Vitis **39** (4), 159-161 (2000)

Analysis of genetic relationships among Muscat grapevines in Apulia (South ltaly) by RAPD markers

G. FANIZZA, M. G. CORONA and P. RESTA

Dipartimento di Biologia e Chimica Agroforestale ed Ambientale, Universita di Bari, Ttalia

Summary

Two hundred 10-mer primers of arbitrary nucleotide sequence were used to study the genetic relationships among Muscat grapevines in Apulia. The coefficients of similarity of these genotypes were determined with 484 polymorphic RAPD bands (profiles). The bootstrap sampling analysis revealed that the number of RAPD bands was suitable to estimate the coefficients of similarity. The pattern of aggregation among genotypes (cluster analysis, principal coordinate analysis) indicates a diversity among the Muscats in Apulia except for Moscato Reale and Moscato Canelli, which were closely related.

K e y w o r d s : PCR, coefficient similarity, cluster analysis, principal coordinate.

Introduction

Muscat grapes have a long history and are nowadays scattered all over Italy under different names or under the generic name of Moscato followed by the denomination of the town where they are grown such as Moscato di Trani, Moscato di Siracusa, Moscato Canelli. In his ampelography MOLON (1946) reports a list of about one hundred Muscat varieties but most of them are synonymies or homonymies.

In Apulia (southern Italy) several Muscat grapevines of unknown origin are grown under different names. Thus a study of the relationships among the Muscat grapevines of Apulia is important not only for germplasm conservation, plant breeding but also to avoid confusion of names when vegetative material is distributed. The relationships among genotypes can be evaluated by morphological characters but repeated measurements are required due to variation by environmental factors. Recently molecular markers have been applied for genetic studies and variety characterization. For *Vitis* restriction fragment length polymorphisms (RFLP) have been used (STRIEM *et al.* 1990; MAURO *et al.* 1992; BOURQUIN *et al.* 1993; BOWERS *et al.* 1993; GUERRA and MEREDITH 1995) as well as microsatellites (THOMAS *et al.* 1994; CIPRIANI *et al.* 1994; BOWERS *et al.* 1996; SEFC *et al.* 1998) but these techniques are time and labor intensive and not easily accessible to many grape breeders.

Random amplified polymorphic DNA (RAPD) markers have gained popularity as a molecular technique in fruit species including *Vitis* because of their lower cost and technical difficulty (GORGOCENA *et al.* 1993; JEAN-JAQUES *et al.* 1993; BÜSCHER *et al.* 1994; GRANDO *et al.* 1995; MULCAHY *et al.* 1995; XIANPING *et al.* 1996; LODHI *et al.* 1997; VIDAL *et al*. 1999). A large number of data sets can be generated because different RAPD primers are commercially available. The objective of this study was to analyze the genetic relationships among the Muscat genotypes of Apulia by RAPD markers.

Material and Methods

S o u r c e m a t e r i a l, D N A e x t r a c t i o n, R A P D p r o c e d u r e s : The Muscat varieties (*Vitis vinifera*) of Apulia are listed in the Table. Most of them were provided by the CRSA 'B. Caramia' Locorotondo (Bari), only the genotype Moscato Nero (M10) was provided by a grower of the Bari viticultural area and Moscato Canelli (M11) by the Istituto Coltivazioni Arboree, University of Torino. Young apical leaves from these genotypes selected during spring were used for DNA isolation. Details of all RAPD procedures have been described previously (FANIZZA *et al.* 1999); 200 primers (Operon Technologies, Alameda, Calif.) were used in this analysis.

D a t a a n a l y s i s : The positions of scorable RAPD bands were transformed into a binary character matrix ('1' for the presence and '0' for the absence of a band at a particular position); only polymorphic bands (profiles) were used for the determination of the simple matching coeffi-

T a b l e

List of Muscat varieties

Name	Code
Moscato Selvatico	M1
Moscato Giallo	M2
Moscatellone Bianco	M3
Moscato Amburgo	M4
Moscatello	M5
Moscato Reale	M6
Moscato Terracina	M7
Aleatico	M8
Moscato Saraceno	M9
Moscato Nero	M10
Moscato Canelli	M11
Moscatellone Nero	M12
Moscardella	M13
Marchione	M14

Correspondence to: Dr. G. FANIZZA, Dipartimento di Biologia e Chimica Agro-Forestale ed Ambientale, Via Amendola 165/A, 70126 Bari, Ttaly. Fax: +39-80-544-2813. E-mail: fanizza@agr.uniba.it

cient of similarity (SNEATH and SOKAL 1973) between any pair of genotype. A bootstrap sampling procedure (EFRON and TIBSHIRANY 1991; TIVANG *et al.* 1994), executed by a computer program written in "c" by the authors, was carried out to evaluate the number of bands suitable for a better estimation of the coefficient of similarity. The cluster UPGMA (unweighed pair group method arithmetic average) analysis, based on the coefficients of similarity, was used to study the relationship of the Muscats. Further evaluation of similarities or dissimilarities among these genotypes was carried out by principal coordinate analysis. The NTSYS pc version 1.8 (ROHLF 1992) was used for multivariate analyses.

Results and Discussion

Of the 200 primers, 42 did not amplify or produced only monorphic bands. The remaining 158 primers produced 263 monomorphic and 484 polymorphic bands (Fig. 1 shows the polymorphism of two primers). The monomorphic bands were excluded from the estimation of the coefficients of similarity. Although the reproducibility of RAPDs is questionable, they can be considered as suitable markers in genotype diversity studies if the method has been standardized. Because of the very high number of commercially available primers, several RAPD bands can be obtained and the estimation of coefficients of similarity will have a greater reliability. The influence of the number of RAPD bands on the estimation of the coefficients of similarity among the Muscat genotypes was evaluated by the bootstrap sampling procedure. Fig. 2 shows the distribution of the coefficients of variation (CV) relative to the coefficients of similarity of each bootstrap sample; it reveals that the mean coefficient of variation decreases as the number of bands increases; this had been observed previously for *Vitis* (FANIZZA *et al.* 1999) and other species (TIVANG *et al.* 1994; NIENHUIS *et al.* 1995). A coefficient of variation of 10 % is obtained with about 120 bands while a lower CV (5 %) might be obtained with a larger number of bands (about 300).

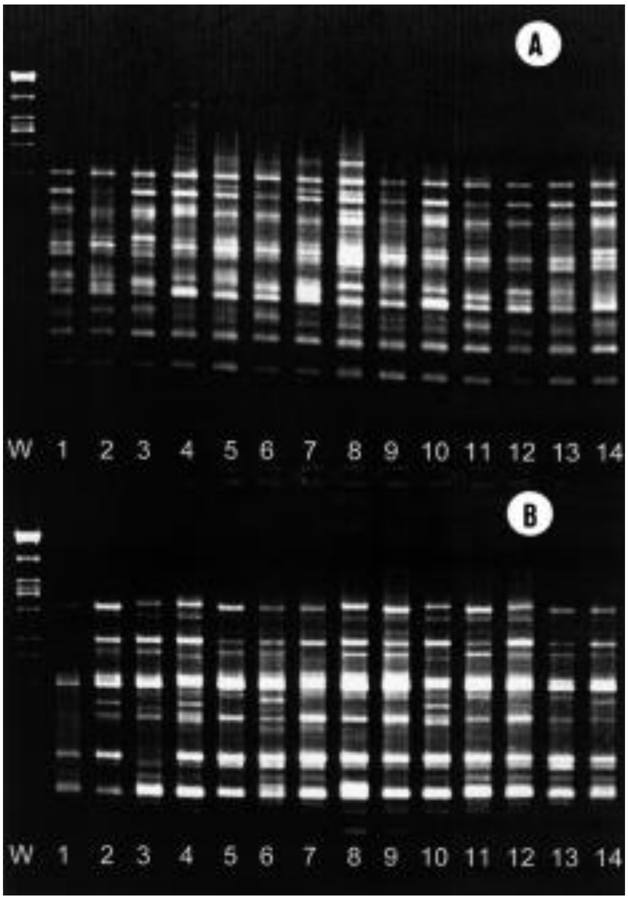

Fig. 1: RAPD electrophoretic pattern from 14 Muscat genotypes generated by primers OPK-12 (**A**), OPK-13 (**B**). Lanes (1-14) correspond to genotypes as indicated in the test; W is Lambda DNA digested with *EcoR*T, *BamH*T, *Hind*TTT.

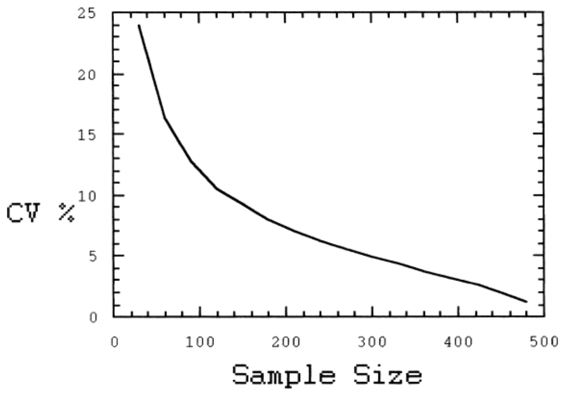

Fig. 2: Plot of the mean coefficient of variation (CV) *vs*. sample size for coefficients of similarity of Muscat genotypes.

The dendrogram of the cluster analysis (Fig. 3), based on similarities of RAPD fragments, reveals diversity among the Muscat grapevines taken in consideration; if truncated at 80 % of the coefficient of similarity only two clusters are formed: one includes Moscato Nero (M10) and Moscato Amburgo (M4) and the other includes Moscato Canelli (M11) and Moscato Reale (M6) with coefficients of similarity of 0.93 and 0.87, respectively. Moscato Nero, provided by a grower in Apulia, is phenotypically very similar to Moscato Amburgo; it is likely that this grapevine is derived from Muscat Amburgo and propagated by growers under the generic name of Moscato Nero (black). Moscato Canelli, the well known cultivar of Piemonte, from where we got the leaf samples, presents a large number of RAPD fragments similar to Moscato Reale grown in Apulia; the lack of his-

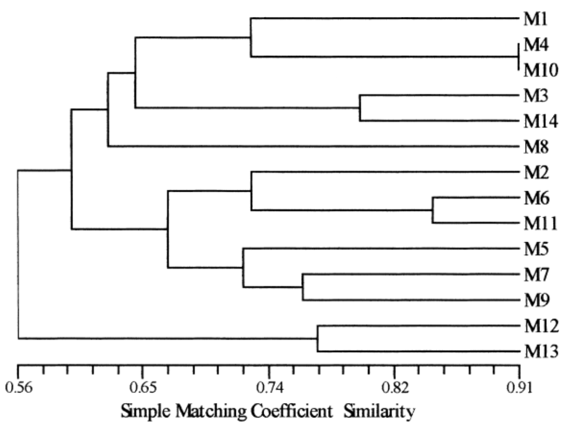

Fig. 3: Dendrogram of Muscat genotypes from cluster analysis based on RAPDs.

torical data makes it difficult to explain the origin of Moscato Reale, however, it seems closely related to Moscato Canelli. Apart from these two groups of Muscat genotypes, most of the Apulian Muscats aggregate into clusters at lower similarity values, ranging from 0.56 to 0.78, (Aleatico (M8) joins into a cluster at the lower value of similarity while Moscatellone Bianco (M3) and Marchione (M14) at the higher value). Even though the formation of clusters at a higher level of similarity might suggest an origin of some genotypes from less divergent parents, the general pattern of aggregation into clusters (Fig. 3) indicates no close relationship among the Muscat grapevines of Apulia. The diversity might be the result of selection for the specific environmental conditions in various regions of Italy. Usually fruit tree varieties with some valuable characteristics are dispersed from one region to another in Italy under different names. As far as the Apulian Muscats are concerned some of them have maintained the name of origin, *e.g*. Moscato of Terracina (from the Latina-Roma area) but most of them have names known only in Apulia and not in other regions of Italy such as Moscato Selvatico, Moscato Reale; these Muscats have been maintained in Apulia because the growers handed them down from generation to generation either for their muscat flavor or as part of the local cultural traditions. No cluster or ordination analysis of the Apulian Muscats provided evidence that these genotypes were seedlings from close parents or from half or full sib families. A better view of the relationships among the Muscat genotypes is shown in Fig. 4; this plot, which presents the result of the principal coordinate analysis, confirms the pattern of aggregation among genotypes obtained from cluster analysis.

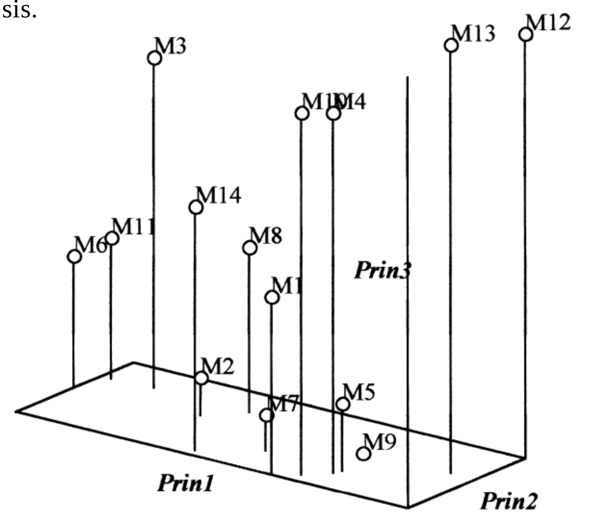

Fig. 4: Plot of Muscat genotypes from principal coordinate analysis based on RAPDs.

Thus little genetic relationship exists among the Muscat genotypes except for *e.g.* Moscato Nero and Moscato Amburgo, Moscato Reale and Moscato Canelli. The differences in RAPD fragments and the pattern of aggregation (cluster analysis) indicate a diversity among the Muscats present in Apulia. The preservation of these grapevines is important not only for germplasm and breeding but also for social-economic reasons and for the local cultural tradition.

References

Bourquin, J. C.; Sonko, A.; Otten, L.; Walter, B.; 1993: Restriction fragment length polymorphism and molecular taxonomy in *Vitis vinifera* L. Theor. Appl. Genet. **87**, 431-438.

Bowers, J. E.; Bandman, E. B.; Meredith, C. P.; 1993: DNA fingerprint characterization of some wine grape cultivars. Amer. J. Enol. Viticult. **44**, 266-274.

- -; Dangl, G. S.; Vignani, R.; Meredith, C. P.; 1996: Isolation and characterization of new polymorphic simple sequence repeat loci in grape (*Vitis vinifera*). Genome **39**, 628-633.

Büscher, N.; Zyprian, E.; Bachmann, O.; Blaich, R.; 1994: On the origin of the grapevine variety Müller-Thurgau as investigated by the inheritance of random amplified polymorphic DNA (RAPD). Vitis **33**, 15-17.

Cipriani, G.; Frazza, G.; Peterlunger, E.; Testolin, R.; 1994: Grapevine fingerprinting using microsatellite repeats. Vitis **33**, 211-215.

Fanizza, G.; Colonna, G.; Resta, P.; Ferrara, G.; 1999: The effect of the number of RAPD markers on the evaluation of genotypic distances in *Vitis vinifera*. Euphytica **107**, 45-50.

Efron, B.; Tibshirani, R.; 1991: Statistical analysis with computer age. Science **253**, 390-395.

Gogorcena, Y.; Arulsekar, S.; Dandekar, A. M.; Parfitt, D. E.; 1993: Molecular markers for grape characterization. Vitis **32**, 183-185.

Grando, S. M.; De Micheli, L.; Biasetto, L.; Scienza, A.; 1995: RAPD markers in wild and cultivated *Vitis vinifera*. Vitis **34**, 37-39.

Guerra, B.; Meredith, C. P.; 1995: Comparison of *Vitis berlandieri* x *Vitis riparia* rootstock cultivars by restriction fragment length polymorphism analysis. Vitis **34**, 109-112.

Jean-Jaques, I.; Defontaine, A.; Hallet, J. N.; 1993: Characterization of *Vitis vinifera* cultivars by Random Amplified Polymorphic DNA markers. Vitis **32**, 189-190.

Lodhi, M. A.; Weeden, N. F.; Reich, B. I.; 1997: Characterization of RAPD markers in *Vitis*. Vitis **36**, 133-140.

Mauro M. C.; Strefeler, M.; Weeden, N. F.; Reisch, B. I.; 1992: Genetic analysis of restriction fragment length polymorphisms in *Vitis*. J. Hered. **83**, 18-21.

Molon, G.; 1946. Ampelografia. Vol. II. Ed. Hoepli.

Mulcahy, D. L.; Cresti, M.; Liskens, H. P.; Intrieri, L.; Silvestroni, O.; Vignani, R.; Pancaldi, M.; 1995: DNA fingerprinting of Italian grape varieties: A test of reliability in RAPDs. Adv. Hort. Sci. **9**, 185-187.

Nienhuis, J.; Tivang, J.; Skroch, P.; Dos Santos, J. B.; 1995: Genetic relationship among cultivars and landraces of lima bean (*Phaseolus lunatus* L.) as measured by RAPD markers. J. Amer. Soc. Hort. Sci. **120** (2), 300-306.

Rohlf, F. J.; 1992: NTSYS-pc-Numerical Taxonomy and Multivariate Analysis System. Exeter Software, Setauket, New York.

Sefc, K. M.; Regner, F.; Glossl, J.; Steinkellner, H.; 1998: Genotyping of grapevine and rootstock using microsatellite markers. Vitis **37**, 15-20.

Sneath, P. H. A.; Sokal, R. R.; 1973: Numerical Taxonomy. WH Freeman and Company. San Francisco.

Striem, M. J.; Spiegel-Roy, P.; Ben-Hayyim, E.; Beckmann, J.; Gidoni, D.; 1990: Genomic DNA fingerprinting of *Vitis vinifera* by the use of multiloci probes. Vitis **29**, 223-227.

Thomas, M. S.; Cain, P.; Scott, N. S.; 1994: DNA typing of grapevines: A universal methodology and database for describing cultivars and evaluating genetic relatedness. Plant Mol. Biol. **25**, 937-949.

Tivang, J. G.; Nienhuis, J.; Smith, O. S.; 1994: Estimation of sampling variance of molecular marker data sites using the bootstrap procedure. Theor. Appl. Genet. **89**, 259-264.

Vidal, J. R.; Moreno, S.; Gogocena, Y.; Masa, A.; Ortiz, J. M.; 1999: On the genetic relatioships and origins of six grape cultivars of Galicia (Spain) using RAPD markers. Am. J. Enol. Vitic. **50**, 69-75.

Xiamping, Q.; Lu, J.; Lamikanra, O.; 1996: Genetic diversity in Muscadine and American bunch grapes based on random amplified polymorphic DNA (RAPD) analysis. J. Amer. Soc. Hort. Sci. **121**, 1020-1023.

Received June 19, 2000