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SHORT COMMUNICATION

A multivariate approach for the ampelographic discrimination of grapevine (*Vitis vinifera*) cultivars: application to local Syrian genetic resources

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Abstract Due to its unique historical and geographical emplacement, grapes have been cultivated in the Syrian Arab Republic for more than 5000 years, so the characterization of its local genetic resources is paramount for understanding grapevine natural diversity. In this work, different local Syrian table grape cultivars were characterized for 42 traits related to plant phenology, shoot, leaf, cluster, berry and juice composition. A series of multivariate analyses were sequentially performed, and five highly-discriminant traits were identified as the most discriminant ones (shoot internode length, berry weight, berry elongation, 100-seed weight and juice titratable acidity). The clustering of the cultivars according to these five traits revealed that some local Syrian cultivars share similitude with some worldwide grown cultivars, suggesting their potential as new genetic resources for the development of new high-quality table grape varieties, and indicating the needing of specific preservation programs aimed to avoid the loss of endangered genetic local resources. Besides, the statistical multivariate pipeline followed in this work is proposed as an efficient one for the selection of ampelographic traits for the discrimination of grapevine cultivars.

Keywords Conservation · Descriptors · Genetic resources · Phenotypic diversity · Syrian Arab Republic · *Vitis vinifera* L.

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most ancient cultivated fruit trees in the world. It is suggested that the earliest domestication processes and the beginning of winemaking took place in the region comprised between the Black and Caspian seas between the seventh and the fourth millennia BC (McGovern et al. 1996). This fact is supported by archeological findings reporting the recovering of grape remains (mainly seeds, but also grape skins and grapevine wood) and jars for wine storage in settlements of Anatolia, the Zagros mountains of Iran, the Caucasus and northern Syria (McGovern et al. 1997). Climatic and geological conditions on northern Syria were specially well suited for viticulture development, with hills and

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mountains providing the cool winters and long hot summers required for optimal grape ripening (Lutz 1922; Unwin 1991). Consequently, relevant centers for grape cultivation, wine making and wine trading arose in this region (Maraqten 1993), and textual and artistic evidences indicate that Syrian wines were highly appreciated in antiquity because of their excellent quality (Unwin 1991). As a result, ancient commercial Syrian cities like Palmyra made of local wines an important commodity for trading with close and distant regions (Lutz 1922; Maraqten 1993), an activity that extended during Greek and Roman periods (Zuchowska 2013). Later on, Muslim influence led to the abandonment of winemaking practices, and farmers focused on the growing of local grape cultivars for fresh fruit and raisins production, which are still cultivated by the Syrian population. Nowadays, the Syrian Arab Republic has a gross production of ca. 200,000 tonnes in 2014 (FAOSTAT) from 46,000 ha of vineyards spread all along the country, particularly in southern and central regions like Homs, Al Suwayda, Al Qunaytirah, Dara'a, Aleppo, Damascus, Hama and the coastal area (Mslmanieh et al. 2006).

Local Syrian grapevine resources are interesting reservoirs for grape quality and possible stress resistance traits. So, their preservation and evaluation is paramount for understanding grapevine natural diversity and for the selection of plant material for modern grapevine breeding programs (Abdullateef et al. 2011). Nonetheless, these traditional local resources are threatened by various anthropogenic pressures and by their replacement by selected international cultivars. Conscious of the relevance of preserving these invaluable local genetic resources, and looking for the development of better adapted new grapevine cultivars and clones, the Arab Center for the Studies of Arid Zones and Dry lands (ACSAD) established in 1996 a Gene Bank collection in the Jillin Research Station after the prospection of locally-grown traditional varieties. Despite its wide interest, and in contrast to local genetic resources from nearby regions (Aroutiounian et al. 2015; Basheer-Salimia et al. 2014; Chalak et al. 2016; Drori et al. 2017; Ekhvaia et al. 2014; Ergül et al. 2002; Eyduran et al. 2015; Fatahi et al. 2003; Khadivi-Khub et al. 2014; Lamine et al. 2014), few local Syrian grapevine cultivars have been adequately described so far (Sawaf et al. 1976).

Ampelography is the science for the identification of grapevines through the analysis of phenotypic traits. The *Précis d'Ampélographie Pratique* by Galet (1952) can be considered the reference manual in ampelography, and many of the descriptors described there have been adopted by international organizations (like IPGRI and OIV) for the official description of grape varieties and *Vitis* species. Although the use of molecular markers is recommended for grapevine identification, ampelography provides relevant morphological and agronomical information for characterization studies, breeding programs and conservation purposes (Barth et al. 2009; García-Munoz et al. 2011; Lamine et al. 2014). Nevertheless, the use of diverse (but sometimes highly correlated) ampelographic descriptors comprising shoot, leaf, cluster and berry traits yield large datasets that need to be treated in a proper way. The sequential use of univariate and multivariate statistical approaches has been proposed to aid in the selection of the most informative ampelographic characteristics for the classification of different grapevine varieties from a certain region. In this regard, Lamine et al. (2014) indicated that the number of ampelographic traits contributing to the efficient characterization of a series of Tunisian autochthonous varieties could be reduced from 70 to 12, and García-Munoz et al. (2011) highlighted 3 out of 57 qualitative and quantitative ampelographic as the most discriminating ones for the clustering of minor grapevine varieties from the Balearic Islands (Spain).

The main purpose of this work is to provide useful ampelographic information of a series of local Syrian table grape cultivars by a wide number of descriptors. Diverse multivariate statistical analyses were sequentially applied to analyze the performance of these traits for the discrimination and further clustering of these unexplored genetic resources.

Materials and methods

Plant material

Ten grapevine (*Vitis vinifera* L.) cultivars commonly grown in the Syrian Arab Republic for the obtaining of table grapes and raisins were evaluated in this study. They include eight local cultivars (Baladi, Bhoth-209, Bhoth-2-Zinni slale, Black Anuni Sweida, Black

Turky Homs, Halawani Kanawat, Hffersli Bhoth and Salti red Sweida) and two international cultivars (Cardinal and Muscat of Alexandria). Cultivars belong to the Grapevine Collection maintained in the Jillin Research Station (ACSAD), located in Jillin, southern Syria (Lat. 32°45′16″N, Long. 35°59′23″E). Plants are maintained following the same practices in terms of grafting, training system, row orientation and agronomical practices (pruning and disease control).

Genotyping

To assess their genetic distinctness, local cultivars were screened at 9 nuclear SSR loci described in previous studies: VVS4 (Thomas and Scott 1993), VVMD6, VVMD7 (Bowers et al. 1996), VVMD28, VVMD31 (Bowers et al. 1999), SCU07VV, SCU10VV, SCU14VV and SCU15VV (Scott et al. 2000). Briefly, genomic DNA was extracted from young leaves using the CTAB-based method proposed by Lodhi et al. (1994), and 2 µL of DNA (about 50 ng/µL) were added to 23 µL of a PCR mix containing 1.5 µL of dNTPs (4 mM), 1.25 µL of each primer (10 pM), 1.6 µL of $MgCl_2$ (25 mM), 2.5 µL of $(NH_4)_2SO_4$ buffer (10×), 14.7 µL of pure water and 1U of Taq DNA polymerase (0.2 µL; 5U/µL). Reactives were obtained from Fermentas-ThermoScientific (Germany). PCR was run on a thermocycler (Mastercycler-pro, Eppendorf, Germany) programmed for an initial denaturing step of 7 min at 95 °C, followed by 36 cycles at 95 °C for 45 s, 52–57 °C for 45 s, and 72 °C for 90 s, and a final step at 72 °C for 7 min. PCR products were independently subjected to electrophoresis in ethidium bromide-stained polyacrylamide gels (6%; PlusOne, GE Healthcare Bioscience, Sweden) along with a ready-to-use 100 bp DNA ladder (Fermentas-ThermoScientific, Germany), used as a reference for the visual scoring of fragments size.

Plant material evaluation

Forty-two qualitative and quantitative traits comprising phenology (1 descriptor), shoot (5 descriptors), leaf (9 descriptors), cluster (10 descriptors), berry (13 descriptors) and juice composition (4 descriptors) were evaluated. Unless otherwise stated, recommendations included in the OIV, IPGRI or ACSAD lists for grapevine evaluation were used (Table 1) (Al-Rayes et al. 2002; IPGRI et al. 1997; OIV 2007). Descriptions were carried out in 2009.

Phenology, shoot and leaf traits

Phenology, shoot and leaf traits were evaluated in five different plants per cultivar. Phenology was recorded as the time from flowering [modified E-L 23 stage (Coombe 1995)] to harvest time [modified E-L 38 stage (Coombe 1995)], as previously suggested by Jones and Davis (2000) (PH_TFH). Shoot tips were evaluated when they were 10–30 cm long [(between modified E-L stages 12 and 15 (Coombe 1995), approximately], and young leaf traits were evaluated at the same moment in the first four distal leaves of 10 different shoots. Lastly, mature leaf descriptions were obtained between berry set [modified E-L 27 stage (Coombe 1995)] and the beginning of veraison [modified E-L 35 stage (Coombe 1995)] on leaves above the cluster and within the medium third of the shoot.

Cluster traits

Cluster traits were evaluated at harvest time [modified E-L 38 stage (Coombe 1995)] in 10 different clusters from 3 different plants. Cluster theoretical volume (CL_TV) was estimated according to Shavrukov et al. (2004), cluster elongation (CL_E) as suggested by Tello et al. (2016), and cluster compactness (CL_CI12) according to the objective index CI-12 proposed by Tello and Ibáñez (2014), which relativizes cluster weight (g) to cluster length squared (cm^2). The number of berries per cluster (CL_BC) was calculated according to Sun et al. (2012).

Berry traits and juice composition

Berry (and seed) traits were evaluated at harvest time using 100 berries per cultivar, considering 10 non-deformed and normally-sized berries from the middle part of each of the 10 evaluated clusters. Berry elongation (BE_E) was evaluated as suggested by Marzouk and Kassem (2011).

For juice composition analyses, ACSAD methods were followed (Al-Rayes et al. 2002). Briefly, around fifty grapes per plant were crushed in a blender, and juice was filtered to remove skin and pulp. Juice pH (JU_pH) was evaluated in 10 mL of grape must using

Table 1 List of the 42 qualitative and quantitative descriptors used in this work

Type	Category	Description	Method	Acronym[a]	PCA[b]	StLDA[c]
Qualitative	Shoot	Shoot attitude (before tying)	OIV 006	SH_A	n.i.	n.i.
	Shoot	Number of consecutive tendrils	OIV 016	SH_T_N	n.i.	n.i.
	Shoot	Form of shoot tip	IPGRI 6.1.1	SH_F	n.i.	n.i.
	Leaf	Petiole sinus base limited by vein	OIV 081-2	LE_PSLV	n.i.	n.i.
	Leaf	Teeth in the petiole sinus	OIV 081-1	LE_TPS	n.i.	n.i.
	Leaf	Shape of teeth	OIV 076	LE_ST	n.i.	n.i.
	Leaf	General shape of petiole sinus	IPGRI 6.1.30	LE_SPS	n.i.	n.i.
	Leaf	Shape of blade	OIV 067	LE_SB	n.i.	n.i.
	Leaf	Number of lobes	OIV 068	LE_NL	n.i.	n.i.
	Cluster	Cluster shape	OIV 208	CL_S	n.i.	n.i.
	Cluster	Cluster compactness	OIV 204	CL_C	n.i.	n.i.
	Berry	Flesh firmness	OIV 235	BE_FF	n.i.	n.i.
	Berry	Berry shape	IPGRI 6.2.6	BE_SH	n.i.	n.i.
	Berry	Formation of seeds	OIV 241	BE_FS	n.i.	n.i.
	Berry	Color of berry skin	OIV 225	BE_C	n.i.	n.i.
Quantitative	Phenology	Time from flowering to harvest (days)	Jones and Davies (2000)	PH_TFH	PC-1	–
	Shoot	Length of shoot internodes (cm)	OIV 353	**SH_I_L**	PC-1	8
	Shoot	Length of tendrils (cm)	OIV 017	SH_T_L	PC-1	–
	Leaf	Petiole length (cm)	OIV 093[a]	LE_PL	PC-1	–
	Leaf	Middle vein length (cm)	OIV 601	LE_MVL	PC-1	–
	Leaf	Length of petiole compared to length of middle vein	OIV 093	LE_PLCMVL	PC-1	10
	Cluster	Peduncle of primary cluster length (cm)	OIV 206	CL_PPCL	PC-2	–
	Cluster	Cluster weight (g)	OIV 502	CL_We	PC-2	–
	Cluster	Berries per cluster	Sun et al. (2012)	CL_BC	PC-7	–
	Cluster	Cluster length (cm)	OIV 202	CL_L	PC-1	11
	Cluster	Cluster width (cm)	OIV 203	CL_Wi	PC-2	–
	Cluster	Cluster elongation	Tello et al. (2016)	CL_E	PC-1	–
	Cluster	Cluster theoretical volume (mL)	Shavrukov et al. (2004)	CL_TV	PC-2	–
	Cluster	Cluster compactness index (g/cm^2)	Tello and Ibanez (2014)	CL_CI12	PC-1	–
	Berry	Pedicel length (cm)	OIV 238	BE_PL	PC-3	5
	Berry	Single berry weight (g)	OIV 503	BE_We	PC-3	–
	Berry	Berry length (cm)	OIV 220	BE_Le	PC-2	6
	Berry	Berry width (cm)	OIV 221	**BE_Wi**	PC-3	3
	Berry	Berry elongation	Marzouk and Kassem (2011)	**BE_E**	PC-2	2
	Berry	Must yield (%)	OIV 233	BE_MY	PC-5	9
	Berry	Seeds per berry	ACSAD	BE_SB	PC-2	–
	Berry	Seed length (cm)	OIV 242	BE_SL	PC-2	7
	Berry	100 seeds weight (g)	OIV 243	**BE_100SW**	PC-4	1
	Juice	pH	Al-Rayes et al. (2002)	JU_pH	PC-3	–
	Juice	Total soluble solids (°Brix)	Al-Rayes et al. (2002)	JU_TSS	PC-6	–
	Juice	Titratable acidity (%)	Al-Rayes et al. (2002)	**JU_TA**	PC-5	4
	Juice	Vitamin C (ascorbic acid) (mg/100 mL)	Al-Rayes et al. (2002)	JU_VC	PC-2	–

The method and the acronyms used in this work are indicated

n.i. not included

[a] Variables selected for UHCA are indicated in bold

[b] For each quantitative variable, the PC with the highest Eigen-value is indicated

[c] The order of variable selection by stepwise-LDA (StLDA) is indicated as a ranking. Variables with no number were excluded by stepwise-LDA

a calibrated digital pH-meter (CyberScan pH 1100, Eutech Instruments). Titratable acidity (JU_TA) was measured by titration of 5 mL of grape juice with NaOH (0.1 N) to pH 8.2, measured with pH meter, and total soluble solids (JU_TSS) were measured with a digital pocket refractometer (Atago PAL-1, Atago). Lastly, ascorbic acid (JU_VC) was determined using an RQ-Easy reflectometer and Merck test strips (Merck). JU_VC is expressed as mg ascorbic acid/ 100 mL of juice. All analyses were carried out by triplicate, and average values are reported.

Statistical analyses

Phenotypic distribution of the traits considered in this study was assessed using histograms. To better fit the assumption of normality in the statistical analyses, the variables BE_LE, PH_TFH and SH_I_L were square-root transformed, whereas the variables BE_100SW, BE_PL, BE_We, BE_Wi and CL_CI12 were logarithmically transformed (Tabachnick and Fidell 1996). Analyses were performed using SPSS v.21.0 (IBM, Chicago, IL, USA), unless otherwise stated.

Kendall's τb coefficients were calculated to evaluate the bivariate correlation between the different traits included in this work. Coefficients were considered significant if $p \leq 0.05$. The correlation plot was obtained with the *corrplot* package for R v.3.2.5 (http://www.r-project.org/). A Principal Component Analysis (PCA) was performed to identify the underlying relationships between the 27 quantitative traits evaluated in this work (Table 1), retaining those PCs whose eigenvalues were higher than 1. Then, a stepwise linear discriminant analysis (stepwise-LDA) was conducted as previously indicated (Tello et al. 2015) to select a reduced set of variables with a high capability to discriminate the different grapevine cultivars used in this study. The predictive capability of the canonical functions released was assessed both by direct and by leave one-out cross-validation. The different samples used in this work were further grouped by means of an unsupervised hierarchical clustering analysis (UHCA) (Ward's method, Euclidian distance) on the basis of the five most predictive quantitative variables, which were selected according to PCA and stepwise-LDA results. Lastly, one-way ANOVA followed by Fisher's LSD post-hoc tests was calculated for the five selected variables to assess significant differences in the phenotype of the cultivars in the different clusters identified by the UHCA. Values of $p \leq 0.05$ were considered statistically significant. Boxplots were obtained with the *easyGgplot2* package for R.

Results and discussion

In this work, a set of local cultivars prospected in the Syrian Arab Republic and maintained in the Grapevine Collection of the Jillin Research Station (ACSAD) have been analyzed. The non-redundancy of these cultivars was revealed by the use of 9 nuclear SSR markers (Online Resource 1). Unfortunately, only two of these markers (VVMD7 and VVMD28) are part of the core set of SSRs adopted as DNA-based descriptors by the OIV (OIV 2007) and/or routinely reported. Consequently, this genetic information was insufficient to compare our data with other published genotypes or public international databases (like the *European Vitis database* and the *Vitis International Variety Catalogue*) to detect potential cases of synonyms.

Values of the different ampelographic traits analyzed in this study can be found in the Online Resource 2 and observed in the Online Resource 3. We found a high level of diversity for highly relevant traits, including the time from flowering until harvest (PH_TFH, from 56 ± 0 to 97 ± 0 days, for cvs. Cardinal and Baladi, respectively), cluster weight (CL_We, from 315.6 ± 61.1 to 516.7 ± 89.6 g for cvs. Muscat of Alexandria and Halawani Kanawat, respectively), seed number (BE_SB, from 1.3 ± 0 to 3.7 ± 0 seeds, for cvs. Baladi and Muscat of Alexandria, respectively) and berry elongation (BE_E), including cultivars with spherical (cvs. Muscat of Alexandria, Cardinal, Halawani Kanawat) and very elongated berries (cv. Baladi). The average weight of berries was also highly variable, from 3.3 ± 0.2 (cv. Muscat of Alexandria) to 6.2 ± 0.2 g (cv. Black Turky Homs). Agreeing with previous results (Khadivi-Khub et al. 2014), we observed a high degree of variation for juice composition: total soluble solids (JU_TSS) ranged from 17.1 ± 0.7 (cv. Bhoth-2-Zinni slale) to 22.9 ± 0.2°Brix (cv. Muscat of Alexandria) and titratable acidity (JU_TA) was found to be between 0.4 ± 0.0 (cv. Halawani Kanawat) and 1.1 ± 0.1% (cv. Cardinal). Vitamin C content (JU_VC) ranged between 9.7 ± 1.2 (cv. Muscat of

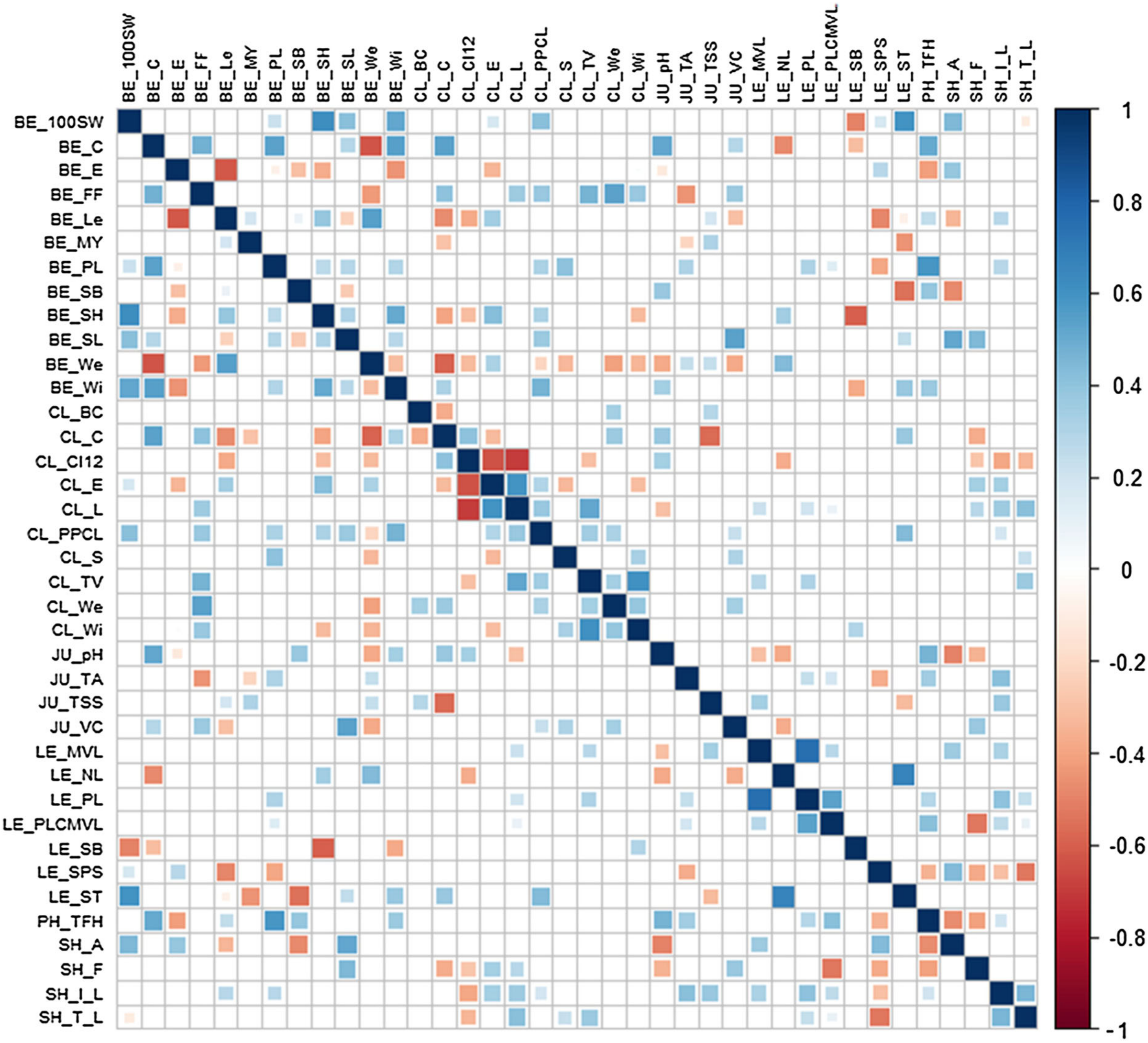

Fig. 1 Correlation plot based on Kendall's τb correlation coefficients obtained between 38 variables included in this study. Variables BE_FS, LE_PSLV, LE_TPS and SH_T_N were not included in the analysis due to their lack of variance. Only significant ($p \leq 0.05$) correlations are shown as *colored squares* (according to adjacent *color bar*). Variables are named according to Table 1. (Color figure online)

Alexandria) and 30.9 ± 0.3 mg/100 mL (Halawani Kanawat), a similar range of variation to that previously reported for a set of autochthonous table grape cultivars from Turkey (Eyduran et al. 2015). Besides, some of the qualitative traits analyzed did not show any variance in our set of cultivars: BE_FS (all cultivars were seeded), LE_PSLV (all cultivars presented veins in both sides of the leaf petiole sinus), LE_TPS (none of the cultivars presented teeth at the petiole sinus) and SH_T_N (all cultivars had two or less consecutive tendrils in the shoot). Consequently, these variables were not considered in further analyses.

As expected, significant correlations ($p \leq 0.05$) between many of the traits analyzed were observed, but, in general, coefficients were low (Fig. 1). As previously reported in different intergenotypic works (Leão et al. 2010, 2011; Khadivi-Khub et al. 2014; Tello et al. 2015), the highest absolute correlations were obtained between traits of the same category (i.e. leaf, cluster and berry traits, Table 1). In this sense, berry traits like weight (BE_We), length (BE_Le),

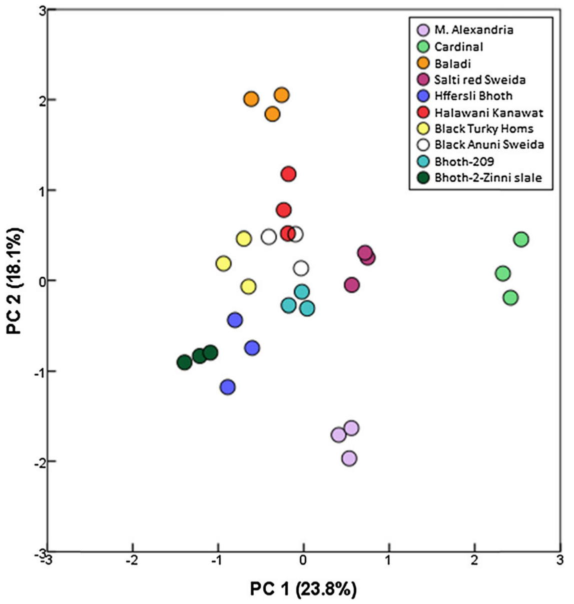

Fig. 2 PCA plot determined for the first two principal components (PC1 and PC2). Each sample is indicated as a *color dot*, according to the legend. (Color figure online)

width (BE_Wi), elongation (BE_E) and shape (BE_SH) were highly correlated, and so happened with some cluster traits like length (CL_L), width (CL_Wi), elongation (CL_E), volume (CL_TV) and weight (CL_We). As observed in different grapevine cultivars (Gatti et al. 2016; Ivorra et al. 2015), the objective compactness index CI-12 proposed by Tello and Ibáñez (2014) correlated significantly with the visual OIV rating in our sample, supporting the usefulness of this indicator for the quantitative estimation of this trait. Interestingly, seed number (BE_SB) did not correlate significantly with berry dimension variables (BE_We, BE_Wi) or its value of significant correlation was low (BE_Le). It is generally accepted that berry dimensions are directly affected by seed number at a intragenotypic level (Walker et al. 2005), but it is not that obvious at an intergenotypic level (Houel et al. 2013; Tello et al. 2015). In this regard, Doligez et al. (2013) have indicated the lack of colocalization between QTLs for berry and seed traits, suggesting that the genetic control of both traits may be partly dissociated.

A PCA analysis was performed to obtain information about the underlying relationship among the 27 quantitative variables included in this work (Table 1). The eight principal components retained were able to explain 92.5% of the variance, and the PC number in which each variable obtained its highest loading can be found in Table 1. Interestingly, the time from flowering to harvest (PH_TFH), and all shoot (SH_I_L and SH_T_L) and leaf variables (LE_MVL, LE_PL and LE_PLCMVL) obtained their highest loadings in PC-1, which explains 23.8% of total variance. In PC-2 (18.1%), different cluster size variables (CL_TV, CL_We and CL_Wi) obtained their highest loadings. Berry dimensions variables (BE_E, BE_Le, BE_We and BE_Wi) showed their highest correlations with components PC-2 and PC-3.

Figure 2 shows the scattering of the thirty grapevine samples analyzed on the bi-dimensional space determined by PC-1 and PC-2. PC-1 was able to separate the cv. Cardinal samples from the other cultivars, due to its high relationship with the variables linked to PC-1 (Table 1). In our sample, cv. Cardinal is the one with the largest leaf petioles (15.4 $\pm$ 1.6 cm), tendrils (26.3 $\pm$ 0.9 cm), and shoot internodes (12.9 $\pm$ 1.1 cm), the shortest cycle from flowering to harvest time (56 $\pm$ 0 days) and it has the largest (29.5 $\pm$ 0.7 cm) and loosest clusters (CI-12 $= 0.4 \pm 0.0$ g/cm^2). On the other hand, PC-2 could separate the cvs. Muscat of Alexandria and Baladi samples from the rest of genotypes. These two cultivars present the most extreme values for some of the variables linked to PC-2, including berry length, berry elongation and seed number (1.6 $\pm$ 0.0 and 2.5 $\pm$ 0.0 cm; 1.0 $\pm$ 0.0 and 1.7 $\pm$ 0.0; 3.7 $\pm$ 0.0 and 1.3 $\pm$ 0.0 seeds, for cvs. Muscat of Alexandria and Baladi, respectively).

A stepwise LDA was then used to identify the variables with the highest capability to discriminate the cultivars used in this work. As a result, eleven variables were selected as the most discriminant predictors for sample classification (BE_100SW, BE_E, Be_Le, BE_MY, BE_PL, BE_SL, BE_Wi, CL_L, JU_TA, LE_PLCMVL and SH_I_L). According to PCA analysis, these variables obtained their highest loadings in the first 5 retained PCs, and they represent diverse shoot, leaf, cluster, berry and juice traits (Table 1), suggesting that the different plant organs evaluated in this work harbor independent information of our set of cultivars for their successful discrimination. In fact, the discriminant model was able to classify correctly 100% of samples in their original cultivar class in both direct and leave-one-out cross-validation approaches (data not shown),

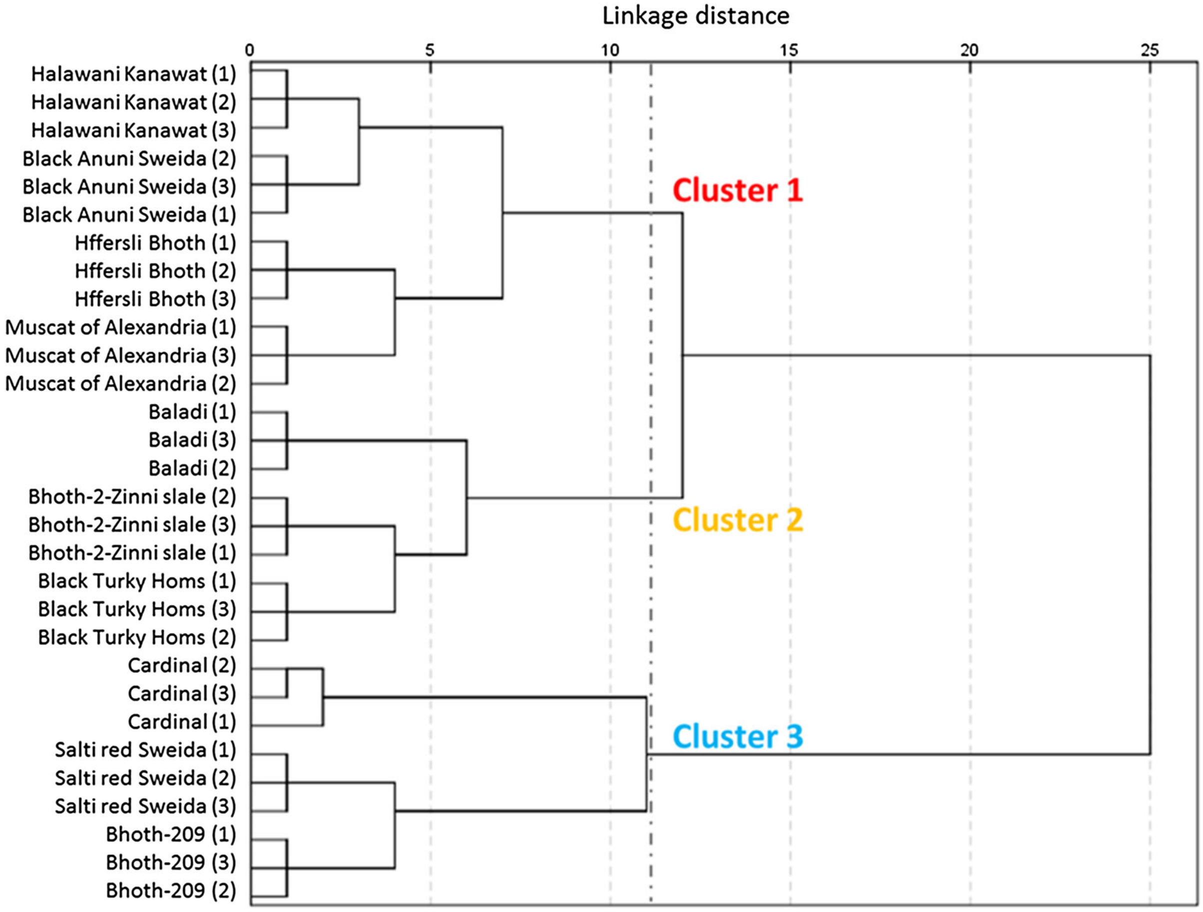

Fig. 3 Dendrogram for the ten table grape cultivars analyzed in this work obtained from the unsupervised hierarchical clustering analysis (UHCA) based on 5 traits (BE_100SW, BE_Wi, BE_E, JU_TA and SH_I_L). Sample number is indicated between *brackets*. (Color figure online)

confirming the high discriminant capability of these variables.

Considering PCA and stepwise-LDA results, a reduced set of variables was then selected to evaluate their clustering capability (Table 1). For their selection, only the 11 variables identified by the stepwise-LDA approach were considered, and only one variable per PC were selected. If two (or more) variables were found to be linked to the same PC, the variable that was first selected by the stepwise-LDA analysis was prioritized. As a result, the variables SH_I_L (representing PC-1), BE_E (PC-2), Be_Wi (PC-3), BE_100SW (PC-4) and JU_TA (PC-5) were finally selected. These variables not only represent different and independent variance segments, but also highly appreciated traits for table grape consumers and growers, including berry size, shape and seediness (BE_100SW, BE_E and Be_Wi), grape composition (JU_TA) and plant vigor (SH_I_L) (Dragincic et al. 2015; Wang et al. 2017; Zhou et al. 2015). In fact, these are key parameters usually considered in table grape breeding programs for the development of new premium varieties (Wei et al. 2002).

The UHCA based on these five selected variables allowed the assessment of similarity between the table grape cultivars included in this study. Samples were adequately clustered according to their genotype, and genotypes were distributed into three different clusters (Fig. 3). Cluster 1 grouped four cultivars (Black Anuni Sweida, Hffersli Bhoth, Halawani Kanawat and Muscat of Alexandria), whereas Cluster 2 clustered three (Baladi, Bhoth-2-Zinni slale and Black Turky Homs). The remaining three cultivars (Bhoth-209, Cardinal and Salti red Sweida) were

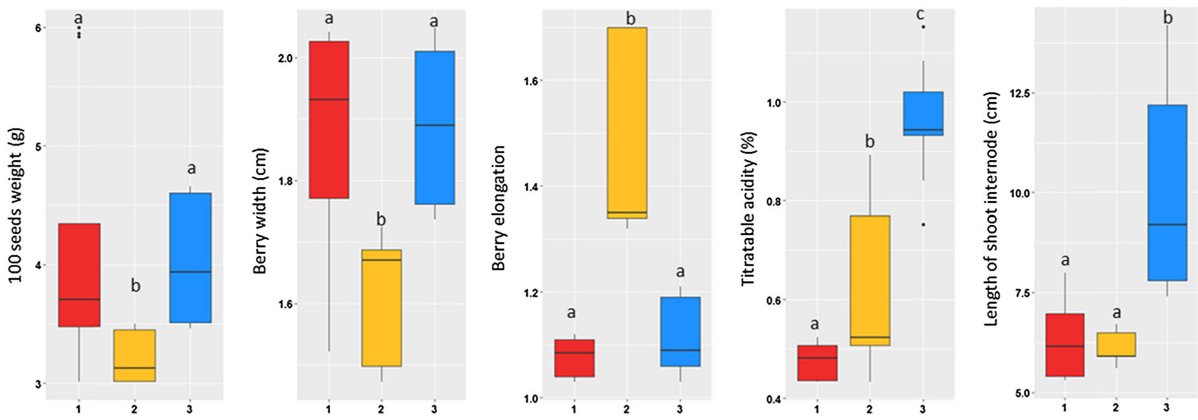

Fig. 4 Boxplots showing the phenotypic distribution of the five most discriminant traits [100-seeds weight (g), Berry width (cm), Berry elongation, Titratable acidity (%) and length of shoot internode (cm)] for the three clusters identified by UHCA. Cluster 1, 2 and 3 are shown in *red*, *yellow* and *blue*, respectively. *Different lowercase letters* within a plot indicate significant difference among clusters according to Fisher's LSD tests ($p \leq 0.05$). (Color figure online)

grouped in Cluster 3. The cultivars grouped in these three clusters showed significant differences in the five selected traits (Fig. 4). Accordingly, cultivars in Cluster 1 were low vigorous and presented large, spherical and highly seeded berries with the lowest values of acidity. Cultivars in Cluster 2 were also low vigorous, but berries were small, elongated and lowly seeded, and they presented intermedium values of titratable acidity. Lastly, cultivars in Cluster 3 were highly vigorous and presented large, spherical and highly seeded berries and the highest values of titratable acidity. Interestingly, some of the local Syrian varieties were grouped with the table grape cvs. Cardinal and Muscat of Alexandria, suggesting some similitude (at least for these five attributes) to two of the most worldwide grown table grape cultivars (Fig. 3). It highlights the potential of the local Syrian genetic resources for the development of new table grape varieties, as well as the necessity of developing specific programs for their management, multiplication, preservation, and further characterization.

Conclusion

The ampelographic description of grapevine phenotypic traits is the first step towards the evaluation of endangered local varieties. Although preliminary, our results indicate the existence of significant variation for a large number of relevant yield and quality traits for grape consumers and growers, suggesting the high potential of traditional Syrian grapevines for the development of new high-quality table cultivars and the needing of specific preservation programs aimed to avoid the loss of local genetic resources. This work improved the knowledge of the grapevine germplasm of the region, but should be repeated in other locations and conditions prior their consideration in future table grape breeding programs. In addition, specific genetic analyses are needed to evaluate their diversity and their relationship with other local and foreign cultivars, as well as for correct identification of potential synonyms. Besides, the multivariate approach here reported allowed the successful identification of a reduced set of ampelographic traits for the adequate discrimination of the cultivars, and it could be potentially applied to the analysis of diverse grapevine genetic resources.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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