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Full Length Research Paper

Genome mapping in F_1 population of crossbred Italia and Mercan grape varieties: Establishment of AFLP and SSR linkage groups towards significant morphological characters and fungal diseases

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This research aims at the establishment of a genome map for F_1 population developed by crossbreeding of Italia and Mercan grape varieties. A co-dominant marker SSR (Simple Sequence Repeats) and a dominant marker AFLP (Amplified Fragment Length Polymorphism) were used in the study. Double-pseudotestcross mapping technique was used for genome mapping. The study analyzed 60 plants selected from the F_1 population as well as the parental grape varieties, which differed in regards to resistance to diseases and some morphological characteristics. Amplification products were evaluated as "available" and "not available" at the end of silver staining for SSR and radioactive marking for AFLP. Polymorphic loci were determined according to their segregation ratios ranging between 1: 1 and 3: 1 at the end of χ2 tests. 3.0 LOD value was used in Mapmaker/Exp 3.0 package program for mapping. Two separate genetic linkage maps (maternal and paternal) were observed which included 6 and 1 linkage groups, respectively. Linkage of the loci located on the linkage groups to the observed diseases and morphological characters were analyzed with regression and variance analyses. A total of 818 primer pairs were tested on the two parents (Italia and Mercan), 60 F_1 (Italia × Mercan population) and two reference grape cultivar (Cabarnet Sauvignon and Merlot), successfully amplifying 112 markers. When the resistance traits to fungal diseases were analyzed during the study, no markers related with resistance to *Botrytis cinerea* and downy mildew could be found. However, it was found that the markers (VrZAG29a, VrZAG29c) related with the control of resistance to powdery mildew were on a locus which was in linkage group.

Key words: *Vitis vinifera* L., grape, SSR, AFLP, mapping.

INTRODUCTION

Vine growing, the art of cultivating healthy grapevines and gaining qualified grapes in accordance with various purposes is the sign of an advanced agricultural civilization (Fidan, 1985). Works on grapevine improvement have been started by giving priority to gaining breeds which are resistant to powdery mildew, downy mildew and *Botrytis cinerea*. In due course, these works have included the areas of increasing yields, improving quality, gaining new breeds bearing fruits both early and late and resistant to drought and cold.

The features that determine the selection criteria such as yield, having seedless, maturation time, berry colour, cluster shape, growing power of the mature and resistance to biotic and abiotic stress conditions take many years. Having a variety of grapevine breeds requires a F_1 population gained by crossbreeding the parents. Progeny

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Abbreviations: SSR, Simple sequence repeats; **AFLP,** amplified fragment length polymorphism; **LOD,** likelihood of odds; **QTL,** quantitative trait locus; **PCR,** polymerasechain reaction; **EST,** expressed sequence tag.

are heterozygote because the parents are also highly heterozygote. While the progeny act at some loci like an F_2 (3: 1), they act like a backcross population (1: 1) at others. The selection of the superior genotypes is made within such kind of populations. The most challenging disadvantage of the crossbreeding improvement is that the selection of the appropriate F_1 takes many years. Traditional improvement techniques require too much effort (Reisch et al., 1994). Among other problems encountered during this process are autogamy (self-fertilization) depression and somatic mutations. Lack of the appropriate genetic supplies and having limited information about the grapevine genome are the most important problems encountered during the grapevine improvement. Researchers must integrate modern biotechnology tools in conventional breeding to improve the most important crop such as grape. With the use of the opportunities of biotechnology in the field of grapevine improvement, all these negative conditions have been minimized and today we obtain effective results in a shorter time than ever. Thus, the concept of improvement has recently entered into a changing process.

During the past decade, several groups have put their efforts into the development of 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. F_1 population is used for such study (Adam-Blondon et al., 2004; Doligez et al., 2002; Fischer et al., 2004).

Plant improvement, either by natural selection or through the efforts of breeders, has always relied upon creating, evaluating and selecting the right combination of alleles. The manipulation of a large number of genes is often required for improvement of even the simplest of characteristics. With the use of molecular markers, it is now a routine to trace valuable alleles in a segregating population and mapping them. Moleculer markers, especially SSR (Simple Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism) have been found to be extremely useful in this regard. Owing to their quality of following clear Mendelian inheritance, they can be easily used in the construction of index maps, which can provide an anchor or reference point for specific regions of the genome. SSR and AFLP are extremely useful in detection of polymorphism between closely related genotypes (Sprague, 1966; Paterson et al., 1991; Troggio et al., 2007).

At the turn of the last century, many diseases were introduced into Turkey vineyards raising the need for breeding new varieties showing resistance to pathogens such as *Uncinula necator* (powdery mildew), *Plasmopara viticola* (downy mildew) or Phylloxera vastatrix. The aim of this research study within the scope of the national project themed “Producing New Grape Types with Standart Specifications and Resistant against Downy Mildew and Powdery Mildew, by Means of Cross Breedding Method”, is to make up AFLP (amplified fragment length polymorphism) and SSR (simple sequence repeats) linkage groups, oriented towards the important morphological traits and fungal diseases by utilizing the individuals selected from the F_1 population gained by crossbreeding the Italia and Mercan grape varieties.

MATERIAL AND METHODS

Plant material

A hybrid population of ‘Italia’ x ‘Mercan’ (60 plants) were used for mapping and two reference grape cultivar (Cabarnet Sauvignon and Merlot) were also included. Italia is a standard table grape cultivar grown in many regions. This variety produces large golden fruit with a slight muscat flavor, very vigorous and productive vine; however, downy mildew and powdery mildew can also be a problem. Mercan is a down mildew and powder mildew resistant variety that a white, small berried juice variety grown in Black Sea region and thought to be a *Vitis labrusca* variety.

Trait observations

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

DNA extraction

Cuttings (standard size, have 5 - 6 buds and rooted) brought in from the Tekirdağ Viticultural Research Institute were potted in perlite: soil: peat moss (1: 1: 1) into pots (pot size: radius 20 cm and height 25 cm) in a greenhouse located at the Department of Horticulture, Faculty of Agriculture, Ankara University. Parents (Mercan and Italia), two reference grape cultivar (Cabarnet Sauvignon, Merlot) and 60 F_1 plant materials were obtained from the greenhouse under controlled conditions. Young leaves were harvested in spring and summer and stored at -20°C. DNA extraction was made in accordance with the method modified by Lodhi (1994) and Lefort et al. (1998). DNA was extracted from young leaves because DNA extraction from grapevine has been difficult due to the presence of contaminants such as polyphenols and polysaccharides. Grapevine DNA can be extracted from any part of the plant, although the preferred material is fresh young leaves. Concentration and purity of the DNA extractions have been quantified with the spectrometric methods by using NanoDrop® ND-1000 (NanoDrop Technologies, Wilmington, DE, USA).

PCR Amplification

SSR (Simple Sequence Repeats) and AFLP (Amplified Fragment Length Polymorphism) primers were tested to screen DNAs from Mercan, Italia, two reference grape cultivar (Cabarnet Sauvignon, Merlot) and sixty F_1 (example, F_1, F_2,…..,F_{60}) plant. SSR and AFLP primers were synthesized at RAFFI MISKCIYAN, IDT (Integrated DNA Technologies, Inc., Coralville, IA, USA). γ^{33} P was synthesized at IZOTOP (Institute of Isotopes Co., Ltd., Budapest). The agreeable multiplication of 24 AFLP combinations (Table 1) and 20 SSR primers developed within the Vitis Microsatellite Consortium (VMC). [VVMD32, VVMD26, VVMD5, VVMD6, VVMD7 (Bowers et al.,1996); VVMD28, VVMD36, VVMD31, VrZAG29, VVMD17 (Bowers et al.,1999); VVS3, VVS5, VVS1, VVS4, VVS2, VVS29 (Thomas and Scott, 1993); VrZAG21, VrZAG79, VrZAG112, VrZAG67 (Sefc

Table 1. The enzyme/primer conditions used for AFLP selective amplification.

Primers	Selective amplifications	
AFLPa + M/SEL6	ESEL22 + M/SEL1	ESEL32 + M/SEL1
AFLPa + M/SEL2	ESEL22 + M/SEL2	ESEL32 + M/SEL2
AFLPc + M/SEL2	ESEL22 + M/SEL3	ESEL32 + M/SEL3
AFLPc + M/SEL5	ESEL22 + M/SEL4	ESEL32 + M/SEL4
AFLPc + M/SEL7	ESEL22 + M/SEL5	ESEL32 + M/SEL5
AFLPd + M/SEL2	ESEL22 + M/SEL6	ESEL32 + M/SEL6
AFLPd + M/SEL4	ESEL22 + M/SEL7	ESEL32 + M/SEL7
AFLPd + M/SEL6	ESEL22 + M/SEL8	ESEL32 + M/SEL8

Table 2. Binary logistics regression analysis results showing the significant trait-marker associations.

Marker	Trait	P (≤0.05)	Linkage group
** VVS2c	Bunch size	0.027	1
**VVS2c	Bunch weight	0.005	1
**VVS2d	Leaf form	0.028	1
** VVS2d	Mature leaf: general shape of petiole sinus	0.041	1
**VVS2d	Bunch size	0.027	1
**VVS2d	Bunch weight	0.005	1
*VVS1c	Hairs on petiole	0.017	2
*VVS1c	Colour of shoot	0.026	2
*VVS1c	Time of flowering	0.044	2
*VrZAG29a	Hairs on petiole	0.026	3
*VrZAG29a	Berry size	0.007	3
*VrZAG29c	Hairs on petiole	0.010	3
*VrZAG29c	Berry size	0.018	3
*AFLPa+MSEL6/17	Leaf form	0.045	5
*AFLPa+MSEL6/17	Bunch form	0.031	5
*AFLPa+MSEL6/19	Berry: uniformity of size	0.005	5
*AFLPc+MSEL7/15	Time of maturity	0.035	7
*ESEL22+MSEL5/13	Colour of shoot	0.046	8
*AFLPd+MSEL6/30	Time of maturity	0.047	9
*AFLPd+MSEL6/32	Leaf form	0.018	9
*AFLPd+MSEL6/32	Berry: classification of flavour	0.036	9
*AFLPc+MSEL2/14	Berry: uniformity of size	0.014	11
*AFLPc+MSEL7/16	Colour of shoot	0.029	8
* AFLPc+MSEL7/16	Bunch form	0.029	8

* The genetic linkage maps of Italia (maternal). ** The genetic linkage maps of Mercan (paternal).

et al., 1999)] was applied.

SSR reactions were performed in a 25-µl reaction mixture containing 10-ng template DNA, 10 pmol of each primer, 0.25 mM of each of 4 dNTPs, 0.5 unit Taq DNA polymerase (Promega, WI, USA) and 1.5 mM $MgCl_2$. Amplification conditions were optimized individually for each marker.

SSR amplifications were performed using a thermocycler (PTC-100; MJ Research Inc., Waltham, MA,USA) programmed as follows: 4 min at 94°C followed by 36 cycles of 1 min at 94°C, 1 min at 56 or 60°C, 1 min at 72°C followed by a final hold of 8 min at 72°C. All amplifications were confirmed by running 5.0 µl of the PCR reaction product on 2% agarose gels. PCR products were mixed with 2x sequencing dye (98% formamide, 10 mM EDTA, 0.05% bromphenol blue and xylenecyanol), denatured at 94°C for 2 min and 3 µl was loaded onto 6% polyacrylamide gels and run in1x TBE buffer at 70 W for 3 h. Gels were visualized by silver staining technique (Promega, Magison, Wis., USA).

AFLP markers were generated using the protocol described by Vos et al. (1995) with the following modifications: 500 ng genomic DNA was digested with EcoRI (10 u) for 3 h at 37°C and Tru 9 (5 u) for 3 h at 65°C. DNA was linked to EcoRI and MseI adaptors with T4 DNA ligase (1 u) for 12 h at 16°C. After 10 times dilution, pre-amplification was performed by 20 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 60 s followed by an extention reaction for 72°C for 60 s. The selective amplication was carried out under the following conditions: 1 cycle at 94°C for 30 s, 65°C for 30

Table 3. Summary of the linkage analysis in a hybrid population of Italia (maternal) x Mercan (paternal).

Parameters	SSR	AFLP	TOTAL
Number of primers	20	24	44
Primer with at least one potentially polimorphic locus	19	24	43
Polymorphic markers	47	771	818
Number of loci segregating 1:1	23	89	112
Ratio of loci segregating 1:1 to polymorphic primer	1.211	3.708	2.604
Ratio of segregating loci to total number of primers	1.068	17.522	18.591

Table 4. Comparison of the genetic linkage maps of Italia (maternal) x Mercan (paternal).

Parameters	Total	Italia	Mercan
Total distance covered (cM)	-	188	9.1
Markers mapped	16	14	2
Average map distance between markers (cM)	8.985	13.420	4.550
Number of linkage groups	7	6	1
Largest linkage group (cM)		69.3	9.1
Number of markers in the largest group		3	2

s and 72°C for 30 s, followed by 12 cycles in which annealing temperature decreased 0.7°C per cycle. Additionally 23 cycles followed at 94°C for 3 s, 56°C for 30 s and 72°C for 60 s. EcoRI primer was endlabelled with [γ33 P] ATP. After selective amplification, AFLP products were mixed with equal volume (10 µl) of denaturation dye (98% formamide, 10 mM EDTA, 0.025% bromofenol blue, 0.025 Xylene Cyanol) and denaturated by 95°C for 5 min. 3 µl of each sample were loaded and electrophoresed on 6% sequencing polyacrylamide gel and run in 1x TBE buffer at 70 W for 3 h. The gel was dried with a vacuum dryer and exposed to X-ray film (Kodak Biomax MS Film, MS-2 sizes, diameter: 35 cm and length: 42 cm) for autoradiography at room temperature between 2 - 4 days.

Scoring, data organization and analyses

Amplified bands were scored as either present (1) or absent (0). Data were entered in Microsoft® Excel (Microsoft Corp.) spread-sheets. Determination of the linkage groups and locating markers were carried out with the package software MAPMAKER/EXP 3.0 (Lander et al., 1987) by using LOD (likelihood of odds) 3.0 Haldane (1919) mapping function with a maximum 25 cm distance between the markers and QTL (quantitative trait loci) analysis of the loci located on the linkage groups were made on the same maps.

RESULTS

Phenotypic Analysis

Statistical estimate were made, oriented towards 24 morphological and resistance traits analyzed in the population. These estimate results are extremely important to observe how the population behaved in terms of the analyzed traits. In the population rating scales, analyzed traits usually range between narrow limits. For example, traits such as berry shape, bud colour, leaf shape and intensity of anthocyanin of tip between quite narrow ranges.

Quantitative trait values are supposed to be distributed at a normal level. Before using the t-test for the comparison of the loci (0: 1) on their powdery mildew (C1), *B. cinerea* (C2) and downy mildew (C3) traits, whether C1, C2 and C3 traits were distributed at a normal level or not was analyzed with Anderson-Darling normality test (Minitab 13.1, $p \leq 0.05$). So, before the t-test C1, C2 and C3 traits were exposed to logarithmic transformation.

Molecular analysis

20 SSR primers and 24 AFLP primer combinations were used for the research. 43 of total 44 primers turned out to be polymorphic (97%). One of the SSR primers, VVMD31, was not polymorphic. It was understood that a total of 818 (SSR: 47 and AFLP: 771) bands obtained from the polymorphic primers were polymorphic in the population. The ratio of every two primary locus to a polymorphic primer remained at the level 19.023. While the ratio of SSR per polymorphic primer was 2.470 loci, it was 32.125 loci for AFLP (Table 3).

While 72 of total 818 loci had a 1: 1 segregation in the female parent (Italia), this number reached 40 in the male parent (Mercan). 1: 1 segregation in Italia was observed in 12 loci for SSR and 60 loci for AFLP. The heterozygote locus number with 3: 1 distribution was 4 for SSR and73 for AFLP (Table 4).

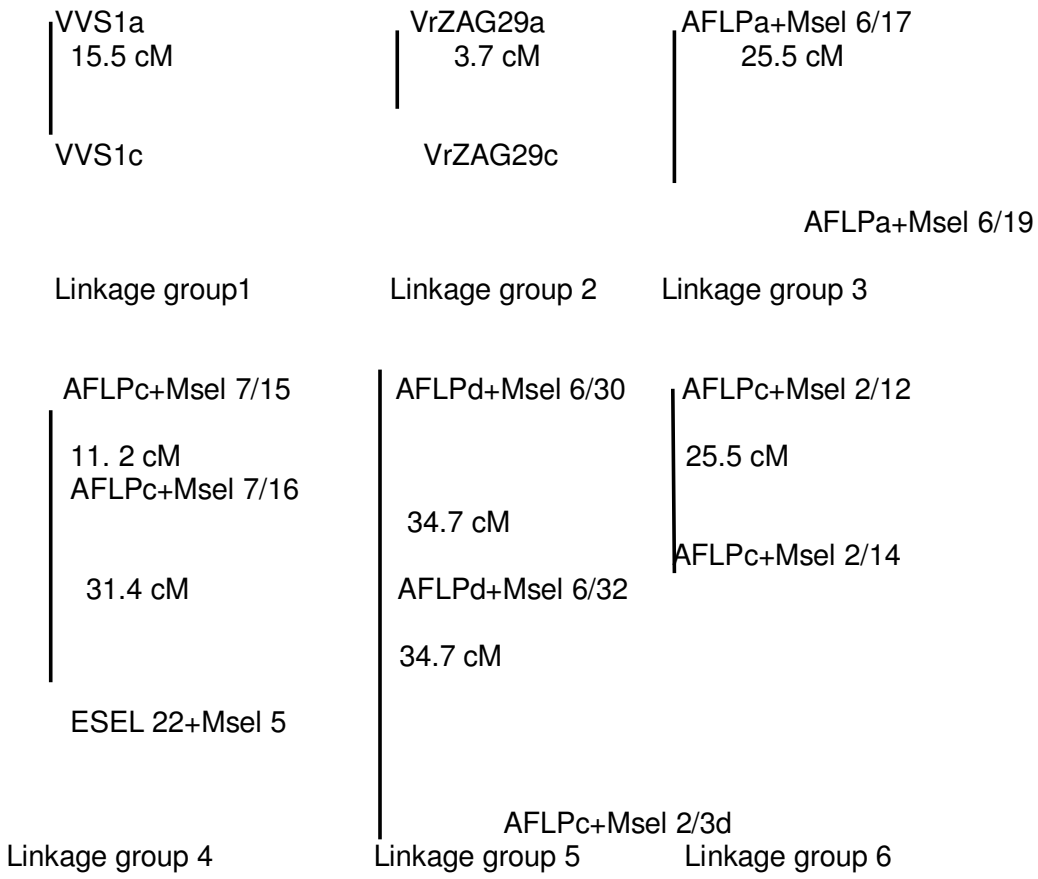

Figure 1. Linkage groups belonging to the Italia (maternal).

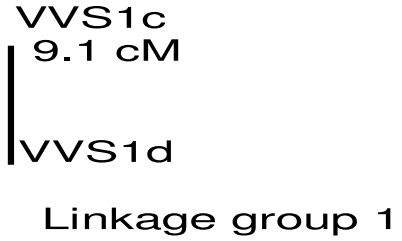

Figure 2. Linkage group belonging to the Mercan (paternal).

Genetic linkage analysis

With the result of the analysis for locating the linkage groups, female parent (Italia) turned out to have 6 linkage groups and male parent (Mercan) to have 1 linkage group. Only 14 of 72 loci that segregated in Italia got located on linkage groups (Fıgures 1 and 2). However, it was found that only 2 of 40 loci that segregated in Mercan with a 1:1 ratio were located on 1 linkage group. 19 loci have been excluded from the analysis, because they did not segregate in accordance with Mendel distribution.

It was seen that there was average of 13.420 cM distance among the 14 loci in the genetic map of Italia. The largest separation with 34.7 cM range in the linkage map of female parent Italia was in the linkage group 5 and the smallest distance of 3.7 cm were in the linkage group 2 between VrZAG29a and VrZAG29c. The largest linkage group of Italia was 5 with 69.3 cM on which 3 loci were located. Average locus distance for male parent genetic map was accounted as 4.550 cM (Figure 1). The only linkage group of Mercan made up this group with two markers with a 9.1 cM distance (Figure 2).

Quantitative trait analysis

The analyses results of resistance traits were given in the Table 5. It was with binary logistic regression technique found that in abnormally distributed traits, 12 marker loci were in relation with 12 morphological traits.

When the resistance traits to powdery mildew (C1), *Botrytis cinerea* (C2) and downy mildew (C3) were analysed, markers related with *Botrytis cinerea* and downy mildew were not found (Table 5). However, it was found that the markers related with resistance to powdery

Table 5. ANOVA test results on disease resistance traits.

Marker	Trait	P (≤ 0.05)	% Phenotypic variance explained	Linkage group distance (cM)
VrZAG29a	Powdery mildew resistance	0.011	0.434 ± 0.708 0.974 ± 0.803	3/3.7
VrZAG29c	Powdery mildew resistance	0.011	0.444 ± 0.695 0.983 ± 0.815	

mildew were located on the locus which was in linkage group 3 in Italia.

DISCUSSION

Modern plant breeding is a dynamic field in the applied sciences. The breeders, by using genetic variations, try to obtain new breeds containing the traits in which cultivators and consumers are interested. Hereditary traits of the plants are called qualitative or quantitative based on how many genes govern these traits. Generally, heritage of the qualitative traits can easily be explained with Mendel principles. However, the quantitative traits are raised as a result of the joint activity of numerous genes such as yield and quality or resistance of cultivated plants. On the other hand, heritage of quantitative traits affected by environmental factors is crucial for the plant breeding.

Development of DNA markers and powerful biometric methods for QTL (quantitative trait loci) description, for over 75 years, has led to very important improvements for mapping of QTL for plants. In this sense, molecular markers serve for the science world as important and strong devices in the fields of searching the genetic structures of plants, mapping of agriculturally important genes including quantitative trait loci (QTL) and analysis of evolutionary correlations (Asins, 2002).

Markers were analyzed with the value of LOD (likelihood of odds) 3.0 by using the package software Mapmaker/Exp 3.0 (Lander et al., 1987) and linkage groups were made up. In the maps of Italia and Mercan, respectively, 6 and 1 linkage groups were obtained. While 6 of 72 loci that segregated in Italia located on linkage groups, 2 of 40 loci of Mercan located on 1 linkage group. With the use of appropriate statistic programmes, the possible correlations of these markers with morphological and resistance traits were set.

In this research, data belonging to the 24 morphological and resistance traits were used for quantitative trait locus (QTL) analysis. Statistical analysis was made with the package software Minitab 13.1 to determine the correlation of the markers, located on the linkage groups, to the traits. To decide whether the traits were distributed normally or not, an Anderson-Darling normality test was made. Because of the fact that traits' distribution was at an abnormal level, after exposing them to logarithmic transformation, a t-test was made on the traits. Analysis results of the resistance traits exposed to a logarithmic transformation and a t-test were given in Table 2. Abnormally distributed traits were realized to Binary Logistic Regression ($p \leq 0.05$). Because of the fact that an efficiently saturated genetic map failed to be produced, the package softwares that were put into service of the scientists with the mapping of QTL remained inefficient to help and forcing us to use these methods.

Several grape linkage maps have previously been published, but most of the mapping populations have been interspecific hybrids, often chosen because the parents are sources of disease resistance (example, Lodhi et al., 1995; Dalbó et al., 2000; Grando et al., 2003; Zyprian et al., 2003). Our map is based on Italia x Mercan. Mercan is a down mildew and powder mildew resistant variety that a white, small berried juice variety grown in Black Sea region and thought to be a *V. labrusca* variety. Italia is one of world's most widely distributed standard grape cultivar. This variety produces large golden fruit with a slight muscat flavor, very vigorous, however, downy mildew and powdery mildew can also be a problem.

The aim of the present work was based on 20 microsatellite markers (as SSR markers are highly transferable co-dominant markers) for *V. vinifera* genome (n = 19) and 24 AFLP combinations (Table 1). A total of 818 primer pairs were tested on the two parents (Italia and Mercan), 60 F_1 (Italia × Mercan population) and two reference grape cultivar (Cabarnet Sauvignon and Merlot), successfully amplifying 112 markers (Table 3). Italia covers an average distance between markers of 13.420 cM and Mercan 4.550 cM (Table 4).

When the polymorphism of total 38 traits in Italia x Mercan population is analyzed, it was seen that male and female individuals were alike in 11 traits and breed population did not segregate in terms of these 11 traits. When a general genetic map is aimed, male and female individuals are supposed to vary in as many morphological traits as possible and transmit these traits to the mapping population. That is the only way to observe high levels of expansion in the breed population. The main purpose of mapping is to locate the loci, related with the traits being analyzed, to the narrow chromosomal areas and so that to increase the reliability and accuracy of the map. While planning a mapping programme, one should bowl along the basic things such as the way of applying, the type and size and DNA marker type of the expansion population.

For gaining the highest level of expansion, the most important criterion is that male and female individuals should be different from each other in terms of the trait(s) being analyzed (polymorphism). It was the most important problem in this research that one-third of the traits, analyzed in Italia x Mercan population, were not polymorphic.

Gökbayrak et al. (2006) analysed the same Italia x Mercan F_1 population with 300 RAPD primers and found a very low level of polymorphism (37%). In the maps of Mercan and Italia obtained from LOD (likelihood of odds) 3.0 with 8 and 6 linkage groups respectively, were found markers varying between 2 and 3. According to the mapping principles, markers closer than 10 cM to each other are regarded to have a correlation (Barevitz and Chory, 2004; Kearsey and Farquhar, 1998). In this study of Gökbayrak et al. (2006), the correlations between OPI4c-OPI4d (5.0 cm) and OPDBa – OPD4a (9.2 cm) in Italia map sc108266 and OPM2a (6.8 cm) in Mercan map were found significant. As a result of taking the importance threshold value (p) as 0.05 in the regression and ANOVA tests, more marker-QTL correlations were obtained. Researchers reported that with further analysis that will be made with more informative primers other linkage groups could be ascertained in detail. Regarding this research, it can be said that only VrZAG29a and VrZAG29c (3.7 cm) in Italia and VVS1c and VVS1d (9.1 cm) in Mercan had real correlations. For a more detailed analysis of the linkage groups, the number of primers should be increased.

It is quite hard to find all the possible QTLs that govern any trait and to map these QTLs. It is reported that the maximum number of the important QTLs, that can be available for any trait, is 12 (Lodhi et al., 1995). Regarding the importance of this study and the facts that hundreds of genotypes may be used to find many more and reliable QTLs and multi-locational and multi-annual experiments may be carried on, it is known that this study will cost too much (Kearsey and Pooni, 1996). Studying with a very low number of F_1 individuals was another factor that limits the success of this study.

One should pay close attention to selecting the male and female parents to solve all the problems that can be encountered in a mapping study. The first mapping studies used populations of 50 - 80 individuals (Lodhi et al., 1995; Dalbo et al., 2000; Grando et al., 2000) with American genotypes such as 'Cayuga White' and 'Aurore', or with Chinese wild species (Luo et al., 2002), or with the breeds focusing on having no seed (Doligez et al., 2002). Riaz et al (2004) had constructed a framework linkage map based on microsatellite markers for *V. vinifera* L., the European wine grape. The mapping population consisted of 153 progeny plants from a cross of *V. vinifera* cvs. Riesling x Cabernet Sauvignon. One hundred fifty-two microsatellite markers and one polymorphic EST marker had been mapped to 20 linkage groups (2n = 38).

Low number of individuals in the population, marker number and polymorphism of the markers caused low saturation in this study. It can be concluded also from the study carried out with the purpose of making a genome map with 60 F_1 plants chosen from the F_1 population which was developed by crossbreeding the Italia and Mercan in terms of resistance and other morphological traits. It is supposed in a mapping study that the number of haploid chromosomes should be equal to the number of linkage groups. In this study, this requirement was not met. Only 6 and 1 linkage groups were obtained in the maternal and paternal map, respectively. The low number of the markers located on these linkage groups made it difficult to reach the targets declared in the aim of the study section. Even though the low number of individuals in the population was intended to be eliminated by using SSR and AFLP primers with very high levels of polymorphism, it was not sufficient to obtain the highest possible information from the study.

When the resistance traits to fungal diseases were analyzed during the study, no markers related with resistance to *B. cinerea* and downy mildew could be found. However, it was found that the markers (VrZAG29a, VrZAG29c) related with the control of resistance to powdery mildew were on a locus which was in linkage group.

This study is important in terms of being the first study which was carried out by using SSR and AFLP primers on the grapevines in our country. However, the Italia x Mercan population that was used as a mapping population was not as polymorphic as a mapping population was supposed to be. Because of this situation, the study was not efficient to reach the targets (to explain the marker-OTL correlation).

Generally, QTL studies can be successfully used in the marker assisted selection. In this way, by enabling selection in early phases, one can both save time and increase the chance to succeed by minimizing the population. On the other hand, there are some conditions to meet to reach this goal, which are; trait being easily searchable, high level of heredity and correlation between assisting traits and target traits. In addition, use of many or enough genotypes, data obtained from multi-annual and multi-recursive experiments, covering genome with as many markers as possible and mapping programmes' being suitable are also required (Haensel, 1976; Falconer and Mackey, 1996).

All the results obtained from this study include some other very important results that will lead further studies. In order to reach the intended goals of a genome mapping study, selecting the most heterozygote male and female individuals, increasing the individual number as much as possible and using the heterozygote and homozygote primary types with suitable rates are absolutely necessary. The maps that will be obtained in the end can become more saturated and QTL (quantitative trait loci) information can be more detailed and reliable.

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