Association Analysis and Estimation of Breeding Values of Iranian Table Grapevine Germplasm by Integrating Pomological and Molecular Data

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Abstract

Genetic resources in each country are valuable assets for sustainable development. An accurate knowledge of genetic behavior and identification of genomic loci associated with important economic traits will help breeders to run efficiently their breeding programs. Fourteen important pomological traits were measured on 45 Iranian table grape cultivars during three successive years. Molecular profile of studied cultivars was prepared with 39 (Simple sequence Repeat) SSR primers pairs. Genetic structure analysis based on the SSR markers revealed two sub-populations ($K = 2$) in the association panel. In association analysis using mixed linear model seven loci were found to be significantly associated with the studied traits at 5% probability level. Breeding values were also estimated for the pomological traits using the best linear unbiased prediction (BLUP). "Saghal Solian", "At Ouzum", "Garmian", "Rishbaba Qermez", "Taifi", "Shahroudi", "Sahebi Qermez", "Lal Qermez", "Alhaghi", "Sarghola", "Chava Ga", "Qzl Ouzum" and "Agh Shani" table grape cultivars showed high and positive breeding value for cluster length, width and weight. Whereas "Garmian", "Rishbaba Qermez", "Fakhri", "Agh Shani", "Lal Sefid" and "Shirazi" had positive and high breeding value for pollen germination, fruit set in open pollination and fruit set under controlled pollination. Finally, Sarghola" and "Qzl Ouzum" showed the positive and highest breeding value for berry weight, flesh weight, cluster length, cluster width and cluster weight. The cultivar with high and positive breeding value can be used as a good parent for breeding of traits in hybridization programs because they can better transfer the desirable characteristics to the progeny in each case.

Introduction

Table grape (*Vitis vinifera* L.) is one of the most important horticultural crops and it is a human interest in many centuries¹². Archaeological studies have shown that Iran is one of the centers of domestication of grape⁴¹. The size of the grape genome with 38 chromosomes is about 4.75×10^8 bp. This genome is a combination of duplicate and non-duplicate sequences that duplicate DNA makes up more than 95% of the genome²⁶. Investigation of genetic basis of quantitative traits has become possible by the development of molecular markers^{58,50}. In many plants, identification of genomic regions controlling quantitative traits could provide valuable genetic information about the genetic basis and structure of complex traits²⁵. The development of molecular marker technology together with appropriate statistical genomic techniques has facilitated the achievement of this goal. Identification of the genomic regions associated with quantitative inheritance is usually accomplished by two main methods: linkage mapping and association analysis. Recently the use of linkage mapping has been limited due to numerous limitations. For instance, production of artificial populations as a prerequisite is difficult especially in the fruit trees²⁷.

For overcome linkage mapping limitations, association analysis has been widely used in mapping programs in the recent years. In this method, the relationship between genotype and phenotype of individuals coming from natural populations is examined directly to identify the chromosomal regions involved in controlling trait variations⁴⁸. Unlike linkage analysis in which the linkage disequilibrium is only due to physical linkage, in natural populations used in association analysis the linkage disequilibrium is caused in addition to physical linkage by other factors such as small population size, migration and so on. Recent factors, however, led to the identification of false positive markers that are not important from the point of plant breeding view⁷⁰. So, in order to decrease false positive associations, it is necessary to consider population structure and kinship in association models⁶⁸.

On the other hand, several studies were conducted in grape via genome wide association analysis to identify genomic regions involved in important traits such as berry skin^{24,37}, berry proanthocyanidin compound³⁴ and fleshless berry³³. QTLs effective on fruit yield in grape using AFLP (Amplified Fragment Length Polymorphism) and SSR (Simple Sequence Repeat) markers were identified¹⁹. Also, in grapevine some association studies have been conducted via candidate genes approach including Myb^{57,23}, VvDXS¹⁶, VvPel⁶¹, VvGAI⁶⁰, VvTFL1A²¹ and 183 candidate genes associated with cluster architecture⁵⁶. In a study, genetic basis of grapevine leaf shape was reported for the first time by genome wide association analysis (GWAS) including 961 grapevine accessions genotyped with 6114 SNPs¹¹.

Selecting parents for hybridization programs is always a great challenge in front of plant breeders. Several methodologies have been utilized in helping the identification of genotypes with promising and appropriate agronomical characters for hybridization. Parent selection based on the predicted breeding values^{29,28,65} is a method extensively used in animal breeding for a long time¹⁸. Two common methods for predicting the breeding value of parents are selection index and BLUP. In the selection index, it is not possible to estimate the effect of environmental factors, so the data used in this method must be corrected for environmental factors before using. In fact, by this method the best predict of breeding value is estimated in populations with same environment conditions. For this reason, in recent years this method is less used for predicting breeding value. On the contrary, in the BLUP method, corrected data for environmental factors is not required for statistical analysis. Another advantage of this method is the use of all kinship relationships in predicting of breeding value. Genetic change that is occurred in populations with continuous genetic selection, can be estimated by BLUP.

Another feature of this method is its using for multiple traits⁵. The type of the model used depends on the specificity of the data and the purpose of breeding programs. When the phenotypic data come from different conditions, they should be tested using robust statistical procedures for correcting the data for disturbing factors in order to increase the heritability coefficient, which will increase the accuracy of selection. Traditionally, for estimating breeding values (BLUP), pedigree information of lines is used in software package such as Wombat⁴⁰. It is possible to use Kinship matrix instead of genetic relationship matrix (pedigree information) for predicting breeding values in the model⁴. In BLUP context, the main purpose of breeders is to increase the correlation of true genotypic values and predicted genotypic values that the method can maximize this correlation⁴⁹.

The objectives of the present study are to find SSR loci associated with pomological traits using association analysis and to evaluate the breeding values for selecting the best cultivars from Iranian table grape germplasm by integrating the pomological and molecular data. The outputs of the present study are introducing appropriate genotypes as parents based on breeding values for constructing source populations and associated SSR markers that can be used for

selecting desirable individuals from the source populations during grape breeding programs. This has an advantage to progeny test. The major disadvantage of progeny test in fruit trees is their long generation and also necessarily having wide areas for plant evaluation.

Results

Descriptive phenotypic analysis. High levels of phenotypic variation were observed among the studied table grape cultivars for studied pomological traits. The highest mean value based on three years' data was observed in cluster weight (544.95). The highest broad sense heritability (98.46%) was observed in pollen germination rate (Table 1). A negative correlation was observed between fruit pH and TA. A positive correlation was observed between berry weight and flesh weight, as well as between fruit set under controlled pollination and pollen germination (Table 2). In cluster analysis based on agro-morphological characters, the studied genotypes were sub-divided into four main groups (Fig. 1).

Table 1
Descriptive statistics for agronomic traits of grape cultivars during three successive years

Trait	Year 1			Year 2			Year 3		
	Range	Mean	Std. Deviation	Range	Mean	Deviation	Range	Mean	Std. Deviation
TSS	14.8-24.6	18.95	2.64	14.5-26	19.86	3.06	15-26.5	18.86	2.49
Ph	2.22-5.4	3.04	0.68	3.1-5.4	3.56	0.34	2.56-4.76	3.38	0.34
TA	0.09-2.6	0.8	0.51	0.09-1.51	0.59	0.23	0.09-1.12	0.65	0.22
BW	1.31-5.15	3.02	1.08	1.36-5.15	3.01	1.05	1.44-6.5	3.44	1.28
FW	1.23-4.93	2.88	1.05	1.36-5.05	2.91	1.02	1.43-6.4	3.36	1.26
SSW	0-0.15	0.06	0.03	0-0.15	0.05	0.03	0-0.15	0.03	0.02
SN	0.95-3.5	2.13	0.64	1.1-3.4	2.09	0.62	1-3.5	2.2	0.61
JV	31-49	41.32	3.63	30-50	42.13	4.22	32-51.8	43.77	4.73
FSOP	10.6-63.3	32.6	11.57	10-76	31.07	13.42	5.6-64	32.42	11.46
PG	0.5-65.5	28.3	17.03	1.2-65	28.95	17.15	0.4-62.3	26.07	16.16
CL	9-26	18.83	4.48	9-32.7	19.68	4.65	13-26	19.91	3.27
CWid	5-17.5	10.1	2.82	4-20	10.01	3.18	5.5-15.5	10.13	2.25
CW	67.1-874.9	402.37	185.5	300-950	636	132.21	223.3-1387	597.61	268.52
FSCP	0-58.5	24.65	13.14	0-70.9	27.32	16.18	1.8-51	24.34	11.99
Trait	Three years data			PCV		GCV	h ² bs	GA5%	GA5% mean
	Range	Mean	Std. Deviation						
TSS	15.33-23.56	19.22	2.17	11.35		9.58	71.23	3.20	16.66
Ph	2.84-5.03	3.32	0.38	11.41		9.47	68.83	0.54	16.18
TA	0.09-1.38	0.68	0.24	35.46		24.22	46.65	0.23	34.08
BW	1.4-5.06	3.14	1.05	33.31		31.82	91.25	1.98	62.62
FW	1.39-4.92	3.04	1.03	33.65		32.05	90.75	1.92	62.90
SSW	0-0.15	0.05	0.02	50.14		44.12	77.43	0.04	79.98
SN	1.03-3.2	2.13	0.55	25.70		23.87	86.25	0.98	45.67
JV	20.7-50.26	41.96	5.19	9.60		9.41	96.25	8.07	19.03
FSOP	8.7-67.7	31.98	11.02	34.43		32.48	88.95	20.21	63.09
PG	1-64.2	27.27	16.97	59.53		59.07	98.46	33.53	120.74
CL	12.2-24.7	19.44	3.24	16.59		13.51	66.32	4.41	22.66
CWid	5-15.5	10.24	2.38	22.01		18.65	71.77	3.28	32.55
CW	326-905	544.95	145.92	26.75		19.46	52.90	158.96	29.15
FSCP	3.8-52	25.4	10.79	42.54		35.00	67.69	15.09	59.32

PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, h^2_{bs} : Narrow sense heritability, GA 5%: Genetic advance in 5 percent probability level. TSS: Total soluble solids, TA: Titratable acidity, BW: Berry weight, FW: Flesh weight, SSW: Single seed weight, SN: Seed number, JV: Juice volume, FSOP: Fruit set in open pollination, PG: Pollen germination, CL: Cluster length, Cwi: Cluster width, CW: Cluster weight, FSCP: Fruit set under controlled pollination.

Table 2
Correlation coefficients among agronomic traits of grape cultivars in the first (upper diagonal) and second (low diagonal) cropping season

	TSS	Ph	TA	BW	FW	SSW	SN	JV	FSOP	PG	CL	CWid	CW
TSS	1	0.196 ^{ns}	-0.105 ^{ns}	-0.296*	-0.293 ^{ns}	-0.210 ^{ns}	-0.011 ^{ns}	-0.051 ^{ns}	0.188 ^{ns}	0.328*	0.069 ^{ns}	0.110 ^{ns}	0.192 ^r
Ph	0.209 ^{ns}	1	-0.494**	0.194 ^{ns}	0.206 ^{ns}	-0.049 ^{ns}	0.10493 ^{ns}	-0.252 ^{ns}	0.003 ^{ns}	0.112 ^{ns}	-0.105 ^{ns}	-0.258 ^{ns}	0.166 ^r
TA	-0.175 ^{ns}	-0.621**	1	-0.271 ^{ns}	-0.275 ^{ns}	-0.012 ^{ns}	-0.096 ^{ns}	0.123 ^{ns}	0.264 ^{ns}	-0.000 ^{ns}	0.201 ^{ns}	0.205 ^{ns}	-0.015 ^{ns}
BW	-0.242 ^{ns}	0.105 ^{ns}	-0.170 ^{ns}	1	0.997**	0.388**	0.315*	0.119 ^{ns}	-0.071 ^{ns}	-0.274 ^{ns}	-0.047 ^{ns}	0.193 ^{ns}	0.330 [‡]
FW	-0.248 ^{ns}	0.111 ^{ns}	-0.164 ^{ns}	0.998**	1	0.327*	0.283 ^{ns}	0.108 ^{ns}	-0.077 ^{ns}	-0.264 ^{ns}	-0.061 ^{ns}	0.175 ^{ns}	0.324 [‡]
SSW	-0.181 ^{ns}	-0.128 ^{ns}	0.001 ^{ns}	0.375*	0.332*	1	0.063 ^{ns}	0.133 ^{ns}	-0.141 ^{ns}	-0.296*	0.074 ^{ns}	0.091 ^{ns}	0.075 ^{ns}
SN	0.227 ^{ns}	0.038 ^{ns}	-0.177 ^{ns}	0.185 ^{ns}	0.171 ^{ns}	-0.235 ^{ns}	1	0.241 ^{ns}	0.307*	0.112 ^{ns}	0.056 ^{ns}	0.369*	0.308 [‡]
JV	-0.144 ^{ns}	-0.092 ^{ns}	-0.073 ^{ns}	0.185 ^{ns}	0.185 ^{ns}	-0.035 ^{ns}	0.228 ^{ns}	1	0.136 ^{ns}	0.048 ^{ns}	0.203 ^{ns}	0.313*	0.440 [‡]
FSOP	0.294*	0.325*	-0.296 ^{ns}	-0.072 ^{ns}	-0.072 ^{ns}	-0.175 ^{ns}	0.216 ^{ns}	0.012 ^{ns}	1	0.2917 ^{ns}	-0.129 ^{ns}	0.259 ^{ns}	0.142 ^{ns}
PG	0.129 ^{ns}	0.102 ^{ns}	-0.148 ^{ns}	-0.338*	-0.323*	-0.503**	0.274 ^{ns}	0.008 ^{ns}	0.148 ^{ns}	1	-0.235 ^{ns}	0.255 ^{ns}	0.076 ^{ns}
CL	0.045 ^{ns}	0.244 ^{ns}	-0.066 ^{ns}	0.157 ^{ns}	0.164 ^{ns}	-0.141 ^{ns}	0.106 ^{ns}	0.176 ^{ns}	0.053 ^{ns}	-0.090 ^{ns}	1	0.206 ^{ns}	0.177 ^{ns}
CWid	0.018 ^{ns}	0.115 ^{ns}	-0.235 ^{ns}	0.157 ^{ns}	0.164 ^{ns}	-0.234 ^{ns}	0.292 ^{ns}	0.447**	0.390**	0.030 ^{ns}	0.372*	1	0.563 [‡]
CW	-0.207 ^{ns}	-0.198 ^{ns}	0.002 ^{ns}	0.499**	0.497**	0.172 ^{ns}	0.203 ^{ns}	0.417**	0.139 ^{ns}	-0.173 ^{ns}	0.141 ^{ns}	0.364*	1
FSCP	0.071 ^{ns}	0.225 ^{ns}	-0.276 ^{ns}	0.073 ^{ns}	0.077 ^{ns}	-0.190 ^{ns}	0.290 ^{ns}	-0.182 ^{ns}	0.473**	0.168 ^{ns}	-0.034 ^{ns}	0.193 ^{ns}	0.061 ^{ns}

Table 2

(Continuation). Correlation coefficients among agronomic traits of grape cultivars in the third (upper diagonal) and mean of three (low diagonal) cropping sea

	TSS	Ph	TA	BW	FW	SSW	SN	JV	FSOP	PG	CL	CWid	CW	FSCP
TSS	1	0.041 ^{ns}	0.157 ^{ns}	-0.176 ^{ns}	-0.165 ^{ns}	-0.275 ^{ns}	0.026 ^{ns}	0.077 ^{ns}	0.138 ^{ns}	0.350*	0.003 ^{ns}	-0.011 ^{ns}	0.014 ^{ns}	0.24 ^{ns}
Ph	0.149 ^{ns}	1	-0.519**	-0.205 ^{ns}	-0.210 ^{ns}	0.159 ^{ns}	-0.146 ^{ns}	-0.176 ^{ns}	0.014 ^{ns}	0.210 ^{ns}	-0.082 ^{ns}	0.056 ^{ns}	0.013 ^{ns}	0.04 ^{ns}
TA	0.085 ^{ns}	-0.664**	1	-0.047 ^{ns}	-0.047 ^{ns}	-0.062 ^{ns}	-0.152 ^{ns}	-0.014 ^{ns}	-0.068 ^{ns}	-0.235 ^{ns}	-0.016 ^{ns}	-0.242 ^{ns}	-0.039 ^{ns}	-0.1 ^{ns}
BW	-0.339*	-0.004 ^{ns}	-0.259 ^{ns}	1	0.999**	0.147 ^{ns}	0.239 ^{ns}	0.083 ^{ns}	-0.095 ^{ns}	-0.453**	0.085 ^{ns}	-0.156 ^{ns}	0.071 ^{ns}	-0.0 ^{ns}
FW	-0.336*	-0.006 ^{ns}	-0.258 ^{ns}	0.999**	1	0.109 ^{ns}	0.224 ^{ns}	0.081 ^{ns}	-0.107 ^{ns}	-0.454**	0.095 ^{ns}	-0.169 ^{ns}	0.071 ^{ns}	-0.0 ^{ns}
SSW	-0.300*	0.006 ^{ns}	-0.046 ^{ns}	0.420**	0.383**	1	-0.047 ^{ns}	0.052 ^{ns}	0.165 ^{ns}	-0.001 ^{ns}	-0.209 ^{ns}	0.143 ^{ns}	-0.090 ^{ns}	0.09 ^{ns}
SN	0.115 ^{ns}	0.033 ^{ns}	-0.238 ^{ns}	0.234 ^{ns}	0.218 ^{ns}	-0.113 ^{ns}	1	0.149 ^{ns}	0.455 ^{ns}	0.071 ^{ns}	-0.073 ^{ns}	0.255 ^{ns}	0.187 ^{ns}	0.24 ^{ns}
JV	-0.148 ^{ns}	-0.589**	0.269 ^{ns}	0.144 ^{ns}	0.143 ^{ns}	0.139 ^{ns}	0.100 ^{ns}	1	0.229 ^{ns}	-0.036 ^{ns}	0.148 ^{ns}	0.073 ^{ns}	0.232 ^{ns}	0.12 ^{ns}
FSOP	0.287 ^{ns}	0.099 ^{ns}	0.060 ^{ns}	-0.116 ^{ns}	-0.123 ^{ns}	-0.096 ^{ns}	0.385**	-0.002 ^{ns}	1	0.186 ^{ns}	-0.172 ^{ns}	0.242 ^{ns}	0.284 ^{ns}	0.33 ^{ns}
PG	0.366*	0.176 ^{ns}	-0.121 ^{ns}	-0.369*	-0.365*	-0.299*	0.176 ^{ns}	-0.095 ^{ns}	0.212 ^{ns}	1	-0.206 ^{ns}	0.304*	-0.130 ^{ns}	0.37 ^{ns}
CL	0.072 ^{ns}	0.007 ^{ns}	0.137 ^{ns}	0.089 ^{ns}	0.091 ^{ns}	-0.146 ^{ns}	0.030 ^{ns}	0.084 ^{ns}	-0.088 ^{ns}	-0.217 ^{ns}	1	-0.015 ^{ns}	0.428**	-0.1 ^{ns}
CWid	-0.069 ^{ns}	-0.007 ^{ns}	0.01 ^{ns}	0.076 ^{ns}	0.074 ^{ns}	-0.136 ^{ns}	0.318*	0.218 ^{ns}	0.452**	0.097 ^{ns}	0.321*	1	-0.112 ^{ns}	0.04 ^{ns}
CW	-0.036 ^{ns}	0.094 ^{ns}	-0.175 ^{ns}	0.339*	0.342*	-0.053 ^{ns}	0.303*	0.279 ^{ns}	0.249 ^{ns}	-0.179 ^{ns}	0.454**	0.585**	1	-0.0 ^{ns}
FSCP	0.242 ^{ns}	0.137 ^{ns}	-0.141 ^{ns}	0.011 ^{ns}	0.008 ^{ns}	-0.106 ^{ns}	0.489**	-0.083 ^{ns}	0.582**	0.380**	-0.202 ^{ns}	0.221 ^{ns}	0.166 ^{ns}	1

TSS: Total soluble solids, TA: Titratable acidity, BW: Berry weight, FW: Flesh weight, SSW: Single seed weight, SN: Seed number, JV: Juice volume, FSOP: Fruit set in open pollination, PG: Pollen germination, CL: Cluster length, Cwi: Cluster width, CW: Cluster weight, FSCP: Fruit set under controlled pollination.

Marker-trait analyses. A total of 199 alleles were amplified with 39 SSR primer pairs with an average of 5.10 alleles/locus. To identify the level of polymorphism, amount of PIC was calculated for each of the 39 SSR loci which ranged from 0.08 for locus Vmc7c3 to 0.89 for locus VVMD8 with an average value of 0.51. The observed heterozygosity ranged from 0.083 for locus Vmc7c3 to 0.98 for locus ZAG64 with an average of 0.56 across the SSR markers (Table 3). The studied genotypes were grouped in four main groups based on SSR data (Fig. 1). Hierarchical groupings of cultivars by phenotypic and genotypic data were compared and it was found that 67% of the cultivars were grouped into similar position across the two hierarchical clusters (Fig. 1). The genetic structure of studied grape population were also analyzed with Bayesian clustering method. Results revealed two possible subpopulations (K=2) in the studied germplasm (Fig. 2). Based on the Barplot (Fig. 2), from 45 studied cultivars, 34 cultivars (75.56%) were belonged to sub-population 1 and 8 cultivars (17.78%) to sub-population 2. Three cultivars (6.67%) with Q < 0.70 were assigned admixed (Fig. 2).

Table 3
Genetic parameters of SSR loci assayed in the characterization of table grape cultivars

Markers	Primer sequence (5'→3')	Annealing temperature (C°)	Genotype No	Allele No	Gene Diversity	Heterozygosity	PIC
ZAG 62	ggtgaaatgggcaccgaacacacgc ccatgtctctcctcagcttctcagc	53	14	8	0.71	0.73	0.68
VVS2	cagcccgtaaatgtatccatc aaattcaaaattctaattcaactgg	53	22	10	0.86	0.95	0.85
VVMD5	ctagagctacgccaatccaa tataccaaaaatcatattcctaaa	56	21	10	0.83	0.78	0.81
VVMD7	agagttgcggaacaggat cgaaccttcacacgcttgat	54	15	6	0.80	0.78	0.77
G7	caacagaattcaaatgaaatgga caaacagcataaatacacaaagca	50	26	14	0.90	0.93	0.89
G10	catcattcatccaaattatgtag ttagtaggttagggataccagt	51	7	5	0.58	0.40	0.50
D12	ctctctttccgaaattggggt attttccctggaacaaagtgg	54	18	10	0.75	0.91	0.71
UCH29	aaacatgatctgatgcaggatga caacctgttgatgaaagggaaa	62.5	16	9	0.74	0.71	0.71
ISV2	cactggcctgttgggagataat ccttcaactggaaaagcctgtc	58	17	10	0.77	0.69	0.75
ISV3	aaggaggagttgagatgtagta gagtaagagagaagcaagaaaa	58	8	4	0.64	0.82	0.57
ISV4	tgcatagtctgtaggccattg tctgtcattgctgtcccttca	59	12	6	0.73	0.62	0.69
VVS3	tgccctatcaattagttcaccta tcgactttgatattgatgatt	52	3	2	0.48	0.69	0.37
VVS4	ccatcagtgataaacctaattgcc cccaccttgcccttagatgtta	58	6	4	0.46	0.44	0.42
ZAG 47	ggtctcaatacatccgtaagtatat acgggtgtgctctcattgtcattgac	54	11	6	0.75	0.84	0.72
ZAG64	tatgaaagaacccaacgcggcacg tgcaatgtggtcagcctttgatggg	58	17	10	0.83	0.98	0.81
ZAG83	ggcggaggcggtagatgagagggcg acgcaacggctagtaataacaacgg	52	3	2	0.41	0.22	0.33
VVMD8	taacaaacaagaagaggaat agcacatccacaacataatg	56	20	12	0.84	0.89	0.82
VVMD17	tgactcgccaaaatctgacg cacacatatcatcaccacacgg	56	6	4	0.51	0.51	0.44
VVMD21	ggttgctctaggagttgatgttc gcttcagtaaaaagggttgcg	56	7	5	0.66	0.82	0.59
VVMD25	ttccgttaaagcaaaagaaaagg ttggatttgaatttattgagggg	56	14	7	0.78	0.76	0.75
VVMD26	gagacgactggtgacattgagc ccatcaccaccatttctactgc	56	5	4	0.52	0.60	0.41

Markers	Primer sequence (5'→3')	Annealing temperature (C°)	Genotype No	Allele No	Gene Diversity	Heterozygosity	PIC
VVMD27	gtaccagatctgaatacatccgtaagt acgggtatagagcaaacggtgt	56	10	5	0.72	0.78	0.67
VVMD32	tatgatttttaggggggtgagg ggaaagatgggatgactcgc	59	14	8	0.71	0.71	0.67
Scu8vv	cgagaccagcatcgttcaag gcaaaatcctccccgtacaagtc	56	2	2	0.12	0.13	0.12
Scu10vv	tacccccacaaccccttt ttctccgccacctctttcac	56	2	2	0.37	0.49	0.30
UDV047	tgtatgataatccataatgtgc taggcattgcttgactattc	50	3	2	0.50	0.33	0.38
VMC6E10	ctaggtgtgccagagatcaga catttgggtagttgtgagga	53	5	3	0.61	0.38	0.53
VMC7a4	taagggtgattagtttgggtc aaactccaaacgatctgattct	51	6	4	0.73	0.13	0.68
Vmc7b1	cacgcaatctctcatttcacaaa tggtttagggtgacccaaccttta	55	3	2	0.50	0.33	0.37
Vmc7c3	cttggagagttccagaggta actgcttaacagtcctttgct	51	2	2	0.08	0.08	0.08
Vmc7f2	aagaaagttgcagtttatggtg aagatgacaatagcgagagagaa	51	2	2	0.29	0.36	0.25
Vmc7f6	attgcttccaaaagaga acccaaacccaatagat	50	3	2	0.19	0.17	0.17
Vmcng2b7.2	tttggagtgaatagagaccct cagaatttggctccatatttgaa	54	4	3	0.40	0.38	0.33
Vmcng2h7	acgttaaatagaacatgggtccc caacctctttttgaggtagc	51	6	3	0.63	0.68	0.56
VVIV67	aacttgattgaacaaaggccta tattatgcctatccagtttcga	50	3	2	0.37	0.39	0.30
VVIV67.2	attctcatttgggttctcac ttcagtagtcactctcaac	50	3	2	0.45	0.60	0.35
VVs16	tcaaactattattcaaccaaagtac tcgatttcaacaaatttagaaaata	51	3	3	0.11	0.12	0.11
VVs29	ccccaaggctctgaaaacaat tgcaaagcaataaagctcca	56	2	2	0.45	0.68	0.35
UDV015	tgcacatttccctccttag cgggttactgggaagggtat	53	3	2	0.27	0.15	0.23
Mean			8.82	5.10	0.57	0.56	0.51

The r^2 values among SSR markers showed an average value of 20.37 ($r^2 \geq 0.1$, P-value ≤ 0.01) (Fig. 2). Using mixed linear model, seven loci found to be significantly associated with the studied traits at 5% probability level (Table 4). One locus (Scu8vv) was identified to be associated with total soluble solids, one locus (Vmc7f2) with pollen germination, one locus (VVMD17) with seed weight, one locus (Vmc7f2) with cluster weight, one locus (Scu8vv) with fruit set in open pollination, one locus (ISV3) with seed number and one locus (Udv015) with titratable acidity. Results showed that Scu8vv was common for total soluble solids and fruit set in open pollination and also Vmc7f2 was common for pollen germination and cluster weight (Table 4).

Table 4
SSR loci linked to the studied morphological traits in the analyzed grapevine germplasm using mixed linear model (MLM) procedure

Trait	SSR locus	F-marker	P-marker	Trait	SSR locus	F-marker	P-marker
Total Soluble Solids	Scu8vv	5.69	0.022	Cluster weight (g)	Vmc7f2	4.86	0.033
Pollen germination (%)	Vmc7f2	5.08	0.029	Fruit set in open pollination (%)	Scu8vv	4.66	0.037
Seed weight (g)	VVMD17	2.76	0.032	Seed number	ISV3	2.44	0.038
				Titrateable acidity	Udv015	3.47	0.044

Predicting breeding values. The highest (2.4342) and the lowest (-2.976) breeding value for total soluble solids (TSS) was observed in "Klkarevi" and "At Ouzum" cultivars, respectively. The highest (0.2052) and lowest (-0.0773) breeding value for pH was observed in "Tabarze Qermez" and "Garmian", respectively. The highest (0.0942) and lowest (-0.0645) breeding value for TA character was seen in "Klkarevi" and "Shirazi" cultivars, respectively. For berry weight, the highest breeding value (1.4583) was observed in "Qzl Ouzum" and the lowest one (-2.9236) was observed in "Rejin". The highest breeding value (1.4914) for flesh weight was observed in "Qzl Ouzum" and the lowest one (-2.4007) was seen in "Rejin". About single seed weight, the highest breeding value (0.0521) was seen in "Shirazi" and the lowest one (-0.309) was observed in "Askari". The highest (0.6241) and lowest (-0.5183) breeding value for seed number was observed in "Chava Ga" and "Mam Braima" cultivars. For juice volume, the highest breeding value (4.6295) was seen in "Sahebi Qermez" and the lowest one (-4.5384) was observed in "Agh Shani" cultivar. The highest (17.8417) and lowest (-19.7354) breeding value for fruit set in open pollination was observed in "Qara Shani" and "Mam Braima" cultivars. Concerning to pollen germination, the highest (24.9031) and lowest (-20.3949) breeding value were observed in "Fakhri" and "Mam Braima" cultivars, respectively. The highest breeding value (2.8615) for cluster length was observed in "Qzl Ouzum" and the lowest one (-3.3038) was seen in "Lal Sefid" cultivar. The highest breeding value (2.2685) for cluster width was observed in "Lal Qermez" and the lowest one (-1.3292) was seen in "Mam Braima" cultivar. About cluster weight, the highest (211.783) and lowest (-121.986) breeding value were observed in "Shahrودي" and "Yaghoti" cultivars, respectively. For fruit set under controlled pollination, the highest (9.1738) and lowest (-9.7768) breeding value were observed in "Qara Shani" and "Bol Mazu" cultivars, respectively (Table 5).

Considering the breeding values of all studied traits, "Taifi", "Qzl Ouzum", "Rishbaba Qermez", "Garmian", "Agh Shani", "Lal Qermez", "Sahebi Qermez", "Saghal Solian" cultivars were top ranked and "Askari", "Hosseini", "Inah Amjai", "Goi Melki", "Mam Braima", "Sachakh" and "Agh Melhi" cultivars got the worst ranking (Table 5). Narrow sense heritability was calculated for all studied traits based on predicted breeding values. Narrow sense heritabilities were 46.21%, 3.83% and 1.97% for total soluble solids (TSS), pH and titrateable acidity (TA), respectively. For berry and flesh weights, it was estimated around 88.51% and 83.51%, respectively. For seed weight and seed number narrow sense heritabilities were 32.13% and 32.33%. For juice volume it was estimated 17.46%. Concerning to fruit set in open pollination and fruit set under controlled pollination narrow sense heritabilities were estimated 38.25% and 15.37%, respectively. It was 46.19% for pollen germination. For cluster length, cluster width and cluster weight narrow sense heritabilities were estimated 25.37%, 9.32% and 27.34%, respectively (Table 5). The highest heritability was observed in berry and flesh weights and the lowest one was seen in TA, pH and cluster width characters (Table 5).

Table 5
Breeding value of *V. vinifera* cultivars in studying traits

Number	Cultivar	TSS	Rank	pH	Rank	TA	Rank	BW	Rank	FW	Rank	SSW	Rank	SN
1	Rezghi	-0.6385	21	-0.0075	14	0.0168	39	-1.0902	12	-1.0133	12	-0.0041	25	-0.2511
2	Hosseini	-2.4015	5	-0.0085	13	-0.0373	15	0.2769	30	0.4187	31	-0.0116	10	-0.2902
3	Tabarze Sefid	1.5597	40	0.1891	44	-0.0549	5	-0.8497	15	-0.8001	15	-0.0095	16	-0.1594
4	Saghal Solian	1.0926	34	0.1799	42	-0.0463	8	0.0462	28	0.1245	28	-0.0031	26	0.0325
5	At Ouzum	-2.9760*	1	-0.0643	3	0.0023	33	0.6809	39	0.7630	38	-0.0098	15	0.4067
6	Lal Seyah	0.5610	32	-0.0219	10	-0.0113	28	-0.6362	19	-0.6202	18	0.0028	34	0.3193
7	Seyah Sardasht	1.2420	36	0.0183	19	-0.0045	30	-1.7427**	2	-1.6217**	2	-0.0110	13	0.4132
8	Garmian	-0.2857	23	-0.0773	1	0.0219	41	0.7280	40	0.7869	41	-0.0049	23	0.2872
9	Maiemo	-0.9128	16	-0.0143	11	0.0044	35	-1.1177	11	-0.9748	13	-0.0094	17	-0.1049
10	Rishbaba Qermez	0.2513	30	0.0415	26	-0.0293	19	-0.8159	16	-0.6876	17	-0.0075	21	0.3178
11	Taifi	-0.0364	25	-0.0122	12	-0.0402	12	0.6112	36	0.6477	36	0.0029	35	0.6065
12	Keshmeshi Qermez	1.3684	38	0.1285	39	-0.0159	26	-1.1752*	10	-1.1400*	10	-0.0224*	3	-0.1837
13	Fakhri	2.0662	44	0.0929	35	-0.0639	2	-0.5695	20	-0.4567	21	0.0014	32	-0.3179
14	Shahrودي	-2.4115	4	0.0781	32	-0.0308	18	-0.4257	24	-0.3069	25	-0.0101	14	-0.2643
15	Qara Shani	1.2783	37	-0.0431	5	0.0592	44	-0.3569	25	-0.3220	24	-0.0087	19	0.5828
16	Sahebi Qermez	-1.0808	13	0.0186	20	-0.0225	23	0.9778	42	1.0421	42	-0.0024	27	0.1786
17	Inah Amjai	-2.1367	8	0.0395	25	-0.0559	4	0.3703	33	0.4735	34	-0.0067	22	-0.2860
18	Tabarze Qermez	1.3766	39	0.2052	45	-0.0382	14	-0.5135	21	-0.5489	20	-0.0128	9	-0.2468
19	Dastarchin	1.0645	33	0.1732	41	-0.0209	24	-0.7601	17	-0.7148	16	-0.0116	10	-0.3113
20	Rishbaba Sefid	-1.5506	11	0.0068	17	0.0027	34	-1.7275**	4	-1.5865**	3	-0.0081	20	-0.2170
21	Agh Melhi	0.0413	28	-0.0352	6	-0.0003	32	-1.3364*	8	-1.1931	9	-0.0139	8	-0.1902
22	Goi Melki	-1.6139	10	0.0370	24	-0.0429	10	0.3337	32	0.4207	32	0.0091	41	-0.2937
23	Sayani	-0.7039	19	0.0208	21	-0.0466	7	-1.0822	13	-0.9724	14	0.0008	31	-0.1426
24	Kalati	-1.5012	12	0.0476	28	-0.0261	21	-0.1787	26	-0.0832	27	-0.0048	24	-0.0261
25	Mam Braima	-2.4737	3	0.0736	31	-0.0156	27	0.7358	41	0.7742	40	0.0279*	44	-0.5183
26	Bol Mazu	-0.4542	22	-0.0282	9	0.0113	37	0.6793	38	0.7728	39	0.0024	33	-0.2109
27	Lal Qermez	-1.0505	14	0.0512	30	-0.0489	6	0.1149	29	0.1475	29	-0.0015	28	0.5315
28	Sefid Shakh Shakh	1.6054	41	0.1485	40	-0.0240	22	-1.4358*	6	-1.2961*	6	-0.0222*	4	-0.1006
29	Alhaghi	-2.4973	2	0.1064	38	-0.0420	11	-0.7402	18	-0.6033	19	-0.0140	7	-0.4194
30	Askari	-2.1175	9	0.0436	27	0.0217	40	-1.7321**	3	-1.5386*	4	-0.0309*	1	-0.2336
31	Keshmeshi Sefid	1.8917	43	0.0995	36	-0.0181	25	-1.4123*	7	-1.2717*	7	-0.0229	2	-0.1229
32	Rejin	1.8828	42	0.1890	43	-0.0278	20	-2.9236**	1	-2.4007**	1	-0.0187	5	-0.0825
33	Sarghola	0.0062	27	-0.0310	7	-0.0600	3	1.2493*	43	1.3487*	44	-0.0008	30	0.4917
34	Chava Ga	0.3101	31	-0.0731	2	0.0082	36	0.5232	35	0.4403	33	0.0128	42	0.6241
35	Yaghoti	-0.1852	24	-0.0029	16	0.0499	43	-1.3076*	9	-1.2099*	8	0.0089	40	-0.3102

Number	Cultivar	TSS	Rank	pH	Rank	TA	Rank	BW	Rank	FW	Rank	SSW	Rank	SN
36	Qara Gandoma	-0.9326	15	0.0221	22	-0.0028	31	-0.4893	23	-0.4284	22	0.00881	39	-0.1675
37	Gazandaii	-2.2117	7	-0.0045	15	-0.0326	17	1.2770*	44	1.3305*	43	-0.0093	18	0.2204
38	Qzl Ouzum	0.1568	29	0.1033	37	-0.0456	9	1.4583*	45	1.4914*	45	0.0080	37	0.1409
39	Agh Shani	1.1959	35	0.0171	18	0.0148	38	0.3055	31	0.4025	30	-0.0112	12	0.0642
40	Jig Jiga	-0.7944	17	-0.0461	4	0.0308	42	-1.0584	14	-1.0549	11	0.0080	37	0.2285
41	Lal Sefid	-0.6458	20	0.0282	23	-0.0051	29	-0.5030	22	-0.3747	23	-0.0162	6	-0.0306
42	Klkarevi	2.4342	45	-0.0287	8	0.0942	45	-1.6757**	5	-1.5302*	5	-0.0012	29	-0.4108
43	Sachakh	-0.7598	18	0.0499	29	-0.0341	16	0.4987	34	0.5552	35	0.0139	43	-0.3665
44	Shirazi	-2.3412	6	0.0906	34	-0.0645	1	-0.0194	27	-0.0927	26	0.0521**	45	-0.2433
45	Angotka	-0.0311	26	0.0818	33	-0.0400	13	0.6746	37	0.7053	37	0.0071	36	0.2513
	%Narrow sense heritability	46.21		3.83		1.97		88.51		83.51		32.13		32.33

Table 5
(Continuation) Breeding value of *V. vinifera* cultivars in studying traits

Number	Cultivar	JV	Rank	FSOP	Rank	PG	Rank	CL	Rank	CWi	Rank	CW	Rank	FSCI
1	Rezghi	2.8965	42	-12.5984*	3	-8.1994	8	2.3681	41	0.0536	22	-31.423	16	-4.76
2	Hosseini	-0.8817	20	-9.5757	6	3.9173	22	-3.1628	3	-0.9271	3	-78.435	4	1.30
3	Tabarze Sefid	-3.8019*	3	-8.5253	9	12.5161	37	0.0463	15	-0.2859	13	-37.536	15	1.59
4	Saghal Solian	-0.6768	22	-6.4190	18	16.0167	40	0.8406	27	0.4573	35	23.029	34	2.32
5	At Ouzum	0.7094	36	-5.4238	22	-0.1167	17	0.9101	30	0.0831	26	16.473	32	-0.93
6	Lal Seyah	-2.0783	12	8.0225	43	-4.9109	14	-2.1832	6	-0.8408	5	4.068	28	7.82
7	Seyah Sardasht	-0.1224	29	-6.2370	19	8.1469	28	-1.6229	7	-0.4276	11	-65.036	6	1.30
8	Garmian	-1.2243	17	0.3276	34	8.8130	31	0.7535	25	0.6913	39	37.042	37	8.72
9	Maiemo	1.7818	38	-9.3284	7	2.4785	19	1.6377	35	0.7302	40	-31.235	17	-4.27
10	Rishbaba Qermez	0.3599	33	2.4234	37	22.5191*	43	2.2945	38	1.3263	43	35.210	36	3.24
11	Taifi	3.3511	44	-1.7388	30	4.9552	24	0.0794	16	0.7582	41	171.652*	42	3.47
12	Keshmeshi Qermez	-3.4054*	5	-8.2283	10	8.2316	30	0.3833	21	-0.0931	18	-40.898	14	1.51
13	Fakhri	-2.2115	11	2.8535	40	24.9031**	45	-2.3826	4	0.0556	23	-108.296	2	1.25
14	Shahrودي	-0.2711	25	1.7806	36	-8.0582	9	2.5863	43	0.0758	25	211.783*	45	-3.90
15	Qara Shani	-0.1970	28	17.8417**	45	-5.4311	12	-0.4402	10	0.1703	28	-47.266	12	9.17
16	Sahebi Qermez	4.6295*	45	-0.3362	32	-13.8949	4	0.8705	28	2.0291*	44	174.977*	43	-1.23
17	Inah Amjai	-1.0278	18	-8.1471	11	4.9079	23	-3.2069	2	-0.6583	8	-49.957	10	0.61
18	Tabarze Qermez	-3.3934	6	-6.0394	20	11.5504	33	1.1631	34	0.3336	32	-2.446	26	1.32
19	Dastarchin	-2.5875	9	-7.6361	14	10.1940	32	1.1442	31	0.3937	34	-9.197	24	0.50
20	Rishbaba Sefid	2.7125	40	-7.2191	16	8.2271	29	2.3439	40	0.6698	38	-26.010	20	-4.77
21	Agh Melhi	0.4595	34	-4.0021	27	16.0685	41	-1.5739	8	-0.5244	9	-85.569	3	-0.80
22	Goi Melki	-0.3369	24	-12.5808*	4	6.0848	26	-0.3074	11	-0.8063	6	-75.823	5	-5.55
23	Sayani	1.7232	37	-5.3235	23	-4.9499	13	1.1554	33	-0.2325	14	44.736	38	-3.93
24	Kalati	0.0249	30	2.4369	38	-9.3695	7	0.7634	26	0.6215	37	-29.348	19	2.11
25	Mam Braima	-0.3735	23	-19.7354**	1	-20.3949*	1	-0.1071	13	-1.3292	1	-57.176	9	-9.68
26	Bol Mazu	2.0746	39	-10.3485	5	-13.6343	5	0.3451	20	-1.0033	2	-25.115	21	-9.77
27	Lal Qermez	2.7605	41	-1.3236	31	11.7650	34	0.8706	29	2.2685*	45	101.809	41	-1.30
28	Sefid Shakh Shakh	-3.1198	7	-7.4481	15	15.2255*	39	0.5322	24	0.0574	24	-31.013	18	2.58
29	Alhaghi	-0.2127	27	2.6229	39	-12.5528	6	2.7001	44	0.2441	30	208.685*	44	-2.58
30	Askari	-3.9114	2	-9.1625	8	14.0619	38	0.0066	14	-0.3783	12	-59.188	8	-3.61
31	Keshmeshi Sefid	-1.5355	16	-7.6692	12	18.4415*	42	0.0946	17	-0.4602	10	-44.847	13	1.53
32	Rejin	-2.5737	10	-4.4204	26	23.9810**	44	0.4288	22	-0.0803	19	-23.599	22	3.27
33	Sarghola	-0.8607	21	-5.1890	24	3.5831	20	0.1044	18	0.3848	33	23.464	35	-0.34
34	Chava Ga	-1.7135	14	-5.6535	21	-18.3529*	2	0.4452	23	0.2779	31	13.879	31	-3.15

Number	Cultivar	JV	Rank	FSOP	Rank	PG	Rank	CL	Rank	CWi	Rank	CW	Rank	FSCP
35	Yaghoti	-2.9675	8	0.0457	33	7.2376	27	-0.2837	12	0.0392	21	-121.986	1	-4.83
36	Qara Gandoma	3.0149	43	-6.9581	17	-16.8250	3	1.7657	36	0.1327	27	-4.943	25	-0.37
37	Gazandaii	0.2445	32	-7.6455	13	-4.6550	15	2.3883	42	-0.7836	7	6.706	30	-0.78
38	Qzl Ouzum	0.1929	31	-5.0105	25	-6.9770	10	2.8615	45	0.1785	29	82.887	40	0.46
39	Agh Shani	-4.5384*	1	3.1126	41	5.0923	25	2.2979	39	1.0296	42	68.073	39	5.60
40	Jig Jiga	-1.8475	13	-2.5927	29	1.3384	18	1.1496	32	0.4889	36	-47.595	11	0.28
41	Lal Sefid	-3.4780	4	5.7738	42	11.8121	35	-3.3038	1	0.0114	20	4.649	29	1.45
42	Klkarevi	-0.2225	26	10.4248	44	-5.5205	11	2.0237	37	-0.2150	15	0.944	27	-4.69
43	Sachakh	-1.6872	15	-14.0367*	2	-4.2272	16	0.1425	19	-0.9138	4	-64.211	7	-5.48
44	Shirazi	0.4941	35	1.2941	35	12.2134	36	-2.2940	5	-0.1390	17	-20.502	23	5.78
45	Angotka	-0.9521	19	-2.9218	28	3.7512	21	-0.6779	9	-0.2081	16	16.540	33	-3.57
	%Narrow sense heritability	17.46		38.25		46.19		25.37		9.32		27.34		15.3

TSS: Total soluble solids, TA: Titratable acidity, BW: Berry weight, FW: Flesh weight, SSW: Single seed weight, SN: Seed number, JV: Juice volume, FSOP: Fruit set in open pollination, PG: Pollen germination, CL: Cluster length, CWi: Cluster width, CW: Cluster weight, FSCP: Fruit set under controlled pollination.

Synten analysis of distribution of genotypes across dendrograms. Comparing the phenotypic clustering with that produced by estimated breeding values revealed that 26.67% of cultivars were grouped into the same groups across the two hierarchical clusters (Fig. 1). Cluster analysis by breeding values as well as that based on SSR genotypic data revealed the presence of four genetic groups in the studied germplasm (Fig. 1). It was observed that the genotypes distribution pattern in clustering with breeding values is different with that clustered by SSR genotypic data (Fig. 1). By comparing the two dendrograms, it was found that all of cultivars except "Rishbaba Qermez" in group 1 of molecular cluster were grouped into similar position across hierarchical clustering with breeding values (Fig. 1).

Discriminant analysis of principal components (DAPC). To further clarify the genetic relationship between groups resulting from each one of molecular, morphological and breeding value cluster analyses; discriminant analysis of principal components (DAPC) was performed. DAPC results classified obviously the collections into four groups (Fig. 3). Results of molecular DAPC showed high dissimilarity between groups. However, some similarity was observed between groups 1 and 2 and also between groups 1 and 3. Density plot of individuals along the first discriminant function also indicated the presence of four groups based on Bayesian information criterion (BIC). High genetic diversity was observed in group 3. Red and orange colored peaks corresponding to groups 1 and 3 are largely overlap. Blue colored peak corresponding to group 4 is completely distinct. DAPC analysis of morphological and breeding values showed high similarity between groups. Groups 1, 2, 3 and 4 are largely overlap (Fig. 3).

Discussion

Two subpopulations (K=2) were identified in the studied grape germplasm. Based on membership probability of greater than or equal to 70 percent, 75.56% of cultivars were belonged to population 1 and 17.78% of cultivars were belonged to population 2. When the membership probability of a genotype to a cluster is greater than or equal to 0.70, the genotype is assigned to one of subpopulations but if the membership probability is less than that, it is defined as admixed genotype⁵³. The presence of structure in the studied population is a deterrent factor to achieving reliable results. As a result, when population structure and kinship relations exist in association panel and not to be considered in the association analysis, they produce false positive results⁸. Based on r^2 statistics, some SSR locus pairs showed significant level of linkage disequilibrium. The extend of LD depended on different factors, for example some factors such as small population size, inbreeding, population admixture, genetic draft, autogamy and epistasis increase LD level. On the other hand, allogamy, high recombination rate and high mutation rate decrease LD level¹. The description of LD was reported in French wild grapes for first time². It was reported that LD level is twelve times higher in native grapes than in wild grapes².

In this study seven SSR loci were found to be significantly associated with genomic regions controlling studied traits. Several QTLs were reported for berry weight, seed number and seed fresh weight in grape and among of these, the one reported for seedlessness was a major effect QTL and closely linked to VMC7F2 locus⁹. In other study close association of two SSR markers (VVIB23 and VVMD34) with flower sex was reported^{3,39,47}. Several QTLs for sugar content on LGs 1, 2, 3, 4, 7, 9, 11 and some other for total acid on LGs 06, 13, and 18 were reported¹⁰. Sugar and total acid are important factors in the taste of grapes. Hexose (Fructose and glucose) are predominant sugars in grape at mature stage^{52,38}. Some QTLs were common among different traits. Identification of common markers for the studied traits may be due to linkage or pleiotropic effects³⁶. Identification of common markers is important in plant breeding program because it enables simultaneous selection for several traits^{32,59}.

Breeding values were estimated for 14 pomological traits in grape cultivars using the best linear unbiased prediction (BLUP). Developing and introducing new cultivars requires parental selection within large germplasm populations, so estimating the value of genotype is an important step in breeding programs.

Breeding value is an important notion in plant and animal breeding¹⁷. He defined breeding value as the mean value of its offspring. Application of BLUP for estimating individual breeding value has been reported in forest tree^{65,63}. It was reported using of BLUP for estimating breeding values in peach genotypes¹³. BLUP were applied to predict the general combining ability (GCA) and specific combining ability (SCA) for date of ripening in apples⁵⁴.

The organoleptic quality of table grapes depends on the sugar content, organic acid content and the balance between them. Organic acids are present in a small amount compared to sugars, but it generally plays an important role in the taste⁴². A few cultivars with high-sucrose concentration were reported in *Vitis rotundifolia* and hybrids between *V. labrusca* and *V. vinifera*³⁸. In this study "Klkarevi" cultivar has the highest breeding value for TSS and TA. Seedlessness is another important and desirable factor for consumers. Development of seeds in grapes are controlled by a1, a2, a3 and i genes. The i gene is a regulator gene. Seedless expression occurs when a1, a2, and a3 are homozygous as recessive, and the regulator gene is homozygous as 'li' or heterozygous 'li'⁶. In stenospermocarp grape, fertilization occurs but embryo is aborted in earlier stages and seed trace remains⁶⁶. Among seedless studied cultivars, "Rejin" and "Askari" cultivars had the highest and lowest breeding value in terms of single seed weight and seed number. Among seeded cultivars, "Shirazi" and "Lal Sefid" cultivars had the highest and lowest breeding value for single seed weight, respectively. For seed number, "Chava Ga" and "Mam Braima" cultivars had the highest and lowest breeding values, respectively.

Pollination is an important factor that affects the percentage of fruit set. It was reported wide percentage of pollen germination from 11.4% in 'Thompson Seedless' to 39.1% in 'King's Ruby' grapevine cultivars⁵⁵. Among the cultivars investigated in the present study, "Rishbaba Qermez", "Fakhri", "Sefid Shakh Shakh", "Keshmeshi Sefid" and "Rejin" cultivars have a high and positive breeding value for pollen germination. Researchers investigated pollen germination in 14 grape cultivars and reported that Touriga Nacional, Cabernet Franc and Cabernet Sauvignon cultivars had low pollen germination while Castelao, Loureiro, Malbec and Petit Verdot cultivars showed high percent of pollen germination⁴⁴.

The end purpose of most breeders is to combine the appropriate situation of more than one trait in common background. From view of estimated breeding values, "Saghal Solian", "At Ouzum", Garmian", "Rishbaba Qermez", "Taifi", "Shahrudi", "Sahebi Qermez", "Lal Qermez", "Alhaghi", "Sarghola", "Chava Ga", "Qzl Ouzum" and "Agh Shani" cultivars are suitable parents for cluster length, cluster width and cluster weight improvement. They can be used in hybridization programs because they can better transfer their characteristics to the progeny. The best parent for high berry weight, flesh weight, cluster length, cluster width and cluster weight would be "Sarghola" and "Qzl Ouzum" cultivars. "Garmian", "Rishbaba Qermez", "Fakhri", "Agh Shani", "Lal Sefid" and "Shirazi" cultivars are best parent for breeding pollen germination, fruit set in open pollination and fruit set under controlled pollination characters. "Yaghoti", "Fakhri", "Shirazi", "Rishbaba Sefid", "Rezghi" and "Maiemo" cultivars clustered into the same group in both breeding value and molecular clustering dendrograms. These cultivars have negative breeding values for berry weight, flesh weight and cluster weight characters. The wide range of breeding value for most of evaluated traits and the possibility to choose a genotype in term of good situation of more traits propose that breeders should evaluate the germplasm according to their purpose before selecting best individuals. BLUP is an effective option for achieving to this goal¹⁸. There is not any cultivar that high-grade breeding value for all traits. The solution is to intercross individuals with positive traits and then select progenies during several generations.

In the selection process based on phenotypic value, success in changing population characteristics is predictable if the degree of conformity between phenotypic and genotypic values to be high. Measuring the degree of conformity is made by calculating heritability¹⁸. Heritability is influenced by type of trait, studied population, environmental conditions and the method of phenotype measurement²⁰. Berry weight and flesh weight showed high heritability and compared to other studied traits are more under control of the additive effects of genes. Researches has shown that the accuracy of estimating breeding values for traits with high heritability is higher than that with low heritability⁶². Because in traits with high heritability, phenotype of individual is closer to the genetic value and therefore the breeding value of individuals is more accurately estimated⁴⁵. Heritability for berry weight has been seen in the range of 0.49 to 0.92^{15,22,51}. In one study narrow-sense heritability was estimated 0.63 for berry weight, 0.69 for berry width, 0.68 for berry length, 0.58 for seediness, 0.48 for Brix index and 0.36 for acidity⁶⁷.

Conclusions

Results of present study revealed the importance of considering population structure and relatedness factors in table grape association analysis. Seven DNA markers were found to be significantly associated with regions controlling studied pomological traits that can be useful in marker-assisted breeding programs. In addition, breeding values were estimated for Iranian table grapevine germplasm by integrating DNA marker data and pomological traits. Considering the sum of the breeding values of all the studied traits, "Taifi", "Qzl Ouzum", "Rishbaba Qermez", "Garmian", "Agh Shani", "Lal Qermez", "Sahebi Qermez", "Saghal Solian" cultivars had the highest rank. The cultivar with high and positive breeding value can be used as a good parent in hybridization programs because they can better transfer their suitable characteristics to the progeny.

Methods

Germplasm. Forty-five Iranian table grape (*V. vinifera* subspecies *vinifera*) cultivars were kindly provided from the germplasm bank of Kahriz Horticultural Research Station (Urmia, West Azarbaijan) (Table 6).

Table 6
Characteristic of grape genotypes utilized in investigating genetic diversity using SSR markers

Genotype	Use	Seed	Genotype	Use	Seed
Rezghi	Table	Yes	Kalati	Table	Yes
Hosseini	Table	Yes	Mam Braima	Table, Raisin	Yes
Tabarze Sefid	Table	Yes	Bol Mazu	Juice	Yes
Saghal Solian	Table	Yes	Lal Qermez	Table	Yes
At Ouzum	Juice	Yes	Sefid Shakh Shakh	Juice	No
Lal Seyah	Table	Yes	Alhaghi	Table	Yes
Seyah Sardasht	Table, Raisin	Yes	Askari	Table	No
Garmian	Table	Yes	Bidane Sefid	Table, Raisin	No
Maimeo	Juice	Yes	Rejin	Juice	No
Rishbaba Qermez	Table	Yes	Sarghola	Table	Yes
Taifi	Table	Yes	Chava Ga	Juice	Yes
Bidane Qermez	Table	No	Yaghoti	Table	Yes
Fakhri	Table, Raisin	Yes	Qara Gandoma	Juice	Yes
Shahroudi	Table	Yes	Gazandaii	Table	Yes
Qara Shani	Juice	Yes	Qzl Ouzum	Table	Yes
Sahebi Qermez	Table	Yes	Agh Shani	Table	Yes
Inah Amjai	Table	Yes	Jig Jiga	Table	Yes
Tabarze Qermez	Table	Yes	Lal Sefid	Table	Yes
Dastarchin	Juice	Yes	Klkarevi	Juice	Yes
Rishbaba Sefid	Table	Yes	Sachakh	Juice	Yes
Agh Melhi	Juice	Yes	Shirazi	Table	Yes
Goi Melki	Table	Yes	Angotka	Juice	Yes
Sayani	Table	Yes			

Phenotyping protocols. Fourteen pomological traits including total soluble solids (Brix), fruit pH, fruit titratable acidity (TA), berry weight (g), flesh weight (g), juice volume (ml), seed weight (g), seed number per berry, cluster width (cm), cluster length (cm), cluster weight (g), fruit set in open pollination (%), fruit set in controlled pollination (%) and pollen germination rate (%) were measured onto 12 plants per cultivar during three successive years. TSS was determined by refractometer in Brix degrees. TA was measured by the amount of 0.10 N NaOH used to adjust the fruit juice pH to 8.1. Fruit pH values were measured by pH meter. Berry and flesh weight, cluster weight and also single seed weight were determined by digital scale. Fruit juice of 100 g of each cultivar was evaluated with graduated cylinder. To measure the length and width of the cluster, three clusters from each vine were selected from the same positions and the traits were measured. To determine pollen germination, clusters were harvested in 50–70% flowering stage and cultured on medium with 1% agar and 5% sucrose 24 h at 26°C. The number of germinated pollen was counted in seven microscopic areas. Percent of fruit set was calculated with the following formula: Fruit set = Number of berry/number of flower ×100.

Marker identification and analysis. Genomic DNA was extracted by Doyle & Doyle (1990) method¹⁴. Thirty-nine SSR primer pairs were used for preparing the molecular profile of studied cultivars (Table 3). PCR was carried out in a final volume of 20 µl including 20 ng of genomic DNA, 1.75 mM magnesium chloride, 2 µl 10× polymerase chain reaction buffer (500 mM KCl, 500 mM Tris–Hydrogen chloride; pH 8.4), 0.25 mM of each dNTP (Cinagene Co., Tehran, Iran), 10 µM of each primer and 1.1 Unit of Taq DNA polymerase (Cinagene Co., Tehran, Iran), and distilled water in a 96-well Mastercycler Gradient thermal cycler (Type 5331; Eppendorf AG, Hamburg, Germany). The PCR reaction program consisted of an initial denaturation at 94°C for 4 minute, followed by 35 cycles of 94°C for 1 minute, annealing temperature (50 to 59°C) for 1 minute, and 72°C for 2 minute, with a final extension of 7 minute at 72°C. Amplification was checked with 3% (w/v) ultra-pure agarose gel. All PCR amplifications were scored as codominant manners.

Descriptive statistics and correlation coefficients among pomological characters were calculated in SAS 9.4 software. Genetic parameters including number of allele per SSR loci, gene diversity, heterozygosity and PIC were calculated by GenAlEx 6 software⁴³. Cluster and discriminant analysis of principal components (DAPC) analyses for testing genetic relationships between groups were performed by using adegenet package in the R environment³⁵. The find clusters function was carried out to identify the number of subpopulations. It uses K-means clustering algorithm which decomposes the total variance of a variable into within- and between-group components. The best number of subpopulations shows the lowest Bayesian Information Criterion (BIC) value. Accurate

classification of table grape cultivars into appropriate sub-populations and detection of cultivars with mixed structure was performed using Bayesian method in Structure 2.3.4 software⁴⁶. The initial value of K was considered in the range of 1 to 10. For each subpopulation 10 replicates were assigned. Burn-in period length was adjusted to 100,000, followed by 100,000 Markov Chain Monte Carlo (MCMC) replications. Marker-trait association was tested by Q + K mixed model in TASSEL 2.1 software⁶⁹. The linkage disequilibrium (LD) was calculated with TASSEL⁷.

Predicting breeding values were carried out using BLUP in SAS software⁵. The structure of the mixed model for estimating breeding values is as follows:

$$Y = Xb + Zu + e$$

Where, Y is the vector of phenotypic values, b is vector of fixed effects, u is vector of random effects, X and Z are the design matrices, and e is the vector of random residual⁴⁵. The breeding values estimates can be realized by solving the mixed model equations (MME)³¹.

$$\begin{bmatrix} x'R^{-1}x & x'R^{-1}z \\ z'R^{-1}x & z'R^{-1}z + G^{-1} \end{bmatrix} \begin{bmatrix} \hat{\beta} \\ \hat{u} \end{bmatrix} = \begin{bmatrix} x'R^{-1}y \\ z'R^{-1}y \end{bmatrix}$$

$$BLUP(U) = \begin{bmatrix} z'x & z'z + Ia \end{bmatrix}^{-1} \begin{bmatrix} x'y \\ z'y \end{bmatrix} = \begin{bmatrix} u_1 \\ u_2 \\ \vdots \\ u_n \end{bmatrix}$$

Declarations

Author contribution statement

M.R. contributed to the experimental activities, data collection, results explanations and writing-original draft. R.D. designed and supervised the work. H.D.B. involved in preparing the plant materials. R.D. and H.A. contributed to analyse of the data. R.D. contributed to review and edit of manuscript. All authors contributed to the discussion. The authors accept full responsibility for the context of the manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Permission for land study

The authors declare that all land experiments and studies were carried out according to authorized rules.

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Figures

Figure 1

Synteny analysis of distribution of genotypes across dendrograms including comparison of morphological and molecular data hierarchical cluster dendrograms, comparison of morphological and breeding value hierarchical cluster dendrograms and comparison of molecular data and breeding value hierarchical cluster dendrograms

Figure 2

(a) Bilateral charts to determine the optimal number of K identified by Structure program. (b) Population structure indicating the two groups identified with different color bars. (c) LD plot generated by retrotransposon marker pairs in 45 Iranian grape (*V. vinifera* subspecies *vinifera*) cultivars. The upper diagonal shows r^2 among each pair of markers. The lower diagonal shows the levels of significance between each pair of markers

Figure 3

Discriminant analysis of principal components (DAPC) between groups resulting from molecular, morphological and breeding value cluster analysis. (a) Dots represent individuals with colors denoting cluster allocation. Percentages of cumulated variance explained by principal component 1 (PC1) to PC10 are shown in the top right corner. (b) Density plot of individuals along the first discriminant function from the discriminant analysis of principal components (DAPC) for groups 1, 2, 3 and 4