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Preliminary Results on Genome Mapping of an Italia x Mercan Grapevine Population

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Abstract: This research was conducted to construct genetic linkage maps of *Vitis* (2n = 38) from grape populations (60 F_1 + 2 parents) of crossing of Italia and Mercan (*Vitis vinifera* L.) cultivars. RAPD reactions were performed using a total of 300 RAPD primers. The amplification products were scored from negatives as presence or absence. Only 59 loci for Italia and 55 loci for Mercan could be used for linkage analysis. Mapmaker/Exp.3.0 was used for genetic linkage analysis with multipoint ordering among markers at LOD ≥ 3.0 score. The map was developed by using the double pseudo-testcross mapping approach. Italia and Mercan resulted in 8 (maternal) and 6 (paternal) linkage groups, respectively. Loci placed on the linkage groups were further analyzed by regression and analyses of variances in order to determine possible linkages between the loci and morphological and disease resistance characteristics. According to the results, only 2 characters, flowering time and resistance to powdery mildew, were significantly linked (P ≥ 0.01) to 1 and 2 marker loci, respectively. Other significant (P ≥ 0.05) marker-QTL relationships were also found for various traits from regression analysis.

Key Words: Grape, genetic map, linkage analysis, QTL, RAPD

Italia x Mercan Asma Populasyonunda Genom Haritalaması Üzerine İlk Sonuçlar

Özet: Bu araştırmada, *V. vinifera* L. varyetesi olan Italia ve Mercan üzüm çeşitlerinin melezlenmesi ile elde edilen F1 populasyonu kullanılarak (60 F_1 + 2 ebeveyn) genom haritasının çıkarılması ve incelenen morfolojik ve hastalıklara dayanım özelliklerine yönelik bağlantı analizinin yapılması amaçlanmıştır. Araştırmada gerçekleştirilen RAPD reaksiyonlarında toplam 300 adet primer test edilmiştir. Italia çeşidinde 59, Mercan çeşidinde ise sadece 55 adet lokus bağlantı analizlerinde kullanılabilmiştir. Haritanın çıkartılması amacıyla Mapmaker/Exp 3.0 paket programında farklı LOD ≥ 3.0 değeri ve çift yönlü-yalancı melezleme tekniği yaklaşımı kullanılmıştır. Ana ve baba ebeveyne ait 2 ayrı genetik bağlantı haritası elde edilmiş olup, bu bağlantı haritaları sırasıyla 8 (ana) ve 6 (baba) bağlantı grubu içermiştir. Bağlantı gruplarına yerleşen lokusların incelenen morfolojik ve hastalıklara dayanımla ilgili karakterlere olan bağlantılarını tespit etmek amacıyla regresyon ve varyans analizleri yapılmıştır. Analiz sonuçlarına göre P ≥ 0.01 önem derecesinde iki adet karakterin (çiçeklenme zamanı ve küllemeye dayanım) sırasıyla 1 ve 2 markör lokusu ile bağlantılı oldukları tespit edilmiştir. Regresyon analizi diğer anlamlı markör-kantitatif karakter ilişkilerinin olduğunu göstermektedir.

Anahtar Sözcükler: Asma, genetik harita, bağlantı analizi, QTL, RAPD

Introduction

The grape (*Vitis vinifera* L.) has been cultivated for thousands of years for fruit, juice and wine production. Its breeding has mainly relied on selection through the ages of naturally occurring genotypes issued from spontaneous crosses that have been recently traced, and, to a lesser extent, due to conventional breeding during the last century (Adam-Blondon et al., 2004).

At the turn of the last century, many diseases spread to European vineyards, raising the need for breeding new varieties showing resistance to pathogens such as powdery mildew, downy mildew and botrytis bunch rot. To introduce resistance from wild-inferior quality species, many crosses are required to recover high quality *vinifera* cultivars. Breeding is further hampered by a long seed-to-seed cycle and a high susceptibility to inbreeding.

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Moreover, most of these traits are quantitatively inherited (Eibach et al., 1989; Doligez et al., 2002; Fischer et al., 2004).

During the past decade, several groups have attempted to develop maps allowing the location of QTLs for agronomic traits, with the goal of using this information for the development of marker-assisted selection in the grape and thus to improve the efficiency of grape breeding (Lodhi et al. 1995; Dalbó et al., 2001; Doligez et al., 2002; Grando et al., 2003; Fischer et al., 2004). These maps are mainly constructed with RAPD or AFLP markers.

The aim of the study was to develop a genomic map of grape with RAPD markers using 60 progenies of the Italia x Mercan population and correlate this information with observations of vegetative, generative traits and fungal disease resistance traits. This cross was developed at the Tekirdağ Viticultural Research Institute in 1992. It was chosen because the progeny was segregated for disease resistance and other important vegetative and generative traits.

Materials and Methods

Plant Material

The construction of the map was based on the study of 60 progenies from a cross between Italia and Mercan parents. Italia is a standard table grape cultivar grown in many regions. Mercan is a white, small berried juice variety grown in Black Sea region and thought to be a *labrusca* variety. These were crossed in 1992 to obtain genotypes with fungal disease resistance. The parents and the derived progenies have been grown on their roots at the Tekirdağ Viticultural Research Institute, Tekirdağ.

Method

Trait observations

A total of 35 morphological traits along with 3 fungal diseases (powdery mildew, downy mildew and botrytis bunch rot) were observed and scored for 2 consecutive years at the Tekirdağ Viticultural Research Institute.

DNA extraction

Cuttings brought in from the Institute was potted in perlite:soil:peat moss (1:1:1) in a greenhouse located at the Department of Horticulture, Faculty of Agriculture, Ankara University. DNA was extracted from young leaves with a modified CTAB method (Lodhi et al., 1994). DNA quantification and purity were determined with NanoDrop ND1000 (NanoDrop Technologies, Wilmington, DE, USA). Purity of the DNAs extracted varied between 0.99 and 2.77.

RAPD amplification

A total of 300 primers were tested to screen DNAs from Italia and Mercan and their progenies. Primers synthesized at Operon Technologies (Alameda, CA, USA) were the 20-primer sets of A, B, C, D, E, F, H, K, M, N, O and OPI (1-16), OPP-17 and OPG-5 and OPG-6. The primers synthesized at Research Genetics (Huntsville, AL, USA) were UBC series (204, 231, 237, 238, 251), BC series (302, 340, 374), B-352, B-356, B-379, B-389, B-392, P-33, P-35, P-123, P-166, P-210, P-232, P-250, P-255, P-313, P-325, P-382, P-394, P-402, P-437, P-443, Kozak primers (1-8), GT-04, S-34, S-35, S-39 and S-69. Primers obtained from IDT (Integrated DNA Technologies, Inc., Coralville, IA, USA) were RAPD series (1-9), OPU-16 and SC series (1022, 1023, 1038, 1043, 1048, 1059, 1065, 1076, 1077, 1082, 1093).

RAPD reactions were performed in a 25 µl reaction mixture containing 100-200 ng template DNA, 1.5 u Taq DNA polymerase (Promega, WI, USA), 0.25 mM of each of 4 dNTPs, 0.2 µM oligonucleotide primers 10-17 bases long, 500 mM KCl, 100 mM Tris-HCl (pH 9.0 at 25 °C) and 1% Triton® X-100. The reaction mixture was overlaid with a drop of mineral oil (Sigma, M-5904). Amplification was performed on a thermocycler (PTC-100; MJ Research Inc., Waltham, MA, USA) for 35 cycles of 94 °C for 30 s, 35 °C for 60 s and 72 °C for 105 s followed by a final hold of 8 min at 72 °C. The amplification products were resolved on 1% agarose plus 1% Nusieve™ (FMC Corp., ME, USA) agarose gels. Electrophoresis was carried out in 1X TBE (Tris-Boric acid-EDTA) buffer (Sambrook et al., 1989) at 7 V/cm. The RAPD bands were visualized with 0.01 mg ml^{-1} ethidium bromide under UV light (λ = 302 nm) and recorded with Type 65 Polaroid film.

Scoring, data organization and analyses

The amplification products were scored from negatives as either present or absent. Data were entered in Microsoft® Excel (Microsoft Corp.) spreadsheets.

Linkage Analysis was performed with MAPMAKER/EXP 3.0 (Lander et al., 1987). For each parental data set, all markers were individually evaluated

by the chi-square method to detect if there was any deviation of gametic segregation from the expected Mendelian 1:1 ratio ($P \leq 0.05$). Two independent data sets were generated that separately contained the meiotic segregation information from each parent. In the absence of phase information, each segregating locus was paired with a dummy locus, resulting in a double data set (double pseudo-testcross strategy, Weeden et al,. 1994). Linked markers were placed into groups with LOD ≥ 3.0 and 25 cM with Haldane mapping functions (Haldane, 1919). Linkage groups obtained from the doubled data were then divided into 2 symmetrical sets of groups, and one set was chosen for further analysis (Lodhi et al., 1995).

Trait analyses

Seventeen vegetative traits, namely antocyanin intensity on shoot tip, cold resistance of buds, budbreak time, young leaf upper surface color, young leaf upper surface hairiness, young leaf lower surface color, leaf lower surface hairiness, leaf upper surface appearance, leaf form, leaf shape, leaf contour, shape of petiolar sinus, sinus width on mature leaf, dentation of mature leaf, petiole color, petiole hairiness and cane color were recorded. Additionally 18 generative traits, namely flower type, flowering time, cluster number/cane, maturing time, berry number, berry shape, berry weight, berry texture, berry skin texture, cluster length, cluster weight, cluster density, peduncle color, berry juiciness, cluster shape, bursting, aroma, and berry uniformity, and 3 disease resistance traits (powdery mildew, downy mildew and botrytis bunch rot) were measured. All trait characterization was done for the parents and the progeny for 2 growing years. The traits were described according to the methods of IPGRI (Descriptors for Grape), Galet (1979) and Eibach (1994).

The pseudo-testcross mapping strategy initially resulted in 2 maps for each parent owing to separate analyses for the coupling and repulsion phases. Similar linkage groups were identified after the initial linkage analysis and only one copy was retained for further analysis. Data from 38 morphological and disease resistance traits were used for confirmation of the quantitative trait loci (QTL) effects. Analysis of variance (ANOVA) and binary logistic regression analysis were carried out to find the closest individual markers underlying QTL peaks. Only those markers that showed a significant correlation ($P \leq 0.05$) with the studied trait were reported.

Results

Of the 300 primers, 113 yielded an average of 1.94 polymorphic DNA bands each. These primers detected 219 polymorphic bands (Table 1) when used on the progeny. After testing by chi-square test, 114 of the clearly amplified markers followed a 1:1 segregation ratio. The rest of the markers analyzed showed distorted segregation ratios and only 16 had 3:1 ratios.

Table 1. Summary of the linkage analysis in a hybrid population of Italia (maternal) x Mercan (paternal).

RAPD Analysis	Total	Italia	Mercan
Polymorphic markers	219	-	-
Markers segregating 1:1	114	59	55
Markers segregating 3:1	16	-	-
Mapped markers (1:1)	32	19	13
Unmapped markers	82	40	42

The first linkage analysis was done separately with markers heterozygous in Italia or Mercan. Among 119 markers, only 32 were mapped in both parents: 19 distributed to 8 linkage groups in Italia (Figure 1) and 13 distributed to 6 linkage groups in Mercan (Figure 2) (Table 1).

The Italia map consists of 19 markers covering 218.1 cM in 8 linkage groups each with 2-3 markers (Tables 2 and 3). The Mercan map covers a smaller map distance compared with the Italia map. This difference may be due to the involvement of fewer markers on the Mercan map, resulting in decreasing map coverage of the genome. In both maps, markers did not cover the entire genome, as expected with the number of markers attributable to the linkage groups. The overall average map distance between adjacent markers was 11.5 and 11.1 for Italia and Mercan, respectively. The largest gap in the Italia map was 28.0 cM, between OPI15e and OPN13a, and the closest gap was 9.2 cM, between OPD3a and OPD4a. The Mercan map had the closest gap between sc1082b and OPM2a (6.8 cM) and the largest gap between gt04a and OPK19c (26.3 cM).

Trait-marker linkage analyses were performed with binary logistics regression and ANOVA (Tables 4 and 5). ANOVA analysis was only performed for disease resistance traits because only they were normally

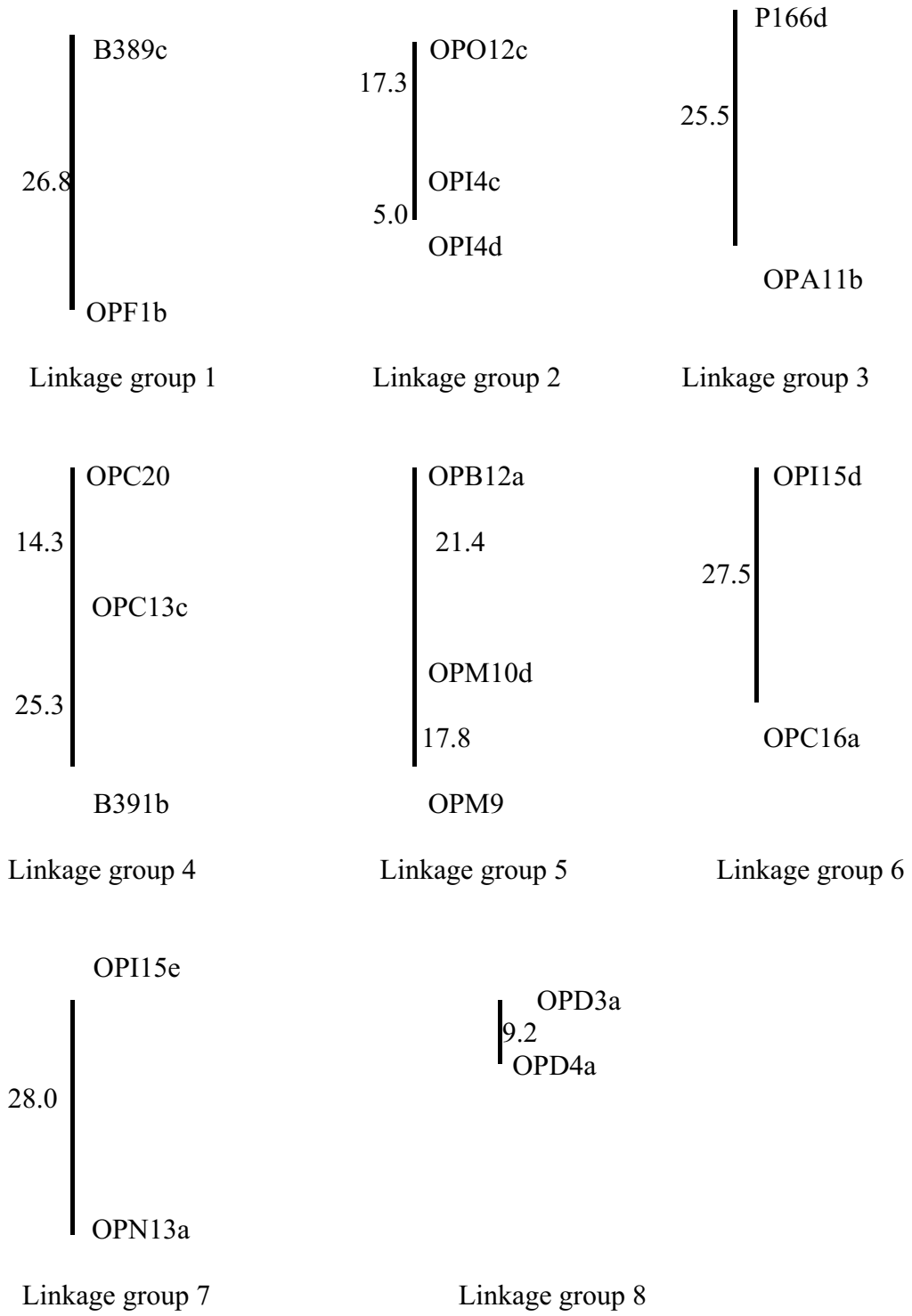

Figure 1. Linkage groups and distances (cM) between markers in Italia (maternal map).

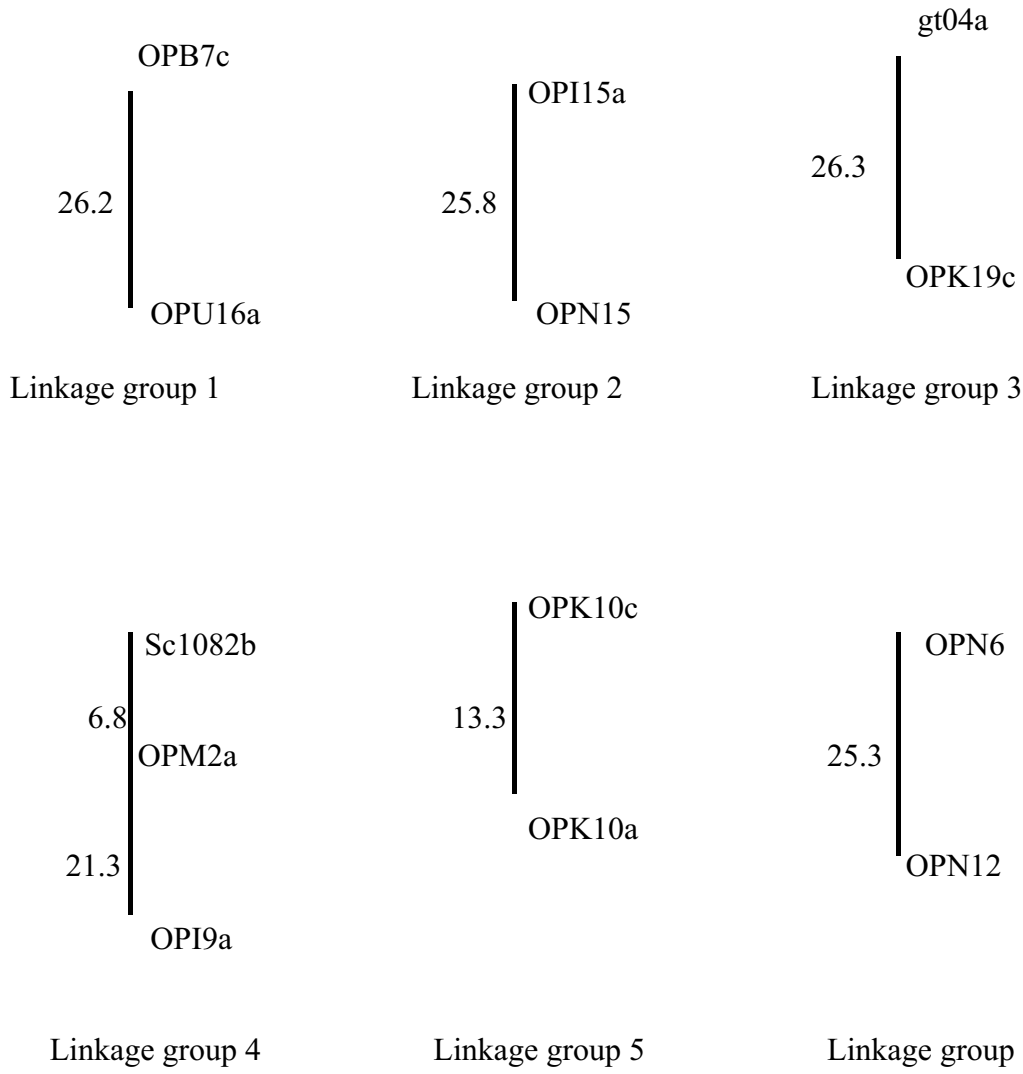

Figure 2. Linkage groups and distances (cM) between markers in Mercan (paternal map).

Table 2. Comparison of the genetic linkage maps of Italia (maternal) x Mercan (paternal).

	Total	Italia	Mercan
Total distance covered (cM)	-	218.1	144.9
Markers mapped	32	19	13
Average map distance between markers (cM)	11.3	11.5	11.1
Number of linkage groups	14	8	6
Largest linkage group (cM)		39.6	28.0
Number of markers in the largest group		3	3

Table 3. Description of individual linkage groups of Italia and Mercan.

	Italia (Maternal map)		Mercan (Paternal map)	
Linkage group	Total distance (cM)	Number of markers	Total distance (cM)	Number of markers
I	26.8	2	26.2	2
II	22.3	3	25.8	2
III	25.5	2	26.3	2
IV	39.6	3	28.0	3
V	39.2	3	13.3	2
VI	27.5	2	25.3	2
VII	28.0	2		
VIII	9.2	2		

Table 4. Binary logistics regression analysis results showing the significant trait-marker associations.

Marker	Trait	P (≤ 0.05)	Linkage Group
IOPA11b	Petiole color	0.037	3
IOPC13c	Time of flowering	0.050	4
IOPM10d	Antocyanin intensity on shoot tip	0.038	5
IOPB12a	Leaf form	0.013	5
IOPB12a	Berry aroma	0.040	5
IOPI15e	Time of flowering	0.016	7
IOPD3a	Leaf form	0.035	8
MOPK19c	Berry size uniformity	0.047	3
MOPN6	Time of flowering	0.023	6
MOPN12	Time of flowering	0.009	6

Table 5. ANOVA test results on disease resistance traits.

Marker	Trait	P (≤ 0.05)	% phenotypic variance explained	Linkage group/ distance cM
IOPI4c	Powdery mildew resistance	0.013	66	2 / 5
IOPI4d	Powdery mildew resistance	0.013	66	

distributed. It showed that IOPI4c had the closest link to the powdery mildew resistance in Italia and explained 66% of the phenotypic variation. No markers were associated with the so-called resistant parent Mercan. Binary logistics regression analysis showed that 7 markers in the Italia parental map had links with 6 traits and 3 in the Mercan map showed linkage with 2 characters. The strongest associations were between IOPB12a and leaf form, IOI15e and time of flowering, and MOPN12 and time of flowering.

Discussion

Population

The molecular mapping of 2 grape varieties, Italia, a white table grape cultivar susceptible to fungal diseases, and Mercan, a so-called *V. labrusca* variety claimed to be resistant to fungal diseases, was attempted. Traits observed in the population of Italia and Mercan were unfortunately not highly segregated, affecting linkage analyses adversely. They also did not fit the requirement of normal distribution among progenies, except for the disease characteristics. This resulted in poor trait-marker linkage. In addition, the number of progenies studied limited the information obtained. Studies have shown that for proper linkage analysis the number of progenies in a population should not be less than 100 (Van Ooijen, 1992; Hyne and Kearsey, 1995; Brar 2002). Having 60 individuals in the Italia and Mercan F_1 population put limitations on the information that could have been obtained.

Marker use and segregation

RAPD markers have been used in genome mapping for their advantages of being able to use a universal primer set and quick scan in the genome and being easy and safe and requiring relatively small amounts of DNA. However, it also has some disadvantages such as sensitivity, dominant heritance and low allele numbers. In addition, phase information (coupling or repulsion) is not available (Tulsieram et al., 1992; Kelly, 1995; Malyshev and Kartel, 1997).

Calculations of goodness-of-fit for the segregating markers using chi-square analysis showed that 187 primers out of 300 had distorted ratios of 1:1. Unfortunately, RAPD markers were not polymorphic enough to detect trait-marker associations. Only 37.7% showed polymorphism with 2 loci average. Furthermore, the mean distance between markers was greater than 10 cM. For a reliable and stable trait-marker association, the distance should be less than 5 cM (Adam-Blondon et al., 2004).

Map genome coverage

A low number of linkage groups was expected due to the low polymorphism of the primers and the population. The fractions of the few linked markers, and therefore few linkage groups, were lower than those of other grape genetic maps published (Lodhi et al., 1995; Dalbó et al., 2000, Doucleff et al., 2004). The total map distances obtained for Italia and Mercan (218.1 cM in the female map and 144.9 cM in the male parent) are much lower than the distances obtained in other grape maps (Doligez et al., 2002; Doucleff et al., 2004; Fischer et al., 2004; Riaz et al., 2004), ranging between 756 and 1728 cM. The larger number of markers linked on their maps resulted in increased genome coverage. The high number of non-informative markers on the Italia and Mercan maps increased the problems associated with mapping.

The problems encountered in mapping were confounded by low segregation ratios in the phenotypic traits obtained for only 2 growing seasons. The number of progenies also limited the information derived. These problems led to an unsaturated genetic map and less reliable and possibly artificial linkages between the traits and the markers. Further analyses on this population with SSR and AFLP markers are warranted and underway.

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