AHS
Advances in Horticultural Science

Genetic and ampelographic characterization of grapevine accessions maintained in the Lebanese national collection

A. Chehade [1], L. Chalak [2], J. Merheb [2], A. Elbitar [3], E. Rmeily [4], N. Madi [2], M. Massaad [2 (*)]

[1] Lebanese Agricultural Research Institute, Tal Aamara, Department of Plant Biotechnology, Plant Genetic Resources Unit, P.O. Box 287, Zahleh, Lebanon.

[2] The Lebanese University, Faculty of Agronomy, Dekwane, Beirut, Lebanon.

[3] Lebanese Agricultural Research Institute, Tal Aamara, Department of Plant Biotechnology, Plant Tissue Culture Unit, P.O. Box 287, Zahleh, Lebanon.

[4] Lebanese Agricultural Research Institute, Tal Aamara, Department of Pomology and Viticulture, P.O. Box 287, Zahleh, Lebanon.

(*) **Corresponding author:**
mmassaad1@gmail.com

Abstract: Safeguarding grapevine biodiversity is one of the main concerns in viticulture today. Management of *ex situ* collections requires a comprehensive characterization of the conserved germplasm to provide basic material for selection and breeding programs. In this study, the local grapevine germplasm conserved at the national collection of the Lebanese Agricultural Research Institute and composed of 43 accessions,was submitted to a genetic and ampelographic study. Nine ISSR primers, previously developed in grapevine, were used in this study. These primers generated a total of 51 bands, of which 77.7% were polymorphic allowing the differentiation of 41 genetic profiles *vs.* one case of synonymy that was recorded among three accessions. Ampelographic characterization was conducted using a set of 33 descriptors established by the International Office of Vine and Wine and related to leaf, bunch and berry. Principal component analysis identified 12 descriptors i.e. veraison date, maturity date, berry length, upper and lower vein pigmentation, bunch density, bunch weight, sugar content at harvesting, flesh of juiciness, berry weight, flesh firmness and color skin, as being the most discriminating descriptors. The correlation between the ISSR clustering and the ampelographic one was not significant (r=0.26) because of the divergence of accessions groups, except for the three accessions synonymy case which was confirmed in both dendrograms. Finally, this comprehensive evaluation of the existing local gene pool of grapevine revealed a substantial diversity. It would further allow the promotion of the valuable accessions directly through multiplication scheme, and their sustainable utilization in genetic improvement programs.

1. Introduction

One of the main concerns for public research in viticulture today is the

need to safeguard grapevine biodiversity by establishing well managed national repositories to ensure germplasm availability to breeders, researchers and farmers (Nass *et al.*, 2012). There are presently around 130 grapevine germplasm collections across the word (Dettweiler *et al.*, 2000). One of the first grapevine germplasm collections is the one established in Spain in 1950, which contains more than 1066 accessions (Ortiz *et al.*, 2004). In France, more than 2,200 varieties originally collected from 35 countries are grouped together at the National Institute of Agronomic Research (Tessier *et al.*, 1999). Similar collections of grapevine were also established USA and in Latin America containing grapevine accessions collected all over the world (Martinez *et al.*, 2003). Grapevine is one of the oldest fruit crops growing in the Eastern Mediterranean region including Lebanon (Zohary, 2003). The country was among the first worldwide to have implemented vineyards which gradually became a traditional culture in Lebanon for the production of both table grapes and wine (Chalak *et al.*, 2016). Today viticulture occupies the eighth rank in the agricultural sector in the country, with a total production area of 9,240 hectares and an annual production of about 89,000 tons of table grapes (FA0, 2010; FAOSTAT, 2019) *vs*. 3,000 hectares dedicated to wine grape with an annual production of approximately 10,000 tones (Rahal, 2015). In addition, about 800 hectares of vineyards are intended for the production of Lebanese Arak (Roby, 2003). Commercial plantations of table grapes have long been constituted of four local varieties, commonly named 'Tfayfihi', 'Baitamouni', 'Maghdouche' and 'Obeidy'. More recently, the new plantations in the country are mostly constituted of improved varieties imported from Europe and the United States. Around 77 varieties are imported to Lebanon. Nevertheless, the major share went mostly to 'Cabernet Sauvignon Blanc', 'Syrah', 'Viognier', 'Chardonnay' and 'Merlot' which represent 65% of the total saplings quantities imported during 2012-2014 (Tabaja, 2015). The long history of viticulture in Lebanon suggests the existence of a large indigenous germplasm associated to a wide range of traditional varieties that are well adapted to the various agro-climatic conditions of the country (Chalak *et al.*, 2016). In Lebanon and despite the increasing interest on the conservation and characterization of plant genetic resources in general, only few studies have addressed the local germplasm of grapevine, although it is threatened by various anthropogenic pressures including in particular the progressive replacement of local varieties by more advantageous improved foreign varieties that are regularly imported into the country (Riachi, 1998; Madi, 2007; Chalak *et al.*, 2016; Rahme, 2016). To face the threat of genetic erosion and avoid the loss of traditional germplasm, the Lebanese Agricultural Research Institute (LARI, Tal Amara station) established in 1998 a national grapevine collection containing numerous local accessions collected from different production areas across the country (Madi, 2007). Within the environmental change and the challenging predictions for the Eastern Mediterranean region including Lebanon (Santillán *et al.*, 2019), there is a growing need to address the local genetic resources such as traditional varieties which are recognized to be more adapted to harsh conditions and having better tolerance to various biotic and abiotic stresses (FAO, 2015). Added to this, the wine industry is witnessing a new trend towards the utilization of the local traditional varieties in the production of typical and well prized wines. Therefore clonal selection, with the respect to specific traits, has become the most important way to improve the quality of grape cultivars.

As a consequence, there is a need for reliable and precise methods of clonal characterization for further use by breeders, nurseries and industries (Galet, 1998; Moreno *et al.*, 1998). Ampelography is a scientific methodology that has long been the single method used for the characterization of grapevine phenotype, based on the description of different morphological, phenological and pomological characters (Galet, 1998; Sabir *et al.*, 2009; Laucou *et al.*, 2011). In addition to the ampelograhic description, in order to discriminate the varieties, synonyms, homonyms and the variation among the accessions, molecular methods has become more frequently used especially markers based on Polymerase chain reaction techniques (Riaz *et al.*, 2012; Madhumati, 2014). Among the molecular approaches, the ISSR (Inter Sequence Simple Repeat) has being evaluated for its usefulness in grapevine cultivar identification and in assessing genetic diversity of grapevine germplasms (Moreno *et al.*, 1998; Dhanorkar *et al.*, 2005; Santiago *et al.*, 2005; This *et al.*, 2006; Sabir *et al.*, 2009; Seyedimoradi *et al.*, 2012; Choudhary *et al.*, 2014; Castro *et al.*, 2016).

This study aimed to characterize the 43 local grape accessions preserved at the Lebanese National Collection of Grapevine at LARI using both ISSR markers and ampelographic descriptors. This work was conducted in an attempt to evaluate the diversity of the local germplasm for further actions of conserva-

tion and sustainable utilization of grapevine genetic resources in diversification strategies.

2. Materials and Methods

Plant material

A total of 43 accessions of traditional grapevine varieties growing in the Lebanese national collection located in Tal Amara, Bekaa (N 33° 49' 59", E 36° 0' 0", 908 m a.s.l.) established in 1998, were considered in this study (Table 1). Accessions initially consisted of farmer's local varieties surveyed and collected across the Bekaa region (Lebanon). The vine cultivars were grafted with four duplications on B41 rootstocks which were planted directly in the collection land a year before the grafting process under the same growing conditions using horizontal trellis training system with the spaces 2.75 x 2.75 m. The site of plantation is characterized by a clay fertile soil and an average of precipitation ranging between 600 to 700

mm/year. For each accession, young leaves were sampled from a single selected plant and stored at -20°C for the DNA extraction and molecular analysis. On the other hand, samples of ten leaves, five clusters and 20 fully mature berries were taken from the same selected plant of each accession between August and October 2020 to undergo the ampelographic characterization.

Molecular characterization

Total genomic DNA was extracted from 300 mg of young leaf tissue after been crushed to a fine powder with liquid nitrogen and stored at -20°C until use. DNA extraction of grapevine landraces was performed according to (Doyle and Doyle, 1990) protocol with minor modifications (Kafkas *et al.,* 2006). DNA concentration was measured by Biospec-nano (Shimadzu corp) and stored at -20 C until further analysis. Nine ISSR primers: UBC807, UBC812, UBC815, UBC816, UBC818, UBC828, UBC864, UBC868 and UBC880 (Table 2) were selected and

Table 1 - Sites of 43 collected samples of traditional cultivars grapevine growing in the national germplasm collection at the Lebanese Agricultural Research Institute (LARI-Tal Amara)

Cultivars growing in the national germplasm collection at LARI	Sites of collected samples	Region
Ainouni	Tamnin	Bekaa Central
Al Mir, Ari, Ashlamish Ahmar, Asouad, Bakhouri, Bourji, Bzaz Al-Anzi, Maksasi, Mariami, Moukh Al-Baghel, Oubaidi, Souri	Nabi Ayla	Bekaa Central
Amarcani Abiad, Amarcani Asouad, Baitamouni, Baitamouni Asouad, Gbaii, Maghdoushi, Nih, Tfaifihi, Zaghzaghani	Niha	Bekaa Central
Amarcani Souri	Ablah	Bekaa Central
Arasani, Souri Zahlaoui	Saghbin	Bekaa Ouest
Ashlamish Abiad, Rins	Rayak	Bekaa Central
Houzairani, Khoudri, Zaitouni	El-Rafid	Bekaa Ouest
Karn Al Ghazal, Maghdoushi Mgaddad, Misky, NABI, Raha, Samaani Abiad, Samani Asouad, Souri Asouad, Souri Mhayyar, Yafaoui, Asali, Boulbouli Asouad, Gnoubi	Unknown Site	Bekaa

Table 2 - The resulting discriminating power, number of polymorphic and monomorphic, the size of bands of ISSR primers, percentage of polymorphism and the polymorphic information content

Primer	Size range (bp)	Number amplified bands	Number of polymorphic bands	Number of monomorphic bands	Polymorphism (%)	PD	PIC
UBC812	350-1000	7	6	1	85.7	0.64	0.33
UBC818	300-700	7	6	1	85.7	0.87	0.59
UBC815	400-650	4	3	1	75	0.62	0.6
UBC828	550-800	3	2	1	66.6	0.44	0.45
UBC864	280-700	7	6	1	85.7	0.9	0.48
UBC868	225-750	4	2	2	50	0.3	0.27
UBC816	300-900	5	4	1	80	0.64	0.64
UBC807	250-800	7	6	1	85.7	0.84	0.47
UBC880	125-600	7	6	1	85.7	0.85	0.58
Total	125-1000	51	41	10	-----	-----	-----
Mean		5.6	4.5	1.1	77.7	0.67	0.49

used in this study based on their good results for amplification and high power of discrimination on grapevine (Seyedimoradi *et al.*, 2012).

The ISSR (Inter Simple Sequence Repeat) amplification was carried out as per (Sabir *et al.*, 2009), using 20 µl reaction mixture containing 2 µl of 10 X PCR buffer (750 mM Tris-HCl pH 8.8; 0.1% Tween-20), 0.2 mM dNTP, 2 mM MgCl$_2$; 200 mM Primer; 50 ng genomic DNA and 1 U Taq DNA polymerase (MBI Fermentas Inc, Hanover, MD-21076, USA). The amplification program consisted of 94°C for 4 minutes fol-

lowed by 35 cycles of 94°C for 1minute, 52°C for 1.05 minutes, 72°C for 2 minutes and a final extension of 8 minutes at 72°C. The amplified DNA was visualized on the 3% agarose gel.

Ampelographic characterization

Thirty-three major morphological descriptors selected from the descriptors list previously developed by the OIV (Organisation International de la Vigne et du Vin) (OIV, 2017) were used in this study (Tables 3, 4, 5). They include 17 qualitative descrip-

Table 3 - Descriptive statistics analysis of quantitative morphological descriptors of 43 grapevine accessions

Descriptors	Mean	Minimum	Maximum	Least significant difference (LSD)
Leaf length (cm)	9.77	7.84	11.26	1.06
Leaf width (cm)	13.29	11.4	15.74	1.35
Petiole (cm)	7.92	4.24	11.12	0.99
Number of lobes	4.89	3.00	5.00	0.09
Bunch weight (g)	576	203	1186	121
Bunch length (cm)	21.76	14.93	30.6	3.60
Bunch width (cm)	13.62	8.15	21.23	2.33
Berry weight (g)	5.7	0.94	9.95	0.47
Berry length (cm)	2.24	1.50	3.40	0.12
Berry width (cm)	1.75	1.16	2.54	0.08
Pedicel length (cm)	1.01	0.49	1.53	0.11
Seed number	2.51	1.00	4.00	0.16
Acidity at veraison (g/L)	2.82	1.35	6.10	0.89
Brix at veraison (°Brix)	15.67	11.2	18.00	1.77
Acidity at harvesting (g/L)	1.98	1.2	3.10	0.43
Brix at harvesting (°Brix)	18.38	13.6	23.00	2.13

Table 4 - Leaf descriptors notation and their frequency distribution among the 43 studied grapevine accessions

Descriptors -Leaf	Notation and frequency (Number of accessions)
Shape of blade	Wedge-shaped (31) - pentagonal (3) - circular (9)
Shape of teeth	Both side straight (3) - both side convex (1) - mixture between both side straight and both side convex (39)
Anthocyanin coloration of main veins on upper side	Absent (25) - only on the petiolar point (10) - up to the 1st bifurcation (5) - up to the 2nd bifurcation (2) - beyond the 2nd bifurcation (1)
Anthocyanin coloration of main veins on lower side	Absent (16) - only on the petiolar point (17) - up to the 1st bifurcation (5) - up to the 2nd bifurcation (1) - beyond the 2nd bifurcation (4)
Prostrate hairs on main veins on lower side	None or very low (21) - low (11) - medium (7) - high (4)
Prostrate hairs on main veins on upper side	Absent (40) - present (3)
Density of prostrate hairs on petiole	None or very low (38) - low (1) - medium (2) - high (2)
Opening of petiole sinus	Wide open (13) - open (26) - closed (3) - overlapping (1)
Leaf Length	Short (13) - medium (30)
Leaf width	Narrow (10) - medium (27) - wide (6)
Petiole length	Short (8) - medium (25) - long (10)
Number of lobes	Three lobes (1) - five lobes (42)

tors and 16 quantitative descriptors related to the leaf (12 descriptors), bunch (5 descriptors) and berry (16 descriptors).

Data analysis

To assess the information given by ISSR markers, the following parameters were calculated as follow: number of alleles per locus, power of discrimination (PD= 1 -Σ gi2, where gi is the frequency of the i_{th} genotype), polymorphism information content (PIC= 1-∑ (Pi)^2, where Pi is the proportion of samples carrying the i_{th} allele of a particular locus) (Botstein *et al.*, 1980). Genetic distances were calculated according to Jaccard. Trees clustering the data with the unweighted pair-group method (UPGMA) with SAHN-clustering and tree programs of PAST software (Kriege *et al.*, 2014).

Qualitative characteristics have been described and scored. For quantitative descriptors, the mean was calculated. To assess the degree of similarity between the units tested and understand the rela-

tionships between them, the data were subjected to a principle component analysis (PCA) in order to condense the quantitative and qualitative descriptors in a small number of synthetic components (Saporta, 1990). Thus, the degree of contribution of each of the characters to the total variation was calculated in order to indicate the most relevant characters.

Hierarchical Cluster Analyses was executed using Euclidean distance to classify cultivars into different groups based on morphological evaluation. LSD test (SAS Institute Inc, 1995) was done in purpose to compare means of quantitative characters between different accessions. The correlation between molecular and ampelographic clustering was studied by performing a Mantel test using past program.

3. Results

ISSR markers analysis

The nine ISSR markers (UBC807, UBC812, UBC815,

Table 5 -   Bunch and berry descriptors notation and their frequency distribution among the 43 studied grapevine accessions

Descriptors	Notation and frequency (Number of accessions)
Bunch descriptors	
Bunch shape	Cylindrical (21) - conical (15) - funnel shaped (7)
Bunch density	Loose (5) -medium (25) - dense (13)
Bunch weight	Very low (4) - low (13) - medium (13) - high (8) - very high (5)
Bunch length	Short (3) - medium (6) - long (26) - very long (8)
Bunch width	Narrow (13) - medium (21) - wide (8) - very wide (1)
Berry descriptors	
Berry shape	Obloid (2) - globose (9) - broad ellipsoid (11) - narrow ellipsoid (6) - cylindric (1) - obtuse ovoid (7) - ovoid (3) - obovoid (3) - horn shaped (1)
Color of skin	Green yellow (27) - rose (2) - red (1) - dark red violet (8) - bleu black (5)
Thickness of skin	Very thin (2) - thin (17) - medium (17) - thick (5) - very thick (2)
Firmness of flesh	Soft (6) - slightly firm (27) - firm (10)
Juiciness of flesh	Slightly juicy (7) - medium juicy (27) - very juicy (9)
Veraison time	Very early (8) - early (3) - medium (2) - late (24) - very late (6)
Veraison acidity (TA)	Low (5) - medium (32) - high (6)
Veraison brix	Low (6) - medium (27) - high (10)
Harvesting time	Very early (3) - early (4) - medium (7) - late (20) - very late (9)
Harvesting acidity (TA)	Low (6) - medium (30) - high (7)
Harvesting brix	Low (6) - medium (31) - high (6)
Berry weight	Very low (4) - low (6) - medium (26) - high (5) - very high (2)
Berry length	Short (7) - medium (16) - long (15) - very long (5)
Berry width	Very narrow (1) - narrow (21) - medium (20) - wide (1)
Length of pedicel	Very short (1) - short (3) - medium (5) - long (7) - very long (9)
Seed number	One (2) - two (24) - three (11) - four (6)

UBC816, UBC818, UBC828, UBC864, UBC868 and UBC880) showed distinct polymorphism among the 43 different grapevine accessions of this study (Table 2). A total of 41 polymorphic bands were detected. The size of amplified products ranged from 125 bp to 1000 bp. The calculated discriminating power (PD) was between 0.3 (UBC868) and 0.9 (UBC864), indicating a high diversity of the loci and confirming the efficiency of these primers in studying the polymorphism of the Lebanese grapevine germplasm. The polymorphic information content (PIC) value varied between 0.27 (i.e. UBC868) and 0.64 (i.e. UBC816) with an average of 0.49. Number of polymorphic bands varied between two (e.g. UBC868) to six (e.g. UBC812). Eight primers had one monomorphic band, while UBC868 generated two monomorphic bands.

Genetic clustering of the accessions

The allelic diversity data was used to produce a dendrogram by using distance matrix-UPGMA, thus revealing the genetic relationship among grapevine accessions (Fig. 1). The dendogram constructed on the base of the amplification product of ISSR primers of the different accessions showed 41 different molecular patterns. Tfaifihi variety indicated a unique marker with the primer UBC812 (305 bp). One case of synonymy was observed between Gbaii, Arasani and Gnoubi cultivars and they are very close for the most discriminating ampelographic traits. The ISSR analysis showed four distinct groups: G1, G2, G3 and G4 at the distance of 0.67 of similarity including 6, 26, 2, and 9 cultivars respectively.

The first group G1 consists of six accessions 'Baitmouni Asouad', 'Zaitouni, 'Asouad', 'Ainouni', 'Moukh Al-Baghel' and 'Karn Al Ghazal' and have a nine alleles in common with six primers (UBC807, UBC812, UBC816, UBC864, UBC868 and UBC880). The Largest group (G2) gathers 26 grapevine accessions, shared only five alleles in common with UBC807, UBC812 and UBC818. This group can be divided into 4 sub-groups: G2.1 contains six accessions, 'Nabi', 'Misky', 'Asali', 'Bourji', 'Oubaidi' and 'Baitamouni'. G2.2 comprises 11 accessions, with two cases of close similarity detected between 'Souri Zahlaoui' and 'Khoudri', and between 'Yafaoui' and 'Souri Mhayyar', at 0.94 similarity levels. Only one case of synonymy was observed between three different accessions initially collected from the south of Lebanon under different vernacular names and presenting the same genetic profile: 'Gnoubi', 'Gbaii', 'Arasani'. G2.3 consists of four accessions, with a close similarity detected between 'Bzaz Al-Anzi' and
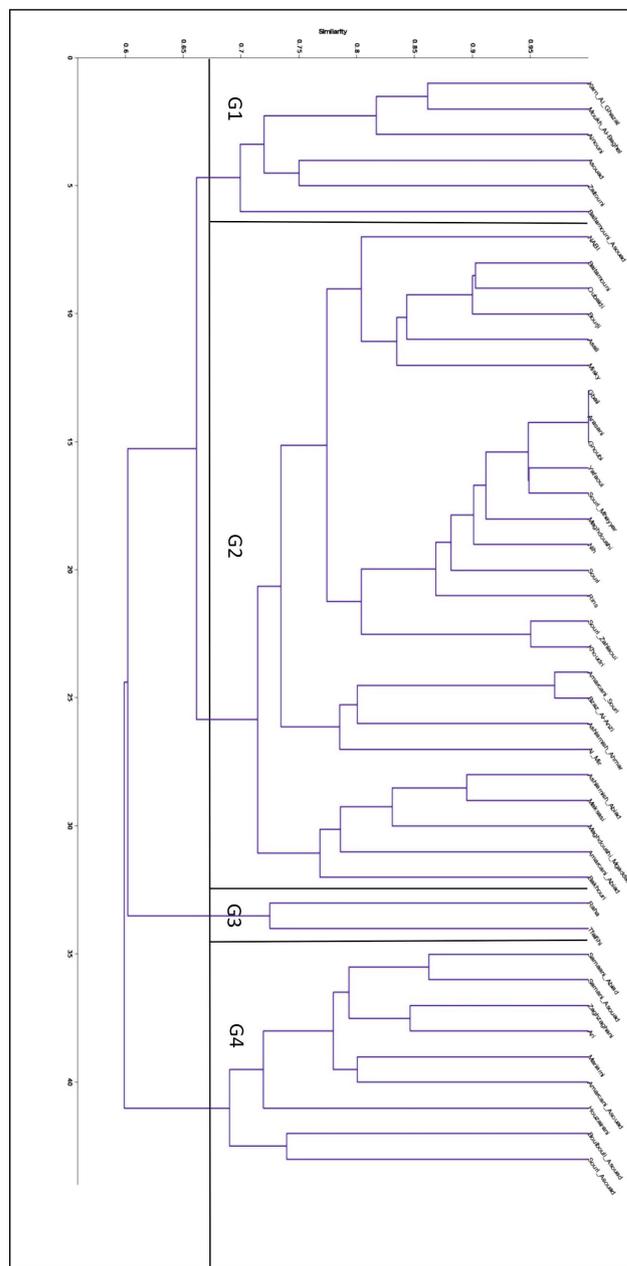

Fig. 1 - Hierarchical clustering analysis of the 43 Lebanese grapevine cultivars, based on 9 ISSR primers using Jaccard distance and UPGMA.

'Amarcani Souri' at a similiraty level of 0.96. G2.4 includes five accessions; 'Bakhouri', 'Amarcani Abiad', 'Maghdoushi Mgaddad', 'Maksasi' and 'Ashalamish Abiad'. The third group (G3) consists only of two accessions: 'Raha' and 'Tfaifihi' had 20 alleles in common with all the nine ISSR markers. The fourth group (G4) consists of nine accessions: 'Souri Asouad', 'Boulbouli Asouad', 'Houzairani', 'Amarcani Asouad', 'Mariami', 'Ari', 'Zaghzaghani', 'Samani Asouad' and 'Samaani Abiad', shared only two alleles in common with UBC815 and UBC864 primers.

Ampelographic description

A total of 43 cultivars were studied. Locale names were commonly given by farmers or nurseries based on berry colour or on the country of origin (Asouad, Souri Aswad, Amarcani Abiad) and maturity date (Houzairani). For each cultivar, a descriptive list was established with 33 morphological traits related to the leaf, bunch and berry (Tables 3, 4, 5).

Leaf description. The majority of accessions have five lobes except 'Souri Zahlaoui' accession which only have three lobes. Thirty-one accessions presented a wedge-shaped leaf form (e.g. 'Ari', 'Bourji', 'Gbaii'), while only nine accessions had circular leaf (e.g. 'Raha', 'Bakhouri'), and the rest had a pentagonal leaf form (e.g. 'Arasani', 'Asali', 'Souri Mhayyar') (Table 4). Most of the accessions shared same teeth form with both sides straight and both sides convex except for the 'Oubaidi' that possesses a both side convex teeth form, and three accessions ('Boulbouli Asouad', 'Nabi' and 'Samani Asouad') which had a both side straight teeth form (Table 4, Fig. 2).

Anthocyanin coloration of main veins on both the upper and the lower side of blade varied from absent (e.g. 'Al Mir'), limited to the petiolar point (e.g. 'Arasani'), extended to the first bifurcation (e.g. 'Khodre'), and beyond the second burifaction (e.g. 'Zaghzaghani').

The opening of the petiole sinus varied from over-lapping sinus (e.g. 'Zaitouni'), closed (e.g. 'Souri Asouad'), open (e.g. 'Misky', 'Rins'), to wide open sinus (e.g. 'Gbaii', 'Souri Zahlaoui').

The density of prostrate hairs between main veins on both sides of the blade and on the petiole varied widely from absent (e.g. Ainouni), low (e.g. 'Bakhouri', 'Zaghzaghani'), medium (e.g. 'Zaitouni', 'Ari'), and high (e.g. 'Tfaifihi', 'Al Mir'). Only three accessions present prostrate hairs on the upper side of the main blade veins ('Rins', 'Souri Mhayyar', and 'Tfaifihi').

Leaves generally presented an average length between 7.84 (i.e. 'Souri Zahlaoui') and 11.26 cm (i.e. Ari*)* with LSD 1.06 and width between 11.4 (i.e.
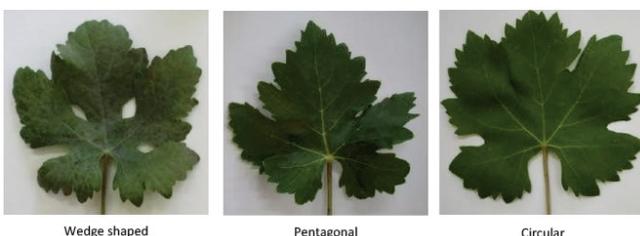
'Rins') and 15.74 cm (i.e. 'Ashlamish Ahmar'). Petiole length average was between 4.24 (i.e. 'Bzaz Al-Aanzi') and 11.12 cm (i.e. 'Baitamouni Asouad') (Table 3).

Bunch description. Bunch characteristics investigated showed a great diversity among the accessions studied. Almost 48.8% of the accessions had cylindrical bunch (e.g. 'Ainouni', 'Houzairani'), 34.9% were conical (e.g. 'Boulbouli Asouad', 'Maksasi') and 16.3% of the accessions had a funnel shaped cluster (e.g. 'Arasani, Raha') (Table 5). Both 'Samani Asouad' and 'Samaani Abiad' had the heaviest bunch weight, with an average of 1150 g approximately. For the remaining accessions, bunch weight ranged from 1090 g (i.e. 'Rins') to 203 g (i.e. 'Amarcani Abiad'). Most of the accessions had a bunch length ranging between 14.93 (i.e. 'Maghdoushi Mgaddad') and 30.6 cm (i.e. 'Rins') with 3.6 of LSD. Bunch width ranged between a minimum of 8.15 cm (i.e. 'Bourji') and a maximum of 21.23 cm (i.e. 'Samaani Abiad') (Table 3, Fig. 3).

Cylindrical   Conical   Funnel shaped

Fig. 3 - Example of variability of bunch shape.

Berry description. Berry's external appearance presented an important diversity among accessions (Table 5). For the shape, 11 accessions had a broad ellipsoid berry shape (e.g. 'Ainouni', 'Arasani', 'Khoudri'), nine accessions had a globose berry form (e.g. 'Ari', 'Tfaifihi'), and the remaining accessions had obvoid (e.g. 'Al Mir', 'Souri Mhayyar'), narrow ellipsoid (e.g. 'Gbaii', 'Souri Asouad'), obloid (e.g. 'Asali', 'Samani Asouad'), ovoid (e.g. 'Misky', 'Ashlamish Ahmar', 'Yafaoui'), obtuse ovoid (e.g. 'Maghdoushi', 'Asouad', 'Souri Zahlaoui', 'Zaitouni'), cylindical (e.g. 'Bzaz Al-Anzi') and horn berry shaped ('Karn Al-Ghazal'). As to skin color, most of the accessions had green yellow berries (e.g. 'Mekssese', 'Yafawi', 'Karn Al Ghazal'). While 'Houzairani' diverged from others with its red berries, eight accessions had a dark red violet berry color and five other accessions shared the bleu black color. Only the rose color was found in 'Ashlamish Ahmar' and 'Tfaifihi' accessions.

Wedge shaped   Pentagonal   Circular

Fig. 2 - Example of variability of leaf shape.

A wide range of variability was also found for the berry quantitative descriptors (Table 3). 'Asouad' and 'Asali' accessions were outstanding with their significantly heavier berries (9.95 g and 9.69 g respectively) while 'Ashlamish Abiad' had only 0.94 g, as berry average weight. For the rest of the accessions, berry weight ranged from 8.3 g in 'Baitamouni Asouad' to 1.71 g in 'Amarcani Abiad'. On the other hand, 'Karn Al Ghazal' accession was distinguished with its long berries (3.4 cm) and its long pedicel (1.53 cm) while 'Asali' accession had the largest berries (2.54 cm).

Principal component analysis

The first three components presented 31.9% of the total variation of the different descriptors (Table 6). The first component consisted of 12.2% of the total variation and included berry skin color, firmness of flesh, bunch weight, harvesting and veraison times, in addition to the brix level at harvesting. Thus, the first component was dominated by the fruit characteristics more than the leaf ones. The second component represented 10.9% of the total variation and included four variables namely berry weight and length, leaf lower vein pigmentation and lower face pilosity. The third component was characterized by a percentage of variation of 8.8% and was dominated by the flesh juiciness and the bunch density. Based on this validation through PCA, this shortlist of discriminating descriptors may be further considered to study the relationships between the grapevine accessions.

Ampelographic accession clustering

The dendogram illustrating the relationship among the 43 studied grapevine accessions was constructed on the 12 most discriminating descriptors as validated by PCA. Four groups were differentiated at the distance -17 of similarity using Euclidean distance (Fig. 4). The first group (G1) consisted of eight accessions of different skin color but sharing same veraison date and the same sugar content at harvesting time. The second and the largest group (G2) consisted of 13 accessions, sharing all the same bunch characteristics in terms of density, juiciness in addition to the common sugar content at harvesting time. Moreover, these accessions started the stage of veraison almost at the same date around the 5th of September. The third group (G3) contained 12 accessions, characterized by medium green berries. A case of close similarity was found between three accessions of different vernacular names 'Gnoubi', 'Gbaii' and 'Arasani'. The fourth group (G4) clustered 10

Table 6 - The first three components of the principal component analysis involving the 33 ampelographic traits and performed for the 43 accessions of the national grapevine germplasm collection

Variable	Factor 1	Factor 2	Factor 3
Leaf form	-0.14042	0.423101	-0.00785
Number of lobes	-0.07649	-0.08669	0.136368
Teeth form	-0.15343	-0.29052	0.301802
Upper vein pigmentation	-0.14288	-0.25638	-0.45432
Lower vein pigmentation	0.069659	**-0.63447**	-0.19775
Lower face pilosity	-0.03692	**0.627619**	-0.00083
Upper face pilosity	0.17382	0.179676	0.009038
Petiole pilosity	0.27927	-0.3332	0.328737
Overlapping of petiole	-0.00076	0.475518	0.226222
Berry shape	-0.0347	-0.00661	0.439309
Color skin	**0.511841**	-0.13872	-0.09665
Skin thickness	0.013082	0.133863	0.46865
Firmness of flesh	**0.519899**	0.152408	0.095108
Juiciness of flesh	-0.27819	0.150394	**-0.55995**
Seeds number	-0.01707	-0.0642	-0.44797
Bunch form	-0.14446	0.507152	-0.03091
Bunch density	0.21315	0.362343	**-0.6217**
Bunch weight	**0.598172**	0.272448	-0.44281
Bunch length	0.344545	0.046891	-0.35337
Bunch width	0.459819	0.390514	-0.32888
Leaf length	0.306676	0.272602	0.398343
Leaf width	0.41474	0.04784	0.18026
Petiole length	0.436157	0.049669	0.154936
Pedicel length	0.269837	-0.46508	0.060371
Berry weight	0.437611	**-0.53381**	-0.29825
Berry length	0.253778	**-0.65709**	0.106556
Berry width	-0.13315	-0.30376	-0.35394
Veraison time	**0.789894**	0.04527	0.01873
Harvesting time	**0.667212**	0.098136	-0.22776
Acidity at veraison	-0.42158	-0.18862	-0.28567
Brix at veraison	-0.49511	0.304928	-0.05688
Brix at harvesting	**-0.57237**	0.156018	-0.1208
Acidity harvesting	-0.44026	-0.21603	-0.20417
Percentage total variation	12.2%	10.9%	8.8%

The characters in bold are discriminant.

accessions of which eight accessions with dark red violet (e.g. 'Asali') to bleu black (e.g. 'Mariami') berries, and two accessions with green yellow berries ('Rins' and 'Samaani Abiad'). All these accessions have common leaf characteristics and intermediate sugar content at maturity time.
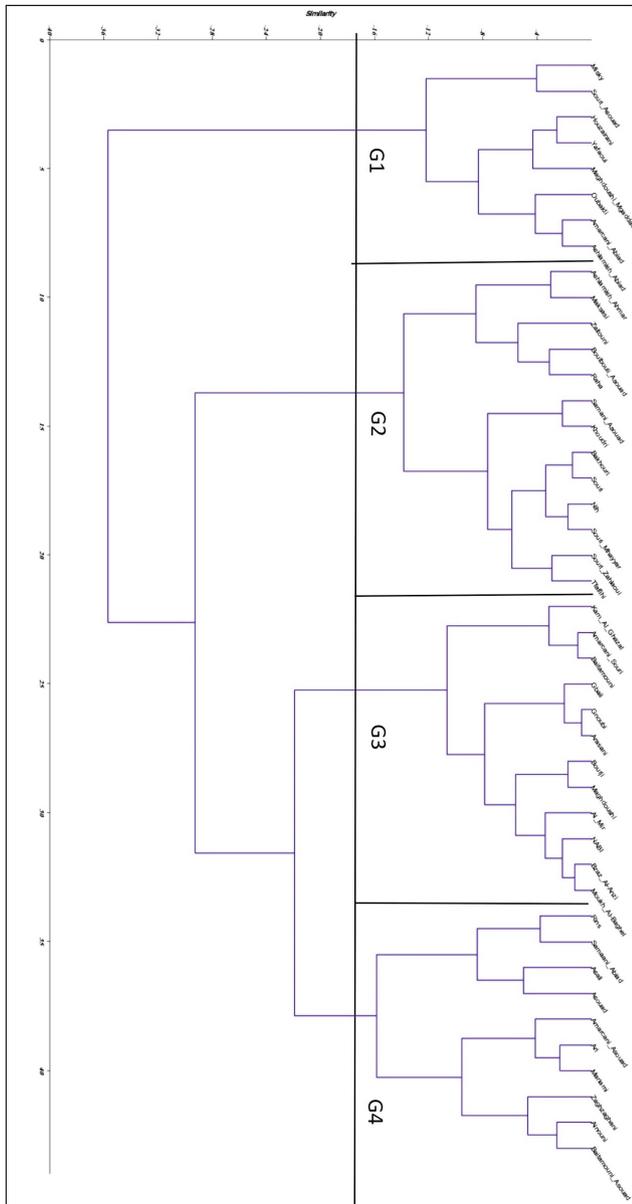
Fig. 4 - Hierarchical clustering analysis of the 43 Lebanese grapevine cultivars, based on the most 12 discriminant morphological traits, using Euclidean distance and Ward method.

4. Discussion and Conclusions

In our study encompassing 43 local accessions, 51 bands were generated from nine primers, out of which 41 were polymorphic. Similarly, 55 polymorphic bands were obtained in Palestine by Basheer-Salimia, (2015) in studying 36 grape accessions using 17 ISSR markers; while 69 polymorphic bands were obtained by Seyedimoradi *et al.* (2012) in studying 21 local Iranian grapevine cultivars using 10 ISSR primers. Our results related to polymorphism rate

(50-85%) and size of amplified bands (125-1000bp) are close to the ones previously reported in Portugal (Castro *et al.,* 2016), Turkey (Sabir *et al.,* 2009), Egypt (Hassan *et al.,* 2011), Iran (Seyedimoradi *et al.,* 2012), and Palestine (Basheer-Salimia, 2015). Larger fragments (300-1500 bp) were generated by the same ISSR markers with values up to 1500 bp in India (Dhanorkar *et al.,* 2005) and 2500 bp in Turkey (Sabir *et al.,* 2009). Moreover, our results indicated a discrimination power value ranging from 0.3 (UBC868) and 0.9 (UBC864) and PIC value varying between 0.27 (i.e.UBC868) and 0.64 (i.e.UBC816) with an average of 0.49, which was similar to the results obtained in Iran by Seyedimoradi *et al.* (2012).

Our ISSR results revealed an important genetic diversity within the national collection of grapevine germplasm. About 41 different molecular profiles were clearly differentiated within the 43 accessions, with only one case of synonymy found between three accessions ('Arasani', 'Jbai' and 'Jnoubi'). These findings certainly confirm one more time the efficiency of these ISSR markers in investigating the genetic variability of grapevine. It also indicates the efficiency of the initial survey and collection of local accessions in establishing this national collection of grapevine germplasm with only 5% synonymy. In Spain, the molecular characterization of grapevine accessions of the national gene bank at Alcalá de Henares allowed the differentiation of 177 accessions (30%) over 621 initially collected (Ortiz *et al.,* 2004). Furthermore, the SSR analysis of the Eastern European cultivars led to the differentiation of only 659 unique profiles over 997 accessions studied (Maul *et al.,* 2015). In Italy, Cipriani *et al.* (2010) reported 200 groups of synonyms vs. 774 unique genotypes out of 1005 grapevine accessions studied. Also, Lopes *et al.* (1999), studying 49 supposed different cultivars from the Portuguese grapevine national collection of Terciera (Azores Island) through microsatellite markers, detected only 36 different profiles after determining the synonym cases.

Along with the genetic differentiation of the grapevine accessions, an ampelographic description was carried out for the 43 Lebanese accessions using 33 descriptors recommended by the OIV, and mostly related to various descriptors including yield components like bunch and berries dimensions and weight (Kara, 1990; Ortiz *et al.,* 2004; Santiago *et al.,* 2005; OIV, 2007; Akram *et al.,* 2019). Mature leaf descriptors did not vary significantly among the Lebanese accessions. Most of these accessions had leaves with

five lobes similarly to the results reported in other studies (Ecevit and Kelen, 1999; Chalak *et al.,* 2016). On the other hand, our results revealed a large variability of bunch shape, weight, density, as well as for the berry shape, color and size, similarly to the results reported in previous works in the Eastern Mediterranean (Sabir *et al.,* 2009; Biniari and Stavrakaki, 2016). On the other hand, the multivariate analysis was efficient to analyze large data generated in our study by qualitative and quantitative descriptors to further identify patterns and relationship among powerful statistical techniques.

PCA allowed to extract the most discriminating descriptors, e.g. veraison date, maturity date, berry length, upper vein pigmentation, lower vein pigmentation, bunch density, bunch weight, brix at harvesting, juiciness of flesh, berry weight and firmness of flesh and skin color. Our results were in accordance with those previously obtained by Riachi (1998), Madi (2007) and Chalak *et al*. (2016) confirming one more the stability of the discriminating descriptors over years. This also in accordance with Leao *et al.* (2010, 2011) who reported the stability of the discriminating characters for two consecutive years. Phenotypic clustering allowed differentiate the 43 accessions studied in four main groups. The synonymy case first revealed between the three accessions 'Gbaii', 'Gnoubi', and 'Arasani' by the ISSR clustering was also confirmed by using ampelographic. This is the minimum rate for verifying synonyms and for clone selection processes (Cervera *et al.,* 2002).

Furthermore, many cases of close phenotypic similarity, first revealed by Madi (2007), were also recorded in this study. It is worthy to note that some of our accessions were also described in other countries. This is the case of 'Bzaz Al Anzi' reported in Egypt by Hassan *et al.* (2011) and presenting almost similar ampelographic characteristics of the bunch and the berries.

When comparing the two dendograms generated apart by the molecular and ampelographic descriptors, the Mantel test indicated a weak correlation (r = 0.26, data not shown) between them. This reflects different structure for the accessions clustering whether it is generated by molecular or ampelographic descriptors. Exception is made to the case of synonymy between the three accessions 'Gnoubi', 'Gbaii', and 'Arasani', which was confirmed by both, ampelographic and molecular clustering. This discrepancy between the two clustering types was also reported in other studies in grapevine collections

(Knezović *et al.,* 2017) and other fruit crops (Talebi *et al.,* 2008; Zdunic *et al.,* 2008).

On the other hand, considering some key ampelographic descriptors, it was possible to categorize the existing diversity of the collected Lebanese germplasm according to the phenological stage, and according to berry skin color. In five groups of maturity time; with 27 accessions had green yellow berries, only one accession with red berries, two accessions with rose berries, five accessions with bleu black berries and eight accessions with dark red violet berries. Surprisingly, similar ratios between green and red accessions were also reported in Canary Islands and Madeira by Marsal *et al.* (2019).

Additionally, most of the accessions studied had crunchy berries with thick skin and very low juice content. This indicates the potentiality of using these accessions for table grapes rather than wine grapes. Only three accessions had thin skin, juicy flesh and high sugar content and, therefore, may be tested for fermentation. Such descriptive ampelographic characterization of the berries is hopefully necessary for evaluating grapevine accessions conserved in field genebanks with respect to their usage as table grape or wine grape (Sabir *et al.,* 2009; Ates *et al.,* 2011; Basheer-Salimia, 2015).

This diversity upon the 43 grapevine accessions conserved at the Lebanese national collection of grapevine as revealed by ISSR markers and ampelographic descriptors indicate the efficiency of sampling/collecting strategy conducted in 1998 in the Bekaa and Chouf areas.

Nevertheless, additional grapevine varieties growing locally under different vernacular names, which do not appear in the national grapevine germplasm collection, were recently assessed in their growing site by (Chalak *et al.,* 2016). Therefore, it is strongly recommended to further extend the survey and mission collection to cover multiple grapevine production areas, particularly the North, the South and of Mount of Lebanon in order to enrich the national collection. Combining both molecular makers and ampelographic descriptors as it was shown in this study, would be very useful in optimizing the collection of accessions by clarifying the mislabeling cases, understanding the genetic distances among accessions, and setting up a descriptive assessment for these accessions. The comprehensive evaluation of the existing local gene pool of grapevine would further allow the sustainable utilization of the valuable accessions directly for multiplication in certified nurs-

eries and also in further breeding programs.

Acknowledgements

References

AKRAM T., QADRI K., JASKANI J., AWAN S., 2019 - *Ampelographic and genetic characterization of grapes genotypes collected from Potohar region of Pakistan.* - Pak. J. Agri. Sci., 56(3): 595-605.

ATES F., COBAN H., KARA Z., SABIR A., 2011 - *Ampelographic characterization of some grape cultivars (*Vitis vinifera *L.) grown in south-western region of Turkey*. - Bulg. J. Agric. Sci., 17(3): 314-324.

BASHEER-SALIMIA R., 2015 - *Ampelographic characterization of white grapevine cultivars (*Vitis vinifera *L.) grown in Palestine*. - Palestine Technical University Research Journal, 3(1): 1-11.

BINIARI K., STAVRAKAKIS M.N., 2013 - *The 'vine of Pafsanias' and the group of grape cultivars 'Mavroudia'of the vineyard of Peloponnese*. - Proceedings of Ampelos, 3rd International Symposium 'Trends in world vitiviniculture development', 30-31 May, Santorini Island, Greece.

BOTSTEIN D., WHITE L., SKOLNICK M., DAVIS R.W., 1980 - *Construction of a genetic linkage map in man using restriction fragment length polymorphisms*. - Amer. J. Human Genetics, 32(3): 314.

CASTRO I., PINTO-CARNIDE O., MARCIDE J.M.O., FERREIRA V., CLEMENTE J.P.M., 2016 - *A comparative analysis of genetic diversity in Portuguese grape germplasm from ampelographic collections fit for quality wine production.* - Spanish J. Agric. Res., 14(4): 13.

CERVERA M.T., RUIZ-GARCÍA L., MARTINEZ-ZAPATER J., 2002 - *Analysis of DNA methylation in Arabidopsis thaliana based on methylation-sensitive AFLP markers*. - Mol. Genetics and Genomics, 268(4): 543-552.

CHALAK L., RAHME S., AZZI R., GUIBERTEAU F., TOUMA J.A., 2016 - *Assessment of the Lebanese grapevine germplasm reveals a substantial diversity and a high potential for selection*. - BIO Web of Conferences, 7: 10-20.

CHOUDHARY R.S., ZAGADE V., KHALAKAR G.D., SINGH N.K, 2014 - *ISSR based genotypic differentiation of grape (*Vitis vinifera *L.).* - Bioscan, 9(2): 823-828.

CIPRIANI G., MARCONI R., TESTOLIN R., 2010 - *Microsatellite markers isolated in olive (*Olea europaea *L.) are suitable for individual fingerprinting and reveal polymorphism within ancient cultivars*. - Theor. Appl. Genetics, 104(2-3): 223-228.

DETTWEILER E., JUNG A., ZYPRIAN E., TOPFER R., 2000 - *Grapevine cultivar Muller-Thurgau and its true to type descent.* - Vitis Geilweilerhof, 39(2): 63-66.

DHANORKAR M., TAMHANKAR A., PATIL G., RAO S., 2005 - *ISSR-PCR for assessment of genetic relationships among grape varieties cultivated in India*. - Vitis: J. Grapevine Res., 44(3): 127-131.

DOYLE J.J, DOYLE J.L., 1990 - *Isolation of plant DNA from fresh tissue.* - Focus, San Francisco, CA, 12(1): 13-15.

ECEVIT F.M., KELEN M., 1999 - *Determination of ampelographical characters of grape cultivars grown in Isparta (Atabey).* - Turkish J. Agric. For., 23(5): 511-518.

FAO, 2010 - *Resultats globaux du module de base du recensement de l'agriculture 2010. Projet 'Observatoire Libanais de Développement Agricole'* - FAO, Liban, pp. 119-120.

FAO, 2015 - *Coping with climate change - the roles of genetic resources for food and agriculture*. - FAO, Rome, Italy.

FAOSTAT, 2019 - *FAOSTAT*. - FAO, http://www.faostat.com/statistic.

GALET P., 1998 - *Grape varieties and root-stock varieties*. - Oenoplurimédia, Chaintré, France.

HASSAN N.A., EL-HOMOSANY A., GOMMA A.H., SHAHEEN M.A., 2011 - *Morphological and ISSR polymorphisms in some Egyptian grapes (*Vitis vinefera *L.) collection*. - World Appl. Sci. J., 15(10): 1369-1375.

KAFKAS S., OZKAN H., AK B.E., ACAR I., ATLI H.S., KOYUNCU S., 2006 - *Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: Comparison of AFLP, ISSR, and RAPD markers.* - J. Amer. Soc. Hort. Sci., 131(4): 522-529.

KARA Z., 1990 - *Determination of the ampelographic characters of grape varieties grown in Tokat*. - Ph.D. Thesis, Graduate School of Natural and Applied Sciences, Ankara University, Ankara, Turkey.

KNEZOVIĆ Z., MANDIĆ A., PERIĆ N., BELJO J., ŽULJ MIHALJEVIĆ M., 2017 - *Morphological and genetic characterization of vine grape cultivars of Herzegovina.* - Croatian Review of Economic, Business and Social Statistics, 3(2): 1-9.

KRIEGE N., MUTZEL P., SCHAFER T., 2014 - *Practical SAHN clustering for very large data sets and expensive distance metrics*. - J. Graph Algorithms Applic., 18(4): 577-602.

LAUCOU V., LACOMBE T., DECHESNE F., SIRET R., BRUNO J.P., DESSUP M., THIS P., 2011 - *High throughput analysis of grape genetic diversity as a tool for germplasm collection management.* - Theoretical Applied Genetics, 122(6): 1233-1245.

LEAO S., CRUZ C.D., MOTOIKE S.Y., 2010 - *Genetic diversity*

of a Brazilian wine grape Germplasm Collection based on morphoagronomic traits. - Revista Brasileira Frutic., 32(4): 1164-1172.

LEAO S., CRUZ C.D., MOTOIKE S.Y., 2011 - *Genetic diversity of table grape based on morphoagronomic traits*. - Scientia Agricola, 68(1): 42-49.

LOPES S., SEFC M., DIAS E., STEINKELLNER H., MACHADO C., MACHADO C., 1999 - *The use of microsatellites for germplasm management in a Portuguese grapevine collection.* - Theoretical Appl. Genetics., 99(3-4): 733-739.

MADHUMATI B., 2014 - *Potential and application of molecular markers techniques for plant genome analysis*. - Int. J. Pure App. Biosci., 2(1): 169-188.

MADI N., 2007 - *Caracterisation morphologique d'une collection d'accessions de raisin de table (*Vitis vinifera*) de la bekaa*. - Lebanese University. Department of Plant Production.

MARTINEZ L., CAVAGNARO P., MASUELLI R., RODRIGUEZ J., 2003 - *Evaluation of diversity among Argentine grapevine Varieties Using morphological data and AFLP markers*. - Electronic J. Biotechnology, 6: 3-8.

MARSAL G., MÉNDEZ J.J., MATEO J.M., FERRER S., CANALS J.M., ZAMORA F., FORT F., 2019 - *Molecular characterization of* Vitis vinifera *L. local cultivars from volcanic areas (Canary Islands and Madeira) using SSR markers*. - OENO One 53: 4-12.

MAUL E., TÖPFER R., CARKA F., CORNEA V., CRESPAN M., DALLAKYAN M., DE ANDRÉS DOMÍNGUEZ T., DE LORENZIS G., DEJEU L., GORYSLAVETS S., GRANDO M.S., HOVANNISYAN N., HUDCOVICOVA M., HVARLEVA T., IBÁÑEZ J., KISS E., KOCSIS L., LACOMBE T., LAUCOU V., MAGHRADZE D., MALETIĆ E., MELYAN G., MIHALJEVIĆ M.Z., MUÑOZ ORGANERO G., MUSAYEV M., NEBISH A., POPESCU C.F., REGNER F., RISOVANNA V., RUISA S., SALIMOV V., SAVIN *G.,* SCHNEIDER G., STAJNER N., UJMAJURIDZE L., FAILLA O., 2015 - *Identification and characterization of grapevine genetic resources maintained in Eastern European Collections.* - VITIS, 54 (Special issue): 5-12.

MORENO S., MARTÍN P., ORTIZ J.M., 1998 - *Inter-simple sequence repeats PCR for characterization of closely related grapevine germplasm*. - Euphytica, 101(1): 117-125.

NASS L., SIGRIST S., RIBEIRO C., REIFSCHNEIDER B., 2012 - *Genetic resources: the basis for sustainable and competitive plant breeding.* - Crop Breeding and Appl. Biotechn., 12: 75-86.

OIV, 2007 - *Descriptor list for grapevine varieties and Vitis species*. - OIV, Paris, 2nd edition.

ORTIZ J.M., MARTÍN J.P., BORREGO J., CHÁVEZ J., RODRÍGUEZ I., MUÑOZ G., CABELLO F., 2004 - *Molecular and morphological characterization of a* Vitis *gene bank for the establishment of a base collection.* - Genetic Resources Evolution, 51: 403-409.

RAHME S., 2016 - *Caractérisation morphologique des*

cépages autochtones du Liban. - Lebanese University. Department of Plant Production.

RIACHI I.E., 1998 - *Germplasm characterization and climate zoning for viticulture in Lebanon*. - MSc dissertation, Department of Crop Production and Protection, American University of Beirut.

RIAZ S., DANGL G.S., EDWARDS K.J., MEREDITH C.P., 2012 - *A microsatellite marker based framework linkage map of* Vitis vinifera *L.* - Theoretical Appl. Genetics, 108(5): 864-872.

ROBY J.F., 2003 - *La filiere viticole au Liban, analyse etpropositions pour une evolution*. - ENITA, Bourdeaux, Frances, pp. 119.

SABIR A., TANGOLAR S., BUYUKALACA S., KAFKAS S., 2009 - *Ampelographic and molecular diversity among grapevine (*Vitis spp.*) cultivars*. - Czech J. Genetics Plant Breeding, 45(4): 160-168.

SANTIAGO J.L., BOSO S., MARTÍN J.P., 2005 - *Characterisation and identification of grapevine cultivars* (Vitis vinifera *L.) from northwestern Spain using microsatellite markers and ampelometric methods*. - Vitis - Geilweilerhof, 44(2): 67-72.

SANTILLÁN D., GARROTE L., IGLESIAS A., SOTES V., 2019 - *Climate change risks and adaptation: New indicators for Mediterranean viticulture*. - Mitigation Adaptation Strategies Global Change, 1-19.

SAPORTA G., 1990 - *Probabilités, statistique et analyse des données*. - Éditions Technip, pp. 488.

SEYEDIMORADI H., TALEBI R., HASSANI D., KARAMI F., 2012 - *Comparative genetic diversity analysis in Iranian local grapevine cultivars using ISSR and DAMD molecular markers*. - Environ. Exp. Biol., 10: 125-132.

TABAJA N., 2015 - *Current status of registered fruit tree seedlings in Lebanon and potential impact on germplasm diversity*. - Lebanese University. Department of Plant Production.

TALEBI R., FAYAZ R., MARDI M., PIRSYEDI S.M., NAJI A.M., 2008 - *Genetic relationships among chickpea (*Cicer arietinum*) elite lines based on RAPD and agronomic markers*. - Int. J. Agric. Biol., 10: 301-305.

TESSIER C., DAVID J., THIS P., BOURSIQUOT J.M., CHARRIER A., 1999 - *Optimization of the choice of molecular markers for varietal identification in* Vitis vinifera *L.* - Theoretical Appl. Genetics, 98(1): 171-177.

THIS P., LACOMBE T., THOMAS M., 2006 - *Historical origins and genetic diversity of wine grapes.* - Trends in Genetics, 22(9): 511-519.

ZDUNIC Z., MIJIC A., DUGALIC K., SIMIC D., BRKIC J., JEROMELA A. M., 2008 - *Genetic analysis of grain yield and starch content in nine maize populations*. - Turkish J. Agric. Forestry, 32(6): 495-500.

ZOHARY D., 2003 - *Domestication of grapevine* Vitis Vinifera *L. in the Near East,* pp. 1-8. - In: MCGOVERN P. E., S.J. FLEMING, and S.H. KATZ (eds.) *The origins ancient history of wine*. Routledge, London, UK, pp. 440.