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Study of clonal variation of 'Bidaneh Ghermez' grapevine cultivar in Iran

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Abstract. Grapevine (*Vitis vinifera* L.) is a well-known plant including different cultivars and clones. In spite of the extensive works at the cultivar level, identification and determination of clonal genetic variation has remained as a challenge. To assess the genetic variation between clones of grapevine cv. 'Bidaneh Ghermez', 20 selected clones were analyzed for cluster weight (CW), cluster length (CL), cluster width (CWI), berry weight (BW), berry length (BL), berry width (BWI) and total soluble solids (TSS) in randomized complete block design with three replications. Analysis of variance revealed considerable genetic variation for all measured traits (except cluster width) among clones. Cluster analysis, discriminant function analysis and principal component analysis (PCA) showed same results and all clones assigned in 2 groups. First group was including 9 clones and second group was including 11 clones. Overall, our results indicated C7, C10, C12 and C14 clones were best clones and have potential to introduce promising clones for stablishing new vineyard with high yield.

Keywords: Clone, genetic variation, *Vitis vinifera* L., Bidaneh Ghermez.

ABBREVIATIONS: CW: Cluster weight; CL: Cluster length; CWI: Cluster width; BW: Berry weight; BL: Berry length; BWI: Berry width; TSS: Total soluble solids; PCA: Principal component analysis.

INTRODUCTION

Grapevine is one of the most economically important horticultural crops in the world and mainly used for wine production, fresh fruit, raisins and grape juice. It has been domesticated about 6,000 to 8,000 years ago in the Near East (Iriti and Vitalini, 2012). International trade in wine, its vegetative propagation and distribution in the different climatic conditions has produced great diversity of varieties (Seyedimoradi et al., 2012; Rusjan, 2013). More than 9,600 different grape cultivars are documented in the world (Rao et al., 2014). Moreover, there are a large number of clones that have different morphological characteristics which cause broad adaptability to different environments and planting techniques.

Iran is one of the top countries in grape production. According to the FAO statistic, Iranian vineyards with 215,000 ha cultivated area supplied more than 2 million tons of

world grape market in 2012. Financial contribution of grape production in the economy of Iran has been more than 13 million dollars. Besides the financial performance, due to variable climate, Iranian germplasm has high diversity including cultivars, wild populations and clones (Tafazoli et al., 1993). Vegetative propagation system together with the sole use of a few cultivars has led to decrease in grapevine diversity. In addition pests and diseases attacks have contributed to this genetic erosion (This et al., 2006).

Nowadays with the growing population, the establishment of new vineyards or improvement of old vineyards is unavoidable and requires identifying of high yield and good quality cultivars and clones. In spite of the extensive works at the cultivar level, identification and study of clones has remained as a challenge (Moncada and Hinrichsen, 2007; Baneh et al., 2009; Loureiro et al., 2011). However, due to importance of clones in modern vineyards, identification of their variability is crucial for increasing grape production (Gotor et al., 2008).

Shinde et al. (2013) studied clonal diversity in 'Centennial Seedless' cultivar by 1,093 AFLP markers. Three polymorphic markers were reported that be useful for establishing genetic identity, variety registration and protection of breeder's right. Combination of SSR and AFLP markers were used to investigate the genetic difference between clones of 'Keshmeshi' cultivar. All clones were assigned to 2 groups based on the AFLP data. The first group included white berry skin clones and the second one with red berry skin clones. They concluded that AFLP could only distinguish the red berry clones of 'Keshmeshi' from other white berry clones (Baneh et al., 2009). Genetic variation was found in grapevine clones by many other reports (Moncada and Hinrichsen, 2007; Loureiro et al., 2011; Miotto et al., 2014). Genetic diversity is critical to success in breeding programs (Aremu, 2011). Genetic variations enable plant breeder to create new gene combinations and select best individuals for different breeding objectives (Glaszmann et al., 2010). Due to long history of grapevine cultivation, *Vitis vinifera* L. indicate considerable diversity in morphology, disease resistance, abiotic stress tolerance and etc. Since grape species are maintained by vegetative propagation, so most of them are heterozygous plants. Regarding grape literature review, clone implies asexual propagation without meiotic recombination resulting identical offspring. This definition means clones will not be able to adapt to environmental changes. However, mutations (in genes or genome level) are often source of clonal variation. Mutations occur spontaneously in nature and many desirable clones have arisen by this mechanism (Anhalt et al., 2011). Recent studies demonstrated that white grape has derived from red grape by mutations that affected the anthocyanin synthesis (Walker et al., 2007). Moreover, accumulation of epigenetic mutations and presence of pathogens could be causes of somatic polymorphism in grape clones (Imazio et al., 2002; Espinoza et al., 2007).

Clonality is a dynamic concept and new genetic variation is added by numerous mechanisms to provide an open system for adaptation and facing with environmental changes (Forneck, 2005). The ampelography is a science that concerned with identification and classification of grape genotypes using morphological traits of leaves, shoots, clusters and berries. Berry and cluster characters are important quality parameters that are considered for grape export and are major contributing parameters (Somkuwar et al., 2006). In table grape, the overall flavor is critical index for consumer preference. Among the flavor compositions, total soluble solids (TSS) are associated with market quality including fruit taste (Shiraishi et al., 2010). Khadivi-Khub et al. (2014) studied

sixteen fruit parameters in 23 grape cultivars and found high variability in the evaluated cultivars. Significant differences were detected among the cultivars in fruit yield, cluster size, berry size, TSS and titratable acidity (TA). This evaluation methods based on morphological, agronomical and physiological traits can help breeder to focus on promising clones or genotypes (Cruz et al., 2004). Also genetic diversity assessment is essential to germplasm characterization and genetic resources conservation which in turn are important to improve or substitute present cultivar and genotypes (Khadivi-Khub et al., 2014). The objectives of present research are to investigate the clonal variation of 'Bidaneh Ghermez' grapevine cultivar and to identify best clones that could be used in the new vineyard establishment.

MATERIALS AND METHODS

Twenty clones of 'Bidaneh Ghermez' cultivar were selected for this study from vineyards of Qazvin, Iran. 'Bidaneh Germez' is a seedless, red-skinned and medium maturity cultivar (Nejatian, 2013). Some other important features of 'Bidaneh Ghermez' are shown in Table 1. It is one of the most popularly consumed table grapes in Iran, but it is not well known in other countries. Qazvin is one of the main areas of Iranian vineyards and located in about 153 km west of Tehran (Fig. 1). Vineyards were frequently screened for applied management and sanitary status (control of fungal disease and virus symptoms free plants) during spring and summer 2014. Clones were taken from well management and health vineyards and localized using GPS (Fig. 2). Cluster related traits including cluster weight (gr), cluster length (cm) and cluster width (cm) and berry related traits including berry weight (gr), berry length (mm), berry width (mm) and total soluble solids (%) were measured. All cluster related traits were recorded as average measurement of five clusters. The widest and longest parts of cluster were calculated as cluster width and cluster length respectively. Measuring the berry length and width (based on the average of 10 berries) was done by caliper and total soluble solids (TSS) were calculated by refractometer. Due to our study was in situ, so data were collected from three different directions (west, east and center) of each clone and directions were taken as replications in the randomized complete block design.

Descriptive statistics (average, variance and standard deviation) and normality test (Kolmogorov-Smirnov and Shapiro–Wilk tests) were calculated by SPSS software. Genetic variation between selected clones was determined by the analysis of variance. Data were subjected to multivariate analysis using PAST software (Hammer et al., 2011). Cluster analysis using UPGMA algorithm and Euclidian distances method were carried out and all clones were grouped. Discriminant function and principal component analysis were used to infer clones relationships and to determine promising clones.

Table 1. Some important features of 'Bidaneh Ghermez' grape cultivar

time of bud burst	very late
status of tip	half open
attitude (before tying)	horizontal
density of erect hairs on tip	none or very low
color of dorsal side of internodes	green
color of ventral side of internodes	red

Table 1 (continued)

shape of blade	pentagonal
size of blade	medium
number of lobes	three
petiole sinus limited by vein	not limited
length of teeth	medium
sexual organ	fully developed stamens and fully developed gynoecium
cluster size (peduncle excluded)	medium
cluster density	medium
veraison	medium
berry size	short
berry shape	broad ellipsoid
color of berry skin	red
thickness of skin	very low
firmness of flesh	soft
particular flavor	none
formation of seeds	none

Nejatian & Doulati Baneh, (2016)

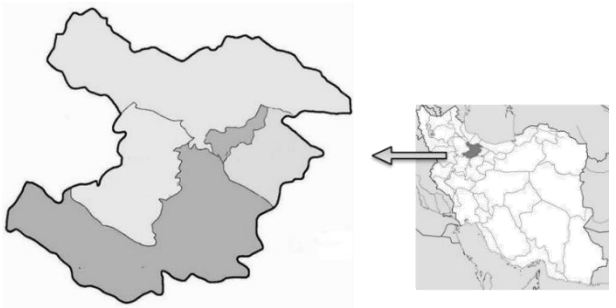

Figure 1. Map of the location of study in Qazvin (right), Iran (left).

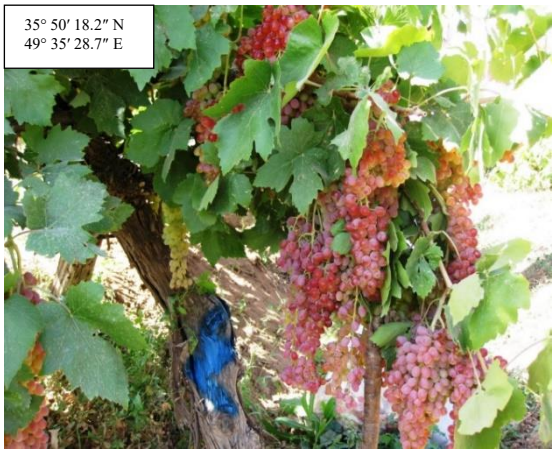

Figure 2. Selected clone and localized by GPS.

RESULTS AND DISCUSSION

Morphological descriptive statistic of measured traits showed cluster weight was more variable (standard deviation was 31.14) characteristic while berry weight had lowest standard deviation (0.33) among recorded traits (Table 2). Due to majority of statistic functions are based on normal distribution, we investigated the normality of experimental data using Kolmogorov-Smirnov and Shapiro–Wilk tests. Non-significance of these tests (especially Kolmogorov-Smirnov test that is designed for large sample size) showed that our experimental data is drawn from a normal distribution and could proceed further to next analyses (Table 3).

Table 2. Descriptive statistics (clones mean, total mean, standard deviation and variance) for recorded traits

Clones	CW (gr)	CL (cm)	CWI (cm)	BW (gr)	BL (mm)	BWI (mm)	TSS (%)
1	68.13	24.33	13.33	1.91	18.17	14.23	25.00
2	55.53	23.00	14.33	1.99	17.50	14.50	25.63
3	104.00	25.00	16.67	1.86	17.47	13.67	23.63
4	74.07	24.33	12.00	1.87	17.50	13.63	23.80
5	56.20	22.67	11.33	2.33	18.67	14.50	21.47
6	63.93	27.00	14.67	1.82	17.20	13.47	24.50
7	105.87	22.00	15.00	2.56	19.57	14.65	25.00
8	60.67	23.67	13.67	1.60	16.47	13.17	25.73
9	127.40	29.00	16.33	1.82	17.07	13.57	28.07
10	118.07	23.00	14.67	2.01	16.33	13.53	25.07
11	74.87	30.33	13.33	1.26	14.53	13.72	21.07
12	93.60	31.33	15.67	1.82	16.43	13.30	19.73
13	107.27	22.33	15.67	1.78	16.23	13.07	21.67
14	111.40	27.00	13.67	2.12	17.07	13.93	25.50
15	116.27	21.50	13.67	1.78	16.83	13.20	23.20
16	45.33	21.00	12.67	1.91	17.60	13.43	24.07
17	78.67	25.50	14.33	1.73	17.50	12.73	23.63
18	75.50	29.00	16.00	2.20	18.27	13.80	18.73
19	99.93	26.67	16.00	1.81	15.60	13.00	21.67
20	88.20	29.67	14.67	1.29	14.97	13.65	21.17
Total Mean	86.3	25.4	14.3	1.87	17	13.6	23.4
Standard deviation	31.14	4.44	2.6	0.33	1.2	0.65	2.52
Variance	970.46	19.7	7.2	0.11	1.6	0.43	6.3

Table 3. Kolmogorov-Smirnov and Shapiro–Wilk tests for normality of data

	Kolmogorov-Smirnov			Shapiro–Wilk		
	Statistics	d.f*	Sig.	Statistics	d.f	Sig.
CW	0.115	58	0.055	0.938	58	0.005
CL	0.090	59	0.200	0.975	59	0.257
CWI	0.095	60	0.200	0.965	60	0.080
BW	0.091	60	0.200	0.982	60	0.505
BL	0.106	60	0.090	0.974	53	0.318
BWI	0.097	53	0.200	0.975	53	0.318
TSS	0.076	60	0.200	0.981	60	0.490

* – Difference in the degree of freedom (d.f) is due to estimation of missing data.

Analysis of variance showed that all traits (except cluster width) were significant indicating considerable genetic variation for all measured traits (except cluster width) among selected clones (Table 4).

Table 4. Analysis of variance for measured traits in randomized complete block design

Sources of variation	Mean Square						
	CW	CL	CWI	BW	BL	BWI	TSS
Replication	95.48	25.04	1.82	0.03	0.135	0.076	0.277
Clones	1,739.64**	30.08*	6.29^{ns}	0.27**	4.34**	0.841**	16.08**
Error	612.76	14.16	8.06	0.04	0.364	0.243	1.849
R^2	0.601	0.542	0.287	0.778	0.857	0.645	0.813

* and ** significant at 5% and 1% probability level respectively. ns – non-significant.

Our research showed difference in studied clones taken from different vineyards. Diversity between different vineyards can be explained by microclimate of each vineyard and on the other hand as a result of different rootstocks, canopy management or fertilization (Spayd et al., 1994; Main et al., 2002; Loureiro et al., 2011). In our study rootstocks and management (including canopy, irrigation and fertilization management) was same in all vineyards. On the other hand, clones taken from one same vineyard were different. We could conclude that existence of genetic factors is main source of observed variation.

Hierarchical cluster analysis clarified the relationships among clones. Cluster analysis indicated 2 groups of similar clones. First group was including nine clones and second group was including eleven clones (Fig. 3). Cluster analysis have been used in many other researches to detect similarity between grape clones (Rakonjac et al., 2010), cultivars (Martínez et al., 2003) and wild populations (Franco-Mora et al., 2008; Ekhvaia & Akhalkatsi, 2010).

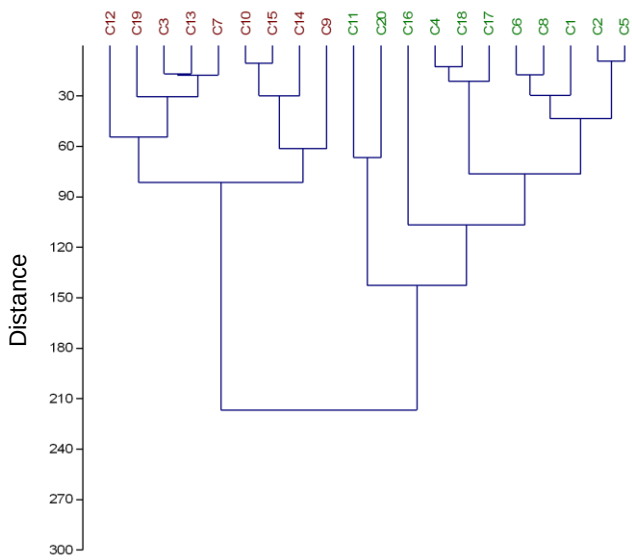

Figure 3. Dendrogram of the grapevine clones using UPGMA algorithm and Euclidian distances method. First group includes 9 clones (red) and second group includes 11 clones (green).

Assuming that created groups by cluster analysis is true; discriminant function analysis based on linear combinations of the predictor variables for 2 groups was fitted to develop a predictive model of group membership. Zero assumed to be cut off point and classification results showed that fitted function assign all clones to correct groups when 2 groups are considered (Fig. 4).

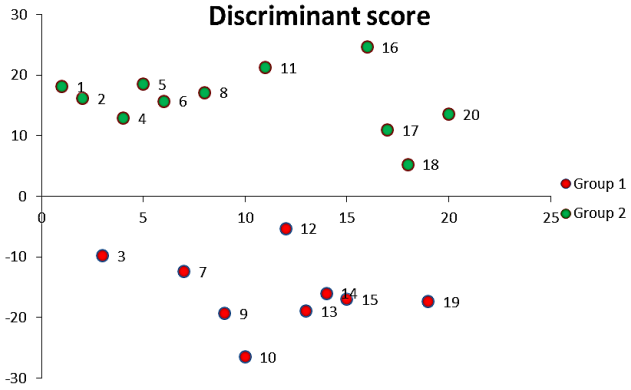

Figure 4. Discriminant score plot shows two groups (is shown with red and green color) in cutoff point (zero).

Principal component analysis (PCA) was performed to estimate morphological differentiation between clones revealed the first 2 components explain 99% of variance. Considering the loading factors indicated first component correspond to cluster related traits (cluster weight, cluster length and width) and second component was correlated with berry related characters (berry weight, berry length, berry width and TSS). PCA analysis separated all clones in 4 regions (Fig. 5). Clones in the first region of coordinate axis (C7, C10, C12 and C14) had highest value of cluster related traits and berry related traits while the rest of the clones had just high value for one out of two components (high value for cluster related characters or for berry related characters).

The PCA, as a powerful tool in statistical investigations, is widely used in the analysis of multivariate data in the agricultural sciences to evaluate genetic diversity and population's classification. For instance, Ekhvaiva & Akhalkatsi (2010) have studied seven wild grape populations from three geographic regions and showed not only PCA and multivariate discriminant analysis are powerful techniques but also could classify the populations correctly, when three geographic regions were considered. In the current study, PCA has separated studied clones in the best possible way. Similar to cluster analysis and discriminant analysis results, PCA showed that C7, C10, C12 and C14 clones are the best ones. Moreover, clones in the first region of coordinate axes, didn't have same value. The C10 clone had high value of the second component and low value of the first component, whereas C7 clone had high value of the first component and low value of the second component. Accordingly it can be concluded that C10 is a distinguished clone when berry related traits are more important than cluster related traits. Conversely, C7 is the preferential clone when cluster related traits are more important than berry related traits. In addition, in cases that berry and cluster related traits have similar importance, C14 can be considered as an alternative clone for selection in breeding programs.

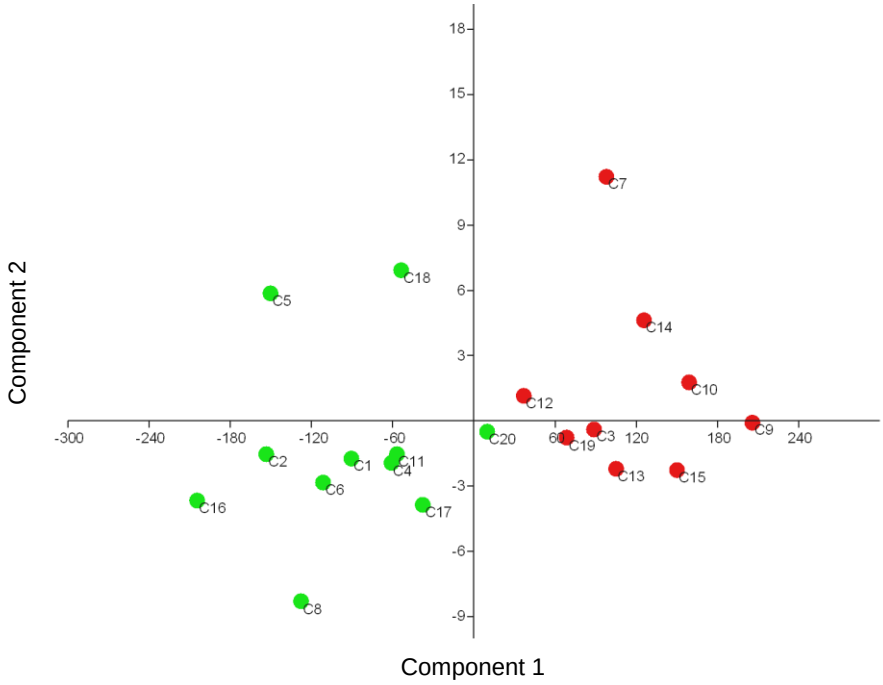

Figure 5. Principal component analysis (PCA) and two separated groups.

CONCLUSIONS

Grape *(Vitis vinifera* L.) is a well-known fruit, botanically a berry, cultivated with different cultivars and clones across the world. The 'Bidaneh Germez' is a local cultivar, consumed as table grape with several diverged clones in Iran. Identifying the grape cultivars and determining their potential is important in improving vineyards and grape quality. The current study indicated a considerable genetic variation among clones that could be used in future breeding programs and clonal selection. According to the results, cluster analysis, discriminant function analysis and principal component analysis showed same results and all clones were assigned in two groups. The low level of divergence might be due to this fact that the studied traits were correlated so further work should be performed to detect more differentiation between the clones. This investigation showed that evaluation of genetic diversity in Iranian grape germplasm, using morphological traits, is highly efficient for future applied and basic researches. According to the results, C7, C10, C12 and C14 clones were found to be distinguished clones and have shown potential to be introduced as promising clones in future breeding programs.

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