



ACCADEMIA ITALIANA
DELLA VITE E DEL VINO

INTRA-VARIETAL GENETIC VARIABILITY IN SOME ITALIAN MALVASIAS

Meneghetti S., Costacurta A., Calò A.

1) The grapevine variability (morphology, physiology) was observed since the Romans ...

2) Italy, for example, is a Country rich of "grapevine Diversity"...

L. JVNII MODERATI
COLVMELLAE
DE
RE RVSTICA
LIBRI XII
CVRANTE
JO. MATTHIA GESNERO
TOMVS PRIMVS



MANNHEMII,
Cura & Sumptibus Societatis literatae
MDCCCLXXXI.

148 COLVMELLAE LIB. III.

tius orbis fruges, adhibito studio colonorum, ferre didicerit. quo minus addubitamus de eo fructu, qui velut indigena, peculiarisque & vernaculus est hujus foli. Neque enim dubium est, Maffici, Surrentinique & Albanæ atque Caecubi agri vites omnium, quas terra sustinet, in nobilitate vini principes esse.

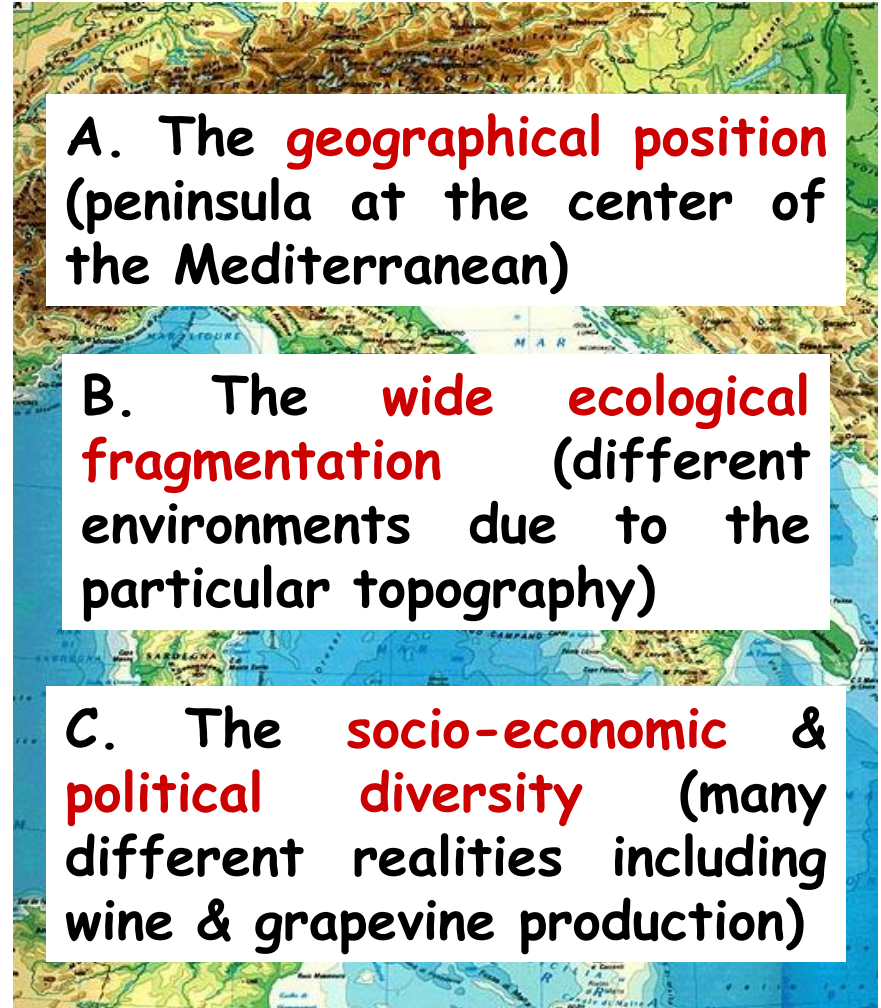
Ut uva complura genera acinorum habeat.

CAP. IX. Foecunditas ab his forsitan desideratur: sed & haec adjuvari potest cultoris industria. Nam si, ut paulo ante retuli, benignissima rerum omnium parens natura, quasque genteis atque terras ita muneribus propriis ditavit, ut tamen caeteras non in uniuersum similibus dotibus fraudaret: cur eam dubitemus etiam in vitibus praedictam legem seruasse? ut quamvis earum genus aliquod praecipue foecundum esse uoluerit, tanquam Bituricum, aut basilicum; non tamen sic Amineum sterile reddiderit, ut ex multis millibus ejus ne paucissimae quidem vites foecundae, tanquam in Italicis hominibus Albanæ illae forores reperiri possint. Id autem cum sit verisimile, tum etiam verum esse nos docuit experimentum, cum & in Ardeatino agro, quem multis temporibus ipsi ante possedimus, & in Carleolano, itemque

A. The **geographical position** (peninsula at the center of the Mediterranean)

B. The **wide ecological fragmentation** (different environments due to the particular topography)

C. The **socio-economic & political diversity** (many different realities including wine & grapevine production)



3) The different growing **environments** during the grapevine evolution increased the differentiation in *Vitis vinifera* with several varieties, biotypes and clones

4) The selection of genotypes and cultivars was made by **farmers** considering the agronomic characters (not only the adaptation to the environment)

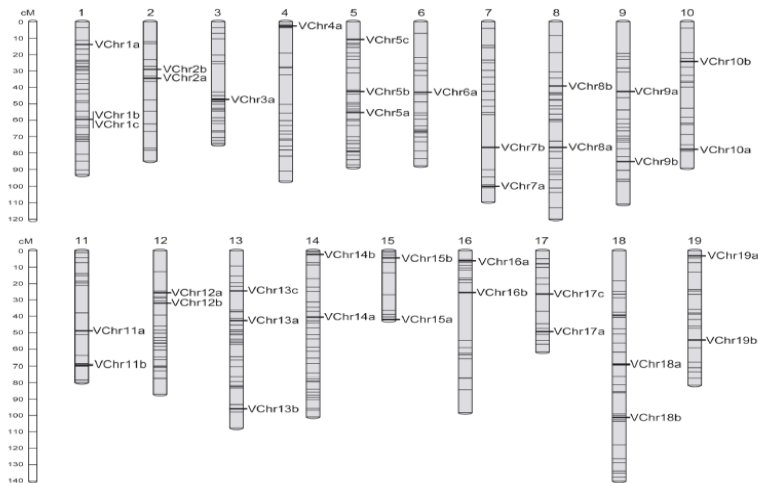
5) Molecular techniques allow to analyze the differences directly at **DNA** level

VITIS GENOME

MOLECULAR MARKERS

BMC Plant Biology 2008, 8:127

<http://www.biomedcentral.com/1471-2229/8/127>



(19 linkage groups, $2n=38$)

SSR (*Simple Sequence Repeat*)

I-SSR (*Inter-microsatellites*)

RAPD (*Random Amplified Polymorphic DNA*)

AFLP (*Amplified Fragment Length Polymorphism*)

SAMPL (*Selective Amplification of Microsatellite Polymorphic Loci*)

SNP (*Single Nucleotide Polymorphism*)

S-SAP (*Specific Sequence Amplified Polymorphism*)

M-SAP (*Methyl-Sensitive Amplified length Polymorphism*)

REMAP (*REtrotransposon-Microsatellite Amplified Polymorphism*)

IRAP (*Inter-Retrotransposon Amplified Polymorphism*)

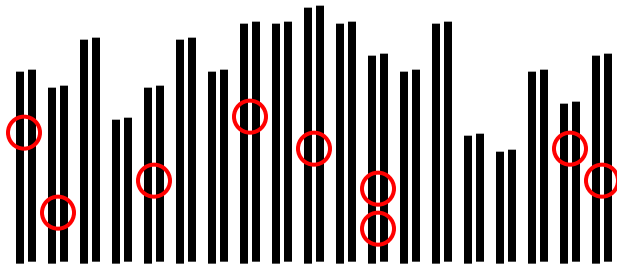
Chloroplast DNA polymorphisms

M-AFLP (*Microsatellites Amplified Fragment Length Polymorphism*)

ANALYSIS of GENOME

Varietal Identification

microsatellite



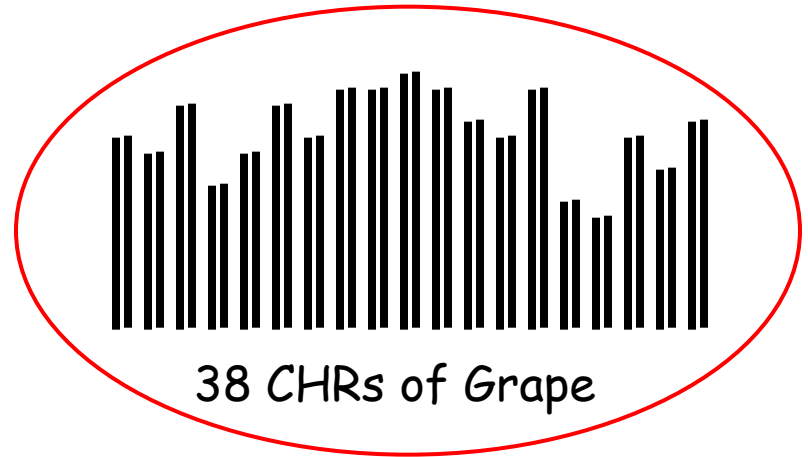
38 CHRs of Grape

SSR analysis (specific genomic regions) identifies the cultivar of grapevine.

Genotypes of the same cultivar showed the same microsatellite profile (SSR alleles).

Intra-varietal variability

Analysis of all Genome



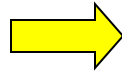
38 CHRs of Grape

Genotypes from a grapevine cultivar (one microsatellite profile) can be discriminated using the different molecular markers of the genome (different biotypes, accessions or clones from a same cultivar).

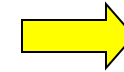
CULTIVAR IDENTIFICATION (SSR)



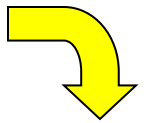
LEAVES



DNA EXTRACTION BY ROBOT



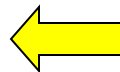
DNA



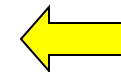
SSR MIX



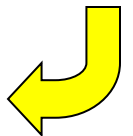
CULTIVAR NAME



AB3130XL SEQUENCER

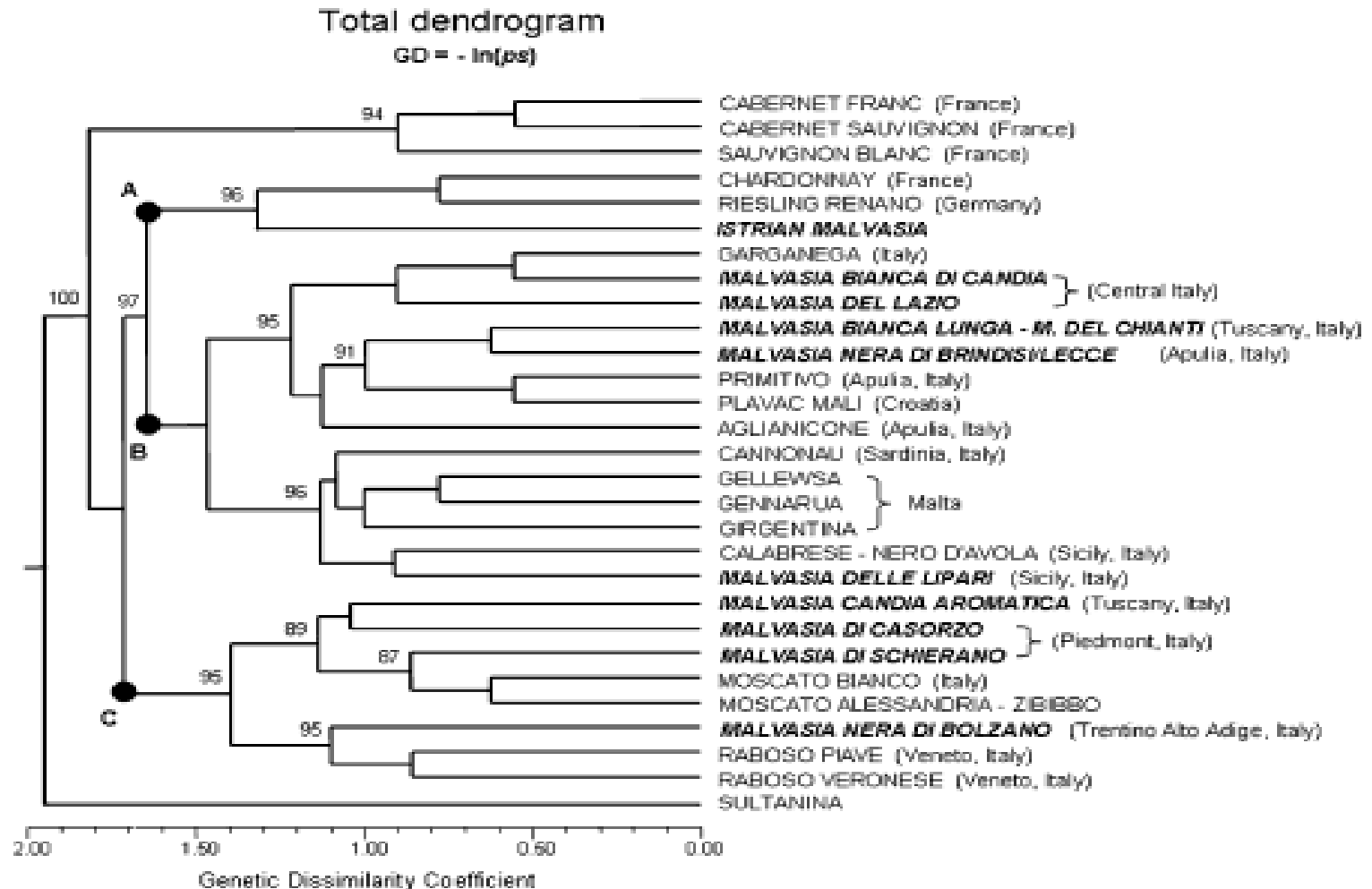


PCR



INTER-VARIETAL GENETIC VARIABILITY of Malvasias

1. microsatellite markers (**SSR**);
2. AFLP-based markers (MAFLP, SAMPL, etc) and I-SSR markers



INTRA-VARIETAL GENETIC VARIABILITY

6) The CRA-VIT - Research Centre for Viticulture of Conegliano - has been developing the molecular protocols for analyzing the genetic variability at intra-varietal level (biotypes and clones) by combining techniques to analyze **large portions of the genome** (AFLP-derived) with markers typical of **hypervariable regions** as:

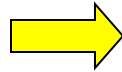
1. Specific microsatellite markers (**SSR**);
2. Regions flanking the microsatellites (**I-SSR**);
3. regions where the sequence of the repeating pattern changes (i.e. AT/AG) - adjacent microsatellites (**ASn**).

In this way it was possible to have a complete analysis on the grape genome and simultaneously to analyze the SSR polymorphisms, important for the *Vitis* taxonomy.

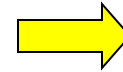
STUDY OF INTRAVARIETAL GENETIC VARIABILITY



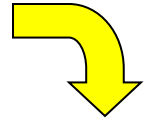
LEAVES OF GENOTYPES



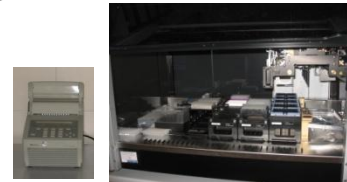
SAME CULTIVAR



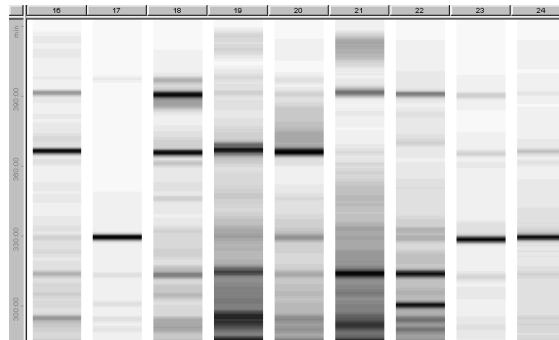
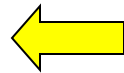
AFLP
SAMPL
I-SSR
M-AFLP



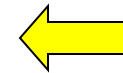
.....



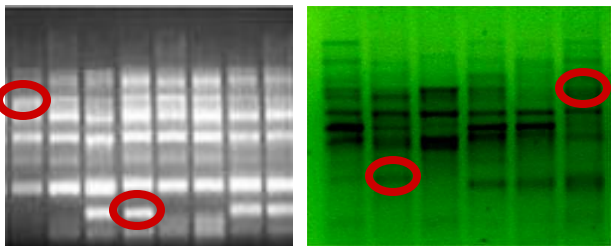
CLONE / BIOTYPE



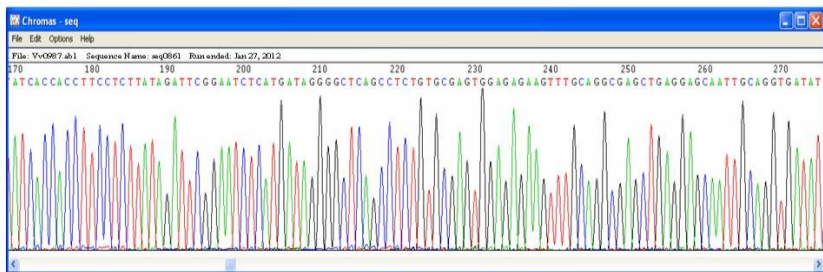
MOLECULAR POLYMORPHISMS



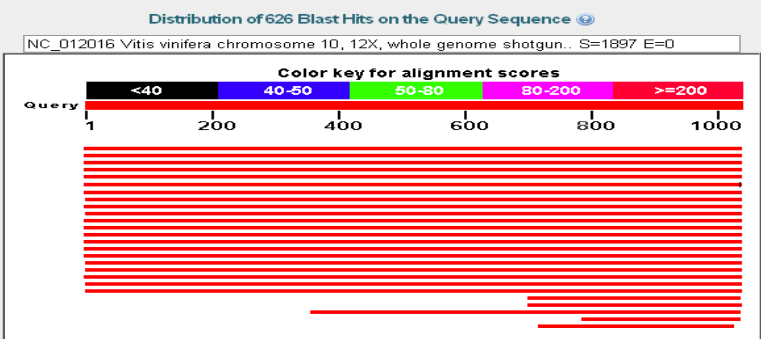
BIOTYPE IDENTIFICATION



DNA POLYMORPHISMS



SEQUENCE OF MARKERS



NUCLEOTIDE DATABASES

```
> [emb|AM457386.1] [D] Vitis vinifera, whole genome shotgun sequence, contig VV78X164817.3,
clone EMYV_115
Length=22664

Score = 171 bits (92), Expect = 7e-39
Identities = 396/536 (74%), Gaps = 56/536 (10%)
Strand=Plus/Plus

Query 1       GGATCAGAAAATGAGAAAATGCACAAAAGTAAAAAGCCAAACCCATTGATCG--TTGAC   58
Sbjct 17652    GGATCAGAAAATGAGAAAATGCACAAAAGTAAAAAGTAAAAAGTAAAGCCATTAATCCGCTTAC   17711

Query 59      CAGGTAGCTGCACAGATCAGGACGATCAGCCGATTCTGCRGG-CTAGCAGATTAATA   117
Sbjct 17712    CAGGCCGCTCAGTGCATCAGGACGATCAGCCGATTCTGCRGG-CTAGCAGATTAATAA   17770

Query 118     C-CTCCCTGACTCTCATTTTCTTCC-CGT-TTCCTT-TGG-TT-CTTCAAGT-TTTT   170
Sbjct 17771    GACTCC-TGCAGGTGTTTGT--CGAC-TATTC-TTCTCAATTTCTTAGG-GTTTTTC   17824

Query 171     TCGATT-CAG-TTGGCTATAAG--TGATTTTTGATCCA--TT-GCAGCAATTTGAGGGG   223
Sbjct 17825    AC-ATTTGAGGATT-CC-A-AGAAT-AGTTC--AAGCAAAGTTTGACCGATT-GAAGGC   17876

Query 224     TTCAGATTG-ATTTTT-GGAGG-ITAG-GGCTTGGATTGGGGAC--GG-CTTCTCCTCC   276
Sbjct 17877    TTTTGATTGGATTTTTGGAGGATTTGGAGGCTAGGG-TTCCGGTTTTGGACTACACTTTC   17935

Query 277     TTCAS-T-GCCTGTAACAACC--GGTTTCAGCTATTCCACRACACCTAGGGTTTTGGG   332
Sbjct 17936    TTCTCTCGCCGCCCAACAACAAAGTTTT--CTCATTCTCACGCACCTAGGGTTTTGGT   17993

Query 333     TGTG--TGATTCCTCTCACCAAGCTTGTCTCCTCATTCTTG-CTTTTTGATGGTTC   389
Sbjct 17994    TGTGAGTGTTTTTCTCTACCAAGCTGTATCTTTATTTTTTGTCTTT-CTCATGGCTC   18052

Query 390     ATAGGAGAGACTR-CTGCCTTAAGGCACAGKGCAGCCCTAT-TGAGCTCTCTCA   447
Sbjct 18053    ATAGGAGAGACTGGGCA-CTCTAAGGCACAGKGCAGCCCT-AGCTGTGCTTTTA   18110

Query 448     GCCTAC-TCACCGAGACTACCGAAATACAGGTTTGAAAGACACCTTTTATGC   502
Sbjct 18111    GC-TCCCTAGTTGGAGGCTCCCAGAAAGCCAGGTTTGACACTACAATTTTTCAGC   18165
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ALIGNMENT SEQUENCES



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No hyb oligo mishyb library specified
Using 1-based sequence positions
WARNING: Unrecognized base in input sequence; Hybridization probe is unacceptable: Tm too low

OLIGO         start  len   tm   gc%  any  3'  seq
LEFT PRIMER   196    20   60.34  50.00  5.00  2.00  cacactgtttgaagcgttgg
RIGHT PRIMER  392    20   60.25  45.00  4.00  2.00  aagaaggcaagcaaacatgg
HYB OLIGO     290    24   56.37  33.33  7.00  4.00  cctcaaatgcagaattataatgc
SEQUENCE SIZE: 1560
INCLUDED REGION SIZE: 1560

PRODUCT SIZE: 197, PAIR ANY COMPL: 6.00, PAIR 3' COMPL: 2.00

1  tcccraggtagagaggcgaaactcacaagtgacatgggtgaattgaaattggtgtca

61  tttcgaagtgatttcaatcataaagttgaatttcgaaatcataagttgaaattcacaat

121  catttcgaaatgacctccaactgcagcagttatctcaaatggtcataacttctcct

181  ttcagctctcaattgacactgtttgaagcgttggactcctgacttctgagcttggaa
      >>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>>

241  cgatataatagatgatatataatagactctataaagtcctcctaaatcttccacagtc
      AAAAAAAAAAAAAA

301  gaattataatgccatcagatcttttggatttcaaatctcatgcaactgaactctgtctc
      AAAAAAAAAAAAAA

361  atgtctctatttccatgttgctgtcctcttttataactccataatggtcatttctctc
      <<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<<

421  ttccaatgcattatcctctctccttctcttctcttggtccatgagcttgcactc
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PCR SPECIFIC POLYMORPHISMS

7) International publications (Theor Appl Genet, Mol Biotechnol; Annals of Applied Biology)

Annals of Applied Biology ISSN 0003-4746

RESEARCH ARTICLE

Clone differentiation and varietal identification by means of SSR, AFLP, SAMPL and M-AFLP in order to assess the clonal selection of grapevine: the case study of Manto Negro, Callet and Moll, autochthonous cultivars of Majorca

E. Cretazzo¹, S. Meneghetti², M.T. De Andrés³, L. Gaforio³, E. Frare² & J. Cifre¹

CULTIVARS FROM MALLORCA

Mol Biotechnol
DOI 10.1007/s12033-011-9403-9

RESEARCH

Study of Intra-Varietal Genetic Variability in Grapevine Cultivars by PCR-Derived Molecular Markers and Correlations with the Geographic Origins

Stefano Meneghetti · Angelo Costacurta · Giacomo Morreale · Antonio Calò

PRIMITIVO, MALVASIA BR/LE, NEGROAMARO

Mol Biotechnol
DOI 10.1007/s12033-011-9423-5

RESEARCH

Inter- and Intra-Varietal Genetic Variability in Malvasia Cultivars

Stefano Meneghetti · Danijela Poljuha · Enrica Frare · Angelo Costacurta · Giacomo Morreale · Luigi Bavaresco · Antonio Calò

MALVASIA ISTRIANA

Mol Biotechnol (2011) 48:244–254
DOI 10.1007/s12033-010-9365-3

RESEARCH

Clones Identification and Genetic Characterization of Garnacha Grapevine by Means of Different PCR-Derived Marker Systems

Stefano Meneghetti · Angelo Costacurta · Enrica Frare · Graziana Da Rold · Daniele Migliaro · Giacomo Morreale · Manna Crespan · Vicente Sotés · Antonio Calò

GARNACHA GRENACHE CANNONAO

Theor Appl Genet
DOI 10.1007/s00122-010-1411-9

ORIGINAL PAPER

The SSR-based molecular profile of 1005 grapevine (*Vitis vinifera* L.) accessions uncovers new synonymy and parentages, and reveals a large admixture amongst varieties of different geographic origin

Guido Cipriani · Alessandro Spadotto · Irena Jurman · Gabriele Di Gaspero · Manna Crespan · Stefano Meneghetti · Enrica Frare · Rita Vignani · Mauro Cresti · Michele Morgante · Mario Pezzotti · Enrico Pe · Alberto Pollicritti · Raffaele Testolin

SSR LONG REPEATS

Mol Biotechnol
DOI 10.1007/s12033-011-9475-6

RESEARCH

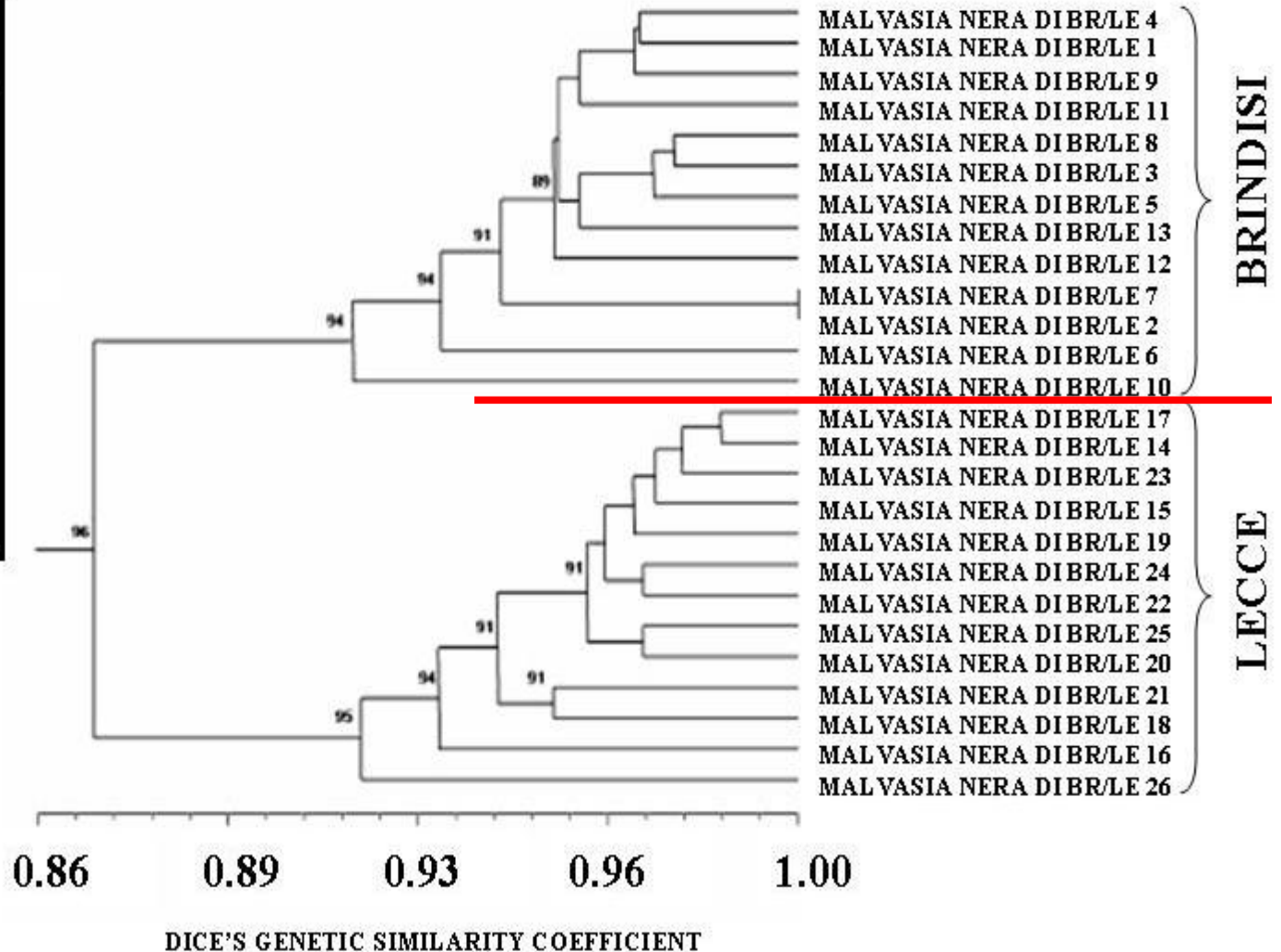
A Strategy to Investigate the Intravarietal Genetic Variability in *Vitis vinifera* L. for Clones and Biotypes Identification and to Correlate Molecular Profiles with Morphological Traits or Geographic Origins

Stefano Meneghetti · Antonio Calò · Luigi Bavaresco

MALVASIA of CANDIA

MALVASIA NERA DI BRINDISI / LECCE

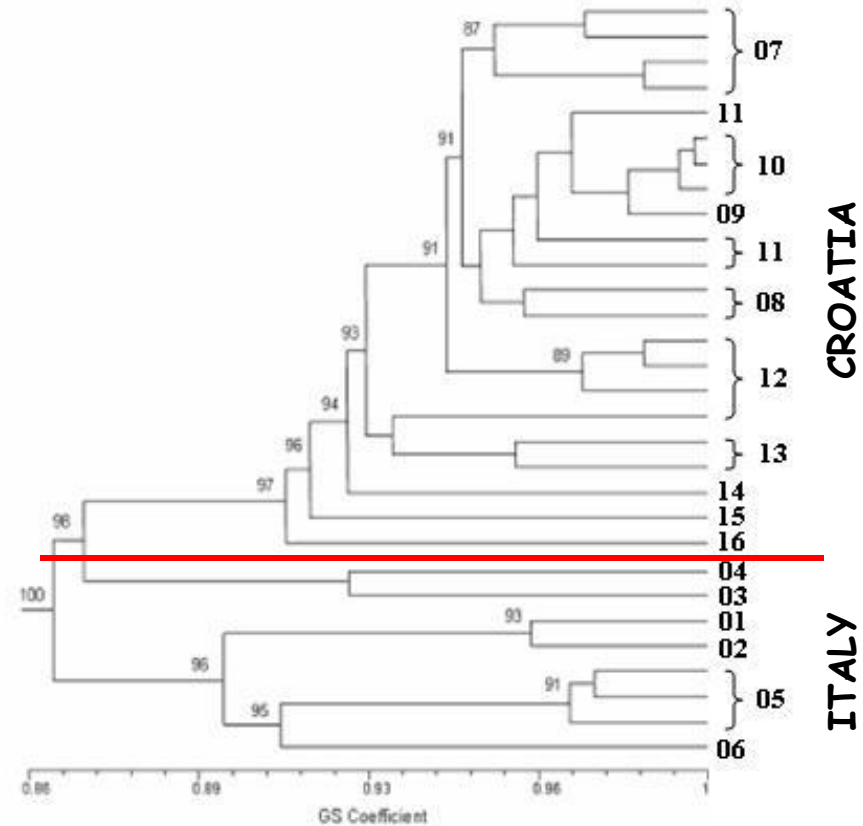
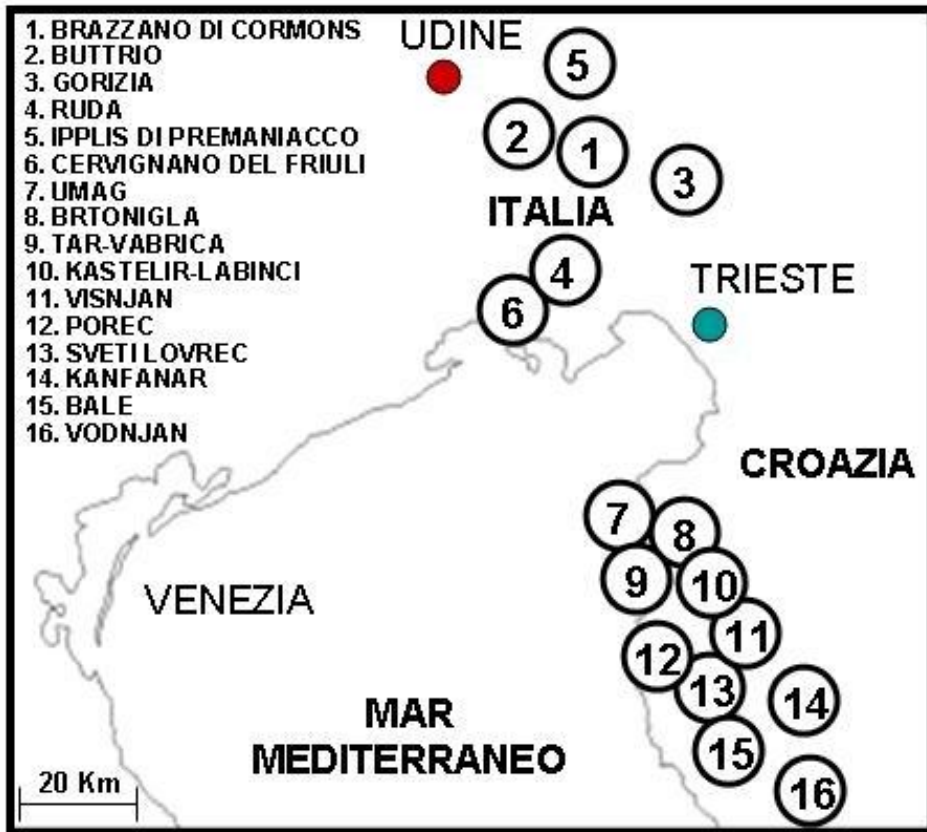
26 accessions of *Malvasia nera* di Brindisi-Lecce from Brindisi and Lecce provinces



MALVASIA ISTRIANA



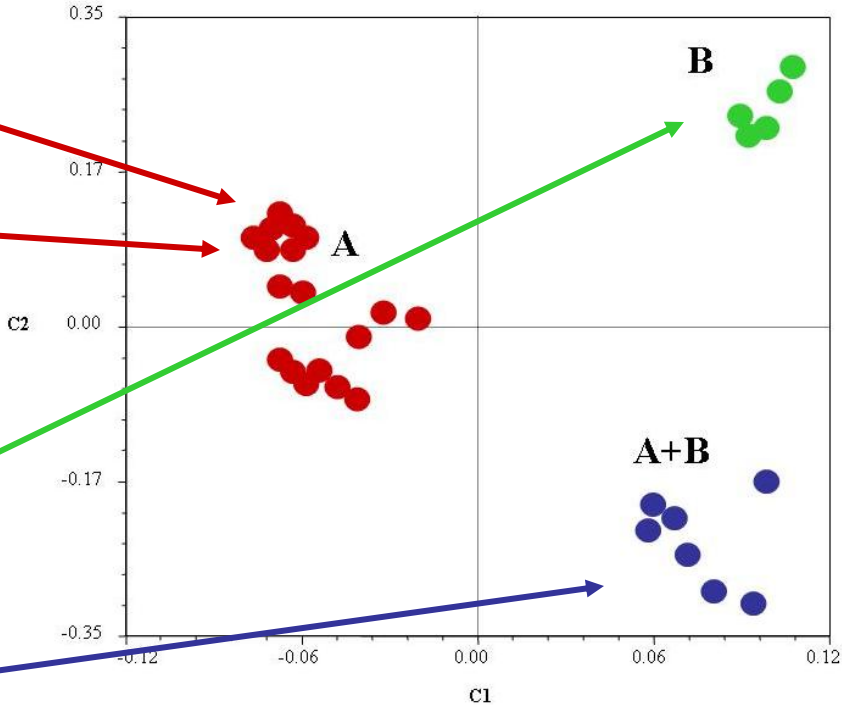
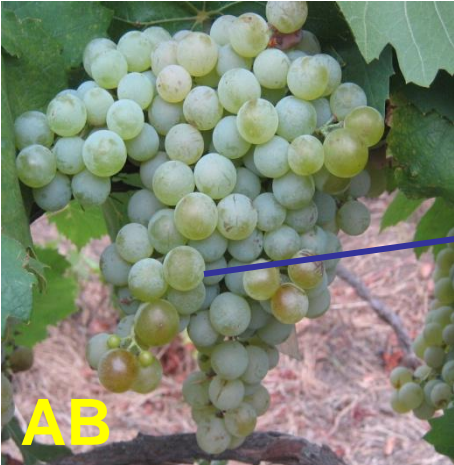
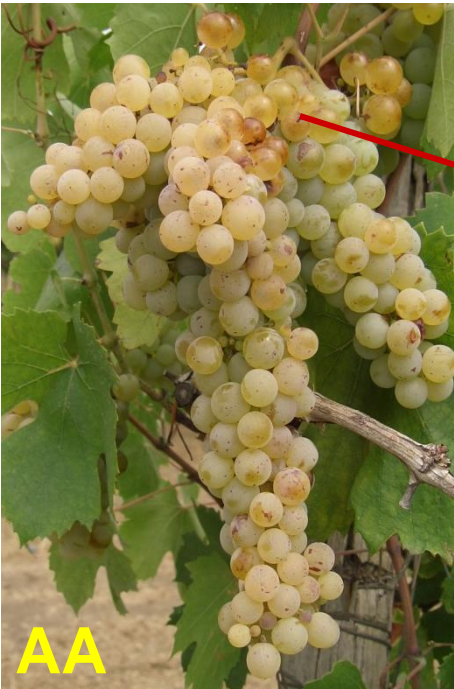
30 accessions of Istrian Malvasia: 8 Italian Clones by VCR, ISV and ERSA Institutes and 22 autochthonous grapevine accessions grown in Croatia.



MALVASIA CANDIA



30 biotypes of Malvasia di Candia S.S. from Frascati DOC - Rome (Italy)



CONCLUSIONS

The molecular strategy here adopted permitted:

1. to identify different accessions, biotypes, and clones of Malvasia cultivars
2. to correlate the genetic differences to their geographical origins or morphological traits.

This genetic variability accumulated during centuries of cultivations and selections should be both recognized and preserved when these claims are corroborated by scientific experimental results.

Thus, it is important not to lose the genetic variability of the biotypes by avoiding the vine nursery practice to propagate the same clone for different cultivation areas.

It would be really useful to promote the propagation of the typical autochthon biotypes, which are possibly already wisely selected by the vine grower because adapted to their environments.

THANKS