVrZAG21, ssrVrZAG47, ssrVrZAG62, ssrVrZAG64, ssrVrZAG79, ssrVrZAG83, ssrVvUCH11 and ssrVvUCH29. Microsatellites are used as genetic markers since they are highly polymorphic and ubiquitous throughout the genome and very stable as indicated by data obtained in different European laboratories (Sefc *et al.* 2000, 2001).

Microsatellites are also reproducible, which opens the door to standardization, which has allowed us to compare Crimean, Moldovan and Russian cultivars with grape genetic resources of western Europe and Greece already characterized at the same loci.

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Implementation in Georgia of the project on "Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region"

Nodar Chkhartishvili
Institute of Horticulture, Viticulture and Oenology (IHVO), Tbilisi, Georgia

Georgia, through its Institute of Horticulture, Viticulture and Oenology (IHVO) is involved in the project of the International Plant Genetic Resources Institute (IPGRI)[24] "Grapevine *(V. vinifera* L.) genetic resources conservation and sustainable use in Georgia", which is a part of the international project "Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black Sea region"

The implementation of the project in Georgia was determined by the importance of the Caucasus region for grapevines. Georgia in particular is recognized as one of the countries of origin and domestication of grapevine on the basis of botanical, ampelographic, archaeological, palaeontological, historical, ethnographical and linguistic investigations carried out in the country (Vavilov 1935; Negrul 1946; Zhukovsky 1971; Ramishvili 2000).

The genetic resources of Georgian grapevines have always attracted the attention of scientists who were studying the biodiversity of cultivated crops. The search for local varieties and their scientific description started in the second half of the 19th century. The "Ampelography of Georgia" (Ketskhoveli *et al.* 1960) contains a list of 524 old local varieties, among which 414 varieties were described in the "Ampelography of the Soviet Union" (Frolov-Bagreev 1946-1956; Negrul 1963-1970). Most of the old Georgian local varieties, according to the classification of Negrul (1946), belong to the Black Sea ecological-geographic group of varieties Proles *Pontica* subproles *georgica* Negr. and a small number belong to the Oriental ecological-geographic group Proles *Orientalis* subproles *caspica* Negr.

Viticulture always played an important role in the economy of Georgia, and it still does: the average yearly harvest of grapes amounts to 200 000 tonnes on 62 000 ha.

Georgian viticulture is mainly directed towards winemaking. The list of standard wine varieties for cultivation in Georgia contains 37 varieties, including 32 old local varieties. They cover most of the vineyard areas in the country and can produce high quality wines, highly rated by wine-tasting panels in different countries of the world and winners of many prizes. Important Georgian varieties are 'Rkatsiteli', 'Saperavi', 'Tavkveri', 'Tsolikouri', 'Ojaleshi', 'Krakhuna', 'Alexandrouli', 'Khikhvi', 'Chinuri' and others.

However, in spite of the increasing use of local varieties, viticulture in Georgia is threatened by genetic erosion: only a small number of local varieties are still cultivated today, the national collections contain only half of the total number of local varieties, and local varieties are rarely included in breeding programmes.

These elements constitute the background of the planning and current implementation of the IPGRI-funded project. Its main objectives are the identification,

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collection, characterization and conservation of the rich germplasm of native Georgian grapevine varieties for sustainable development of national viticulture.

Participants in the project include IPGRI, the Institute of Arboriculture of Milan University, the Georgian Institute of Horticulture, Viticulture and Oenology and the Georgian State Agrarian University.

The main activities identified for Georgia to carry out in the framework of this project are:

- Identification of the diversity of traditionally cultivated and wild grapevines, and their protection from genetic erosion. Planting of a new collection in Georgia including safety-duplicates, where the old local varieties and wild forms will be collected;
- Analysis of the genetic diversity by molecular markers;
- Reinforcement of the National Institute and its active involvement in a European network for long-term conservation and use of grapevine genetic resources;
- Archaeological research on the territory of Georgia;
- Sustainable use of Georgian grapevine genetic resources in local viticulture.

Recent achievements include:

- Inventory of all grapevine collections in Georgia and identification of 248 local varieties;
- Compilation of comparative lists of local varieties from different collections;
- Grafting of 160 Georgian varieties in Italy. Some of these were planted in field collections in Italy, others were returned back to Georgia to be planted in the local field collection;
- During the last winter–spring period the soil was ploughed and fertilized for future planting in Vashlidjvari in the fall of 2003;
- In the spring of 2003, 240 Georgian local varieties were grafted in Kakheti;
- Establishment of a new grapevine field collection in Vashlidjvari Experimental Station on a 1.2 ha testing plot. For this purpose, the soil and climatic conditions of the site were investigated. The varieties were planted in the field according to a design based on geographic criteria, grouping together those from the same origin.

In collaboration with colleagues from Milan, we participated in the preparation of a book which includes one chapter on Georgian viticulture and the ampelographic description of native varieties with photos (Del Zan *et al.* 2004).

Georgian viticulturists believe that this unique project, the first to be concerned with the local conservation of Georgian grapevine genetic resources, is an important and essential step for the revival of our grapevine germplasm.

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Survey on *Vitis* genetic resources

Collecting, maintenance and evaluation of grapevine clones in France in 2003

Thierry Lacombe [1] ***and Laurent Audeguin*** [2]
[1] *Institut National de la Recherche Agronomique (INRA) - UMR 1097 Diversité et Génome des Plantes Cultivées (DGPC), Equipe Génétique Vigne, Montpellier, France*
[2] *Etablissement National Technique pour l'Amélioration de la Viticulture (ENTAV), Domaine de l'Espiguette, Le Grau du Roi, France*

Introduction

Clonal selection has been developed in France since the beginning of the 1950s. The Institut National de la Recherche Agronomique (INRA) was mandated to develop this methodology. In the early 1950s, the main goal was to renew the vineyard with healthy material (mostly safe from the grapevine fanleaf virus, GFLV). Thus, the Domaine de Vassal (INRA) in 1950 and later the Etablissement National Technique pour l'Amélioration de la Viticulture (ENTAV) in 1962 were set up in soils made of pure Mediterranean sand, free from the nematode vectors of GFLV.

The Domaine de Vassal was dedicated to grapevine conservation and became one of the most important ampelographic repositories of the world, with about 7500 accessions (Anonymous 2003). ENTAV developed its selection work on cultivars registered in the official catalogue of grapevine varieties and clones cultivated in France: 228 of *Vitis vinifera* (ENTAV *et al.* 1995). About 4000 clones are conserved in ENTAV's repository, including more than 3000 clones of wine grape varieties.

INRA had also planted the first clonal repositories (e.g. 'Cabernet-Sauvignon' and 'Merlot' in Bordeaux); some of the most famous clones came out of these vineyards, such as 'Merlot N 181'.

From 1971 to 2002, 1065 clones of wine cultivars have been certified, representing about 160 varieties. During the past 30 years, selection criteria and winegrowers' objectives have greatly changed.

In parallel to this work, other French partners have come to understand the importance of grapevine genetic resources preservation. Regional collections are becoming more popular and have been planted for the past 15 years; they have become a priority today.

From collecting to certification of grapevine clones: evolution of the methodological approach

The first objective of clonal collecting in the 1960s was to obtain healthy clones (mostly safe from the fanleaf virus) of "varietal standard types" able to give regular yields. After the phytosanitary selection had been carried out (detection of virus diseases by enzyme-linked immunosorbent assay (ELISA) and indexing), the clones were propagated (Boidron *et al.* 1997).

The clones were certified in 1971. Agronomic behaviour was characterized in the collections of ENTAV and INRA-Bordeaux.

It was only from the late 1970s onwards that local technicians wanted to detail the certified clones' characteristics in their own regions. In 1987 this approach resulted in the development of a national protocol by the National Trial and Demonstration

Network (Réseau National d'Essai et de Démonstration, RNED), which codified experimentation on clones according to the following steps:

- Pre-selection in vineyards or repositories
- Planting of clones in regional "study collections" or "data collections" while the "sanitary selection" is made in ENTAV
- "Certification" of the most interesting clones (healthy and high quality clones)
- "Behavioural plots" in different regions of the certified clones.

The "study collection" contains clones which have passed the sanitary tests for fanleaf and leafroll viruses; it is established in order to define the clone profiles for possible certification: the aims are to evaluate their technological potential.

However, the behavioural plots are set up to compare at a given site the cultural, technological and organoleptic characteristics of certified clones, in order to advise wine-growers.

This method was an opportunity to develop trials in several French vineyards (Audeguin *et al.* 1998) and resulted in the publication of a collective document in 1995: *Catalogue of selected wine grape varieties and certified clones* (ENTAV *et al.* 1995), presenting the characteristics of the "first clonal generation".

Since 1987, the RNED protocol has been updated and adopted by the Permanent Technical Selection Committee (Comité Technique Permanent de Sélection, CTPS) in 1998. Under these rules, the evaluation must continue through until the wine tasting (CTPS 1998). In concrete terms, a new clone cannot be certified without 5 years of collecting wine-growing data and 3 years of collecting oenological data and tasting *in fine*.

We now consider that the main objective is to select clones with low production potential. Then we have to add, according to the cultivar and to the clones already diffused, other criteria to those of the first clonal generation (Audeguin *et al.* 1999; Boidron 2000):

- Phytosanitary status should be more precise about secondary viruses
- Limited fertility
- Limited density of bunch
- Reduced size of berries for black cultivars
- Better aroma characteristics and complexity
- Better polyphenolic potential.

The current work of selection is not only to search for "quality" clones. The goal is rather to offer to French viticulture clones which represent the widest genetic diversity, if possible using the resources of the repositories. This approach, begun in the 1990s, could be called the "second generation of clones". We can give the examples of 'Viognier B 1042', 'Petit Verdot N 1058' or 'Cot N 1061'.

This objective evolution of selection has logically modified the approach of collecting activities which are not only oriented *a priori* to the best standard for a variety but also to collect the existing diversity within each cultivar. After an inventory of the oldest vineyards (more than 40-50 years old) of the area (Fig. 1), vines are tagged giving priority to the number of plots visited rather than to the number of vines tagged within each plot. Sometimes the phenotypic limits of a single variety can be a problem. So, even if ampelography is still very helpful for making collections, molecular markers such as microsatellites are being increasingly

used today. This new tool has been available in a commercial context at ENTAV since 2001 (through technology transfer from INRA-Montpellier).

ENTAV and INRA are the only national institutes in France that are officially recognized and registered to present and obtain clones for certification.

Responding to the interest in French material from foreign countries, the two institutes have created a common trademark, ENTAV-INRA®, which is registered in 50 countries. This trademark underlines the know-how built up over 40 years. It guarantees the origin, authenticity, phytosanitary quality and genetic value of the material. ENTAV is in charge of the national and international dissemination of French certified clones. The income generated through this trademark is used to support selection work with regional partners: study collections, repositories or certification requests.

Fig. 1. Population pyramid of the French vineyard in 1998 (source: DGDDI and ONIVINS 2002).

Grapevine clone maintenance in France in 2003

All French repositories of clones are in open-field vineyards. Only ENTAV has begun to duplicate a part of its collection in insect-proof greenhouses since 2002, with the objective of long-term security. Towards the same goal, INRA and the Institut de Recherche pour le Développement (IRD) in Montpellier are trying to develop cryopreservation protocols for dormant buds. Practical use of this method is not expected soon, but it might be a very useful tool within a few years.

Part of the French system's wealth is the number and status of organizations involved in selection and preservation. Over many years, clone collections have been supported by three types of agents: INRA (centres in Angers, Bordeaux, Colmar and Montpellier), ENTAV, and about 30 professional partners in the regions (Agricultural chambers, Interprofessional committees, Technical associations, Winegrowers' unions). Public and sometimes private partners are also involved in this mission.

The cultivars potentially concerned by clonal work are the 228 clones registered in the Official Catalogue (ENTAV 1995). In this list there are still 20 interspecific hybrids and 27 recent intraspecific genotypes which do not benefit from either clonal or preservation action (except for 'Baco 22 A').

In parallel, 16 cultivars originally registered have disappeared from French vineyards, according to the last vineyard register database (Casier Viticole Informatisé, CVI) (DGDDI and ONIVINS 2002): 'Arbane B', 'Aubin vert B', 'Bachet N', 'Bouquettraube B', 'Colombaud B', 'Franc noir de la Haute-Saône N', 'Genovèse B', 'Grassen N', 'Joubertin N', 'Mayorquin B', 'Morescono N', 'Pagadebiti B', 'Petit Meslier B', 'Rimenèse B', 'Roublot B' and 'Servanin N'. Each variety is represented by at least one accession in the two national collections of Vassal (INRA) and ENTAV.

Although the 228 cultivars are all to be preserved in theory, the specific preservation and selection actions taking place are heterogeneous and depend on the economic importance of the variety.

Today 88 cultivars feature in one (or more) regional specific clone collection. Some of them have a national and international importance (e.g. 'Chardonnay B', 'Merlot N', 'Syrah N'); others are only of local interest (e.g. 'Piquepoul B', 'Petit Courbu B', 'Négrette N'). Some cultivars are present in several collections (e.g. 'Cabernet franc N', 'Mauzac B', 'Petit Verdot N'): this explains the total of 102 repositories in 2003.

The creation of regional repositories began in the late 1950s (ex. 'Grenache N', 'Merlot N'), until finally this practice has become very widespread for many cultivars from the beginning of the 1990s (Audeguin *et al.* 1998) until today (Fig. 2).

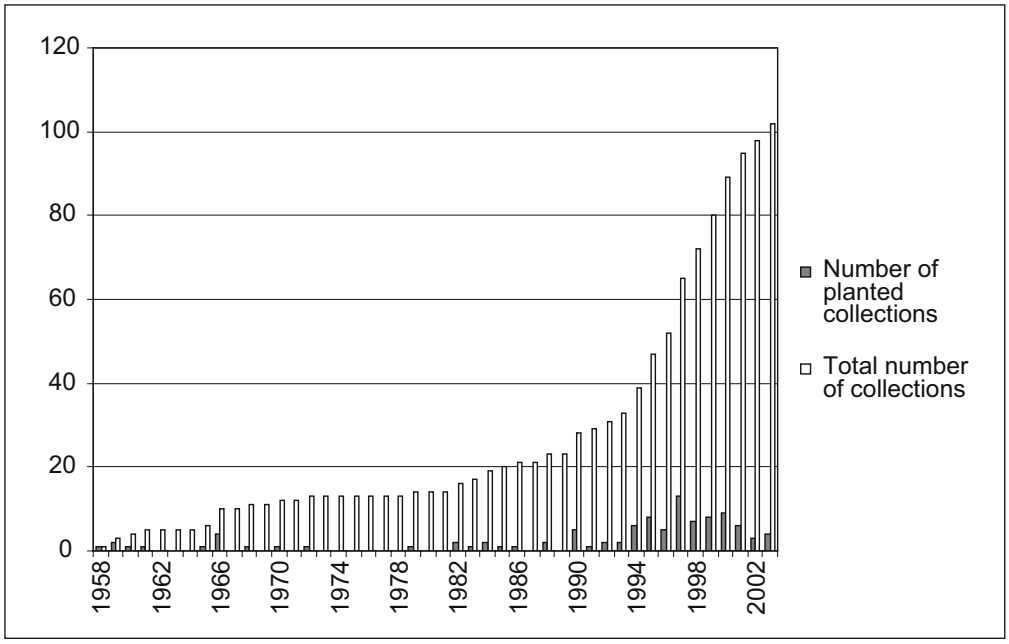

Fig. 2. Distribution of French clonal repositories' creation dates.

The number of clones preserved per variety ranges from 10 (e.g. 'Carmenère N', 'Counoise N', 'Sciacarello N') to more than 300 (e.g. 'Tannat N', 'Merlot N', 'Pinot N', 'Grenache N'). The biggest repository contains 622 clones of 'Syrah N'. The average is about 130 clones per cultivar and per repository (Fig. 3).

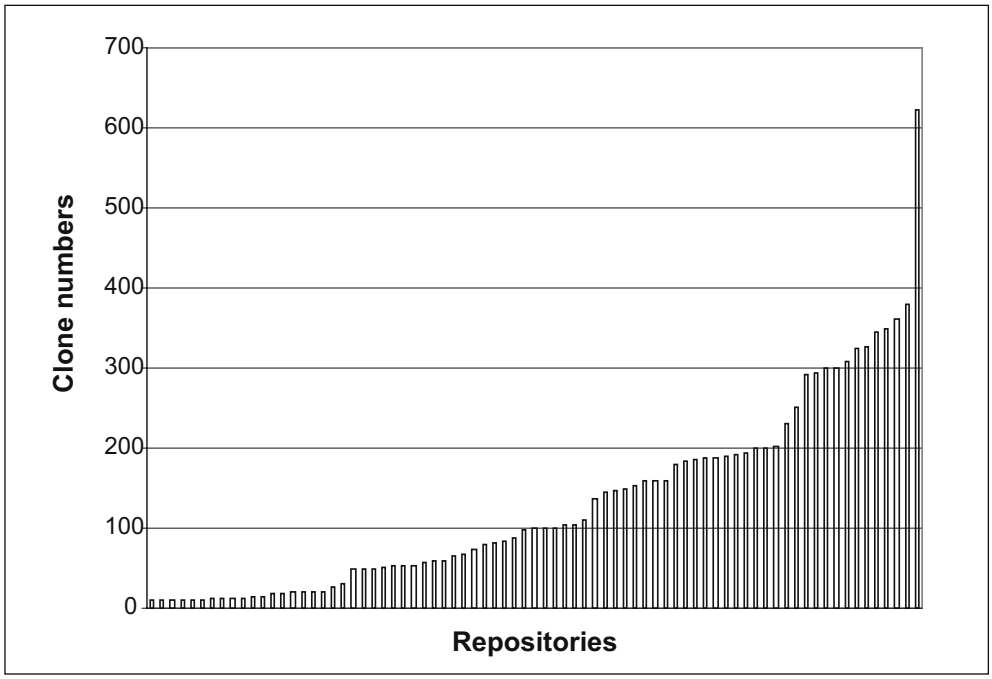

Fig. 3. Distribution of clone numbers per French repository.

In contrast, for 14 cultivars there are few certified clones and no specific regional repository (e.g. 'Alicante Henri Bouschet N', 'Aubun N', 'Jurançon noir N'). Preservation of these clones is only done by ENTAV.

Finally, the number of cultivars registered in the Official Catalogue but without any certified clone at the moment is 70 (e.g. 'Arbane B', 'Béclan N', 'Claverie B', 'Merlot blanc B', 'Terret gris G', 'Valdiguié N'). Within those cultivars, some collecting/conservation/selection/certification work is planned for the coming years (2003-2008) on 31 varieties (e.g. 'Arbane B', 'Baco 22 A', 'Béclan N', 'Cinsaut N', 'Clairette B' and 'Clairette rose Rs', 'Claverie B', 'Franc noir de la Haute-Saône N', 'Mérille N', 'Meslier Saint-François B', 'Milgranet B', 'Orbois B', 'Petit Meslier B', 'Romorantin B' and 'Sacy B'). The other 39 varieties with neither a certified clone nor in local demand are only represented by a few accessions in ENTAV and INRA collections. They can be considered as "orphan" varieties. For those 70 varieties, the problem is the low quantity of material still available in old plots; this limits every present project.

The whole system represents about 15 000 to 20 000 clones (precise inventory and identification of duplicates are in progress) with 30 regional partners. This mobilization of local professional partners with national institutes is essential. The

coordinating position of ENTAV is central and the creation with INRA of a national network on grapevine collections is in progress. This network is established with the support of the Genetic Resources Board (Bureau des Ressources Génétiques, BRG) and its general agreement or "Charter". The resources concerned are not only the clones but every grapevine genetic resource. This network is helpful for the partners in managing and harmonizing information related to selection and preservation and also in the support of new programmes in grapevine genetic resources.

Clonal certification and collection perspectives

Since the late 1950s, French efforts on grapevine clonal collection and preservation have been important and continuous.

For cultivars with economic importance, the present objective is to certify new clones that represent the natural diversity preserved in collections.

For secondary (or endangered) cultivars, the main goal is to create a specific clone collection located in its traditional region and the certification of at least one clone per cultivar.

In association with regional partners, several actions are in progress at this moment. In concrete terms, three or four repositories are supported (creation or extension) each year and three to ten clones are certified.

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Web sites

Etablissement National pour l'Amélioration de la Viticulture (ENTAV)	www.entav.fr
Institut National de la Recherche Agronomique (INRA)	www.inra.fr
Office National Interprofessionnel des Vins (ONIVINS)	www.onivins.fr

Rediscovery of several less known grapevine varieties in Romania

Marius Stoian, Anca Drãgunescu and Marius Tomescu
Wine Growing Research Institute, Jud. Prahova, Valea Calugareasca, Romania

The rediscovery of old varieties that are threatened by extinction and their use into hybridization programmes is of great value to plant genetic resources conservation.

The grapevine varieties described below were planted almost 130 years ago in Romania. The project aims at studying old forgotten grapevine varieties and spontaneously growing wild varieties ('Rupestris Viala') and including them in the ampelographic collections.

The following varieties were identified: 'Agria', 'Boscănată', 'Țâța vacii albă', 'Balaer blanc' and 'Rupestris Viala'. None of them is sensitive to rot or powdery mildew. They are described below.

Țâța vacii albă
White variety for table grapes. White berries quite lax in the bunch, plump pulp, thin skin. Grape yield: 11.1 t/ha. Sugar content: 176 g/l. Acidity: 4.1 g/l. Commercial appearance: quite nice. Bunch weight: 485 g.

Boscănată
Black variety for table grapes and wine grapes. Black berries quite lax in the bunch, juicy pulp, medium-thick skin. Grape yield: 11 t/ha. Sugar content: 172 g/l. Acidity: 5.8 g/l.

Balaer blanc
White variety for table grapes. White berries quite lax in the bunch, fleshy pulp, thin skin. Grape yield: 12 t/ha. Sugar content: 181 g/l. Acidity: 4.9 g/l. It may be used in winemaking for its sugar content and flavour.

Agria
Black variety for wine grapes. Black berries, lax in the bunch, juicy pulp, thick skin. Grape yield: 8 t/ha. Sugar content: 185 g/l. Acidity: 5.2 g/l. Given its sugar content and coloured must, it is used only for red wines.

Rupestris Viala
A rootstock variety differing from 'Rupestris du Lot' by two main ampelographic characters: functional female flower, U-shaped leaf petiole sinus.

These varieties require less work and fewer pesticides (at least one antifungal treatment less than the five that are necessary for other *vinifera* varieties).

These old varieties are genetically less vulnerable to diseases than the currently used *vinifera* varieties.

'Rupestris Viala' roots easily, therefore diminishing the cost of the grafted planting material.

A study of grapevine genetic resources of the Georgian subgroup under Crimean conditions

Vladimir A. Volynkin
Institute for Vine and Wine "Magarach", Yalta, Crimea, Ukraine

Historically, prior to studying new grape varieties under new ecoclimatic conditions, they first had to be introduced to the new zone. This used to be associated with considerable difficulties, including the long duration of the studies. No criterion was available to evaluate on a preliminary basis a variety's behaviour under new ecoclimatic conditions, without introducing the grape to the zone. The concept of "biological zero" (in terms of grapevines, this is represented by a temperature of +10°C) could be used as the initial criterion for starting to describe the performance of a new variety in a new zone, since it determines the beginning of a plant's vegetative growth, irrespective of its geographical origin and parentage. Whatever the date of the "biological zero" onset for a plant of a given variety in each vegetation year, the same number of days will be required to pass through the phenological stages which are determined by the genome of the variety.

Under the conditions of the pre-mountainous zone of the Crimea (Experimental Station "Magarach"), we found considerable variations over the years in the "biological zero" onset in grapevine. This enabled us to use this criterion to observe the onset of a phenological stage (Volynkin 2001, 2002).

Our research was done in 1995-2000, and is based on 160 varieties belonging to the Georgian subgroup of the ecogeographical group of the Black Sea Basin: *Vitis vinifera pontica georgica* Negr. Average sugar accumulation of the varieties under study varied from 14 to 24 g/cm^3. To facilitate the examination of a possible correlation between sugar accumulation and the length and timing of the production period, the varieties were divided into four groups according to the levels of sugar accumulation: (1) low (≤ 16 g/cm^3), (2) medium (17-19 g/cm^3), (3) high (20-22 g/cm^3) and (4) very high (≥ 22 g/cm^3).

It is noteworthy that many researchers see the production period as a whole length of time from budbreak till the onset of industrial maturity, and this parameter is commonly used to define early or late dates of ripening. The production period is also used in models for the breeding of new varieties. However, we suggest, while combining by crossing pairs of varieties with different dates of ripening, that both the whole length and the structure of the production period should be taken into account. The structure of the production period can be described by using the dates of the onset of intermediate phenological stages and the lengths of periods between phenological stages of the grape plant, taking "biological zero" as the starting point for the variety in each case.

The formation of particular biological properties in a definite variety, form or species of grape has been shown to be associated with the existence of isolated centres of origin of grapevine; a set of ecological factors prevailing in these centres influence the formation of these traits (Vavilov 1926, 1987; Negrul 1946a). In this connection one should bear in mind that the development of any crop, including grape, takes place in definite latitudes (Negrul 1946b; Zaitsev 1973, 1983). Within this geographical framework which determines the potential crop distribution, one can draw conclusions about the commercial expediency of introducing various grapevine varieties from one region to another and their potential suitability for new sets of ecogeographic

conditions. One can therefore speak both about the general theory of evolution and the introduction of crops and a possible and promising introduction of certain forms and varieties of grape both to new latitudes and longitudes throughout the globe.

In this paper we describe detailed studies of the development of grape varieties belonging to the Georgian subgroup of the ecogeographical group of the Black Sea Basin under conditions of the Crimea (Experimental Station "Magarach" located in the pre-mountainous zone of the peninsula). The varieties under study are *Vitis vinifera* grapes all originating from the same microcentre, i.e. the Caucasus. The possibility of revealing the two microcentres of grape origin, the Crimea and the Caucasus, is confirmed by expeditions carried out in those two regions (Ramishvili 1988) and the samples of wild grape found there, both of *Vitis vinifera* and *V. silvestris*.

Experimental material presented in Tables 1 and 2 should be considered, however, not from the standpoint of the development and behaviour of the same varieties in different centres of origin but with reference to the development of groups of varieties differing in biological characteristics (such as level of sugar accumulation) depending on the dates of the onset of phenological stages from the biological zero (+10°C for grape), i.e. the structure of the production period.

Table 1. Percent fractions of periods of time from +10°C prior to the dates of the onset of phenological stages in varieties of the Georgian subgroup with different levels of sugar accumulation

Level of sugar accumulation	Budbreak (%)	Onset of flowering (%)	Onset of ripening (%)	Industrial maturity (%)
Low	2.7	27.1	70.2	100.0
Medium	3.0	28.1	68.9	100.0
High	3.1	27.8	69.1	100.0
Very high	3.6	27.4	69.0	100.0
$\bar{x}$	3.1	27.6	69.3	100.0
σ	0.3	0.4	0.6	0.0
V	10.5	1.4	0.8	0.0

Table 2. Percent fractions of periods of time between the dates of the onset of phenological stages in varieties of the Georgian subgroup with different levels of sugar accumulation

Level of sugar accumulation	From 10°C to budbreak (%)	From budbreak to the onset of flowering (%)	From the onset of flowering to veraison[1] (%)	From veraison to industrial maturity (%)
Low	2.7	25.8	43.0	28.5
Medium	3.0	26.7	39.2	31.1
High	3.1	27.3	41.4	28.2
Very high	3.6	26.2	41.2	29.0
$\bar{x}$	3.1	26.5	41.2	29.2
σ	0.3	0.6	1.4	1.1
V	10.5	2.1	3.3	3.9

[1] Veraison: first colour change, (beginning of) ripening in grapes.

It is well known both from the practice and the literature that varieties differ in the level of sugar accumulation and the time needed to attain ripening. Here we compare these two parameters based on the dates of the onset of individual phenological stages calculated in days from +10°C as the biological zero for grape. Such a comparison envisages analysis both of the length of time prior to the date of the onset of a phenological stage (Table 1) and the length of time between phenological stages (Table 2).

Since the whole of the production period, from budbreak to industrial maturity, in all the groups of varieties with different levels of sugar accumulation is taken as 100%, we might expect that the structure of the production period may be different for the four distinct sugar level groups. Nevertheless, as it is seen from Table 1, varieties in all the groups need the same percentage periods of time to achieve budbreak (about 3%), flowering (about 30%) and veraison (about 70%). This is confirmed by data presented in Table 2 which indicate that, irrespective of the level of sugar accumulation, varieties of this well-defined botanical group show the same percentage periods of time between phenological stages: about 3% from the biological zero to budbreak, 27% from budbreak to the onset of flowering, 40% from the onset of flowering to veraison and 30% from veraison to industrial maturity. Therefore, this suggests that this parameter (percentage periods of time between phenological stages) should be considered as a biological characteristic of the structure of the production period for a certain group of varieties belonging to a single taxonomic group based on their geographical origin and botanical properties. No correlation between the level of sugar accumulation and the structure of the production period was established. Against the general biological regularities revealed, it is of interest to consider the information obtained concerning the structure of the production period (in terms of number of days) for individual varieties of the Georgian subgroup of the ecogeographical group of the Black Sea Basin with different levels of sugar accumulation (Table 3).

Table 3. Number of days between phenological stages in varieties of the Georgian subgroup of the ecogeographical group of the Black Sea Basin with different levels of sugar accumulation

Level of sugar accumulation	Varieties	From 10°C till budbreak (days)	From budbreak till the onset of flowering (days)	From the onset of flowering till veraison (days)	From veraison till industrial maturity (days)	Total length of the production period (days)
Low	Mamukas sapere	7	47	72	66	192
	Alexandrouli	3	50	77	42	172
	Aladasturi	6	50	91	45	192
	Chvitiluri	5	47	89	51	192
Medium	Khroghi	4	48	74	66	192
	Argvetuli sapere	5	51	57	79	192
	Rkatsiteli	7	48	66	51	172
	Rko shavi	6	45	77	48	176
	Mtsklarta	3	51	70	48	172
High	Grdzelmtevana	3	48	64	43	158
	Machkvaturi	4	51	81	56	192
	Durgushi	6	49	90	47	192
	Ojaleshi	7	46	80	59	192
	Urishula	6	44	58	59	167
Very high	Shavbarda	7	47	75	63	192
	Buza	6	47	73	41	172

It is noteworthy that, on the one hand, varieties differing in the length of the production period by 20 days or more often fall within the same group which is established on the basis of the level of sugar accumulation. On the other hand, all four groups also contain varieties with the same total length (e.g. 192 days) of the production period. Also noteworthy is the fact that within each group corresponding to the level of sugar accumulation, some varieties have the same total length of the production period but with a different structure.

Therefore, we may conclude that no correlation exists between the level of sugar accumulation and the length and the structure of the production period in varieties belonging to the Georgian subgroup of the ecogeographical group of the Black Sea Basin and that all these parameters are variety-specific. They may be used in combining pairs of varieties with a view to obtaining forms with different desired levels of sugar accumulation and time to ripening, taking into account the structure of the production period of the initial forms.

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APPENDICES

Appendix I. Proposal for the acceptance of a *Vitis* Working Group within ECP/GR

Long-term goal: conservation and sustainable use of *Vitis* Genetic Resources in Europe

Objectives: strengthen European collaboration on genetic resources of *Vitis* through establishing a Working Group within ECP/GR

Grapes are among the most important fruit crops in Europe. In 1999 the area planted in Europe (5.02 million ha) represented 64% of the world's wine-growing area. The production of wine in the same year was 207.5 million hl which was 74% of the world production of wine, and the production of table grapes was 3.1 million tonnes, which was 23% of world production.

The EU project GENRES CT96 81, which started on 1 March 1997 and ends on 28 February 2002, brought tremendous progress to *Vitis* genetic resources. Twelve partners from seven countries of the European Union (Austria, France, Germany, Greece, Italy, Portugal and Spain) and seven partners from non-EU countries (Bulgaria, Croatia, Czech Republic, Hungary, Moldova, Slovenia and Switzerland) participated.

Within the first four years of the project the European *Vitis* Database was established. It now includes passport data of about 27 000 accessions, with primary descriptions of more than 600 and secondary descriptions of nearly 300 accessions. Primary and secondary descriptions focused mainly on old neglected native grapevine varieties. To support variety identification, photos (about 1000) from different parts of the vine of 250 accessions were added. In addition the project turned out to be the appropriate platform for standardizing molecular tools such as Sequence Tagged Microsatellite Analysis (STMS) and the development of descriptors for such markers.

During the last project meeting in September 2001, all project partners expressed a demand to continue the initiative. Huge efforts like the EU project GENRES 81 provide an excellent platform to be continued as an ECP/GR Working Group. After the acceptance of an ECP/GR Working Group on *Vitis* the following outputs will be proposed:

1. Committed experts from a larger number of European countries will collaborate on *Vitis* genetic resources.
2. Sustainable maintenance and updating of the European *Vitis* Database.
3. Involvement of partners from Eastern European countries.
4. Promotion of commonly agreed primary, secondary and STMS marker descriptors.
5. Completion of characterization and evaluation of endangered grapevine cultivars.
6. Improved management of EU *Vitis* collections through use of the characterization results.

The interest in the EU *Vitis* Database established within the GENRES CT96 81 project is high, as witnessed by a frequency of use of up to 200 times per month. On-line changes/additions by each partner on passport and descriptor data is a short- to medium-term objective to maintain the database updated, but has to be monitored by an institution in charge.

The inclusion of partners from Eastern European countries (Albania, Armenia, Cyprus, Macedonia FYR, Romania, Slovakia, Serbia and Montenegro) is of great importance, as they are maintaining in particular the varieties of Eastern European origin.

The 54 primary, 16 secondary and 6 STMS marker descriptors used within the scope of the GENRES CT96 81 project were taken mainly from the "Descriptor List for Grapevine Varieties and *Vitis* Species" (OIV 1983)[25] and partly modified according to the ampelography experts' experience; some descriptors were newly created. The reintegration/addition of these descriptors into the OIV "Descriptor List for Grapevine Varieties and *Vitis* Species" and the official acknowledgement of the 76 descriptors are envisaged. In the revised edition of the "Descriptors for Grapevine (*Vitis* spp.)" (IPGRI *et al.* 1997)[26], some modifications carried out during the first GENRES CT96 81 workshop were already included.

IPGRI, UPOV and OIV are working with differing descriptor lists. With the objective of harmonizing the descriptor definitions of these three different lists, the partners from GENRES CT96 81 decided to contact representatives from IPGRI, UPOV and OIV to organize a round table and to find out how the differences can be overcome.

Initiated through GENRES CT96 81 in all participating countries, efforts on safeguarding, description, identification and evaluation of old endangered cultivars have been intensified. But owing to the large number of homonymous and synonymous designations and the occurrence of 5 to 10% misnamed varieties in grapevine collections, the trueness-to-type and hence the sorting out of grapevine collections and the exchange of true-to-type material is an ongoing problem.

Owing to the problem of synonymy/homonymy and misnaming, the following facts must be stated:

1. It is not known from the 27 000 accessions in the EU *Vitis* Database how often the same variety occurs and which varieties are endangered and should be conserved for security because they occur only once or twice.
2. Within the five project years, accessions of old endangered varieties were described and the data registered in the EU *Vitis* Database, but the variety identity (trueness-to-type) for many of the characterized varieties still has to be assessed.
3. The GENRES CT96 81 participants considered STMS markers as an appropriate tool for trueness-to-type assessment. STMS marker data obtained in different laboratories of the GENRES CT96 81 partners produce reliable results by encoding the allele length with example variety codes. The analysis of a second set of varieties is still in progress and will lead to the completion of already

[25] OIV. 1983. Descriptor List for Grapevine Varieties and *Vitis* Species. Office International de la Vigne et du Vin, Paris.

[26] IPGRI, UPOV, OIV. 1997. Descriptors for Grapevine (*Vitis* spp.). International Union for the Protection of New Varieties of Plants, Geneva, Switzerland/Office International de la Vigne et du Vin, Paris, France/International Plant Genetic Resources Institute, Rome, Italy.

designed STMS marker descriptors. The next steps should be (1) the STMS marker analysis of accessions in the partners' collections, (2) assessment of the trueness-to-type of varieties and (3) the development of an identification procedure, helping to sort out mistakes within collections.

Attention should also be paid to the identity verification of *Vitis* species and their offspring as a source of valuable genes for breeding with very distinct characters for resistance.

Hence the proposed priorities of an ECP/GR Working Group on *Vitis* would be:

1. Database completion (updating passport data, addition of descriptor data, expansion to other European countries)
2. Harmonization of developed descriptors with existing descriptor lists of IPGRI, UPOV and OIV
3. Checking of the trueness-to-type of accessions in grapevine collections by ampelographic and STMS marker descriptors
4. Publication of the results.

As the coordinator of GENRES CT96 81 I submit the proposal to you and hope that it meets with your consideration and approval.

Dr Erika Dettweiler IRZ Geilweilerhof, 17.09.2001

Bundesanstalt für Züchtungsforschung an Kulturpflanzen
Institut für Rebenzüchtung Geilweilerhof
76833 Siebeldingen
Germany

Appendix II. Acronyms and abbreviations

AFLP	Amplified fragment length polymorphism
BAZ	Bundesanstalt für Züchtungsforschung an Kulturpflanzen (Federal Centre for Breeding Research on Cultivated Plants), Germany *(now Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen)*
BGVCAM	Banco de Germoplasma de Vid de la Comunidad de Madrid (*Vitis* Germplasm Bank), El Encín, Spain
BPGRY	Bank of Plant Genetic Resources of Yugoslavia
BRG	Bureau des Ressources Génétiques (Genetic Resources Board), Paris, France
CCDB	Central Crop Database
CIS	Community of Independent States
CNR	Consiglio Nazionale delle Ricerche (National Research Council), Italy
CRA-VIT	Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Centro di Ricerca per la Viticoltura (Agricultural Research Council – Research Centre for Viticulture), Conegliano, Italy
CRI	Crop Research Institute, Prague, Czech Republic
CTPS	Comité Technique Permanent de Sélection (Permanent Technical Selection Committee), France
ECP/GR	European Cooperative Programme for Crop Genetic Resources Networks *(now ECPGR)*
ECPGR	European Cooperative Programme for Plant Genetic Resources
ELISA	Enzyme-linked immunosorbent assay
ENTAV	Etablissement National Technique pour l'Amélioration de la Viticulture (National Technical Association for Viticultural Improvement), France
EPGRIS	European Plant Genetic Resources Infra-Structure
ETSIA	Escuela Técnica Superior de Ingenieros Agrónomos (Higher Technical Scool of Agricultural Engineering), Madrid, Spain
EU	European Union
EURISCO	European Plant Genetic Resources Search Catalogue
EVDB	European *Vitis* Database
FAO	Food and Agriculture Organization of the United Nations
GFLV	Grapevine fanleaf virus
IBPGR	International Board for Plant Genetic Resources *(now Bioversity International)*
IHVO	Research Institute of Horticulture, Viticulture and Oenology, Tbilisi, Georgia
IMIA	Instituto Madrileño de Investigación Agraria y Alimentaria (Institute of Agriculture and Food Research of Madrid), Spain

INAO	Institut national des appellations d'origine (National Institute for the Labels of Origin), France
INRA	Institut National de la Recherche Agronomique (National Institute for Agricultural Research), France
INVV	Institutul National pentru Viticultura si Vinificatie **(**National Institute for Viticulture and Oenology), Moldova
IPGRI	International Plant Genetic Resources Institute *(now Bioversity International)*
IRZ	Institut für Rebenzüchtung (Institute for Grapevine Breeding) Geilweilerhof, Germany
ISO	International Organization for Standardization
ISV	Istituto Sperimentale per la Viticoltura (Experimental Institute for Viticulture), Italy
KSAU	Kuban State Agrarian University, Russian Federation
MCPD	Multi-crop Passport Descriptors (FAO/IPGRI)
MLS	Multilateral System
NCRIHV	North Caucasus Regional Institute for Horticulture and Viticulture, Russian Federation
OIV	Organisation Internationale de la Vigne et du Vin (International Organisation of Vine and Wine), Paris, France
ONIVINS	Office National Interprofessionnel des Vins (Interprofessional Wine Organisation), France
PCR	Polymerase chain reaction
PGR	Plant genetic resources
RNED	Réseau National d'Essai et de Démonstration (National Trial and Demonstration Network), France
SSR	Simple sequence repeat
STMS	Sequence tagged microsatellite site
UPM	Universidad Politécnica de Madrid (Polytechnic University of Madrid), Spain
UPOV	Union Internationale pour la Protection des Obtentions Végétales (International Union for the Protection of New Varieties of Plants), Geneva, Switzerland
VIR	N.I. Vavilov Research Institute of Plant Industry, St. Petersburg, Russian Federation
VIVC	*Vitis* International Variety Catalogue
ZADI	Zentralstelle für Agrardokumentation und -information (Centre for Agricultural Information and Documentation), Germany

Appendix III. Agenda

***First Meeting of the ECP/GR Working Group on* Vitis**
12–14 June 2003, Palić, Serbia and Montenegro

Wednesday 11 June

Arrival of participants

Thursday 12 June

9:00-10:00 **Introduction**

Opening remarks, welcome address (*local organizers*)

ECP/GR

Information on ECP/GR and current international PGR events (*L. Maggioni, 15 min*.)

GENRES Project

GENRES 081: A basis for the preservation and utilization of *Vitis* genetic resources (*E. Dettweiler, 15 min.*)

Discussion

10:00-10:30 **Documentation**

- The European *Vitis* Database: Status quo. Part I: Passport data (*E. Dettweiler, 15 min.)*

10:30-11:00 *Coffee break*

11:00-11:20
- The EPGRIS project and the new Multi-crop Passport Descriptors *(L. Maggioni, 15 min.)*

Discussion

11:20-12:30
- GENRES 081 descriptors for *Vitis*/ Priority primary descriptors *(A. Schneider, 30 min.)*
- Harmonization of IPGRI, OIV and UPOV descriptors for *Vitis* *(E. Dettweiler, 20 min.)*
- The European *Vitis* database: Status quo. Part II: Characterization / evaluation data *(E. Dettweiler, 15 min.)*

12:30-14:00 *Lunch*

14:00-15:30 **Presentation of national collections**
(brief updates on the status of national collections - 5-7 minutes each)
Albania, Armenia, Austria, Azerbaijan, Czech Republic, Croatia, Cyprus, France, Georgia, Germany, Greece, Hungary, Italy, Macedonia FYR

15:30-16:00 *Coffee break*

16:00-17:30 **Presentation of national collections** *(continued)*
Malta, Moldova, Portugal, Russian Federation, Serbia and Montenegro, Spain, Ukraine

Updating the European *Vitis* database with collection data from new partners
(*Discussion introduced by E. Dettweiler*)

19:30 *Wine-tasting*

Friday 13 June

9:00-10:30 **Differentiation and identification of grapevine varieties**

- Synonymy, homonymy, misnaming, etc. - Obstacles for a system for an international network on the conservation of *Vitis* germplasm in Europe (*E. Dettweiler, 30 min.*)
- Developing a common standard for uniform labelling of microsatellite profiles using reference cultivar based allele codes (*A. Jung, 30 min.*)
- Combined use of STMS markers plus ampelographic characters in order to detect synonyms, homonyms and misnaming (*J. Ortiz, 15 min.*)
- Documentation of biodiversity within varieties : genetic differences within the grapevine variety 'Traminer' (*H. Kaserer, 10 min*)

10:30-11:00 *Coffee break*

11:00-12:30

- Development of a database of germplasm of Ukrainian, Moldovan and Russian *Vitis vinifera* cultivars using microsatellite markers (*S.M. Gorislavets)*

Development of a common STMS marker database
(Discussion introduced by E. Dettweiler)

12:30-14:00 *Lunch*

14:00-15:30 **Conservation and sustainable use of grapevine genetic resources in the Caucasus and Northern Black sea region**
(L. Maggioni and D. Maghradze)

Survey on *Vitis* genetic resources
Brief presentation of attending members about:

- number of varieties existing in former times/today
- number of recommended varieties, respectively number of clones available/mainly used in viticulture
- collecting in old vineyards
- maintenance of grapevine variety clones in conservatories (e.g. France, Switzerland)

15:30-16:00 *Coffee break*

16:00-17:30

- Collecting, preservation and evaluation of clones in France (*T. Lacombe, 15 min.*)
- Investigation of genetic resources of the Georgian subgroup of grape under Crimea conditions (*S.M. Goryslavets, 15 min.*)

Saturday 14 June

9:00-16:00 **Drafting of the report**
Excursion for participants not involved in report drafting: *visit to the Institute of Fruitgrowing and Viticulture and sightseeing of the town of Sremski Karlovci*

16:00-18:00 **Closing session**

- Discussion and approval of the report
- Election of Chair and Vice-Chair
- Closing remarks

Social dinner

Sunday 15 June

Departure of participants

Appendix IV. List of participants

***First Meeting of the ECP/GR Working Group on* Vitis**
12–14 June 2003, Palić, Serbia and Montenegro

N.B. Contact details updated at time of publication. The composition of the Working Groups is subject to changes. The latest update for the *Vitis* Working Group can be found on the Web page (http://www.bioversityinternational.org/networks/ecpgr/contacts/ecpgr_wgvit.asp).

Working Group Members

Adriatik Çakalli
National Seed and Seedling Institute
Albanian Genebank
Enti Shteteror i Frerave dhe Fidaneve
Rr. Siri Kodra
Tirana
Albania
Tel: (355-42) 30324
Fax: (355-43) 62419
Email: adri_cristi@hotmail.com

Samvel Gasparyan
International Academy of Viticulture and Wine-making
33 Pushkin Street, Apartment 10
Erevan
Armenia
Tel: (374-1) 233279
Fax: (374-1) 232441
Email: agrpress@arminco.com

Herwig Kaserer
Höhere Bundeslehranstalt und Bundesamt für Wein- und Obstbau
Abteilung Rebenzüchtung
Wienerstrasse 74
3400 Klosterneuburg
Austria
Tel: (43-2244) 2286
Fax: (43-2244) 29554
Email1: herwig.kaserer@hblawo.bmlfuw.gv.at
Email2: reb.hblawo@eunet.at

Edi Maletić
Department of Viticulture and Enology
Faculty of Agriculture
University of Zagreb
Svetosimunska 25
1000 Zagreb
Croatia
Tel: (385-1) 2393 918/2301 189
Fax: (385-1) 2343 658
Email: emaletic@agr.hr

Savvas Savvides
Agricultural Research Institute
Fruit Trees/Viticulture Section
Ministry of Agriculture, Natural Resources and Environment
PO Box 2016
1516 Nicosia
Cyprus
Tel: (357-2) 2403220
Fax: (357-2) 2316770
Email: s.savvides@arinet.ari.gov.cy

Olga Mercedes Jandurová
Research Station of Viticulture
Karlštejn 96
Czech Republic
Tel: (420) 311681131
Fax: (420) 311681131
Email: jandurova@vurv.cz

Thierry Lacombe
Institut National de la Recherche Agronomique (INRA)
UMR 1097 – Diversité et Adaptation des Plantes Cultivées (DiAPC)
Equipe Génétique et Génomique Vigne
2 Place Pierre Viala
34060 Montpellier cedex 1
France
Tel: (33) 499 612253
Fax: (33) 499 612064
Email: lacombe@supagro.inra.fr

Erika Maul *(ex Dettweiler)*
Julius Kühn-Institut (JKI)
Bundesforschungsinstitut für Kulturpflanzen
Institut für Rebenzüchtung
Geilweilerhof
76833 Siebeldingen
Germany
Tel: (49-6345) 41122
Fax: (49-6345) 919050
Email: erika.maul@jki.bund.de

Massimo Gardiman
(Representing Angelo Costacurta)
Consiglio per la Ricerca e la Sperimentazione in Agricoltura
Centro di Ricerca per la Viticoltura
CRA-VIT
Viale 28 Aprile, 26
31015 Conegliano (TV)
Italy
Tel: (39) 0438 738058
Fax: (39) 0438 738489
Email: massimo.gardiman@entecra.it

Klime Beleski
Department for Vitis and Winery
Institute of Agriculture - Skopje
Ul. Natanail Kuchevishki bb
1000 Skopje
Macedonia (FYR)
Tel: (389-2) 2621980
Fax: (389-2) 2621434
Email: beleskik@freemail.com.mk

Randall Caruana
Viticulture and Oenology Unit
National Agricultural R&D Centre
Ghammieri, Marsa
Malta
Tel: (356-21) 248802
Fax: (356-21) 231517
Email: randall.caruana@gov.mt

José Eduardo Eiras Dias
Estação Vitivinicola Nacional
Quinta da Almoinha
2565-191 Dois Portos
Portugal
Tel: (351-261) 712106/712124
Fax: (351-261) 712426
Email: evn.eiras.dias@mail.net4b.pt

Petar Cindrić
Faculty of Agriculture
Institute for Fruitgrowing and Viticulture
Trg Dositeja Obradovica, 8
21000 Novi Sad
Serbia
Tel: (381-21) 350366/881765
Fax: (381-21) 450123
Email: cindric@polj.ns.ac.yu

Jesús María Ortiz
Escuela Técnica Superior de Ingenieros Agrónomos (ETSIA)
Universidad Politécnica de Madrid
Ciudad Universitaria s/n
28040 Madrid
Spain
Tel: (34-91) 3365658
Fax: (34-91) 3365656
Email: jesusmaria.ortizm@upm.es

Observers

Jasminka Karoglan Kontić
Department of Viticulture and Oenology
Faculty of Agriculture
University of Zagreb
Svetosimunska 25
1000 Zagreb
Croatia
Tel: (385-1) 2343657/2301189
Fax: (385-1) 2343658/2393893
Email: jkkontic@agr.hr

David Maghradze
Department of Grapevine and Fruit Crops
Germplasm Research, Genetics and Breeding
Research Institute of Horticulture, Viticulture and Oenology (IHVO)
6, Marshal Gelovani Ave.
0159 Tbilisi
Georgia
Tel: (995-32) 534822(+123int); 524611/523011/526908
Fax: (995-32) 001368
Email: d_maghradze@geo.net.ge

Andreas Jung
Office for Ampelography and Clone selection
Heidengasse 13
67363 Lustadt
Germany
Tel: (49-6347) 700294
Fax: (49-6227) 7803462
Email: andreas.jung@online.de

Anna Schneider
Consiglio Nazionale delle Ricerche (CNR) Istituto Virologia Vegetale
Unit of Grugliasco
Via Leonardo da Vinci 44
10095 Grugliasco (TO)
Italy
Tel: (39) 011 6708745
Fax: (39) 011 6708658
Email: a.schneider@ivv.cnr.it

Gheorghe Savin
Department of Grapevine Genetic Resources and Amelioration
National Institute for Viticulture and Oenology
59, Vierul street
2070 Chişinău
Republic of Moldova
Tel./Fax: (373-22) 285003
Email1: gsavin@mail.md
Email2: ghsavin@yahoo.com

Alexander Smurygin
N.I. Vavilov Research Institute of Plant Industry (VIR)
Bolshaya Morskaya Street 42-44
190000 St Petersburg
Russian Federation
Tel: (7-812) 3119901
Fax: (7-812) 3118762
Email1: lptroshin@mail.ru
Email2: s.alexanian@vir.nw.ru

Ivana Dulić–Marković
(at the time of the meeting)
Federal Department for Plant and Animal Genetic Resources
Federal Ministry of Agriculture
Omladinskih brigada 1
11070 Belgrade
Serbia and Montenegro

Maja Jecmenica
Ministry of Agriculture, Forestry and Water Management
Nemanjina 22-26
11000 Belgrade
Serbia
Tel: (381-11) 3117588
Fax: (381-11) 3117591
Email1: maja.jecmenica@minpolj.sr.gov.yu
Email2: mjecmenica@yahoo.com

Vladimir Pekić
Maize Research Institute "Zemun Polje" (MRIZP)
Gene Bank for Maize
Slobodana Bajica 1
11081 Belgrade-Zemun
Serbia
Tel: (381-11) 3756704
Fax: (381-11) 3756707
Email1: vpekic@mrizp.co.yu
Email2: jmuminovic@mrizp.co.yu

Nada Korać
Faculty of Agriculture
Trg D. Obradovica 8
21000 Novi Sad
Serbia
Tel: (381-22) 350366
Fax: (381-22) 450123
Email: cindric@polj.ns.ac.yu

Marina Radonić
Ministry of Agriculture of the Republic of Serbia
Omladinskih brigada 1
11070 Belgrade
Serbia
Tel: (381-11) 3117588
Fax: (381-11) 3117591
Email: fed.pagri@www.yu

Svitlana Goryslavets
National Institute of Vine and Wine "Magarach"
Department Breeding, Genetic of Grape and Ampelography
31 Kirov Street
98600 Yalta, Crimea
Ukraine
Tel: (380-50) 2853258
Fax: (380-654) 230608
Email: goricvet_2@rambler.ru

ECPGR Secretariat

Lorenzo Maggioni
ECPGR Coordinator
Bioversity International
Via dei Tre Denari 472/a
00057 Maccarese
Rome
Italy
Tel: (39) 06 611 8231
Fax: (39) 06 619 79661
Email: l.maggioni@cgiar.org

Unable to attend / Corresponding members

Mirza Musayev
Institute of Genetics Resources
National Academy of Sciences
155 Azadlyg prospect
370106 Baku
Azerbaijan
Tel: (994-12) 4424907
Email: m_musayev@yahoo.com

Penka Abracheva[27]
Institute of Viticulture and Enology
Kala Tepe str.
5800 Pleven
Bulgaria
Tel: (359-64) 22423/22468/22161
Fax: (359-64) 26470
Email: ilv@el-soft.com

Antonios Mattheou
Agricultural Research Centre of Makedonia and Thraki (ARCMT)
Greek Gene Bank
570 01 Thermi, Thessaloniki
Greece
Tel: (30-31) 471544
Fax: (30-31) 471209
Email: kgeggb@otenet.gr

Erzsebet Kiss
Research Institute for Viticulture and Enology
Pázmány Péter u.4
7634 Pécs
Hungary
Tel: (36-72) 511132
Fax: (36-72) 517936
Email: vitivin@axelero.hu

Pal Kozma
Research Institute for Viticulture and Enology
Pázmány Péter u.4
7634 Pécs
Hungary
Tel: (36-72) 511132
Fax: (36-72) 517936
Email: vitivin@axelero.hu

Avihai Perl
Department of Fruit Tree Breeding
Agricultural Research Organisation (ARO)
The Volcani Center
PO Box 6
50250 Bet Dagan
Israel
Tel: (972-3) 9683720
Fax: (972-3) 9669583
Email: perlx@int.gov.il

Angelo Costacurta
Consiglio per la Ricerca e la Sperimentazione in Agricoltura
Centro di Ricerca per la Viticoltura
CRA-VIT
Viale 28 Aprile, 26
31015 Conegliano (TV)
Italy
Tel: (39) 0438 73264/738058
Fax: (39) 0438 738489
Email1: angelo.costacurta@entecra.it
Email2: amg@ispervit.it

Giulia Tamai
Dipartimento di Produzione Vegetale, Sezione Coltivazioni Arboree
Università degli Studi di Milano
Via Celoria 2
20133 Milano
Italy
Tel: (39) 02 50316 563
Fax: (39) 02 50316 553
Email: giulia.tamai@unimi.it

Darius Ryliskis
Vilnius University Botanical Garden
Kairenu 43
2040 Vilnius
Lithuania
Tel: (370-2) 317944
Fax: (370-2) 317429
Email: darius.ryliskis@gf.vu.lt

[27] (now retired)

Roman Houlubowicz
Katedra Nasiennictwa i Szkolkarstwa
Akademia Rolnicza w Poznaniu
Baranowo
62081 Poznań
Poland
Tel: (48-61) 8142291
Fax: (48-61) 8142291
Email: rhseed@owl.au.poznan.pl

Marius Stoian
Wine Growing Research Institute
Valea Calugareasca
Str Valea Mantei nr. 1
2040 Jud. Prahova, Valea Calugareasca
Romania
Tel: (402-44) 236300
Fax: (402-44) 236389
Email: icvv.vie.vin@xnet.ro

Boris Koruza
Fruit and Vine Growing Department
Agricultural Institute of Slovenia
Hacqetova 17
1001 Ljubljana
Slovenia
Tel: (386-1) 2805146
Fax: (386-1) 2805255
Email: boris.koruza@kis.si

Dominique Maigre
Station Fédérale de Recherches en
Production Végétale de Changins
Domaine Viticole de Caudoz
1009 Pully
Switzerland
Tel: (41-21) 7211562
Fax: (41-21) 7289629
Email:
dominique.maigre@acw.admin.ch

Necla Ercan
Aegean Agricultural Research Institute
(AARI)
PO Box 9, Menemen
35661 Izmir
Turkey
Tel: (90-232) 8461331
Fax: (90-232) 8461107
Email: etae@aari.gov.tr

Emma-Jane Allen
National Fruit Collections
Imperial College
Brogdale Road
Faversham ME13 8X2
Kent
United Kingdom
Tel: (44-1795) 590272
Email: e.lamont@imperial.ac.uk

INDEX OF AUTHORS

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