Research Note

Evaluation of genetic diversity of Iranian grapevine accessions using microsatellite markers

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Introduction: Grapevine (*Vitis vinifera* L.) is economically the most important fruit crop in the world. There are 8,000 to 10,000 grape cultivars existing worldwide today. Characterization and determination of grapevine cultivars are sometimes difficult using conventional methods. Molecular approaches might be implemented in cultivar identification and breeding programs, since morphological markers are time-consuming and prone to equivocal interpretations (MARGIT *et al.* 2005). The high polymorphism of microsatellites (or SSR - simple sequence repeats), a special class of tandem repeat loci involving a base motif of 1-6 bp of DNA repeated up to 100 times, allows cultivar identification, and their co-dominant Mendelian inheritance allows the reconstruction of crosses (VOUILLAMOZ *et al.* 2003).

Material and Methods: A total of 44 grapevine accessions were used in this study as follows: 34 accessions from Iran, six accessions from Russia, three accessions from USA and one accession from France. Total DNA was extracted from young, fully expanded leaves, using modified CTAB method. The purified total DNA was quantified by gel electrophoresis and its quality was verified by spectrophotometry.

Nine SSR primer pairs including VVS2, VVMD14, VVMD27, VVMD28, VVMD32, VVMD36, VrZAG21, VrZAG47 and VrZAG79 were selected for genotyping assays through preliminary screening of 23 primer pairs, which were generated consistently reproducible clear fragments with high polymorphism. Touch-down PCR was performed on a 20 µl volume, with 30 ng of genomic DNA. All amplification reactions were repeated two times under identical conditions accompanied by a negative control. Amplification reaction products were separated on 6 % denaturing polyacrylamide gels and were detected using the silver staining method.

Polymorphic fragments were coded using one and zero to mean presence or absence, respectively. The number of alleles (NA), probability of identity (PI), polymorphism information content (PIC), expected heterozygosity (He), Shannon's information index (I), G_{ST} and Nm were calculated. The relationship between accessions was inferred using the UPGMA clustering method on the basis of Jaccard's coefficient of genetic distance (MOHAMMADI and PRASANNA 2003).

Results and Discussion: All of the nine loci displayed polymorphism among 44 grapevine accessions with a total of 75 alleles identified. The number of alleles per locus ranged from 6 to 11, with an average of 8.3. Polymorphism information content (PIC) ranged from 0.65 to 0.88, indicating that these loci were highly informative (Table). The expected heterozygosity over all loci was high and ranged from 0.701 (VrZAG47 and VVMD27) to 0.884 (VrZAG21). Locus VrZAG21, with the highest number of alleles, exhibited the highest level of polymorphism (He = 0.884, I = 0.493 and PIC = 0.876), while loci VrZAG47 and VVMD27 with only six alleles in 44 accessions, ex-

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Characterizations of markers

Locus	NA total	MNA(Std)	He(1-∑Pi²)	Ho	I	PIC	PI	G_{ST}	NM
VrZAG21	11	9 (1.73)	0.884	0.953	0.493	0.876	0.025	0.133	3.259
VrZAG47	6	4 (1.73)	0.701	0.721	0.366	0.658	0.136	0.135	3.204
VrZAG79	10	7 (2.64)	0.826	0.953	0.401	0.804	0.053	0.273	1.332
VVMD14	8	6 (2.64)	0.844	0.884	0.398	0.825	0.43	0.185	2.203
VVMD27	6	4 (1.73)	0.701	0.721	0.366	0.658	0.136	0.135	3.204
VVMD28	8	6.7 (1.53)	0.794	0.767	0.359	0.778	0.070	0.056	8.429
VVMD32	8	5.3 (2.31)	0.791	0.86	0.377	0.768	0.071	0.107	4.173
VVMD36	10	6.3 (2.51)	0.807	0.977	0.352	0.783	0.61	0.174	2.374
VVS2	8	5.7 (2.51)	0.807	0.744	0.334	0.783	0.063	0.137	3.150
Mean	8.3	6	0.795	0.842	0.383	0.770	0.177	0.217	1.808

Number of alleles (NA), mean number of alleles (MNA), expected heterozygosity (He), observed heterozygosity (Ho), Shannons information index (I), polymorphism information content (PIC), probability of identity (PI), gene flow (NM) and coefficient of gene differentiation (G_{ST}). Values in parenthesis are standard deviations.

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hibited the lowest level of polymorphism (He = 0.701, I = 0.366 and PIC = 0.658). In fact, a positive correlation was observed between the number of alleles and the level of polymorphism (r = 0.870 between NA and He, r = 0.867 between NA and PIC and r = 0.579 between NA and I, Table is not shown). When calculated across all accessions, the PI was low for all loci (PI values from 0.025 to 0.136) except for loci VVMD36 (PI = 0.61) and VVMD14 (PI = 0.43).

Using molecular data, clustering analysis assigned the 44 accessions into five groups (Figure). Group one included 20 accessions from Zarghan and Khorasan (two important areas of grapevine planting in Iran). Two accessions, Askari Nishabor and Askari Mashhad, showed identical profile and appear to be synonymous in this group. In a similar assessment of genetic diversity for some of the Iranian and European grapes, NAJAFI *et al.* (2006) had also studied some accessions from the afore-mentioned regions and clustered the accessions in one group. Group two included eight accessions from Shiraz (the most important area for grapevine planting in Iran) and Oroomieh. All of the accessions in this group are seeded with oval berry. In group three, there were four seeded accessions from Ghoochan and three accessions from USA ('Flame Seedless', 'Perlet' and 'Beauty Seedless'). All of the accessions in this group have round berry, except 'Beauty Seedless'. These US seedless accessions are the results of crosses between 'Sultanina' (a cultivar of oriental origin) and one or two other cultivars; 'Muscat' and 'Cardinal' for 'Flame Seedless', 'Reine des Vignes' for 'Perlet' and 'Reine des Vignes' x 'Black Kishmish' for 'Beauty Seedless' (GALET 2000). FATAHI *et al.* (2003) had also used these US accessions in a similar analysis and reported the same results. Unfortunately, the pedigrees of the four Ghoochan accessions are ambiguous, and the appearance of 'Sultanina' is unclear in their pedigree. Group four included three seeded round berry accessions from Abade region, Fars. Group five included five Russian accessions. The 'Mukhchaloni' accession from Russia belonged in group one together with 19 Iranian accessions. Considering the geographical proximity of Iran to Russia, it can be concluded that this accession has been transferred from Iran to Russia. DONGLE *et al.* (2001) also clustered Russian and Middle Eastern genotypes into two different groups. 'Pinot Noir' genotype, which we had used as a reference genotype (for the production of transferable information among labs), formed an independent cluster. In fact, it was the only wine grape genotype that was used in this research project. All of the other genotypes were table grapes. Principle Coordinate Analysis also confirmed the observed pattern of genetic diversity (data not shown).
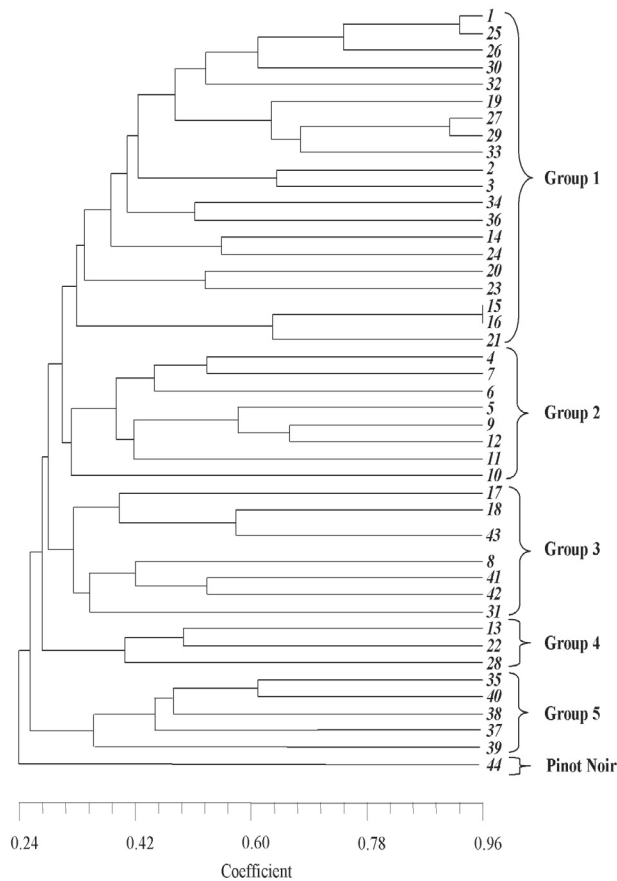

Figure: A UPGMA dendrogram based on Jaccard's distance showing genetic relationships among the 44 accessions of grapevine. 1 = 'Rajabi Aghighi Zarghan', 2 = 'Kaleii Zarghan', 3 = 'Yaghoti Zarghan', 4 = 'Rishbaba Siah Bavanat', 5 = 'Rishbaba Oroomie', 6 = 'Monagha Shiraz', 7 = 'Rishbaba Arsenjan', 8 = 'Shahpasand Ghoochan', 9 = 'Rishbaba Siah Shiraz', 10 = 'Gieii Ghalat', 11 = 'Rishbaba Siah Doodaj', 12 = 'Sahebi Oroomie', 13 = 'Askari Abade', 14 = 'Sahebi Nishabor', 15 = 'Askari Mashhad', 16 = 'Askari Nishabor', 17 = 'Dooshabe Ghoochan', 18 = 'Laele Ghoochan', 19 = 'Ite Riz Zarghan', 20 = 'Divane Kashmar', 21 = 'Askari Lirak Zarghan', 22 = 'Siah Ghoochan', 23 = 'Askari Kashmar', 24 = 'Sorkhak Nishabor', 25 = 'Rajabi Siah Zarghan', 26 = 'Rotabi Zarghan', 27 = 'Rishbaba Sefid Zarghan', 28 = 'Sefide Abade', 29 = 'Rishbaba Siah Zarghan', 30 = 'Rotabi Sefid Zarghan', 31 = 'Alhaghi Siah Abade', 32 = 'Domrobahi Zarghan', 33 = 'Ite Siah Zarghan', 34 = 'Keshmeshi Ghoochan', 35 = 'Goudovng Pendji kentsky', 36 = 'Moukhtchaloni', 37 = 'Druzhba', 38 = 'Noulizok', 39 = 'Volgo Don', 40 = 'Tuy Tish Gngchinskii', 41 = 'Flame seedless', 42 = 'Perlet', 43 = 'Beauty seedless', 44 = 'Pinot Noir'.

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