INTERNATIONAL JOURNAL OF ENVIRONMENT

Volume-8, Issue-2, 2019 ISSN 2091-2854

Received:1Feb 2019 *Revised:4 Apr 2019* *Accepted: 3Aug 2019*

GENETIC DIVERSITY AMONG OLD LOCAL GRAPE CULTIVARS IN SOUTH OF SYRIA

Bayan M. Muzher* and Ola T. Al-Halabi

General Commission for Scientific Agriculture Research (GCSAR), Sweida, Syria

*Corresponding author: bmuzher@hotmail.com

Abstract

This investigation was carried out in Sweida governorate of Syria from 2014 to 2015. The purpose of this investigation was to estimate the genetic diversity among 17 old local grape cultivars using morphological traits and SSR molecular markers. Principal Component Analysis (PCA) of morphological traits revealed that four main components were accountable for 50.34 % of variance, with Eigen values of 16.77%, 13.31%, 10.59% and 9.67%, respectively. While distribution of cultivars was found significantly dependent of first and second components, which separated Aswad Sharar and Derbly cultivars rather than other studied cultivars. Hierarchical cluster of morphological traits showed high diversity among studied cultivars. On the other hand, molecular characterization was conducted using eight informative SSR primer pairs, polymorphism was detected by seven SSR's primers. As a result, 18 polymorphic alleles were revealed with a polymorphism percentage of 100%, which reflected the genetic variation among studied cultivars. Genetic similarity was achieved, where 0.875 was the highest between Zeiny and Khedry cultivars, while the other two cultivars Ebeidy and AhmarMokamaa showed the lowest genetic similarity as 0.077. Cluster analysis of SSR markers grouped the studied cultivars into three clusters as per Jaccard coefficient. The first cluster grouped Ebeidy, Aswad Helwany and Derbly cultivars together, the second cluster contained two cultivars Beiady and Alb Atair, while the third cluster contained the remaining cultivars. Consequently, the results showed high diversity among studied cultivars, and some morphological traits were useful for cultivar discrimination.

Key words: Grape cultivars, Morphological traits, PCA, SSR.

DOI: http://dx.doi.org/10.3126/ije.v8i2.25507

Introduction

The genus *Vitis* which belongs to the family *Vitaceae* is widely distributed in Asia, Europe and North America (Emanuelli *et al*., 2013). According to historical studies, the genus *Vitis* is native to Qauqaz and the Black sea region, Minor Asia, Iran and Syria.

Many local grapevine cultivars are distributed in different regions in Syria, which amounts to 46,987 ha and produces 212,834 metric tons (MoA, 2016). Sweida governorate has been considered as the main area of grapevine agriculture as there are many evidences indicated that grapevine agriculture has been established since 3,000 BC. Identification and characterization of grape varieties are achieved as per IPGRI (1997) and OIV (2009) descriptors. Boselli *et al*.(2000) studied some of white grape cultivars using morphological traits depending on OIV descriptor. Russo and Liuzzi (2007) characterized two old local genotypes depending on bunch and berry characters, in addition to juice content. Morphological characters were widely used to identify grapevine cultivars in Italy, Spain and Brazil (Pommer *et al*., 2000; Weihl and Dettweiler, 2000; Nieddu *et al*., 2007; Russo *et al*., 2007). Molecular markers were developed to overcome the drawbacks of morphological traits. Since then, SSRs (Simple Sequence Repeats) were applied for identification, and breeding programs among different fruit species (Muzher and Sharaf, 2014). Likewise, the SSR technique was used for grape genetic diversity, genetic identification, genetic relationship, and genetic characterization in different countries such as Italy, Iran, Russia, Romania, and Hungary (Martínez *et al*., 2008;Ramezani*etal*., 2009; Galbacs *et al*., 2009; Ghetia *et al*., 2010).

Conservation and preservation of genetic diversity of old cultivars are the main priority of the twenty-first century agenda of the United Nations (Konrad, *et al*., 2009). Different species were undergone the genetic erosion due to climate change and human activities, which led to the loss of many ancient grape cultivars in different regions (Gago *et al*., 2009). However, old local grapevine cultivars in Syria are extremely important due to their adaptability to environmental conditions and their resistance against several pests, as well as their broad usage in industry field. Most of the cultivars are used for certain industries or just as table cultivars. This investigation was carried out to characterize and estimate the genetic diversity among some local grapevine cultivars using PCA, hierarchical cluster and SSR markers.

Materials and Methods

This investigation was carried out from 2014 to 2015 in farmers' grapevine orchards and at Pome and Grapevine Division- GSAR in Sweida governorate, which is located in south of Syria at 1200-1500 m altitude. The annual average rainfall is 525 mm. The soil is classified as a clay with a low content of organic material and nitrogen, high content of phosphorous and moderate content of potassium, with a pH 6.5 to 6.8.

Seventeen old local grape cultivars which belong to *Vitisvinifera*, wereused for different purposes; 12 white cultivars: Ebeidy (Eb), Shammouty (Sh), Zeiny (Z), Beyadi (B), Ras Al- Asfour (RA), ZeinyRafeea (ZR), Feddy (F), Khedrey (KH), Leyat Al- Kharoof (LK), Derbly (D), Kasoufy (K) and Soury (S) which were used as multi purposes cultivars, three red cultivars: AhmarMkammaa (AM), Alb Al-Tayr (AT) and AhmarDoumany (AD) which were used as table cultivars; and two black cultivars: Aswad Helwany (AH) and Aswad Sharar (Ash) which are used for wine-making.

Morphological characterization

45 morphological and agronomic traits were studied according to IPGRI (1997) and OIV (2009) descriptors including the following:

- Young shoots (4 traits): Form of tip, anthocyanin coloration of shoot tip, density of prostrate hairs on tip, and density of erect hairs on tip.
- Shoots (11 traits): Color of dorsal side of internode, color of ventral side of internode, color of dorsal side on node, color of ventral side on node, density of prostrate hairs on internode, density of erect hairs on internode, density of prostrate hairs on node, density of erect hairs on node, number of consecutive tendrils, tendrils length, and number of inflorescences per shoot.
- Young leaf (5 traits): Color of upper surface, density of prostrate hairs between veins, density of erect hairs between veins, density of prostrate hairs on main veins, and density of erect hairs on main veins.
- Mature leaves (10 traits): Shape of blade, blade length, blade width, number of lobes, general shape of petiole sinus, anthocyanin coloration of main veins on upper side of blade, petiole sinus limited by veins, shape of upper lateral sinus, shape of teeth, and length of petiole compared to middle vein.
- Bunch (5 traits): Length of bunch, width of bunch, density of bunch, length of peduncle, and weight of bunch.
- Berries (10traits): Length of berry, width of berry, berry shape, skin color, weight of berry, weight of seeds, pedicel length, must yield, total soluble solids, and tetratable acidity.

Data analysis

Means for each trait were used to perform a Principal Component Analysis (PCA) using SPSS 17 software, and Hierarchical cluster analysis using unweighted pair group method with arithmetic mean (UPGMA) based on Euclidean distance using PAST software.

Molecular characterization

Young green leaves of the seventeen grapevine cultivars were collected for DNA extraction using CTAB protocol (Porebski*et al*., 1997). DNA estimation was carried out using bio-photometer plus (Eppendorf, Germany). Eight informative grape SSR primer-pairs were used (Table 1).The PCR reaction was carried out using 1 unit Go-*Taq*polymerase in 25 µl reaction volume containing 1X PCR buffer, 2 mM of each dNTPs,

10 P mol of forward primer, 10 P mol of reverse primer and 50 ng genomic DNA. Hot Starting and denaturation step at 95 °C for 5 min was followed by 35 cycles at 95 °C for 45 seconds, annealing temperatures according to each primer sets (45 Seconds) depending on melting temperature as shown in Table 1, and primer extension at 72 °C for 1 min, then followed by an extension cycle for 5 min and cooling at 4 °C.

Table1: List of SSR flanking primers. The corresponding melting temperatures, and the expected Allele size

SSR Name	Primer sequence (5'------3')	Tm
Vmc8A7 F	GCAGCAACTCTCTTACACACCG	68
Vmc8A7 R	GTGGGAGCACTGGTTGCTTTAG	68
Vmc8B12 F	AGAGCACGCTGGACCTTCTTC	66
Vmc8B12 R	GCACTGCGCGATTTCACTC	60
Vmc8D11 F	TGTTGAAGCTAGCATTGTCTCC	64
Vmc8D11 R	ATTCGTCTTTATGCCCATTGTT	60
Scu5vv F	CAAGCAGTTATTGAAGCTGCAAGG	70
Scu5vv R	TCATCCATCACACAGGAAACAGTG	70
Scu6vv F	CCTAATGCCAGGAAGGTTGC	62
Scu6vv R	CCCTAGTCTCTCTACCTATCCATG	72
Scu8vv F	CGAGACCCAGCATCGTTTCAAG	68
Scu8vv R	GCAAAATCCTCCCCGCCTACAAGTC	70
Scu15vv F	GCCTATGTGCCAGACCAAAAAC	66
Scu15vv R	TTGGAAGTAGCCAGCCCAACCTTC	74
Scu16vva F	CAAAGACAAAGAAGCCACCGAC	66
Scu16vva R	ACCCTCTAAAGCACACACAGGAAC	72

Tm: Melting temperature for each primer sequence

Amplified PCR products were detected on 2% agarose gel and visualized by Gel documentation system (VILBER LOURMOT Germany). The amplified alleles were scored either as present (1) or absent (0). Alleles of the same mobility were scored as identical. The genetic similarity coefficient (GS) between two genotypes was estimated according to Jaccard coefficient (Jaccard, 1908). Dendrogram was clustered using the unweighted pair-group method with arithmetic averages (UPGMA) based on SSR data.

Results

Principal Component Analysis (PCA)

Using PCA over the correlation matrix of the 43 morphological studied characters (Number of consecutive tendrils and length of petiole compared to middle vein were excluded due to the similarity among studied cultivars), the first 4 principal components accounted 50.34% of variance with Eigen values of 16.77%, 13.31%, 10.59% and 9.67% respectively (Figure 1).Variability within the highest Eigen vectors in each PC were as follows:

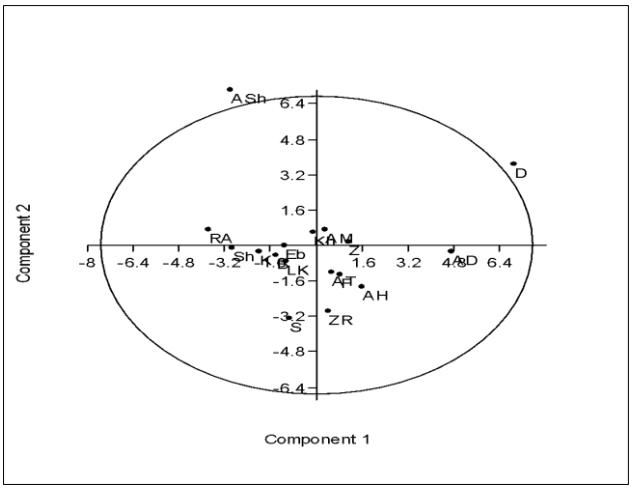

Figure 1: Distribution of studied cultivars according to the first and the second principal components of the studied traits.

PC1: The most important characters are correlated with a high mean value of density of prostrate hairs between veins (0.90), density of prostrate hairs on main veins (0.83), density of erect hairs between veins (0.77), density of prostrate hairs on tip (0.74), density of prostrate hairs on internode (0.72), erect hairs on internode (0.62), density of erect hairs on tip (0.59), density of erect hairs on main veins (0.57), tendril length (0.54), anthocyanin coloration of main veins on upper side of blade (0.54) and leaf shape (0.54).The characters density of prostrate hairs on tip and erect hairs on internode discriminated D, Eb and AD cultivars, while the character density of prostrate hairs on main veins differentiated D, AD and ASh cultivars, the character density of prostrate hairs between veins discriminated D, ASh and AH cultivars, the character anthocyanin coloration of main veins on upper side of blade distinguished RA, ZR and K, and the character LSh differentiated Kh cultivar.

PC2: Mature leaf width (0.84), tetratable acidity (0.74), mature leaf length (0.69), berry width (0.53) and general shape of petiole sinus (0.51) were positively associated, whereas color of dorsal side of node (-0.66) and bunch density (-0.55) were negatively correlated. PC2 discriminated ASh cultivar which revealed the highest leaf length and width, and very loose bunch density; on the other hand, S cultivar was distinguished by the lowest leaf length and width, in addition to very small berries. In addition, Sh cultivar discriminated by tetratable acidity, while E cultivar distinguished by very dense bunch.

PC3: Included seven characters which were positively correlated (Berry pedicel length 0.71, peduncle length, 0.64, berry length, 0.6, dorsal side of internode, 0.59, berry width, 052, and density of erect hairs on node with color of dorsal side of internode 0.51). PC3 discriminated Kh cultivar by short to medium peduncle length. Eb and D cultivars distinguished by the completely green color of dorsal side of internode.

PC4: Two characters were included in PC4; the form of the young shoot tip (0.61) was positively correlated while seed weight was negatively correlated (-0.63). PC4 discriminated F and RA cultivars by fully open and

wide open shoot tip respectively, while the other cultivars ranged between closed to half open shoot tip, and distinguished K cultivar by low to medium seed weight, at the time that all other studied cultivars ranged between medium to very high seed weight.

Hierarchical cluster

The studied cultivars were clustered into two clusters and three separate cultivars "ASh, D and S" (Figure 2). The first cluster contains four white cultivars, which grouped into two sub clusters, the first one contains K and Eb, which has the same berry shape, berry skin color, and berry pedicel length, and the second sub cluster includes Sh and RA cultivars, which revealed the same berry length and width, berry shape and seed weight. The second cluster divided into four sub clusters, AM laid in a separate sub cluster, while the second sub cluster contains three cultivars " AD,F and AH". The third sub cluster includes four cultivars, which grouped into two groups "Z and ZR which have the same berry shape, length, width, skin color and bunch density, and AT with LK which have the same berry length and weight, bunch width and weight, peduncle weight, and leaf shape ". The fourth sub cluster contains only two cultivars B and Kh cultivars which revealed the same berry width, length and shape, number of bunches, and the form of young shoot tip.

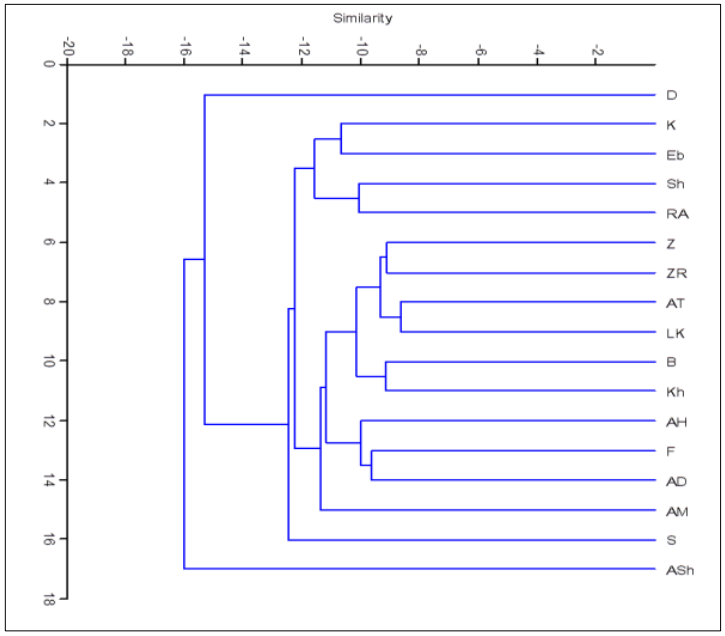

Figure 2: Hierarchical cluster analysis of studied cultivars revealed by morphological traits.

Molecular characterization

In this study 8 SSR primer pairs were used, seven out of them were able to detect the polymorphism which revealed 18 polymorphic alleles, with polymorphism percentage 100%, this reflected the genetic variation among studied cultivars. The highest number of amplified alleles was four with Vmc8D11 and Scu8vv (Figure 3), while the other primer pairs revealed two polymorphic alleles. The average number of alleles per

primer across the seventeen cultivars was 2.57 alleles per locus (Table 2). The size of all alleles exhibited by these polymorphic loci ranged between 130- 179 bp.

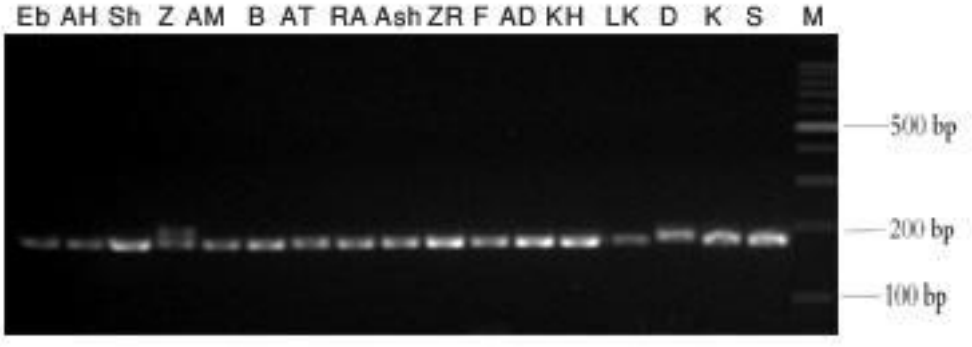

Figure 3: SSR profile of the 17 old local grape cultivars amplified with SSR primerScu8vv. M: 100 bp ladder marker.

Table 2.Total number of allelic forms obtained among the seventeen grape cultivars.

Primers	Total allelic forms	Polymorphic alleles	Polymorphism %	Allele size/bp
Vmc8A7	2	2	100	130-140
Vmc8D11	4	4	100	130-135-140-155
Scu5vv	2	2	100	135-145
Scu6vv	2	2	100	161- 172
Scu8vv	4	4	100	146- 154-161-179
Scu15vv	2	2	100	159-165
Scu16vva	2	2	100	170-178
Total	**18**	**18**	**100**	

The genetic similarity based on Jaccard coefficient for the seventeen local grape cultivars ranged from 0.071 between B and D cultivars, to 0.875 between Z and Kh cultivars as shown in Table 3. Eb cultivar revealed low genetic similarity (less than 0.50) with all studied cultivars except AH and D, while ZR showed high genetic similarity with most of studied cultivars (11 cultivars). Dendrogram based on SSR data using the UPGMA clustered the seventeen grape cultivars into three clusters (Figure 4), the first cluster contains three cultivars; Derbly cultivar which clustered in a separate sub cluster and Eb with AH which grouped in the second sub cluster. The second cluster includes two cultivars B and AT, while the third cluster grouped all the other cultivars into four sub clusters; The first sub cluster contains only one cultivar AM, the second one includes three cultivars, the third includes two cultivars and the fourth sub cluster contains four cultivars.

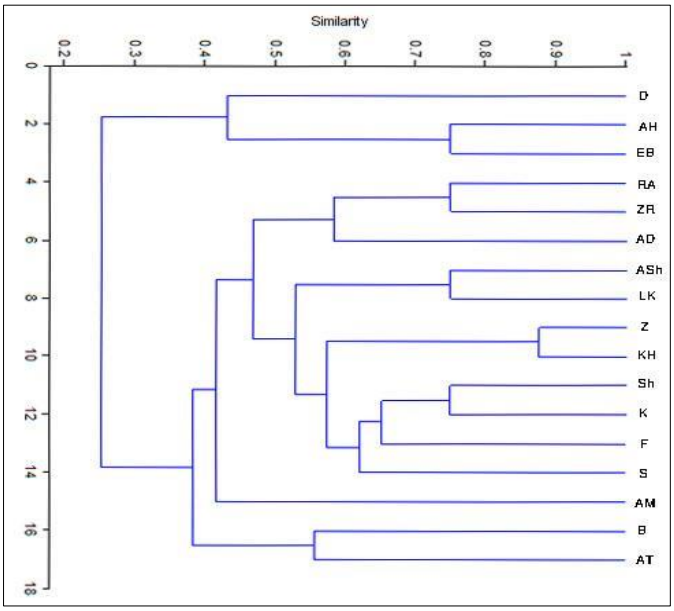

Figure 4: Cluster analysis based on SSRs data.

Table 3. Genetic similarity among studied cultivar depending on Jaccared coefficient.

	Eb	AH	Sh	Z	AM	B	AT	RA	ASh	ZR	F	AD	Kh	LK	D	K	S
Eb	1.00																
AH	.750	1.00															
Sh	.167	.167	1.00														
Z	.364	.364	.500	1.00													
AM	.077	.167	.556	.250	1.00												
B	.167	.167	.273	.500	.273	1.00											
AT	.167	.167	.556	.500	.273	.556	1.00										
RA	.167	.273	.400	.364	.400	.400	.400	1.00									
ASh	.400	.556	.400	.364	.400	.167	.167	.556	1.00								
ZR	.167	.167	.556	.500	.273	.556	.556	.750	.400	1.00							
F	.273	.273	.556	.500	.400	.273	.273	.556	.556	.556	1.00						
AD	.154	.154	.500	.231	.500	.250	.500	.667	.364	.500	.364	1.00					
Kh	.400	.400	.556	.875	.273	.556	.556	.400	.400	.556	.556	.250	1.00				
LK	.273	.400	.556	.500	.556	.273	.273	.750	.750	.556	.750	.500	.556	100			
D	.500	.364	.250	.231	.154	.071	.071	.250	.500	.250	.500	.231	.250	.364	1.00		
K	.273	.273	.750	.667	.400	.400	.400	.556	.556	.750	.750	.364	.750	.750	.364	1.00	
S	.167	.167	.556	.500	.556	.556	.273	.400	.400	.556	.556	.250	.556	.556	.250	.750	1.00

Discussion

Syria is one of the most important countries concerning old local grape cultivars that revealed high diversity among and within cultivars classified as manufactured and table grapes. Genetic diversity depends on the application of morphological and molecular characterization to establish integrated knowledge about distributed species. Multivariate analysis such as Principal Component Analysis (PCA) and hierarchical cluster are widely used to resolve complex problems related to definition and classification in viticulture and other crop accessions (Manjunath*et al*., 2007; Borges *et al*., 2008). The present results shows high genetic diversity among old local grape cultivars in south of Syria depending on the main morphological characters, as described in IPGRI and OIV descriptors, in addition to SSR molecular markers, which contribute to build a beneficial platform around the origin and description for grapevine germplasm.

Principal Component Analysis (PCA) revealed that thirteen out of 45 characters contain hairiness, shoot tip, leaf, bunch density, berry size and seeds characters probably formed the most important characters to

discriminate between studied cultivars. Thus, PC1 and PC2 significantly separated ASh and D cultivars from other studied cultivars. These results confirmed that principal component analysis is a powerful tool in grape diversity studies, and that the ability to use informative traits and reduce the number of studied traits for evaluation purposes, as mentioned by Rakonjac*et al*. (2014). Lamine*et al*. (2014) who stated that PCA is a useful assay to discriminate among grape cultivars depending on a few differentiation characters. However PCA is widely used in grape studies (Burin *et al.*,2010; Leao*et al*., 2010; Vilanova*et al.,* 2013; Chalak*et al*.,2016).

The results showed that hierarchical cluster discriminates between cultivars depending on some morphological traits especially berry traits (berry shape, berry length and width, berry weight, and seed weight), in addition to bunch width and weight, leaf shape and the form of shoot tip, which was in agreement with (Chalak*et al*., 2016). While the color or chemical contents weren't efficient to differentiate among studied cultivars. Ampelographic characters revealed clear morphological discrimination among Syrian old local grape cultivars. However, hierarchical cluster successfully used in genetic diversity studies on different species (Aljane and Ferchichi, 2009; Leao*et al*., 2011; Al-Halabi and Muzher, 2015).

Molecular Characterization

Cluster analysis revealed high tendency between morphological traits and molecular study, some of informative traits like anthocyanin coloration of shoot tip, number of lobes, and the color of upper surface in young leaves, somehow played an important role in clustering the studied cultivars into main clusters. On the other hand, another traits of berry, hairiness, and some bunch traits contributed in grouping the studied cultivars into separated sub clusters, which prove a coherent relation between morphological and molecular characterization to differentiate the studied cultivars. The obtained data reflected the ability of SSR marker to discriminate between individuals and identify the genetic diversity among the studied grape cultivars. As well, application of SSR markers in the grapevine identification have increasingly become the ideal tool for genetic studies due to their high polymorphism and simple detect ability (Akkak *et al*., 2010). Ramezani *et al*.(2009) stated that the polymorphism percentage ranged between 0.658 -0.880 and the number of alleles per locus ranged between 6 to 11 alleles when they compared among thirty for grapevine accessions from Iran with six accessions from Russia and three accessions from USA. Wang *et al*.(2015) found that the grouping of studied cultivars into different clusters may have been because of the collection of these cultivars from different regions and may have been end to uniformity through a long time to adapt to climate and human activities through the long-time of cultivation.

Conclusion

Our research shows the genetic diversity as revealed by principal component analysis and hierarchical cluster depending on morphological characters and the polymorphism of SSR marker provided a sufficient information about the old local grapevine cultivars, which demand serious preservation and conservation management.

References

Al-Halabi, O. and Muzher, B., 2015. Genetic diversity of some apple cultivars in the south of Syria based on morphological characters. *International Journal of Environment*, 4 (4), 86- 99.

Akkak, K., Boccacci, P., Lacombe, T. and Botta, R., 2010. Relationship and genetic diversity of grapevine (*Vitis vinifera* L.) grown in Algeria and in Mediterranean Basin, Università degli Studi di Torino, p. 3.

Aljane, F. and Ferchichi, A., 2009. Assessment of genetic diversity among some Southern Tunisian Fig (*Ficus carica* L.) cultivars based on morphological descriptors. *Jourdan Journal of Agriculture Sciences*, 5 (1), 1-16.

Annual statistical abstract. 2016. Ministry of agriculture. Damascus. Syria.

Borges, R. M. E., Goncalves, N. P. S., Gomes, A. P. O. and Alves, E. O. S., 2008. Divergencia phenotypica entre acessos de uvas de mesa no semi-aridobrasileirio. *Pesq. Agropec. Bras.*, 43, 1025-1030.

Boselli, M., Iannini, C., Corso, C., Monaco, A., Iannelli, D. and Cottone, C., 2000. Analysis of variability in the Aglianico grapevine (*vitis vinifera*) in Campania. *Acta Horticulturae,* 528, 47-50.

Burin, V. M., Falcão, L. D., Gonzaga, L. V., Fett, R., Rosier, J. P. and Marilde Terezinha Bordignon-luiz, M. T.,2010. Colour, phenolic content and antioxidant activity of grape juice. Ciênc. *Tecnol. Aliment., Campinas*, 30(4), 1027-1032.

Chalak, L., Touma,S., Rahme, S.,Azzi, R., Guibereau, F. and Touma JA., 2016. Assessment of the Lebanese grapevine germplasm reveals a substantial diversity and a high potential for selection. 39th World Congress of Vine and Wine, p 6.

Emanuelli, F., Lorenzi, S., Grzeskowiak, L., Catalano, V., Stefaniani, M., Troggio, M., Myles, S., Martinez-Zapater, J., Zyparian, E., Moreira, F. and Grando, M., 2013. Genetic diversity and population structures assessed by SSR and SNP markers in large germplasm collection of grape. *BMC Plant Biol*., 13 (1),1-17.

Gago, P., Santiago, J.L., Boso, S., Alonso-Villaverde, V., Grando, S. and Martinez. C., 2009. Biodiversity and characterization of twenty-two *Vitis vinifera* L. cultivars in the Northwestern Iberian Peninsula. *Am J Enol Viticult*,60, 293-301.

Galbacs, Z., Molnar,S., Halasz,G., Kozma,P., Haffmann, S., Kovaks, L.,Veres, A.,Galli,Z.,Szoke,A.,Heszki,L. and Kiss, E., 2009.Identification of grapevine cultivars using microsatellite –based DNA barcodes. *Vitis,* 48 (1), 17-24.

Ghetia, L. G., R. Motoc,M., Popescu,C. F.,Barbacar,N., Ianco,D., Cnstantinescu,C .and Barbarii,L. E., 2010. Genetic profiling of nine grapevine cultivars from Romania based on SSR markers. *Romanian Biotechnological Letters,*15 (1), 116-124.

IPGRI, 1997. Descriptors for Grapevine (*Vitis* spp.). International Plant Genetic Resources Institute, Rome, Italy. p.58.

Jaccard, P. 1908. Nouvelles recherches sur la distribution flora. *Bull. Sac. Nat.,*44, 223-270.

Konrad, H., Schmid, J., Lindner,B., Prüm, S., Rühl, E., Weihl, T. and Porten, M., 2009. How To Maintain Genetic Diversity Of Traditional German Varieties. *Acta Horticulturae*, 827, 199-202.

Lamine, M., Zemni, H., Ziadi, S., Chaabane, A., Melki, I. Mejri, S. and Zoghlami, N., 2014. Multivariate analysis and clustering reveal high morphological diversity in Tunisian autocthonous grapes (*Vitis vinifera*): Insights into characterization, conservation and commercialization. *J. Int. Sci*, *VigneVin*, 48,111-122.

Leao, P. C., Cruz, C. D. and Motoike, S. Y., 2010. Genetic diversity of a Brazilian wine grape germplasm collection based on morphoagronomic traits. *Rev Bras Frutic* – SP,1164-1172.

Leao, P. C., Cruz, C. D. and Motoike, S. Y., 2011. Genetic diversity of table grape based on morphoagronomic traits. *Scientia Agricola*, 68 (1), 42-49.

Manjunath, T., Bisht, I. S., Bhat K. V. and Singh, B. P., 2007. Genetic diversity in barley (*Hordium vulgare* L.) landraces from Ulttaranchal Himalaya of India. *Gen. Resour. Crop Eval*, 54, 55-65.

Martínez, L., Cavagnaro,P.,Boursiquot, J. M. and Agüero,C., 2008. Molecular Characterization of Bonarda-type Grapevine (*Vitis vinifera* L.) Cultivars from Argentina, Italy, and France. *Am. J. Enol. Vitic*, 59(3), 287-291.

Muzher, B. M. and Sharaf, A. N., 2014. Molecular characterization of some Syrian pears (*Pyrus syriaca* Boiss) genotypes using SSR marker. *Jordan Journal of Agricultural Sciences*, 10 (4), 661-672.

Nieddu, G., Nieddu, M., Cocco,G.F., Erre, P. and Chessa, I., 2007. Morphological and Genetic Characterization of The Sardinian 'Bovale' Cultivars. *Acta Horticulturae*, 754,49-54.

OIV. 2009. Code des caracteres descriptifs des varieties et especes de vitis. Organisation Internationale de la Vigne et du Vin, Paris, France.

Pommer, C.V., Ferri ,C.P., Martins, F.P., Passos, I.R.S., Terra, M.M., Pires, E.J.P., 2000. Agronomic and phenological characterization of grape genotype kept in collection at Jundiaí. *Brazil* (abstract), 523,211-223.

Porebski, S., Bailey, G. L. and Baum, B. R., 1997. Modification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. *Plant Molecular Biology Reporter*, 15(1), 8-15.

Rakonjac, V., Korac, N., Todic, S., Medic, M., Belsic, Z, I. Kuljanicic, I., Ivanisevic, D. and Popov, M., 2014. Genetic diversity of a Serbian grapevine germplasm collection based on morphoagronomic characteristics. *Genetika*, 46 (3), 719-730.

Ramezani, A., Haddad, R. and Dorostkar, M., 2009. Genetic diversity of grapevine accessions from Iran, Russia and USA using microsatellite markers. *Pak. J. Biol. Sci.*, 12, 152-157.

Russo,G. And Liuzzi, V., 2007. Evaluation, Conservation and Valorization of Local 'Maruggio' Grapevine In Southern Italy. *Acta Horticulturae*, 754,55-58.

Russo, G., Liuzzi,V. And D'Andrea, L., 2007. Ampelographic Characteristic and Wine Composition Of Three Grapevines Varieties With Black Grapes. *Acta Horticulturae*, 754, 59-64.

Vilanova, M., Genisheva, Z., Graña, M. and Oliveira, J.M., 2013. Determination of odorants in varietal wines from international grape cultivars (*Vitis vinífera*) grown in NW Spain. *S. Afr. J. Enol. Vitic.*, 34(2), 212- 222.

Wang, L., Zhang, J., Liu, L., Zhang, L., Wei, L. and Hu, D., 2015.Genetic diversity of grape germplasm as revealed by microsatellite (SSR) markers. *African Journal of Biotechnology*, 14 (12), 990-998.

Weihl, T. and Dettweiler, E., 2000. Differentiation and identification of 500 grapevine *(Vitis* L.) cultivars using notations and measured leaf parameters, in Bouquet, A and Boursiquot, J.-M. (Eds.). *Proceedings of the Seventh International Symposium on Grapevine Genetics and Breeding.* Montpellier, France. *Acta Horticulturae*, 528, 37-44.