MICROSATELLITE ANALYSIS TO DEFINE GENETIC DIVERSITY OF GRAPEVINES (*VITIS VINIFERA* L.) GROWN IN CENTRAL AND WESTERN MEDITERRANEAN COUNTRIES

ANALYSE DE LA DIVERSITÉ GÉNÉTIQUE DE CULTIVARS DE *VITIS VINIFERA* L. DU BASSIN MEDITERRANÉEN CENTRAL ET OCCIDENTAL AU MOYEN DE MARQUEURS MICROSATELLITES

M. LABRA[1], O. FAILLA[2], G. FORNI[3], A. GHIANI[1], A. SCIENZA[2] and F. SALA[1]

1: Department of Biology, University of Milano, Via Celoria 26, 20133 Milano, Italy.
2: Department of Crop Science, University of Milano, Via Celoria 2, 20133 Milano Italy
3: Lombard Museum of History of Agriculture, Sant'Angelo Lodigiano, Italy

Abstract : PCR-based microsatellite analysis has been applied to define the relationships among 238 grapevine cultivars selected as representative of local germplasm in the Central and Western Mediterranean regions.
The estimation of genetic distances among the five grapevines groups, based on cluster analysis, c2 tests and Principal Component Analysis, was coherent with a common geographic origin of cultivated grapes and of their primordial varietal radiation. In fact, genetic similarity was high among French and Iberian cultivars and among Greek and Balkan cultivars. The Italian grapes clustered in an intermediate position, reflecting its geographical location. Results show that the geographical distribution is consistent with a pattern of viticulture based on the origin of primordial varieties from regions in the Near East, and subsequent and multiple variety flows that linked different viticultural regions through different routes. This is coherent with the model of varietal diffusion proposed on the bases of archaeological and historical evidence, in which population movements and cultural exchange contributed to the phenomenon.

Résumé : L'analyse des loci microsatellites a été utilisée pour définir les rapports de similitude génétique entre 238 cultivars de *Vitis vinifera ssp*. sativa cultivés dans les pays du bassin méditerranéen central et occidental.
L'élaboration statistique des données obtenues, effectuée par analyse des clusters, a permis de grouper les cultivars en cinq groupes principaux, parmi lesquels, le groupe 1, qui comprend 50 p. cent des échantillons avec des valeurs de similarité génétique comprises entre 28 et 87 p. cent et le groupe 3 qui comprend les échantillons à provenance géographique différente. Les autres groupes, avec assez peu d'échantillons, ont montré un haut degré de spécificité géographique. L'élaboration statistique, par tests $\chi 2$ et par analyse des composants principaux (PCA), a montré d'importantes relations de similitude génétique entre les cultivars français et ibériques et entre les cultivars grecs et ceux de la zone balkanique. Ces données concordent avec la position géographique occupée divers cultivars étudiés.
Cela fait penser à une origine monocentrique de la vigne cultivée et donc d'un pool génétique dominant qui aurait subi une intense circulation variétale parmi les pays méditerranéens
Sur la base des données archéobotaniques, on a pu définir un modèle d'origine médiorientale de la vigne permettant de suivre sa diffusion en Europe, de l'orient vers l'occident. Les résultats de l'analyse de l'ADN effectuée au moyen des marqueurs microsatellites sont cohérents avec les données archéobotaniques et confirment le modèle décrit qui prévoit une diffusion de la vigne par vagues successives correspondant aussi bien à des déplacements de populations qu'à des échanges culturels.

Mots clés : SSR (Simple Sequence Repeat), Microsatellites, *Vitis vinifera* L.
Key words: SSR (Simple Sequence Repeat), Microsatellite, *Vitis vinifera* L.

INTRODUCTION

The number of grapevine cultivars (*Vitis vinifera spp.* sativa) around the world has been estimated at more than 8-10,000 (ALLEWELDT, 1988).

Their origin is still a matter of discussion.

In particular, there is no agreement as to the localisation of the primary domestication centre from wild grapevine and on possible secondary centres.

*Correspondance : massimo.labra@unimi.it

J. Int. Sci. Vigne Vin, 2002, **36**, n°1, 11-20

On the basis of phyto-geographical considerations, ZEVEN and ZHUKOVSKY (1975) proposed three independent centres of primary domestication: Near East, Central Asia and the Mediterranean Basin. Phyto-geographical and archeo-botanical data convinced ZOHARY and SPIEGEL-ROY (1975) and ZOHARY and HOPS (1993) to propose a single centre, the Near East region, while OLMO (1976) confined this site to the Transcaucasian region. The latter agrees with former hypothesis put forward by NEGRUL (1946) and LEVADOUX (1956).

FORNI (1996), on the basis of archeo-botanical findings, proposed that domestication occurred first in the second half of the 4th millennium B.C., in two neighbouring regions: the Northern Circum Mesopotamian region (i.e., Southern Anatolia, Syria, Northern Lebanon, Kurdistan, Western Iran) and in the Western Transcaucasian one (Georgia).

At first, domesticated grapevine expanded in the South-eastern Mediterranean regions (Palestine, Southern Lebanon and Jordan) (MILLER, 1991; WASILYKOWA *et al.*, 1991; ZOHARY and SPIEGEL-ROY, 1975). Thereafter, domesticated grapevines appear during the first half of the 3rd millennium B.C. in Minor Asia, Southern Greece, Crete and Cyprus. At the beginning of the 2nd millennium B.C. grapevines are found in Southern Balkans (KROLL, 1991; RENFREW, 1973; LOGOTHETIS, 1970), while they make their first appearance in Southern Italy in the second half of the 2nd millennium B.C. and in Northern Italy, Southern France, Spain and Portugal in the second part of the 1st millennium.

Besides localising the primary centre of domestication attention has also been focused on the possible existence of secondary centres of domestication, i e. of centres in which at different times wild varieties have been domesticated (OLMO, 1995). No evidence for the existence of such centres has yet been produced, although the presence of these centres outside the Near East regions cannot be excluded (NUÑEZ and WALKER,1989). In support of this, we should consider that grapes have also been used as food before domestication: archaeological remains showed that ancient populations included wild grapes in their diet even before the 4th millennium B.C.

Grapevine propagation has been traditionally performed by cuttings (vegetative propagation), although occasionally seeds have been propagated for example in the case of migrations and colonisations.

Natural crosses between local wild grapes and introduced grape varieties produced and selected in different periods of the viticultural history may also have determined the appearance of new genetic assortments could also be selected (RIVES, 1974).

On the basis of the above considerations, research on each local grapevine varietal assortment should consider different intersecting events based on:

a. direct domestication from local wild vine;

b. introduction from different places and at different times during the first steps of the establishment of viticulture and/or during the following historical periods;

c. intentional or accidental local breeding and selection;

d. genetic mutation occurring in varieties introduced by mechanisms a, b and d.

The use of historical, cultural, morphological and amphelographical informations to validate these hypothesis when studying the phylogeny and the genetic relationship among grapevine cultivars is considered helpful but insufficient to give conclusive evidences (LABRA *et al.*, 1999; BISSON, 1999; FOSSATI *et al.*, 2001).

In recent years, the use of molecular markers based on PCR amplification of specific or random genomic sequences has been proposed as a more direct and effective tool to study genomes (KARP *et al.*, 1998).

In the case of grapevine, AFLP (based on amplification of DNA restriction products) and SSR (based on amplification of specific microsatellite DNA loci) have been preferentially used (BOTTA *et al.*, 1995; REGNER *et al.*, 1998; LOPES *et al.*, 1999; SEFC *et al.*, 2000). Molecular analysis have already been used to study (a) grapevines varietal assortment (REGNER *et al.*, 1998; LOPES *et al.*, 1999; LABRA *et al.*, 2000; REGNER *et al.*, 2000; SEFC *et al.*, 2000), (b), phylogeny (LABRA *et al.*, 1999) and (c) pedigree (BOWERS and MEREDITH, 1997; SEFC *et al.*, 1998).

In the present study we applied SSR analysis to estimate genetic diversity and relationships among 238 cultivars randomly selected as representative of traditional or minor germplasm growing in Central and Western Mediterranean regions. Results give a substantial contribution to the rationalisation of our knowledge on the expansion pattern of domesticated grapevines, as well as, on the genomic relationships among local germplasm accessions.

MATERIALS AND METHODS

I - PLANT MATERIAL

The 238 grapevine (*Vitis vinifera* L.) accessions used in this study have been selected from different ampelographic collections with the aim to sample Greek, Balkan, Italian, Iberian and French (including Corsica) germplasm. The complete list is given in table I.

II - DNA EXTRACTION

Young leafs (1-2 cm long) were harvested from rooted cuttings, frozen in liquid nitrogen and ground to fine powder. Genomic DNA was extracted in 5 ml of « CTAB buffer » (2 p. cent CTAB, 100 mM Tris-HCl pH 8.0, 20 mM EDTA pH 8.0, 1.4 M NaCl, 1 p. cent w/v polyvinylpyrrolidone, 0.1 p. cent v/v b-mercaptoethanol) as described by DOYLE and DOYLE (1990).

III - SSR ANALYSIS

DNA was analysed at the following 7 microsatellite loci: VVS1, VVS2, VVS3, VVS4, (THOMAS and SCOTT, 1993), VVMD5, VVMD6 and VVMD7 (BOWERS *et al.*, 1996). The analysis was performed by adding 50 ng of genomic DNA to a 25 ml PCR mixture containing 0.25 mM of the DNA primer specified for each microsatellite locus by THOMAS and SCOTT (1993) or BOWERS (1996), 200 mM of each of the 4 dNTPs, 0.5 U Dynazyme and Dynazyme buffer

TABLE I

List of grapevine accessions with their geographical origin

Liste des cultivars de vigne analysés avec indication de leur origine géographique

Area (n° accessions)	Accessions
Balkan (23)	Aniretac; Babic; Blatina; Blau Affentaler; Coarna alba; Coarna negra; Crevatina; Debina N.; Debit; Furmint; Kallmet; Klarnica; Kriqës; Kunbullor; Plavin; Plavina; Plovdina; Rush R.; Ruzija; Shesh B.; Stanusina; Veltliner rot; Zlaratika;
France (16)	Altesse; Bouboulenc; Calitor blanc; Chardonnay; Clairette; Folle B.; Grenache; Gros Manseng; Marsanne; Mourverdre; Petit Manseng; Piquepoul; Roussanne; Sauvignon; Sicilien; Syrah;
Greece (47)	Aggianniotiko; Agiorgitiko; Amfioni (1); Amfioni (2); Ampelakiotiko Mavro; Asprouda Patron (1); Asprouda Patron (2); Assyrtico; Athiri; Bakouri; Begleri; Debina; Fileri; Fokiano; Iatrou (1); Iatrou (2); Kacotrygis; Karvouniaris; Kokkinorobola; Korfiatis; Kotselina; Koutsoulbeli; Liatiko; Mandilaria; Mavro bulgarias; Mavro kalabriton; Mavro kosmas; Mavro messenikola; Mavro styfo; Mavrodafni; Mavrotragano; Mavroudi arachovis; Nerostafylo; Pavlos; Prekniariko; Psilomavro kalavriton; Robola; Roditis; Savatiano; Sefka (1.); Sefka (2); Sideritis; Skylopnichtis; Vartzami; Vilana; Vossos; Votsichi;
Iberia (22)	Albarin; Arino; Bobal; Espadero; Godello; Graciano; Jaen; Laurerio; Macabeo; Malvasia di Sitges; Mataro; Monastrell; Mourisco; Palomino; Parellada; Pedro Ximenes; Tempranillo; Tinta de toro; Torrontes; Trexiaduri; Viura; Xarello;
Italy (130)	Aglianico Galluccio; Albaranzeuli N.; Aleatico; Aleatico Castelvenere; Altulina; Ansonica; Arneis; Barbera; Barzemino; Bellone; Bianco d'Alessano; Bombino N.; Bovale Sardo; Bucalò; Buchholzer Vernatsch; Cagnulari; Canaiolo B.; Canaiolo N.; Carignano; Cesanese; Chiavennasca; Cla; Coda di volpe B.; Cortese; Corvinone; Corvinone San Felice; Croà R.; Croatina; Durassa del Gel; Elbin weiss; Erbaluce; Erbamat; Favorita; Fiano; Forastera; Frappato; Freisa; Furner; Gafforella; Gaglioppo; Garganega; Geschlafene; Grecanico; Grechetto; Greco; Greco B.; Greco di Tufo; Grisa bianca; Grossvernatsch; Heunisch rot; Heunisch weiss; Inzolia; Jungferlen; Kleinvernatsch; Lacrima di moro d'alba; Lagarino; Lagrein; Magliocco; Malvasia del Chianti; Malvasia di Asolo; Malvasia di Candia; Malvasia d'Istria; Malvasia nera di Brindisi; Malvasia nera di Lecce; Mangiaguerra; Marzemino; Mataosso; Minella B.; Montonico; Montuni; Moscato bianco; Moscato giallo; Muskat Blatterle; Muskat Trollinger; Nasco; Nebbiolo; Negroamaro; Nerello Mascalese; Neretto; Nero d'Avola; Nieddera; Ottavianello; Pampanuto; Pecorino; Perricone; Picolit; Piedirosso; Pigato; Pignola (1); Pignola (2); Pignolo; Platterle; Primitivo; Prosecco; Raboso; Refosco Boton; Refosco di Rauscedo; Refosco Faedis; Refosco Guarnieri; Refosco Uccelli; Retagliado; Ribolla; Riesling italico; Rollo; Rossola; Rossolino; Sangiovese; Schiava grossa; Siriaca; Tabernello; Terrano; Timorasso; Trebbiano d'Abruzzo; Trebbiano di Soave; Trebbiano di Spagna; Trebbiano spoletino; Trebbiano valtenesi; Uva di Troia; Uva Rara; Verdicchio; Vermentino; Vermentino N.; Vermiglio; Vernaccia di Orosei; Vernaccia Oristano; Vernaccia S. Gimignano; Vernatsch kleinbeerig; Vespolina; Weiss Vernatsch; Weisser Hortling

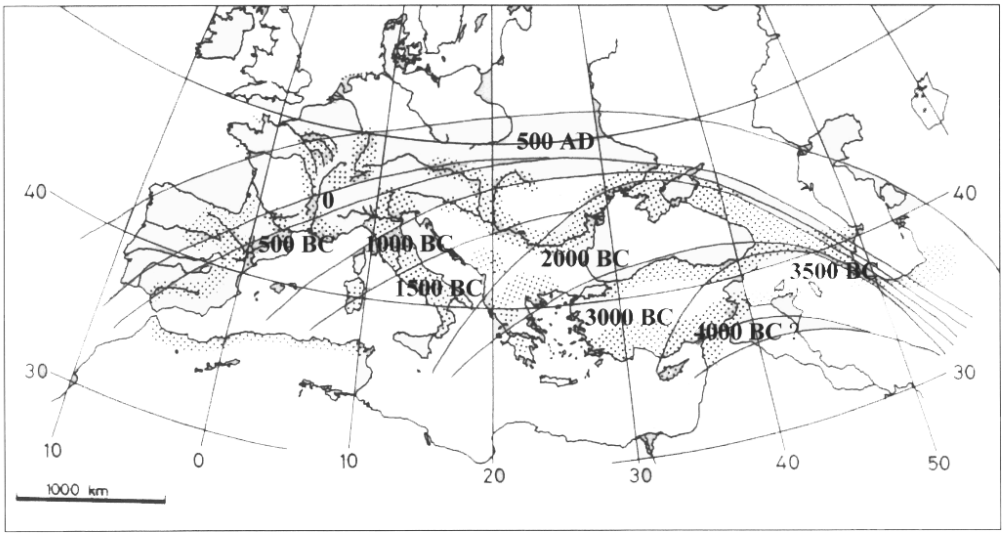

Figure 1 – Possible expansion of domesticated grapevine from East to West on the basis of archaeological and paleontological evidence.

Propagation de la vigne domestique de l'Orient vers l'Occident obtenue sur la base des données archéobotaniques et paléontologiques.

(Celbio, Italy) as specified by the supplier. PCR amplification was performed with a programmable thermal controller (PTC 100, MJ Research Inc., USA) with the following thermal cycles: 7 min at 94 °C; 35 cycles of denaturation (45 s at 94 °C), annealing (30 s at 52 °C) and extension (1 min at 72 °C); then a final step for 7 min. at 72 °C.

IV - ANALYSIS OF THE DNA AMPLIFICATION PRODUCTS

10 ml of the PCR-amplified mixture was added to an equal volume of loading buffer (80 p. cent formamide, 1 mg.ml^{-1} xylene cyanol FF, 1 mg.ml^{-1} bromophenol blue, 10 M EDTA, pH 8.0). Ten ml of this mixture were analysed by electrophoresis on 10 p. cent polyacrylamide gel in TBE buffer (50 mM boric acid, 1 mM EDTA, pH 8.0) for 16 h at 100 mV. After staining in a 5 p. cent ethidium bromide solution, the gel was recorded and analysed in a Gel Doc 2000 (Biorad, USA). The length of different alleles was assigned based on commercial molecular weight markers (pBr322-MspI markers, Celbio)

V - STATISTICAL ANALYSIS

Experimental data were analysed by determining:

1) Genetic distance among accessions. This was measured on the bases of shared alleles (BOWCOCK *et al.*, 1994) and on proximity measures based on dichotomic characters. To this purpose, each microsatellite allele was scored as a binary character for absence (0) or presence (1). Presence was scored as (1) independently of the heterozygous or homozygous state. The resulting data were analyzed both using Jaccard's and Dice's coefficients.

2) Genetic distance among regional population. This was defined by applying the c2 distance and the Principal Component Analysis (PCA) to data on allele frequency of each SSR locus.

In all cases, usefulness of SSR as genomic markers was estimated by calculating gene diversity as $1-\sum p^2_{ij}$ (ANDERSON *et al.,* 1993), were p_{ij} is the frequency of the j allele for the i microsatellite.

All statistical analysis were processed with the SPSS (V. 8.5) software.

RESULTS

I - SSR ANALYSIS

The grapevine cultivars listed in table I (for a total of 238) were selected from different ampelographic collections in the mediterranean area: Italy, Greece, Spain, Portugal, France and Balkan region. These mostly included traditional or minor germplasm accession and excluded only recent accessions produced by breeding.

DNA was extracted from young leaflets and the purified DNA was analysed for polymorphism at 7 microsatellite loci. PCR amplification was performed in the presence of the two oligonucleotide primers specific for each locus. Following gel electrophoresis it was verified that all tested SSR loci were highly polymorphic. Table II summarises the results by showing, at each locus, the numbers of alleles (from 19 to 26),

TABLE II
Range of allele size, number of alleles, and gene diversity verified at seven microsatellite loci among the 238 grapevine accession listed in table I.

Dimension des allèles, nombre d'allèles et diversité génétique correspondant aux sept microsatellites utilisés pour analyser les 238 génotypes de *Vitis vinifera* décrites dans le tableau I.

Locus	Range of allele sizes (bp)	n° of alleles	Gene diversity
VVS1	185 – 260	19	0.80
VVS2	124 – 160	21	0.89
VVS3	202 – 266	26	0.88
VVS4	165 – 223	27	0.91
VVMD5	230 – 264	27	0.91
VVMD6	190 – 220	21	0.86
VVMD7	220 – 267	26	0.89

the range of allele size and the values of gene diversity (from 0.80 to 0.91). The latter substantiate the usefulness of the selected genomic markers.

II - GENOTYPE GROUPING

In order to define genetic relationship among the analysed cultivars, the frequency of shared SSR alleles was measured. The resulting matrix was analysed by using both Jaccard's and Dice's coefficient. Figure 2a shows that these are strictly correlated (r ª 1). High correlation (r ª 0.98) was also found between the Jaccard's coefficient values and percent of shared alleles (figure 2b). In this case, the evident stepwise pattern in the diagram can be explained by considering that the two distance criteria show an intrinsic peculiarity: the percent of shared alleles, but not the Jaccard's (as well as the Dice's) coefficient is influenced by the

TABLE III
Five main varietal groups identified by cluster analysis using Jaccard's coefficient as described in Material and Methods. Within any group, every pair of genotypes had at least 10 p. cent of common SSR alleles.

Les cinq principaux groupes identifiés utilisant le coefficient de Jaccard comme décrit dans Matériel et méthodes. Chaque paire de génotypes a au moins 10 p. cent des SSR allèles en commun.

Group (n° of accessions)	Accessions
1 (129)	Aggianniotiko; Albaranzeuli N.; Aleatico; Aleatico Castelvenere; Altulina; Ampelakiotiko Mavro; Aniretac; Asprouda Patron (1); Athiri; Babic; Bakouri; Barbera; Barzemino; Begleri; Blatina; Blau Affentaler; Bombino N.; Bovale Sardo; Buchholzer Vernatsch; Cagnulari; Carignano; Cesanese; Chardonnay; Cla; Coarna alba; Coda di volpe B.; Corvinone; Corvinone San Felice; Crevatina; Croà R.; Croatina; Debina; Debina N.; Durassa del Gel; Fiano; Fileri; Fokiano; Frappato; Freisa; Furner; Geschlafene; Graciano; Grecanico; Grechetto; Greco; Greco di tufo; Grenache; Grisa bianca; Grossvernatsch; Heunisch rot; Heunisch weiss; Jungferlen; Kacotrygis; Kallmet; Klarnica; Kleinvernatsch; Korfiatis; Kotselina; Kriqës; Kunbullor; Lacrima di moro d'alba; Lagrein; Liatiko; Malvasia nera di Brindisi; Malvasia nera di Lecce; Marzemino; Mataosso; Mataro; Mavro bulgarias; Minella B.; Monastrell; Mourisco; Mourverdre; Muskat Platterle; Muskat Trollinger; Nebbiolo; Negroamaro; Nero d'Avola; Nieddera; Ottavianello; Pampanuto; Pavlos; Pecorino; Perricone; Pignola (1); Pignola (2); Pignolo; Platterle; Plavin; Plavina; Plovdina; Prekniariko; Primitivo; Prosecco; Raboso; Refosco Boton; Refosco Faedis; Refosco Uccelli; Riesling italico; Rossola; Rossolino; Rush R.; Ruzija; Sangiovese; Savatiano; Schiava grossa; Sefka (2); Shesh B.; Sideritis; Siriaca; Skylopnichtis; Stanusina; Syrah; Tabernello; Tempranillo; Terrano; Tinta de toro; Uva di Troia; Uva Rara; Veltliner rot; Vermiglio; Vernaccia di Orosei; Vernaccia Oristano; Vernatsch kleinbeerig; Vossos; Votsichi; Weiss Vernatsch; Weisser Hortling; Zlaratika;
2 (20)	Asprouda Patron (2); Coarna negra; Gafforella; Gaglioppo; Iatrou (1); Iatrou (2); Kokkinorobola; Mandilaria; Mavro kalabriton; Mavro kosmas; Mavro messenikola; Mavro styfo; Mavrodafni; Mavrotragano; Mavroudi arachovis; Nerostafylo; Psilomavro kalavriton; Robola; Sefka (1.); Vespolina;
3 (53)	Agiorgitiko; Albarin; Amfioni (1); Amfioni (2); Arino; Assyrtico; Bellone; Bucalò; Canaiolo B.; Canaiolo N.; Cortese; Debit; Elbin weiss; Espadero; Favorita; Forastera; Furmint; Godello; Gros Manseng; Jaen; Koutsoulbeli; Lagarino; Laurerio; Malvasia di Asolo; Malvasia di Sitges; Marsanne; Montonico; Moscato bianco; Moscato giallo; Nasco; Neretto; Palomino; Parellada; Pedro Ximenes; Petit Manseng; Picolit; Piedirosso; Pigato; Retagliado; Rollo; Roussanne; Sauvignon; Trebbiano d'Abruzzo; Trebbiano di Spagna; Trebbiano spoletino; Trebbiano valtenesi; Trexiaduri; Verdicchio; Vermentino; Vermentino N.; Vernaccia S. Gimignano; Vilana; Viura;
4 (27)	Altesse; Ansonica; Arneis; Bianco d'Alessano; Bobal; Bouboulenc; Calitor blanc; Clairette; Erbaluce; Erbamat; Folle B.; Garganega; Greco B.; Inzolia; Macabeo; Malvasia del Chianti; Malvasia di Candia; Malvasia d'Istria; Mangiaguerra; Montuni; Piquepoul; Ribolla; Roditis; Sicilien; Timorasso; Torrontes; Trebbiano di Soave; Vartzami; Xarello;
5 (7)	Aglianico Galluccio; Chiavennasca; Karvouniaris; Magliocco; Nerello Mascalese; Refosco di Rauscedo; Refosco Guarnieri;

frequency of homozygous loci in the compared genotypes.

Altogether, the three different approaches give comparable results. Values based on the Jaccard's index were preferred for the analysis of genetic similarity among the grapevine varieties of this work because, at variance from values of percent shared alleles, it account for both number and size of shared alleles.

A dendrogram was built on the data produced by analysing the 238 grapevine cultivars of table I at the 7 microsatellite loci specified in Materials and methods.

Due to its large dimension, the dendrogram is not reproduced here. Cutting the dendrogram at the genetic distance of 0.1 Jaccard's index value, five main similarity groups were identified. These were named group 1 to 5. In table III accessions are listed for each group. The original dendrogram is available upon request to the authors of this article (LABRA, 2000).

The comparison of the verified genomic similarity values and geographical origin of the analysed cultivars, as summarized in figure 3, showed that: (a) Balkan accessions are essentially clustered in group 1, with

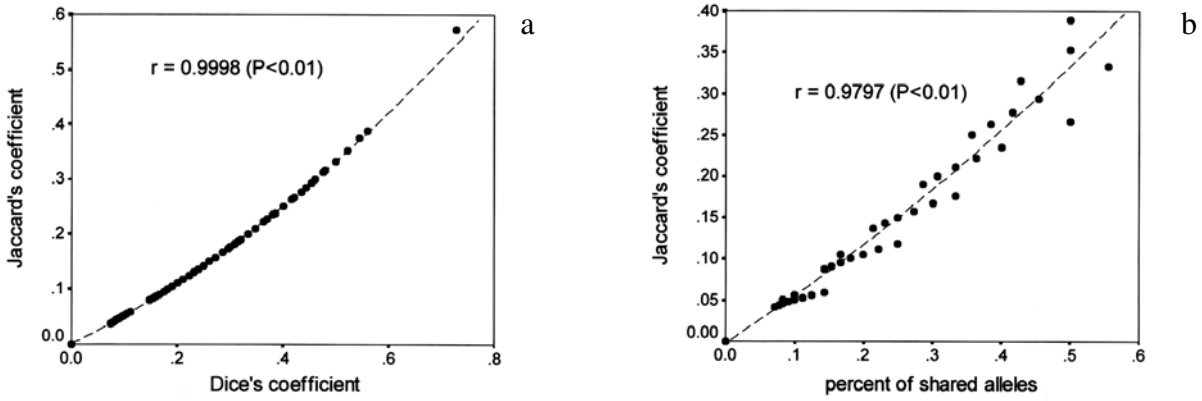

Figure 2 – Correlation between Jaccard's and Dice's coefficient (upper diagram) and between Jaccard's and percent of shared alleles (lower diagram).

The correlation has been computed on a sample of 238 pair of accessions randomly chosen within the analysed germplasm.

Corrélation entre le coefficient de Jaccard et le coefficient de Dice (fig. 2a) et entre le coefficient de Jaccard et le pourcentage d'allèles partagés (fig. 2b).

La corrélation a été calculée sur la base de 238 couples des génotypes choisies au hasard parmi celles qui ont été analysées.

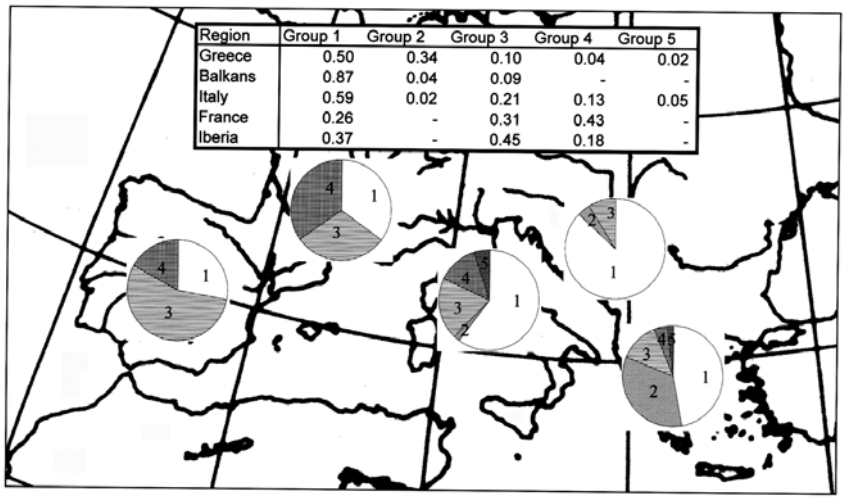

Figure 3 – Frequency distribution of the accessions from the different regions into the five main groups determined by cluster analysis.

Within each group the genetic distance according to Jaccard's coefficient was ≥ 0.1. The diagrams show the number corresponding to different groups.

Distribution des fréquences des génotypes de différentes origines géographiques dans les cinq principaux regroupements de variétés obtenus par analyse de cluster.

Dans chaque regroupement, la distance génétique selon le coefficient de Jaccard était ≥ 0.1. Dans les diagrammes, les chiffres correspondent aux groupes variétaux.

few cases in group 2 and 3; (b) the French ones are equally represented in groups 1, 3 and 4; (c) the Greek ones are most abundant in groups 1,2 and 3, with a few accessions in the other two groups; (d) the Iberian ones are most abundant in group 3, with other accessions distributed in group 1 and 4; (e) the majority of the Italian grapevines cluster in group 1, this is followed by group 3, while few genotypes are placed in group 2 and 5.

In order to determine genetic distance among the 5 regional populations, and thus to study the expansion pattern of grapevine in the Mediterranean area, the χ^2 distance test was applied to the experimental data of figure 3 and a similarity dendrogram was built (figure 4). This showed close relationship between French and Iberian cultivars and placed Balkan, Italian and Greek cultivars in peculiar positions.

III - ANALYSIS OF ALLELE FREQUENCY

Data of figure 4 were verified by analysing allele frequency, i.e., by measuring the frequency of each microsatellite allele in the analysed cultivars. This was performed by applying the χ^2 distance test and the Principal Component Analysis (PCA) to data on allele frequency of each SSR locus.

The dendrogram of figure 5 summarises the result of the χ^2 distance test. These agree with those of figure 4, except that the distance between the Italian cultivars and the cluster of France-Iberian cultivars was narrowed. The Balkan and Greek cultivars were confirmed to cluster in separate position. Results are analysed in details in table IV, where the χ^2 value and its significance is determined for each pair of geographical region. Altogether, the results confirmed the large genetic similarity between French and Iberian cultivars (only 2 significant χ^2 values out of 7), the high genetic dissimilarity between the Western (French and

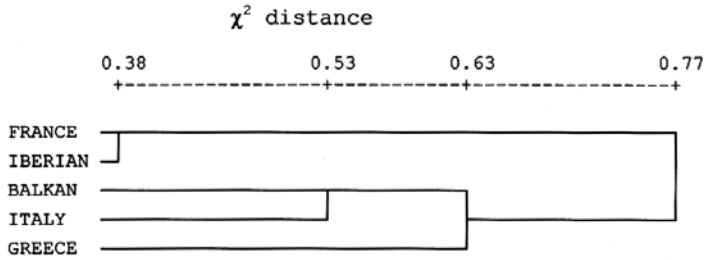

Figure 4 – Dendrograms, obtained by data of table III, using χ^2 distance showing the relations among the Mediterranean regions on the basis of the frequency distributions of their accessions into the main five cluster groups obtained by Jaccard's index.

Dendrogramme montrant la relation entre les populations variétales des différentes régions du bassin méditerranéen.
Le dendrogramme a été obtenu sur la base de la distribution de fréquence des génotypes de toute origine, dans les cinq principaux groupes variétaux obtenus sur la base du coefficient de Jaccard, comme le montre le tableau III.

TABLE IV
Comparisons among different Mediterranean regions based on χ^2 ratio of the allele frequency for each SSR locus.

Conparaison entre les différentes régions méditerranéennes par analyse du χ^2 calculé sur la fréquence allélique de chaque locus microsatellite.

Comparisons		VVS1		VVS2		VVS3		VVS4		VVMD5		VVMD6		VVMD7	
		χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.
Balkan	Italy	49.90	***	37.13	**	35.88	*	33.02	ns	33.3	ns	52.23	***	22.43	ns
Balkan	France	24.76	**	25.93	**	21.88	ns	27.54	**	39.37	**	35.23	***	35.98	***
Balkan	Greece	26.07	*	30.43	**	16.41	ns	21.89	ns	43.20	***	61.08	***	40.58	***
Balkan	Iberian	23.88	*	30.87	**	32.68	**	40.04	**	35.67	**	30.06	**	45.05	***
France	Greece	33.06	***	28.75	**	32.43	**	45.23	***	45.85	***	67.08	***	41.34	***
France	Iberian	6.94	ns	10.10	ns	22.03	*	23.17	ns	23.92	*	19.12	Ns	9.66	ns
France	Italy	22.54	ns	21.67	ns	43.43	**	25.21	ns	30.31	ns	34.45	**	44.92	***
Greece	Iberian	30.07	***	21.02	*	33.45	**	61.32	***	65.76	***	55.42	***	40.23	***
Greece	Italy	35.79	**	47.67	***	66.47	***	67.66	***	71.82	***	110.39	***	55.46	***
Iberian	Italy	8.35	ns	44.52	***	41.18	**	36.93	ns	52.18	***	35.64	**	61.26	***

ns = not significant; * = P≤0.05; ** =P≤0.01; *** =P≤0.001

Iberian) and eastern (Greek and Balkan) grapevines. Italian grapes are confirmed in an intermediate position.

The above conclusions were substantiated by a PCA analysis of the allele frequency of each SSR locus. Four functions able to explain 100 p. cent of total variance for the cultivars of each geographical region were calculated. These values, together with percent of variance, are shown in table V.

The first principal component, which explained one third of total variance, distinguished the Balkan and Greek cultivars (similar and positive values) from the French and Iberian cultivars (similar and negative values). The Italian cultivars were intermediate (close to zero value). The second component, which explained almost 30 p. cent of total variance, clustered the Italian and Greek cultivars and showed intermediate values for the others. The third component essentially distinguished Greek and Balkan cultivars, while the fourth component distinguished the French from the Iberian ones.

DISCUSSION

Genomic analysis of grapevine genomes has become an experimental tool only in recent years. First applications based on RAPD-PCR and RFLP markers allowed to solve cases of homonyms and synonymies and to fingerprint varieties. More recently SSR markers were used to search or to confirm parents of prominent grapevines varieties, like for example Cabernet Sauvignon (BOWERS and MEREDITH, 1997) or Müller Thurgau (DETTWEILER *et al.*, 2000). LABRA *et al.* (1999) recognised in the Greek variety Roditis a possible ancestor of several western Mediterranean white varieties, including Inzolia and Ansonica. SEFC *et al.* (2000) showed that microsatellite analysis can be used to determine the geographical origin of cultivars with an unknown pedigree. FOSSATI *et al.* (2000) used AFLP and SSR analysis to demonstrate that grapevines collectively referred to as « Schiave », in the Southern and Northern slopes of the Eastern Alps, refers to a similar cultivation practice in contiguous regions rather than to a common genetic background. The SSR analysis was also used to verify the origin of sub-varietal genetic variability (SILVESTRONI *et al.*, 1997).

The problem of the origin of today's cultivars is under discussion. Search for direct ancestors of local varieties (local wild vines or cultivated wines from other regions) is still an open issue, as is their genetic history. Vegetative propagation, seeds produced by self-pollination or breeding may have played intersecting roles in defining the present genomic constitution of each cultivar.

In the present work we addressed the problem of defining the genetic relationships existing among the most representative grapevines traditionally cultivated in the Central and Western Mediterranean regions. This knowledge is essential in order to offer experimental evidence and add details to the model of grapevine

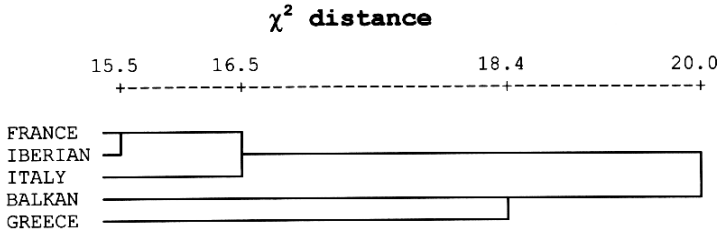

Figure 5 – Dendrograms obtained by χ^2 distance computed on the basis of the SSR allele frequency within each region.

Dendrogramme obtenu par analyse des valeurs du χ^2 calculées sur la base de la fréquence allélique de chaque population variétale régionale.

TABLE V
Principal component analysis performed on the allele frequency distribution of the SSR loci and component values of each region.

Analyse des composantes principales effectuée sur la distribution des fréquences alléliques de chaque locus microsatellite et des valeurs de chaque composante pour chaque région.

Component	Eingvalues	Variance (%)	Balkan	France	Greece	Iberian	Italy
F1	51.23	32.44	1.00	-1.02	0.88	-1.06	0.18
F2	46.42	28.63	-0.08	-0.19	0.97	-0.43	1.68
F3	32.14	21.34	-1.47	-0.01	1.19	-0.28	0.58
F4	27.23	17.32	-0.15	-1.47	0.59	1.35	0.22

expansion of domesticated grapevine from the proposed site of primary domestication (figure 1). The results presented in this paper are consistent with this model. In fact, by analysing the genetic pool of seven SSR loci in 238 local accessions from five different geographical origins, we produced evidence for the expansion of viticulture from East (Greek and Balkan regions) to West (Italian, French and Iberian regions).

Clustering analysis on the basis of genetic distance showed the existence of five varietal groups. Group 1 of figure 3 collected more than 50 p. cent of the sampled accessions from all five origins, with Jaccard's similarity values ranging from 28 to 87 p. cent. This is consistent with the hypothesis of a common and monocentric origin, and/or of an intense varietal flowing of a dominant gene pool. Group 3 of the same table clustered accessions from all the geographical origins. This group is possibly represented by varieties that crossed the Mediterranean basin at different times and in different ways, as compared with varieties of group 1. The other three groups displayed high site specificity. In fact, group 2 clustered almost exclusively Greek accessions, group 4 western Mediterranean and group 5 Italian and Greek.

The geographical distribution assessed by microsatellite analysis is consistent with the pattern of viticulture origin and varietal circulation proposed on the bases of archaeological and historical evidence. Altogether, the available data are consistent with (1) the radiation of primordial varieties from the Near East regions, and (2) the subsequent and multiple variety flows that linked different viticultural regions through different routes. In both cases, population movement and cultural exchanges contributed to the phenomenon.

The estimation of genetic distances among the five grapevines groups, based on the comparison of c2 ratios with their allelic frequencies, was coherent with this model and, in particular, with the hypothesis of a common geographic origin of cultivated grapes and of their primordial varietal radiation. In fact, genetic similarity among French and Iberian cultivars turned out to be high, while genetic similarity was low comparing French and ibrian to Greek and Balkan cultivars. The latter two clustered in the same group. The Italian grapes clustered in an intermediate position, reflecting its geographical location.

The results of the Principal Component Analysis of the microsatellite allelic frequency distributions was also coherent with the conclusions drown from the cluster analysis and with the c2 tests.

Acknowledgements : We thank the NAGREF Vine Institute, Lykovrissi (Greece), the Laboratory of Viticulture, Dept. of Agriculture, Thessaloniki (Greece), CIVIFRUCE, Ricca Gioia, Pavia (Italy) for supplying the biological material. Thanks are also given to Marco Gibin for technical assistance. Research was partially supported by Slow Food - Agricola, Torino (Italy).

REFERENCES

ALLEWELDT G., 1996. Genetics of grapevine breeding. *Progress in Botany*, **58**, 442-454.

ANDERSON J.A., CHURCHILI G.A., AUTRIQUE J.E., TANKSLEY S.D. and SORRELLS M.E., 1993. Optimizing parental selection for genetic linkage maps. *Genome*, **36**, 181-186.

BISSON J., 1999. Essai de classement des cépages francais en écogéogroupes phénotypiques. *J. Int.Sci. Vigne Vin*, **33**, 3, 105-110.

BOTTA R., SCOTT N.S., EYNARD I. and THOMAS M.R., 1995. Evaluation of microsatellite sequence-targetted site markers for characterizing *Vitis vinifera* cultivar. *Vitis*, **34**, 2, 99-102.

BOWCOCK A.M., RUIZ-LINARES A., TOMFOHRDE J., MINCH E., KIDD R. and CAVALLI-SFORZA L., 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature*, **368**, 455-457.

BOWERS J.E., DANGL G.S., VIGNANI R. and MEREDITH C.P., 1996. Isolation and characterisation of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera* L.). *Genome,* **39**, 628-633.

BOWERS J.E. and MEREDITH C.P., 1997. The parentage of a classic wine grape, Cabernet Sauvignon. *Nature genetics*, **16**, 84-87.

DETTWEILER E., JUNG A.E. and TOPFER R., 2000. Grapevine cultivar Müller-Thurgau and its true to type descent. *Vitis,* **39**, 2, 63-65

DOYLE J.J. and DOYLE J.L., 1990. Isolation of plant DNA from fresh tissue. *Focus*, **12**, 13-15.

FORNI G., 1996. Genesi e protodiffusione della viti-vinicoltura dal Mediterraneo orientale alla Cisalpinia. Aspetti ecologici, culturali, linguistici e tecnologici. *In* G. Forni and A Scienza eds. *2500 anni di cultura della vite nell'ambito alpino e cisalpino*, Trento, Ist. Trentino del Vino, pp. 19-183.

FOSSATI T., LABRA M., CASTIGLIONE S., FAILLA O., SCIENZA A. and SALA F., 2001. The use of AFLP and SSR molecular marker to deciher homonyms and synonims in grapevine cultivars: the case of the varietal group known as « Schiave ». *Theoret. Appl. Genet.*, **102**, 200-205.

KARP A., ISAAC P. and INGRAM D., 1998. *Molecular tools for screening biodiversity*, 1st ed. Chapman & Hall, London, p 181-213.

KROLL H., 1991. SÜDOSTEUROPA IN W. VAN ZEIST,K. WASYLIKOWA, KE BEHRE (eds), *Progress in Old World Palaeoethnobotany*, Rotterdam/Brookfield, Balkema, p. 161-177.

LABRA M., FAILLA O., FOSSATI T., CASTIGLIONE S., SCIENZA A. and SALA F., 1999. Phylogenetic analysis of cv. Ansonica growing on the island of Giglio, Italy, by AFLP and SSR markers. *Vitis*, **38**, 161-166.

LABRA M., 2000. Origine, distribuzione geografica e variabilità varietale ed intravarietale di alcuni vitigni mediterranei valutati attraverso il polimorfismo del DNA. *Thèse Doctorat*, Università di Milano.

LEVADOUX L., 1956. Les populations sauvages et cultivées de *Vitis vinifera*. *Ann. Amelior. Plantes*, **6**, 59-110.

LOGOTHETIS B.C., 1970. *The development of the vines and of viticulture in Greece*, Tessalonica, Aristotelion Panepistemion Tessalonikis (in Greek with summary in French) p. 76-79.

LOPES M.S., SEFC K.M., EIRES DIAS E., STEINKELLNER H., LAIMER DA CAMARA MACHADO M. and CAMARA MACHADO A., 1999. The use of microsatellites for germplasm management in a Portuguese grapevine collection. *Theoret. Appl. Genet.*, **99**, 733-739.

NEGRUL A.M., 1946. Origin of cultivated grapevines and its cultivation. *Ampelography of USSR*, vol. I, Moscow.

NÚÑEZ D.R. and WALKER M.J., 1989. A review of paleobotanical findings of early *Vitis* in the Mediterranean and of the origin of cultivated grape-vines, with special reference to new pointers to prehistoric exploitation in the western Mediterranean. *Paleobotany Palynology*, **61**, 205-237.

OLMO H.P., 1976. Grapes. *In:* N.W. Simmonds (ed.), *Evolution of crop plants*, London, Longman, p. 294-298.

OLMO H.P., 1995. The origin and domestication of vinifera grape. *In The origin and ancient history of wine*. P.Mc Govern, S.J. Fleming and S.H. Katz eds. Gordon & Breach, Luxembourg. p. 31-43.

RENFREW J.M., 1973. Paleoethnobotany. *The prehistoric food plants of the Near East of Europe*, London, Methuen, p. 125-131.

RIVES M., 1974. Les vignes sauvages comme sources de gènes pour l'amélioration. *Vitis*, **13**, 186-197.

SEFC K.M., LOPES M.S., LEFORT F., BOTTA R., ROUBELAKIS-ANGELAKIS K.A., IBANEZ I., PEJIE H.W., WAGNER J., GLOSSL H. and STEINKELLNER H., 2000. Microsatellite variability in grapevine cultivars from different European region and evaluation of assignment testing to assess the geographic origin of cultivar. *Theoret. Appl. Genet.*, **100**, 489-505.

SEFC K.M., REGNER F., GLOSSL J. and STEINKELLNER H., 1998. Genotyping of grapevine and roodstock cultivars using microsatellite markers. *Vitis*, **37**, 15-20.

SILVESTRONI O., DI PIETRO D., INTRIERI C., VIGNANI R., FILIPPETTI I., DEL CASINO C., SCALI M. and CRESTI M., 1997. Detection of genetic diversity amongs clones of cv. Fortana (*Vitis vinifera* L.) by microsatellite DNA polymorphism analysis. *Vitis*, 3**6**, 147-150.

SNEATH P.H.A. and SOKAL R.R., 1973. *Numerical taxonomy. The principles and practice of numerical classification*. W. H. Freeman, San Francisco, p. 35-120.

THOMAS M.R. and SCOTT N.S., 1993. Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequenced-tagged site (STSs). *Theoret. Appl Genet.*, **86**, 985-990

WASILYKOWA K.E., 1991. East central Europe. *In: Progress in Old World Palaeoethnobotany,* W. Van Zeist, K. Wasylikowa, K.E. Behre (eds), otterdam/ Brookfield, Balkema, p. 161-177.

ZEVEN A.C. and ZHUKOVSKY P.M., 1975. *Dictionary of cultivated plants and their centres of diversity*. Wageningen, Centre for Agricultural Publishing and Documentation.

Reçu le 30 avril 2001
accepté après révision le 25 janvier 2002

ZOHARY D. and HOPF M., 1993. *Domestication of plants in the old world*, Oxford, Clarendon Press. p. 143-150.

ZOHARY D. and SPIEGEL-ROY P., 1975. Beginnings of fruit rowing in the old world. *Science*, **187**, 319-327.