GMR

Genotyping and morphometric analysis of Kazakhstani grapevine cultivars versus Asian and European cultivars

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ABSTRACT. Grapes are an important fruit crop in Kazakhstan. Modern viticulture requires inclusion of traditional morphological and agronomical methods in the development and sustaining of grapevine culture as well as molecular approaches. We used molecular markers and leaf shape analysis to characterize grapevine cultivars developed in Kazakhstan compared with European and Asian varieties. A set of 17 landmarks describing grapevine leaf shape were analyzed using generalized procruste and principal component analyses in order to evaluate shape variation among cultivars. Mean leaf shapes were obtained for 94 cultivars. Outer tip pattern had the most impact on leaf shape variation. Principal component analysis revealed the complex nature of grapevine leaf shape variation and demonstrated the differential impact of leaf landmarks on shape variation. The standard set of six simple sequence repeat markers used for molecular genotyping was sufficient to differentiate each of the cultivars by a distinct allelic profile. Kazakh grapevine cultivars were found to be an admixture of European and Asian varieties. However, for some cultivars, there was no confirmation of identity with respect to pedigree either by bibliography or comparison with the Vitis International Variety Catalogue. Also, 11 markers for resistance-associated alleles in corresponding loci (*RUN1, REN1, REN3, Rpv3, Rpv10,* and *Rpv12*) were applied to characterize Kazakhstan and some parent cultivars with different levels of resistance to mildew pathogens.

Key words: Grapevines; Kazakhstan; Simple sequence repeat (SSR); Mildew resistance; Oidium; Leaf shape

INTRODUCTION

Grapevines are one of the most important fruit plants in the world, especially in Kazakhstan. The fall of the USSR caused a period of economic instability in Kazakhstan, significantly damaging its grapevine culture. Government programs and regulations aimed at restoring viticulture in Kazakhstan include addition of new and replacement of old vineyards. Viticulture recovery depends on evaluation of available cultivated varieties as well as standardization and certification of Kazakhstan selected cultivars, which includes assessment of genetic diversity and common pathogen resistance. A lack of correct and precise cultivar information can be a significant problem for long-term viticulture development.

The morphological characterization of *Vitis* taxa (ampelography) that has been used for grapevine characterization for centuries is still a useful approach (Chitwood et al., 2014). The International Organization of Vine and Wine (OIV) descriptor uses a series of morphological characteristics of grapevine plants, including leaf parameters, as diagnostic criteria for cultivar description and identification. Despite the influence of environmental conditions on leaves (Bodor et al., 2013; Sack and Scoffoni, 2013) leaf shape is mostly dictated by genetics, whereas parameters related to size are influenced by both internal and external factors (Welter et al., 2007; Alba et al., 2014; Chitwood et al., 2014). Thus, a promising approach to grapevine characterization is based on methods that allow separation of size and shape components (Viscosi and Cardini, 2011). Along with ampelography description of grapevine varieties, data based on molecular markers have increasing use in grapevine cultivar certification (Aradhya et al., 2003; Welter et al., 2007; Hvarleva et al., 2009; Emanuelli et al., 2013; Riaz et al., 2013; Marrano et al., 2015). Molecular characterization of grape germplasms has shown that their genetic diversity is strongly influenced by cultivar putative origin and distribution of diverse underlying genetic resources (Bacilieri et al., 2013, Emanuelli et al., 2013). Among all molecular markers, simple sequence repeats (SSR) are a reliable, highly reproducible, and relatively cheap way to classify grapevine varieties and resolution of ambiguous cultivar assignment (Sefc et al., 2009; Pokhriyal et al., 2012). A set of six SSR markers was proposed as a standard by the OIV and recognized by the international community (This et al., 2004). These markers were included in the second edition of the OIV descriptor list for grape varieties and *Vitis* species as OIV 801–806. Previous studies have also demonstrated that some morphological parameters can be sufficient for correct cultivar discrimination in combination with molecular methods in ambiguous cases (Alba et al., 2014; Alba et al., 2015). An important problem in worldwide viticulture is the susceptibility of grapevines to pathogens, leading to severe economic damage, including yield loss and indirect negative effects of pesticides. Among the most destructive diseases of grapevines are powdery mildew (PM) caused by the ascomycete *Erysiphe necator* [syn. *Uncinula necator*] and downy mildew (DM) caused by the oomycete *Plasmopara viticola* (Gessler et al., 2011; Armijo et al., 2016). International efforts are being made to identify genetic sources of resistance to these diseases as well as heritability and stability of this resistance in the field in order to introduce these sources into cultivars by crossbreeding. For instance, the PM resistance locus *RUN1* has been introduced by pseudo-backcross into the *Vitis vinifera* (2n = 38) genome from the wild North American species *Muscadinia rotundifolia* [syn. *V. rotundifolia*; 2n = 40] (Merdinoglu et al., 2003; Barker et al., 2005). In certain accessions of *V. vinifera* from Central Asia, including Uzbekistan cultivars Kishmish vatkana and Dzhandzhal kara, a major R-gene in the *REN1* locus was discovered. Though less effective than *RUN1*, it can still significantly restrict PM growth and sporulation relative to susceptible *V. vinifera* cultivars (Hoffmann et al., 2008; Coleman et al., 2009; Agurto et al., 2017). SSR markers linked with the resistant *REN1* allele (143 bp) allowed identification of 10 new mildew resistant genotypes,

eight of which are *V. vinifera* cultivars (three related to Kishmish vatkana and Karadzhandal) and two are *V. vinifera* subsp. *sylvestris* (Riaz et al., 2013). The *REN3* locus was discovered in the cultivar Regent, a full sibling of Villard blanc, the resistant ancestors of which were *V. aestivalis, V. berlandieri, V. cinerea, V. lincecumii,* and *V. rupestris* (Eibach and Töpfer, 2003; Welter et al., 2007). In cultivar Bianca, which has common wild ancestors with Regent through Villard blanc, the *Rpv3* locus has been detected as a major quantitative trait locus responsible for DM resistance (Bellin et al., 2009). Wild *V. amurensis* is the source of DM resistance locus *Rpv10* (LG 09), which was introgressed into grapevine varieties of Western Europe (Schwander et al., 2011). A quantitative trait locus analysis revealed the dominant gene *Rpv12* for DM resistance was inherited from *V. amurensis* independent of other resistance genes (Venuti et al., 2013). Researchers have also demonstrated the additive effect of *Rpv12* and *Rpv3* in conferring resistance to strains that were virulent to *Rpv3* plants.

Kazakhstan is not a native region for grapes, and only the Kuldzhinskyi cultivar is indigenous. Most common cultivars in vineyards of Kazakhstan are European or Caucasian (Rkatsiteli, Saperavi, Cabernet franc, Cabernet sauvignon, etc.) and Central Asian (Taifi rozovyi, Kuldzhinskyi, Akhalili, Khusaine belyi). Local grapevine cultivars were bred from these Eurasian cultivars (Nurmuratuly et al., 2012).

We examined phylometric and molecular markers of grapevine characteristics from cultivars developed in Kazakhstan and compared them with a number of European and Central Asian cultivars. Generalized procrustes analysis (GPA) with principal component analysis (PCA) were performed to evaluate shape variation, and the standard set of six SSR markers (OIV 801–806) was used for molecular genotyping. Furthermore, 11 markers for loci *RUN1, REN1, REN3, Rpv3, Rpv10*, and *Rpv12* were applied to characterize Kazakhstan cultivars as a prospective source of genotypes resistant to mildew pathogens.

MATERIAL AND METHODS

Plant material collection

All investigated cultivars were developed by the Institute of Horticulture and Viticulture (Almaty region, Kazakhstan) and collected in the Pomological Garden of the Institute. Information about local cultivars provided by the Institute of Horticulture and Viticulture is shown in Table Supplementary 1. Two sets of material were collected depending on their availability. For morphometric analysis, mature leaves were collected from the middle third of several shoots and preserved in an herbarium. For molecular analysis, leaves up to 25 mm in width were stored at -80^0C.

Morphometric analysis

Mature leaves were digitized by scanning at 150 dpi and saved as bitmap files. Coordinates of 17 key landmarks of leaves (Figure 1) were obtained using the ImageJ software. GPA was performed using the R package Geomorph (Adams et al., 2017). For visualization of results, the ggplot2 package was used (Wickham, 2009).

Microsatellite analysis

Analysis was conducted in laboratory facilities of the Institute of Plant Biology and Biotechnology (Almaty, Kazakhstan). Genomic DNA was extracted according to a previous

protocol (Doyle and Doyle, 1990), with minor modifications. Loci were amplified via PCR using primer sets for six standard loci markers (VVS2, VVMD5, VVMD7, VVMD27, VrZAG62, and VrZAG79) combined with three universal unspecific oligonucleotides (D8S1132, D12S1090, and DYS437) that were labeled with fluorescent dyes (VIC, NED, and FAM, respectively) used as "tails" (Table 1). PCR was conducted in a 20 μL total volume, containing 20 ng of genomic DNA, 2.5 mM MgCl2, 0.2 mM of each dNTP, 0.2 mM forward primer, 0.2 mM labeled primer, 0.1 mM primer with a "tail," and 1 U of Taq-polymerase (Thermo Scientific) in conventional 1× ammonium TaqBuffer. The cycling profile was as follows: 94^0C for 2 min followed by 7 cycles at 94^0C for 1 min, 60^0C for 2 min, 72^0C for 2 min; 20 cycles at 94^0C for 1 min, 54^0C for 2 min, 72^0C for 2 min; and final extension at 72^0C for 10 min. Aliquots of the amplification products were checked by 1% agarose gel electrophoresis. The amplification products of all six markers per sample were combined and mixed with formamide and the size standard LIZ (Size Standard 500 LIZ, Applied Biosystems) so that in a 10 μL total volume, PCR products labeled by VIC, NED, and FAM were diluted 540-, 120-, and 360-fold, respectively. Samples were run on a 310 Genetic Analyzer (Applied Biosystems). Data were processed by GeneMapper Software 4.0 (Applied Biosystems) and alleles defined by their size (in bp). Analysis of genotypic data included distance-based unweighted pair-group method with arithmetic mean (UPGMA) and Bayesian clustering. Statistical summary of genotyping results was obtained using GenAlex 6.5 for Excel. Bayesian clustering was performed using STRUCTURE 2.3.4 as follows: 10 values of K were run 10 times with 100,000 burn-in and 100,000 analyzing iterations, using an allelic admixture model with correlated alleles. Ten independent runs were aligned, and mean cluster configuration was calculated using CLUMPP [greedy algorithm, 100 random permutations tested]. Results were visualized using Distruct 1.1 (Rosenberg, 2003). UPGMA clustering based on Bruvo genetic distances was performed using R packages poppr and ape.

Table 1. Sequences of primers used for SSR genotyping and resistance-associated marker amplification in *Vitis vinifera*.

Locus	Forward primer	Reverse primer
SSR markers; "tails" are designated by codes of corresponding fluorescently labeled primers		
VVS2	5'-AAATTCAAAATTCTAATTCAACTGG-3'	5'-D8S1132-CAGCCCGTAAATGTATCCATC-3'
VVMD5	5'-TATACCAAAAATCATATTCCTAAA-3'	5'-D8S1132-CTAGAGCTACGCCAATCCAA-3'
VVMD7	5'-AGAGTTGCGGAGAACAGGAT-3'	5'-DYS437-CGAACCTTCACACGCTTGAT-3'
VVMD27	5'-GTACCAGATCTGAATACATCCGTAAGT-3'	5'-D8S1132-ACGGGTATAGAGCAAACGGTGT-3'
VrZAG62	5'-GGTGAAATGGGCACCGAACACACGC-3'	5'-D12S1090-CCATGTCTCTCCTCAGCTTCTCAGC-3'
VrZAG79	5'-AGATTGTGGAGGAGGGAACAAACCG-3'	5'-D12S1090-TGCCCCCATTTTCAAACTCCCTTCC-3'
Resistance-associated markers for oidium and mildew		
GF12-22	5'-GCCAAGTTAGACGAAGTGCAA-3'	5'-ACGGTTTCCTTTTCAACCAGT-3'
GF12-07	5'-AGGGTTTGTATTGTTTGGGAGA-3'	5'-GTGGAAGTGTGAAGTAACGTGG-3'
GF15-28	5'-TGCACACAATCACAGAGAGAGA-3'	5'-TGCGGTTAATTTTGACTCCTTC-3'
GF18-08	5'-GACAATAGCGAGAGAGAATGGG-3'	5'-AGTTGGCTAAAACCCTAGAGGC-3'
GF15-30	5'-TCACAGTATGCAGTAACCTGGC-3'	5'-AAAGGGAAAATGAGCAGTTGAG-3'
GF09-46	5'-GAGAGATTTGAGGGATTGTTGG-3'	5'-ATCCACGTTTGTAGCCTTTTGT-3'
GF09-48	5'-TCTGGAAAGCACAGTAGAGAAGTG-3'	5'-ATGGAAGGAACCAATGCTAAGA-3'
GF14-28	5'-TTGGTTCATGGTTGATGCTTAC-3'	5'-ACCACATGCAGACAGGTTAGTG-3'
GF18-06	5'-GGTCTCCTAGAAAGCCAAGCAA-3'	5'-TCCCTTTTCCCCTTGTTCTCG-3'
GF13-13	5'-GTGCATCTTCTTCTTCCCAACC-3'	5'-GCATTTGTCAAAGTCGTGTACTTC-3'
VMC9H4.2	5'-GCAGTTGATGCAAAACAACAGT-3'	5'-CACATCATTCATTGATGAGGCT-3'

Amplification of resistance loci to powdery and downy mildew

Analysis of resistance loci was conducted in the Julius Kühn Institute for Grapevine Breeding (Siebeldingen, Germany). Molecular markers used to characterize loci related to oidium and mildew resistance are presented in Tables 2 and Table Supplementary 2. In the analysis of Kazakhstan varieties, parental varieties were used as a control to confirm the inheritance of sequences cosegregated with resistance.

Table 2. List of markers used to identify alleles in corresponding loci correlated with resistance to oidium and mildew in *Vitis vinifera*.

Marker	Pathogen	Locus	LG	Allele, bp	Source of resistance	Reference
GF12-22	oidium	*RUN1*	12	187	VRH	Schwander, 2011, JKI
GF12-07	oidium	*RUN1*	12	284/288	VRH	Zyprian et al., 2016, JKI
GF13-13	oidium	*REN1*	13	214	*'Kishmish vatkana'*	Zhang et al. 2009
VMC9H4.2	oidium	*REN1*	13	283	*'Kishmish vatkana'*	Hoffmann et al., 2008
GF15-28	oidium	*REN3*	15	342	'Regent'	Schwander, 2011, JKI
GF15-30	oidium	*REN3*	15	452	'Regent'	Schwander, 2011, JKI
GF18-06	mildew	*Rpv3*	18	389	'Villard blanc'	Zyprian et al., 2016*
GF18-08	mildew	*Rpv3*	18	399	'Villard blanc'	*Zyprian et al., 2016*
GF09-46	mildew	*Rpv10*	09	416	'Solaris'	Schwander et al., 2011
GF09-48	mildew	*Rpv10*	09	359	'Solaris'	Schwander et al., 2011
GF14-28	mildew	*Rpv12*	14	150	V. amurensis	Schwander, 2011, JKI

Notes: VRH – vinivera×rotundifolia hybrid; JKI: Institut für Rebenzüchtung; *- In 'Table of Loci for Traits in Grapevine Relevant for Breeding and Genetics' (last update November 2016)

Multiplex PCR was conducted in 25-µL reactions containing 1 µL of genomic DNA (1 ng/µL), 2.5 µL of QIAGEN Master Mix, 0.01 µL (100 pmol/µL) of each forward and reverse marker primer, 1.38 µL of deionized sterile water. Markers were combined into two groups. In the first group, GF12-22, GF12-07, GF15-28, GF15-30, and GF18-08; in the second group, GF13-13, VMC9H4.2, GF18-06, GF09-46, GF09-48, and GF14-28. The amplification profile was as follows: an initial heat activation step at 95^0C for 15 min; 30 cycles at 94^0C for 30 s, 60^0C for 1.5 min, 72^0C for 1 min; and final cycle at 72^0C for 30 min. Amplification products were diluted 2-fold, and 2 µL of the products were mixed with a size standard (Size standard 500 LIZ, Applied Biosystem) according to manufacturer's instructions. Electrophoresis of PCR products was performed on an ABI 3130 DNA analyzer (Applied Biosystems).

RESULTS AND DISCUSSION

Leaf shape analysis

Landmark-based methods allow neglect of nonperforming parameters and focus on the shape of the object expressed as a set of points corresponding to its key morphological traits (Viscosi and Cardini, 2011). GPA was performed to test variability of leaf shape among cultivars. Mean shapes for each cultivar were calculated and used for further analysis (Figure Supplementary 1). Three patterns were defined describing veins' first branching points (pattern 1), sinuses (pattern 2), and outer tips (pattern 3) (Figure 1).
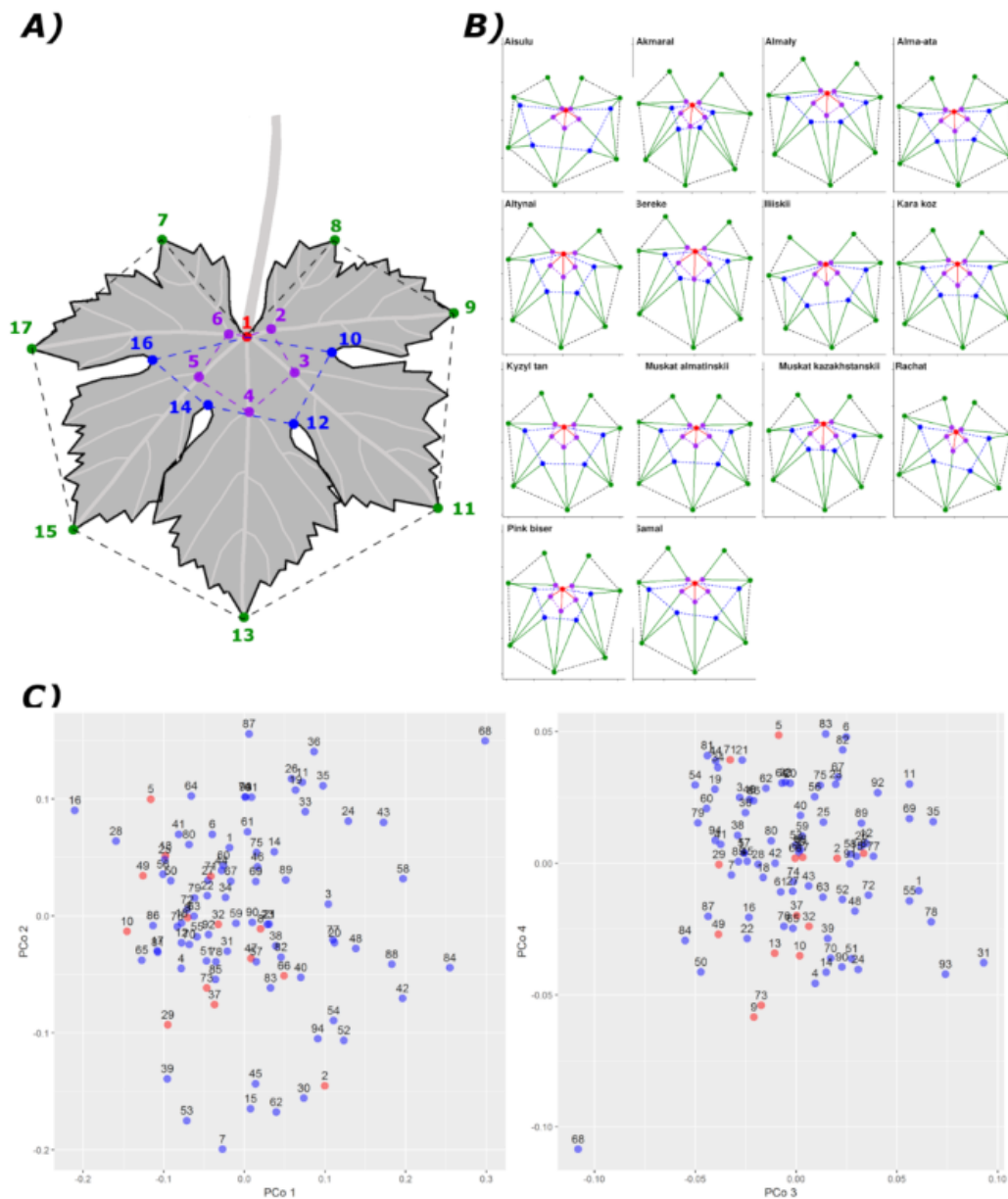
Figure 1. A: Landmarks of grapevine leaves used for Generalized Procrustes analysis (GPA). B: Mean configurations of 14 Kazakhstan grapevine cultivars obtained by GPA. C: Principal component analysis of Riemannian shape distances between cultivars. Kazakhstan cultivars are highlighted in red; numeration according to Table 1.

PCA was performed for each of these patterns and overall landmarks set with function "plotTangentSpace()" (Table 3; Figures 2 and Figure Supplementary 2). The first four principal components were sufficient to explain 98–99% of the variation of

different patterns and nearly 98% for the full set of landmarks. Shape variation of each pattern is expressed in standard deviations of each principal component. The first two components of variation show a nonlinear relation. Pattern 3, describing leaf lobe tips, has the most impact on this variation and stronger nonlinearity than others. However, patterns 1 and 2 also show the relation. By summarizing the first two components of shape variation among the three patterns, the overall structure has an exceptionally strong nonlinear relation. These results may be evidence that grapevine leaf shape has too complex a nature, and optimization of more advanced approaches lay beyond the scope of the current study.
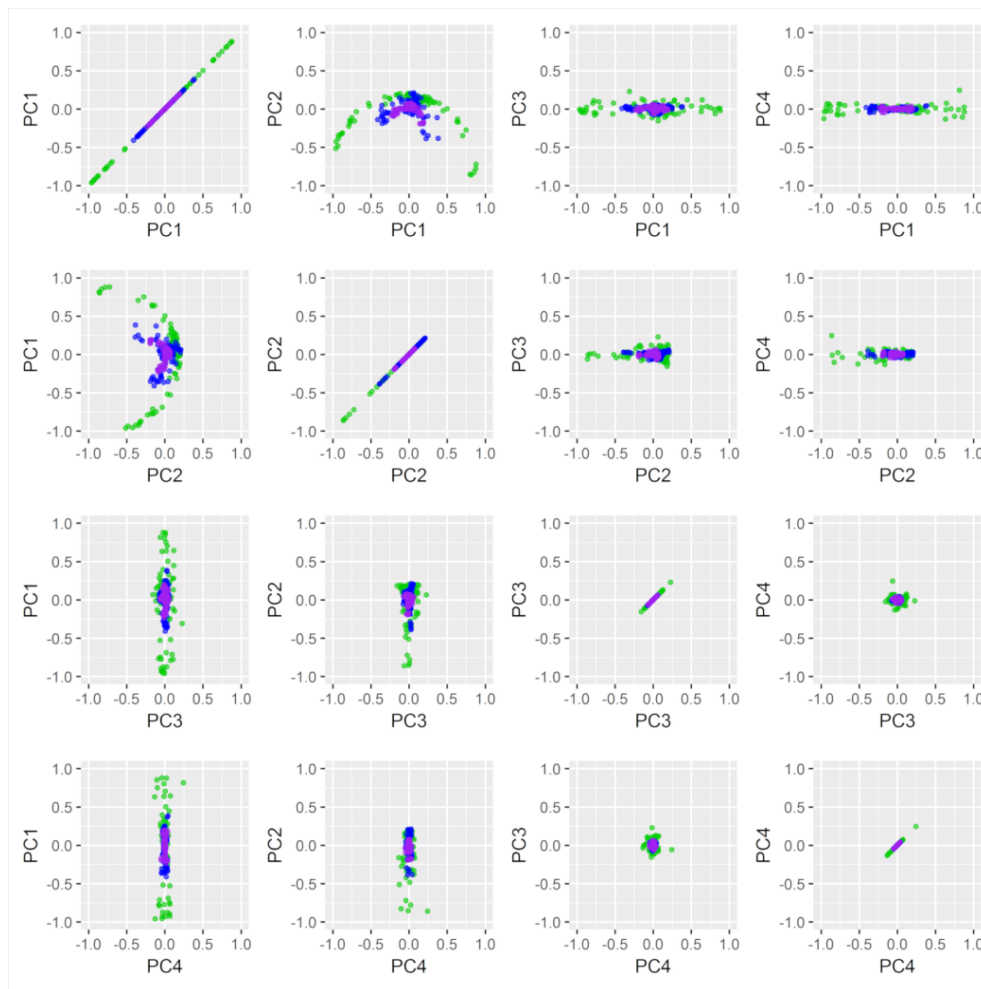

Figure 2. Plots of the first four principal components of variation for three leaf shape patterns among 94 cultivars (purple, blue, and green dots for patterns 1, 2, and 3, respectively). See also Figure Supplementary 2 for separate plots of patterns and summary of leaf shape variation.

Table 3. Results of PCA of leaf shape variation among 94 grapevine cultivars.

		PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10
Pattern 1 (landmarks 1-6)	Eigenvalues	00.01004	00.003677	00.000548	00.000162	$09.24\cdot10^{-5}$	$04.32\cdot10^{-5}$	$03.53\cdot10^{-5}$	$02.95\cdot10^{-5}$	$01.57\cdot10^{-5}$	$01.22\cdot10^{-5}$
	Variance explained (%)	68.41	93.481	97.22	98.321	98.951	99.245	99.486	99.687	99.794	099.878
Pattern 2 (landmarks 1, 10, 12, 14, 16)	Eigenvalues	00.028527	00.014738	00.000859	00.000303	00.000253	00.000169	00.000157	00.000101	$06.26\cdot10^{-5}$	$02.74\cdot10^{-5}$
	Variance explained (%)	63.12	95.73	97.626	98.3	98.856	99.231	99.578	99.801	99.939	100
Pattern 3 (landmarks 1, 8, 9, 11, 13, 15, 17, 7)	Eigenvalues	00.209215	00.068278	00.00425	00.002141	00.000555	00.000375	00.000353	00.000237	00.000207	000.000163
	Variance explained (%)	73.09	96.96	98.441	99.189	99.382	99.513	99.637	99.72	99.792	099.849
All landmarks	Eigenvalues	00.244135	00.081796	00.005495	00.003858	00.00232	00.001253	00.000595	00.000545	00.000376	000.000295
	Variance explained (%)	71.33	95.22	96.825	97.953	98.631	98.997	99.171	99.33	99.44	099.526

Genotyping

The microsatellite profiles of the accessions were compared with available representative profiles in the database http:/www.vivc.de/ (Table S2). Summary of SSR genotyping is available in Table S3. In our experiment, allele sizes were 2 nucleotides less for locus VVMD5(-2), 1 nucleotide less for locus VVMD27(-1), and 1 nucleotide more for VrZAG62(+1). In most data from various laboratories, allele size increments were 2 bp due to the nature of these markers, and 1-bp increment differences for VVMD27 and VrZAG62 were also identified (This et al., 2004). Such results are interpreted as a stutter or extra base additions by some Taq polymerases (Brownstein et al., 1996). In our study, six markers turned out to be sufficient to differentiate each of the cultivars. However, for a number of cultivars, there was no confirmation of identity with respect to pedigree either by bibliography or comparison with an international database, such as the Vitis International Variety Catalogue (VIVC); some confusion in field collection is assumed. On the other hand, for example, the pedigree of cultivar Muskat susanna (by breeder/bibliography Malingre S.P. × Muskat rozovii) was not confirmed when genotyping. In our study of marker profiles, one of the parents of Muskat susanna should be Madeleine angevine; the same is indicated in cultivar passport data in VIVC.

Kazakhstan cultivars and the same number of randomly selected European and Asian ones were compared to identify the features of allele representation (Table Supplementary 3). The total number of alleles was 77, with 22 private alleles (56 and 3 alleles for Kazakhstan cultivars, 55 and 5 alleles for European cultivars, 66 and 13 alleles for Asian cultivars). In order to distinguish groups of cultivars in accordance with their genetic similarity, two methods based on different approaches were used: a UPGMA tree with Bruvo genetic distances (Bruvo et al., 2004) and Bayesian Monte Carlo Markov Chain simulation implemented in STRUCTURE (Pritchard et al., 2009). Configurations were tested with a number of clusters (K) from 2 to 10 and combined results with the UPGMA tree (Figure 3). With K = 4, distinct structure patterns corresponding to clusters on the

UPGMA tree were observed. Three large groups were noted (Alma-Ata—Riesling; Altynay—Akhalili; Khindogny—Mushketnyi). In the first group, four cultivars (Alma-Ata, Rizamat, Kyzyl tan, Volgodon) were united in subclusters by Katta kurgan in their ancestry. The other 13 varieties belonged to the European cultivars. The second group united 12 cultivars of the eastern origin. In the third group, more than 1/4 were eastern cultivars; 3/5 cultivars were related in the pedigree due to Madeleine angevine or Csaba gyoengye (syn. Pearl of csaba). By VIVC, one of the Csaba gyoengye's parents was Madeleine angevine. Many of these cultivars have a second parent of some oriental variety. The fourth, smaller group (Sasun—Neiburger) was mixed.
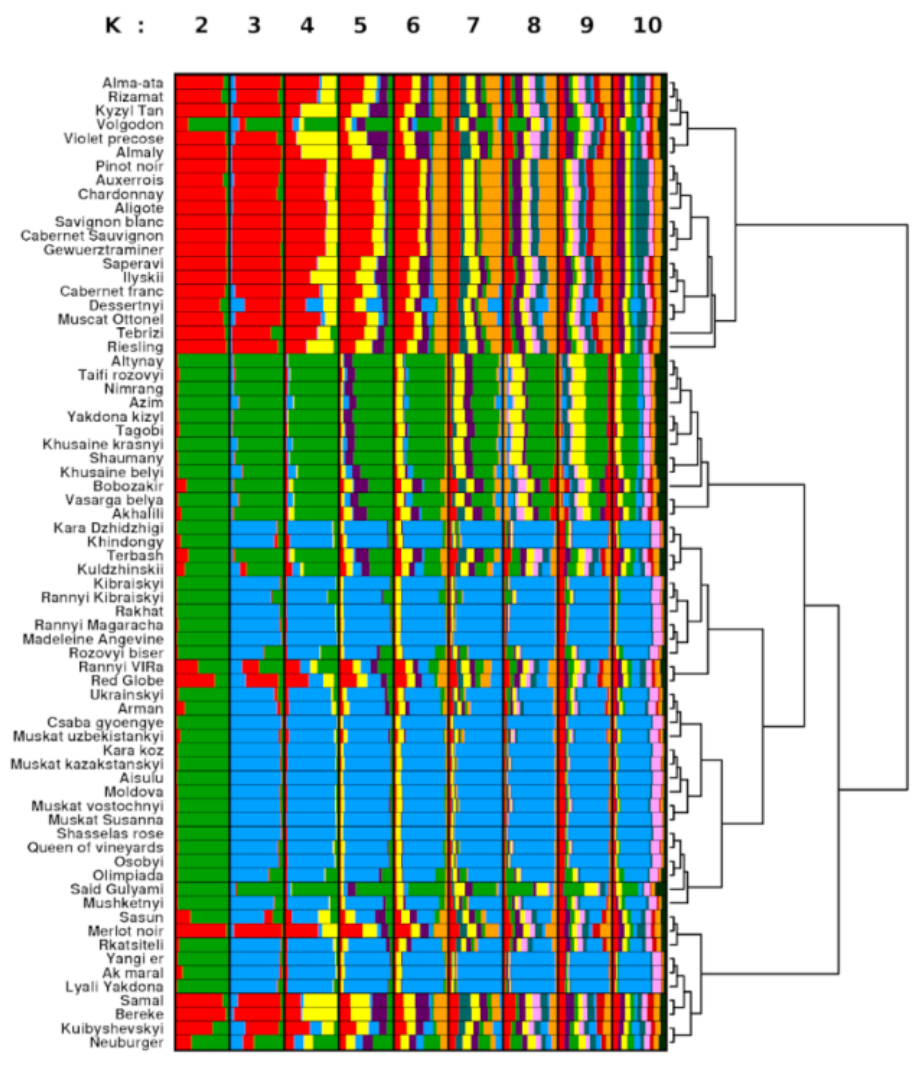

Figure 3. Results of Bayesian clustering of cultivars based on 6 SSR markers using the STRUCTURE software with the UPGMA tree of Bruvo genetic distances.

Identification of loci alleles related to oidium and mildew resistance

In our research, molecular marker analysis revealed certain alleles related to pathogen resistance in several Kazakhstan cultivars (Table 4). In cultivar Bereke, declared highly field-resistant to oidium and mildew, there were 416- and 359-bp alleles of the Rpv10 locus associated with resistance to DM but none associated with PM resistance. Conversely, in cultivar Samal (Bereke's full sibling), declared field-resistant to abovementioned pathogens, a 284-bp allele of the RUN1 locus was associated with PM resistance. In the VIVC, one of the Severnyi cultivar's parents is characterized as having resistance to DM/PM 8/1-leaf/bunch (1 = very low; 9 = very high). Thus, Severnyi could be a source of resistance to DM in cultivar Bereke. Two resistance-associated alleles, 416 and 359 bp, of the Rpv10 locus were revealed in cultivar Almaly and its parent, Fioletovyi rannii. In these cultivars, the 284-bp allele of the RUN1 locus associated with PM resistance was shown as well. Breeders have characterized the Almaly cultivar as sufficiently resistant to both pathogens, whereas its parent based on bibliography (http://vinograd.info/), Fioletovyi rannii, is resistant to DM and sensitive to PM. In contrast, the VIVC degree of resistance to PM in Fioletovyi rannii is indicated as very high (9/9-leaf/bunch). Evidently, resistance to the pathogens in Kazakhstan cultivars Bereke, Samal, and Almaly is more likely due to inheritance from V. amurensis (Rpv10 locus). In view of Bereke and Samal cultivars' resistance to both pathogens, more research is needed following the development of new markers for new putative loci of resistance to each pathogen. Previously, resistance-associated alleles of the REN1 locus in wild and cultivated Central Asian grapes were based on four SSR markers (Riaz et al., 2013). However, two representatives (DVIT1803 and DVIT3351.27) from 40 accessions of V. vinifera subsp. sylvestris were identified as resistant by field test, wherein the former possessed four alleles and the latter had only two. Researchers speculate that alleles at other markers were lost due to recombination. In the other cases, the presence of the resistance-associated allele may be due to size homoplasy. Therefore, even in the presence of alleles of interest, marker analysis allows identification of accessions as potentially resistant that need to be verified in field screening. It should also be noted that hybrid family genetic analysis showed that resistance "is inherited dominantly, but degrees of resistance are defined by polygenic effects".

Resistance-associated alleles 283 bp of *REN1* and 389 bp of *Rpv3* were revealed in cultivar Alma-Ata and its parent Rizamat. The *REN1* locus has Central Asian origin (Hoffmann et al., 2008). The presence of the resistance-associated allele of *REN1* in Alma-Ata based on Asian cultivars is reasonable. Cultivar Muskat uzbekistanskyi has been described as weakly affected by oidium and possesses alleles of *RUN1* (187 bp) and *REN1* (283 bp). In Uzbekistanian Muscat, as well as in Rizamat and Katta-Kurgan, two *REN1* alleles, 260 bp (VMCNg4e10.1) and 249 bp (sc47-18), were revealed (Riaz et al., 2013). However, testing of these cultivars for PM sensitivity was high (4–5), and none of these varieties have been ranked as resistant to oidium. Kazakhstan cultivar Aisulu is characterized by weak resistance to both pathogens and only possesses allele 214 bp of *REN1*, whereas its parent, Pearl of Csaba, "in the middle degree is affected by

DM and sensitive to PM" as it possessed allele 389 bp of *Rpv3* and 214 bp of *REN1*. Kazakhstan cultivars Kyzyl tan, Kara koz, and Muskat kazakhstankyi are sensitive to the pathogens and possessed only allele 214 bp of *REN1*. In some *V. vinifera* cultivars analyzed, the presence of a resistance-associated allele of *RUN1* in *M. rotundifolia* species was identified (Merdinoglu et al., 2003). It is worth noting that the cultivated grapevine *V. vinifera* has a cluster of resistance gene analog sequences (RGA) at the syntenous region on chromosome 12, closely related to MrRGAs at the Mr*RUN1*/Mr*RPV1* locus in *M. rotundifolia* (Feechan et al., 2013). Phylogenetic analysis of coding regions at the syntenous locations have allowed researchers to suggest *V. vinifera TIR-NB-LRR* genes at this locus share a common ancestor with the MrRGAs in *M. rotundifolia* (Feechan et al., 2013). Selection pressure took place in M. rotundifolia due to the presence of the pathogens.

Table 4. Results of amplification of markers associated with powerly mildew and downy mildew resistance loci in grape.

		Powdery mildew (Erysper necator)						Downy mildew (Plasmopara viticola)				
	Locus	RUN1		REN1	VMC9h4.2	REN3		Rpv3		Rpv10		Rpv12
	Marker name	GF12-22	GF12-07	GF13-13		GF15-28	GF15-30	GF18-08	GF18-06	GF09-46	GF09-48	GF14-28
	resistance-related allele lengths	187	284/288	214	283	342	452	399	389	416	359	150
Cultivar	Pedigree											
Aisulu	Pearl of csaba×Ak yakdona	1 8 2	2 2 / 9 9 / 0 6	2 1 4 / -	2 6 2 / 2 7 4	3 5 6 / 3 6 8	4 3 6 / -	3 8 5 / 3 8 6	3 8 2 / 3 8 7	3 9 6 / 4 2 4	3 4 8 / -	1 8 0 / -
Alma-ata	Druzba×Rizamat	2 0 6 / 2 0 8	2 9 0 / 2 9 4	2 0 9 / 2 1 1	2 8 3	3 6 2 / -	4 2 1 / -	3 8 9 / 3 9 3	3 8 2 / 3 9 9	4 2 4	3 8	1 7 0 / 1 8 0
Almaly	Violet precose × Ilyskii	1 9 9 / 2 7	2 8 4 / 2 9 5	1 9 3 / 2 6	2 7 1 / 4 2	3 6 9 / 3 1 4	4 2 0 / 4 7	3 8 7 / 3 1	3 8 9 / 9 7	3 9 6 / 9 6	4 8 / 5 9	3 3 / 8 9
Bereke	Ilyskii×Severnyi	1 8 3 / 2 8	2 9 4 / 2 6	1 7 8 / 2 4	2 7 4 / 4 6	3 7 0 / 3 3	4 3 9 / -	3 8 6 / 3 7	3 8 7 / 5 7	3 6 1 / 6 4	4 2 / 5 9	8 0 / -
Csaba gyoengye	unknown	1 8 3 / 2	2 9 6 / -	2 0 5 / 2 4	2 6 2 / 2 6	3 7 9 / 3 7	4 7 0 / 4 0	3 8 6 / 3 2	3 8 8 / 8 9	3 9 6 / 2 4	4 8	8 0 / -
Ilyskii	Saperavi × Riesling	1 9 8 / 2 8	2 9 4 / 9 6	1 9 4 / 2 6	2 7 2 / 4 2	3 6 2 / 3 0	4 2 1 / 1 3	3 8 6 / 2	3 9 3 / 6	3 9 6 / 4 9	4 2 / 4 0	1 7 2 / 1 0
Kara koz	Madeleine Angevine× Senso	1 8 2 / 2 3	2 9 9 / -	2 0 5 / 2 4	2 6 2 / 1 6	3 7 0 / 3 0	4 2 0 / 0 0	3 8 7 / -	3 7 6 / -	2 3 / 8	4 8	1 6 8 / 0
Kyzyl tan	Legkyi aromat×Rizamat	1 9 3	2 9 0 / 2 4	2 0 5 / 2 9	2 8 3 / 2 9	3 6 7 / 3 2	4 2 1 / 1 9	3 8 5 / 2	3 8 0 / 2 0	3 6 2 / 4	4 8	1 6 0 / 6
Muskat kazakhstankyi	M.A×M.Al ex× M.uz	1 9 3 / 2 8	2 9 0 / 9 6	2 1 9 / 5 3	2 7 2 / 4 9	3 5 6 / 3 2	4 2 4 / 1 0	3 8 5 / 1	3 8 7 / 0	3 6 2 / 4	4 8	1 6 0 / 6 / 2
Muskat uzbekistankyi	Katta-Kurgan×Musk.alex.	**1** 8 7 / 1 3	2 9 9 / -	2 0 9 / 2 6	2 7 3 / **8** 3	**2** 3 3 / 3 4	4 7 6 / 3 7	3 8 6 / 1	3 9 3 / 1	3 9 2 / 3	4 8	**1** 8 0 / -
Rizamat	Katta-Kurgan×Parkent	1 9 3 / 2 8	2 9 0 / 9 6	2 1 4 / 9	2 7 4 / 7 **8** 4	3 6 2 / **3**	4 2 1 / 5	3 8 5 / **9**	3 8 0 / 9 0	3 6 4 / 4	4 8	1 7 0 / 0
Riesling	unknown	1 9 3 / 2 8	2 9 6 / 9 6	2 0 8 / 2 4	2 7 6 / 2 6	3 8 1 / 6 6	4 1 9 / 2 1	3 8 6 / 2	3 9 8 / 2 5	3 6 2 / 4 6	4 2 / 4 8	1 6 7 / 9 1
Samal	Ilyskii×Severnyi	2 0 2 / 2 8	**2** 8 4 / 2 6	2 0 5 / 2 6	2 7 6 / 2 3	3 7 0 / 3 7	4 1 9 / 4 9	3 8 6 / 6 7	3 8 9 / 2 5	3 9 2 / 4 7	4 2 / 4 8	1 6 2 / 0
Saperavi	Black sea region	1 9 7 / 2 6	2 9 9 / 4	1 9 3 / -	2 7 4 / -	3 7 0 / -	4 3 3 / -	3 8 7 / 1	3 9 8 / 2 5	3 9 6 / -	4 8	1 8 0 / -
Fioletovyi rannii	(Severnyi (Malingre seedling x V. amurensis) x Muskat hamburg)	1 9 3 / 1 9	**2** 8 4 / 2 9 0	1 7 8 / 2 6	2 8 1 / 3 6	3 3 9 / 3 9 4	4 8 / -	3 8 7 / 9 3	3 9 9 / 1 7	**4** 1 6 / 1 2 4	3 4 8 / **5** 4	1 6 2 / 1 5

In our study, none of the cultivars tested with markers revealed alleles associated with *REN3* or *Rpv12* (from *V. amurensis*). Thus, in the absence of confirmation, the presence of *Rpv10* or *Rpv12* by marker- assisted selection in the developed grapevine hybrids with a high level of resistance suggests unknown resistance genes (Khafizova and Michlovský, 2016). For high-throughput marker-assisted selection of disease-resistant grapevine cultivars, development of "perfect" genetic markers is the only guarantee. New strategies are yet to be developed to facilitate rapid evaluation of R-gene candidates (Feechan et al., 2013). This will help researchers to better understand the degree to which genes of interest are able to function in different genetic backgrounds (Qiu et al., 2015).

Results of SSR genotyping of collected grapevine cultivars herein show that local varieties in Kazakhstan retain their genetic proximity to their source breeding material. They do not form distinct groups and are distributed among European and Central Asian varieties according to their origin. This corresponds to admixture of cultivars used in the breeding process during the Soviet period. Thus, genetic properties of local cultivars derive directly from their predecessors. However, their interaction with specific environmental conditions of Kazakhstan may be of interest in further studies (e.g., in context of interplay of hybridized European and Asian genotypes or development of pure European varieties in continental climatic zones). Interaction between genetic and external factors in organism development results in its phenotypic properties, and morphometric (ampelometric) features of grapevine varieties cannot be ignored in such studies. Although the present study was not able to analyze associations between genetic and phenotyping data due to inconsistency of corresponding datasets and limited amounts of data, further development of this methodology may be important for both theoretical and applied aspects of grapevine biology.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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