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Morpho-physiological diversity in the collection of sour cherry (*Prunus cerasus*) cultivars of the Fruit Genebank in Naoussa, Greece using multivariate analysis

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ABSTRACT

The phenotypic characterization of sour cherry cultivars provides important information on their attributes, which is of paramount importance for their breeding and germplasm management. This study examined the Hellenic Sour Cherry Genebank, which consists of 27 sour cherry cultivars, located in the Institute of Pomology Fruit Genebank, Naoussa, Greece. The data analyzed in this study were obtained after a ten-year consecutive monitoring of 38 morpho-physiological descriptors of phenology, plant morphology, yield and fruit quality. For the statistical analysis were applied hierarchical cluster analysis and principal component analysis (PCA). According to the analysis, the descriptors demonstrated a high degree of variability, especially, yield, ratio of titratable acidity and soluble solids, number of flower buds, and pit weight. Moreover, the variables fruit equatorial diameter, fruit width, fruit weight, fruit size, fruit shape and lamella shape characters exhibited high discriminating power. Significant positive and negative correlations were detected as well among the studied quantitative traits. The highest significant positive correlation was found between the fruit equatorial diameter and the fruit width (0.974). Whereas, the highest significant negative correlation was found between the ratio of pit volume to fruit volume and the fruit weight (−0.737). An unsupervised hierarchical cluster analysis was performed using the Euclidean distance and the Ward's agglomeration method. The sour cherry cultivars were classified into three main clusters, suggesting that the characterized sour cherry collection has a high potential for specific breeding goals. We discuss the usefulness of the identified correlations among the traits, for potential breeding projects regarding fruit size and quality.

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1. Introduction

Sour cherries are a highly important fruit crop with many health benefits, which contributed to the increase of their commercial importance, especially in the temperate zone (Toydemir et al., 2013). Moreover, sour cherry helped the development of new commercial cultivars of sweet cherry, used as rootstock imparting dwarf and resistant plants (Kappel et al., 2012). A large number of studies have been carried out in order to characterize Genebank collections of Cerasus germplasm and evaluate Cerasus germplasm from native populations (Hillig and Iezzoni, 1988; Khadivi-Khub et al., 2012; Krahl et al., 1991; Nazari et al., 2012; Perez et al., 2010; Rodrigues et al., 2008; Sánchez et al., 2008, among others).

The study of phenotypic traits is precise enough for the identification and analysis of the underlying genetic diversity between

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Table 1
Origin and the main fruit characteristics sour cherries cultivars analyzed.

Number (No)	Cultivar	Origin	Fruit shape	Skin colour	Fruit size
1	Vissino Episkopis	Greece	oblate	light red	very large
2	Kanaris	Greece	circular	light red	medium
3	Florinis	Greece	circular	light red	very small
4	Cacanski Rubin	Czech	circular	medium red	large
5	Cerise Belle Magnifique	France	circular	medium red	small
6	Cigahcica	Hungary	oblate	medium red	very large
7	Dwarf Meteor	U.S.A.	circular	light red	large
8	Dwarf Northstar	U.S.A.	oblate	light red	very small
9	Gelly	–	circular	light red	medium
10	Griotte De Provence	France	oblate	light red	medium
11	Keleriis	Denmark	oblate	light red	small
12	Heimann Rubin	Czech	oblate	light red	very large
13	Ilva	Romania	oblate	medium red	very large
14	Lotova	Russia	circular	blackish	very large
15	Marasca	Bulgary	oblate	light red	large
16	Marasca Moschata	Bulgary	circular	medium red	large
17	Meteor Korai	Hungary	oblate	light red	very large
18	Ministro Bodbieski	Russia	oblate	medium red	medium
19	Montmorency	France	oblate	light red	very large
20	Oblacinska	Serbia	reniform	medium red	very large
21	Pándy 48	Hungary	oblate	medium red	large
22	Randy Mecy	Hungary	circular	light red	very small
23	Rekseler	–	oblate	brown red	large
24	Rubin	Czech	circular	red	very small
25	Stark Montmorency	U.S.A.	elliptic	light red	very small
26	Suda Hardy	Germany	oblate	medium red	very large
27	N15	Greece	oblate	light red	small

the various sour cherry cultivars (IPGRI, 1985; UPOV, 1995), while the evaluation of morphological traits through phenotyping constitutes a quick method to both characterize the sour cherry germplasm and provide useful qualitative information for breeding.

Characterization of phenotypic diversity and structure is paramount in order to discover the phenotypic traits that contribute to the total diversity in a germplasm collection and discover the levels of variation among the cultivars (de Oliveira et al., 2012; Furones-Pérez and Fernández-López, 2009; Mehmood et al., 2014). A powerful statistical technique for analyzing genetic relations from morphological traits –a large data set consisting of many qualitative and quantitative traits- is multivariate data analysis (de Oliveira et al., 2012 Furones-Pérez and Fernández-López, 2009; Mehmood et al., 2014). PCA and cluster analysis are the most popular multivariate techniques for the morphological characterization of genotypes (Mohammadi and Prasanna, 2003; Peeters and Martinelli, 1989). The combination of PCA and cluster analysis could uncover important information concerning morphological traits that contribute to genetic diversity in plants (Khodadadi et al., 2011).

In many countries new breeding material of sour cherry cultivars and traditional local varieties are compared, in order to evaluate their performance in different climatic conditions (Grafe and Schuster, 2014; Schuster et al., 2009, 2014; Siddiq et al., 2011). In plant breeding programs, the attention has been focused on finding the appropriate parents for hybridizations. A large number of studies have been carried out towards the characterization of fruit traits involved in fruit quality. Recently, it has been discovered that some varieties of Hungarian sour cherries had functional properties that enhanced the health benefits of sour cherries (Papp et al., 2010; Veres et al., 2006). For the characterization and preservation of the genetic material of eight autochthonous sour cherry cultivars in Portugal, studies of commercially interesting morphological traits were also carried out; see Rodrigues et al. (2008). Rakonjac et al. (2010) studied the morphological traits of 41 accessions of the autochthonous and commercially important cultivar 'Oblačinska', and found that only two genotypes could be cultivated in Serbia. Moreover, the physicochemical composition and health potential of the cultivar 'Marasca' has been studied on a great scale (see Grafe and Schuster, 2014; Pedisić et al., 2007; Šarić et al., 2009).

The aims of this study were (i) to evaluate the phenotypic diversity in 27 international sour cherry cultivars preserved in a Greek GeneBank collection, (ii) to identify specific traits, and (iii) to detect relationships among the studied cultivars.

2. Materials and methods

2.1. Plant material

The 27 sour cherry cultivars studied originate from the Fruit Genebank of the Institute of Pomology, Naoussa, Greece (Table 1).

The Institute is located at 40°63′ N latitude, and 22°06′ E longitude, at an altitude of 115 m. The trees, propagated on Prunus avium rootstock, dated around 10–14 years old. Each cultivar was represented in the Gene Bank by three trees which were used to produce the data. Thirty-eight variables were selected as sour cherry descriptors and were included in the experimental collection for ten consecutive years (2000–2009). Different horticultural practices, including fertilizer application, spraying and irrigation, among others, were performed at regular intervals each year. At the beginning of the study, (2000), the trees were at fruit-bearing capacity (8 years old), healthy and in cropping condition.

2.2. Analysis of morpho-physiological traits

The 24 quantitative traits evaluated were yield [Yd], number of flower buds [NoFlBu], petiole length [PeLe], petiole width [PeWi], ratio petiole length/width [PeLe/PeWi], blade length [BlLe], blade width [BlWi], ratio blade length/petiole length [BlLe/BlWi], number of nectary glands [NoNeBu], number of flowers per spur [NoFlSp], fruit polar diameter [FrPoDi], fruit equatorial diameter [FrEqDi], fruit width [FrWi], fruit weight [FrWe], soluble solids [SoSo], titratable acidity [TiAc], ratio titratable acidity/soluble solids [TiAc/SoSo], pedicel length [PedLe], pit length [StLe], pit width [StWi], pit thickness [StTh], pit weight [StWe], ratio weight

Table 2
Descriptive statistics for 24 quantitative traits in 27 sour cherry cultivars.

Variable	Minimum	Maximum	Mean	Std. deviation	CV (%)
Yd	20.000	70.000	42.037	15.207	36.17
NoFlBu	1.000	6.500	4.596	1.233	26.82
PeLe	8.300	11.800	9.777	0.775	7.92
PeWi	3.650	6.490	5.153	0.561	10.88
PeLe/PeWi	1.613	2.381	2.055	0.220	10.70
BlLe	1.350	3.780	2.142	0.410	19.14
BlWi	0.130	0.200	0.161	0.016	9.93
BlLe/BlWi	0.143	0.347	0.216	0.035	16.20
NoNeBu	1.000	3.000	1.765	0.441	24.98
NoFlSp	2.500	4.500	3.426	0.583	17.01
FrPoDi	15.300	22.000	17.800	1.434	8.05
FrEqDi	15.700	24.000	20.726	2.002	9.65
FrWi	13.900	21.000	18.322	1.851	10.10
FrWe	2.600	7.000	5.030	1.043	20.73
SoSo	11.600	19.700	14.369	1.938	13.48
TiAc	5.700	24.000	14.917	4.646	31.10
TiAc/SoSo	0.286	1.667	1.052	0.308	29.27
PedLe	1.900	5.500	3.744	0.754	20.13
StLe	9.000	11.800	10.033	0.687	6.84
StWi	7.400	11.000	9.056	0.843	9.30
StTh	5.900	8.500	7.237	0.600	8.29
StWe	0.250	0.520	0.349	0.072	20.63
StWe/FrWe	0.040	0.118	0.072	0.017	23.61
StVo/FrVo	0.059	0.141	0.098	0.018	18.36

pit/weight fruit [StWe/FrWe] and ratio volume pit/volume fruit [StVo/FrVo].

The 14 qualitative traits analyzed were fruit type [FrTy], tree vigour [TrVi], tree shape [TrSh], tree habit [TrHa], tree branching [TrBr], lamella shape [LaSh], lamella size [LaSi], shape of petal [PeSh], arrangement of petals [ArPe], fruit shape [FrSh], skin colour [SkCo], fruit size [FrSi], pit shape [StSh] and pit size [StSi].

These traits were selected from the International Union for the Protection of New Cultivars of Plants descriptors proposed for sweet cherry (UPOV, 1995; IPGRI, 1985). The scoring system is the same as used by UPOV and IPGRI. When possible, all measurements regarding a trait were made on the same date, in order to diminish differences due to environment and developmental stages of the trees.

To minimize the environmental effects, all parameters were averaged over ten years. Fruit was harvested randomly from various parts of trees at the ripening time of each cultivar (varied from the beginning of June to end of July), based both on its skin colour, appearance and taste.

A portable digital refractometer (Palette PR-32, Atago Co. Tokyo, Japan) was used in order to measure the soluble solid content (SoSo), which is expressed as percentage of the juices (%Brix). The titratable acidity (TiAc) was measured as follows: a mixture of 2 ml of juice and 60 ml of distilled water were titrated using 0.1 N NaOH to pH 8.2 (automatic titrator TitroLine alpha, Schott-Geräte GmbH, Ludwigshafen, Germany). Three titrations per sample were performed. The TiAc/SoSo0020ratio was calculated and used as an indicator of taste quality.

2.3. Data scoring and analysis

Data of 27 sour cherry cultivars, including 38 traits, were analyzed via XLSTAT software (version 2014.1). The mean values calculated for each parameter were used and PCA was applied separately for the quantitative and qualitative traits. The missing data were estimated by the mean in the first case and by the mode in the latter. In both cases the correlation matrix was used, since the variables were measured in different units and standardization deemed to be necessary. Within PCA, factor loadings >0.55 were regarded as significant, since the number of observations was 27 (see also Mehmood et al., 2014). In each case, 3D plots were

Table 3
Correlation coefficients (Pearson) among 24 quantitative traits in 27 sour cherry cultivars.

	Yd	NoFlBu	PeLe	PeWi	PeLe/PeWi	BlLe	BlWi	BlLe/BlWi	NoNeBu	NoFlSp	FrPoDi	FrEqDi	FrWi	FrWe	SoSo	TiAc	TiAc/SoSo	PedLe	StLe	StWi	StTh	StWe	StWe/FrWe	StVo/FrVo
Yd	1																							
NoFlBu	0.166	1																						
PeLe	**0.392**	−0.206	1																					
PeWi	**0.383**	0.175	**0.663**	1																				
PeLe/PeWi	−0.005	−0.210	−0.242	**−0.558**	1																			
BlLe	0.303	0.212	**0.415**	**0.620**	−0.293	1																		
BlWi	0.152	0.144	**0.664**	**0.731**	−0.280	**0.542**	1																	
BlLe/BlWi	0.184	0.341	0.050	0.368	−0.283	**0.915**	0.292	1																
NoNeBu	−0.020	**0.397**	−0.116	0.280	−0.341	0.221	0.241	0.317	1															
NoFlSp	0.115	0.157	−0.304	−0.242	**0.543**	−0.108	−0.132	−0.035	0.145	1														
FrPoDi	0.161	0.259	−0.073	0.142	0.153	0.173	0.149	0.213	**0.454**	**0.487**	1													
FrEqDi	−0.010	0.293	−0.146	0.148	0.147	**0.418**	0.217	**0.479**	**0.389**	0.377	**0.774**	1												
FrWi	0.052	0.261	−0.083	0.243	0.133	**0.478**	0.301	**0.511**	**0.456**	0.363	**0.745**	**0.974**	1											
FrWe	0.110	0.281	−0.093	0.185	0.156	**0.460**	0.231	**0.511**	**0.383**	**0.429**	**0.826**	**0.968**	**0.961**	1										
SoSo	−0.081	−0.241	−0.228	−0.373	**0.556**	−0.266	−0.302	−0.282	**−0.415**	**0.605**	0.045	0.115	0.067	0.142	1									
TiAc	−0.094	**−0.403**	−0.029	**−0.389**	−0.037	**−0.504**	−0.295	**−0.500**	−0.370	−0.090	**−0.575**	**−0.612**	**−0.667**	**−0.650**	0.150	1								
TiAc/SoSo	−0.148	−0.294	−0.017	−0.288	−0.261	**−0.458**	−0.213	**−0.421**	−0.179	−0.309	**−0.551**	**−0.631**	**−0.679**	**−0.686**	−0.207	**0.921**	1							
PedLe	−0.097	−0.034	0.003	0.076	−0.051	0.302	0.100	0.314	0.142	0.165	0.166	0.293	0.258	0.220	−0.019	0.129	0.174	1						
StLe	−0.099	0.096	0.021	−0.041	−0.218	−0.051	0.060	−0.003	0.332	−0.066	**0.489**	0.184	0.074	0.159	−0.318	0.005	0.164	0.296	1					
StWi	0.063	−0.066	0.001	0.130	0.134	**0.566**	0.239	**0.597**	0.191	0.228	**0.503**	**0.738**	**0.756**	**0.707**	−0.034	**−0.430**	**−0.440**	**0.406**	0.126	1				
StTh	0.023	−0.035	−0.009	0.048	0.195	**0.536**	0.193	**0.579**	0.143	0.112	**0.449**	**0.680**	**0.692**	**0.659**	−0.115	**−0.451**	**−0.435**	0.313	0.152	**0.932**	1			
StWe	0.034	−0.075	0.018	−0.002	−0.047	0.326	0.079	0.380	0.187	0.092	**0.434**	**0.459**	**0.415**	**0.445**	−0.109	−0.136	−0.105	0.349	**0.519**	**0.715**	**0.751**	1		
StWe/FrWe	−0.071	−0.359	0.091	−0.195	−0.179	−0.250	−0.202	−0.261	−0.235	−0.298	**−0.453**	**−0.595**	**−0.616**	**−0.630**	−0.152	**0.513**	**0.561**	0.070	0.226	−0.125	−0.061	**0.387**	1	
StVo/FrVo	−0.130	−0.288	0.074	−0.243	−0.117	−0.173	−0.115	−0.171	−0.290	−0.359	**−0.635**	**−0.697**	**−0.696**	**−0.737**	−0.252	**0.509**	**0.573**	0.055	0.107	−0.161	−0.060	0.177	**0.885**	1

Values in bold are different from 0 with a significance level alpha = 0,05.

Fig. 1. Fruit features of the main sour cherry cultivars used in this study.

constructed, based on the three most important principal components, to facilitate the visualization of the results. Within correlation analysis, the Pearson coefficient (parametric) was used to measure the correlation among quantitative characteristics and the Spearman coefficient (non-parametric) was used to measure the correlation among qualitative characteristics. The combined data from both the quantitative and qualitative traits were used for dendrogram construction. The chosen distance to estimate the genetic dissimilarity component was Euclidean, and Ward's method was selected for the agglomerative hierarchical clustering (AHC).

3. Results and discussion

3.1. Descriptive statistics and correlations for the quantitative variables

In this survey, 24 quantitative traits were evaluated and descriptive analysis' results are displayed in Table 2, including minima, maxima, means, standard deviations and coefficients of variation (CV). These results revealed extensive morphological diversity, with some quantitative traits displaying high CV, such as Yd (36.17%), TiAc/SoSo (29.27%), NoFlBu (26.82%), StWe/FrWe (23.61%) and StWe (20.63%).

Additionally, strong positive, linear correlations were observed among all 24 tested quantitative traits (see Table 3). The highest significant, positive correlation was observed between FrWi and FrEqDi (0.974). Significant positive correlations were also observed between: FrEqDi and FrWe (0.968), StWi and StTh (0.932), TrAc and TiAc/SoSo (0.921), BlWi and BlLe/BlWi (0.915), FrWe and FrPoDi (0.826), FrPoDi and FrEqDi (0.774), FrWi and FrPoDi (0.745), StWi and FrEqDi (0.738), and StTh and FrWi (0.692). In addition, high significant, negative correlations have been revealed, for example, between: StVo/FrVo and FrEqDi (−0.697), and StVo/FrVo and FrWi (−0.696) (Table 3). Significant correlations were observed between phenotypic traits which contribute to fruit yield and quality; this information might be quite helpful for any future plant breeding program. To attain high yield and superior quality cultivars, for instance, cross combinations could be performed between cultivars with very large fruit size ('Florinis', 'Vissino Episkopis', 'Oblacinska' and 'Montmorency'), low pit weight ('Montmorency', 'Stark Montmorency' and 'Griotte De Provence') and high total soluble solids ('Gelly', 'Griotte De Provence' and 'Kanaris'). Previously, in Grafe and Schuster (2014), 75 sour cherry genotypes were examined and rather low correlation coefficients among the fruit traits were found. Siddiq et al. (2011) suggested that sour cherry cultivars with higher fruit weight tend to reveal relatively higher soluble solids. In sweet cherry, significant correlation between fruit weight and other fruit quality traits, such as soluble solids and titratable acidity, were recently observed; see Ganopoulos et al. (2015). Similarly, significant relationships were revealed (r = −0.330) among fruit weight and titratable acidity in nectarine and peach cultivars; see Cantín et al. (2010). In our study, the observed low correlation coefficient between soluble solids and titratable acidity (r = 0.150) does not imply any tendency. This is consistent with the results of Grafe and Schuster (2014), where a similar correlation coefficient is postulated. Previous surveys targeting either a worldwide collection of 146 sweet cherry cultivars (Ganopoulos et al., 2015) or the collection of sour and duke cherries of the Fruit Genebank in Dresden-Pillnitz (Höfer and Peil, 2015), both revealed a high

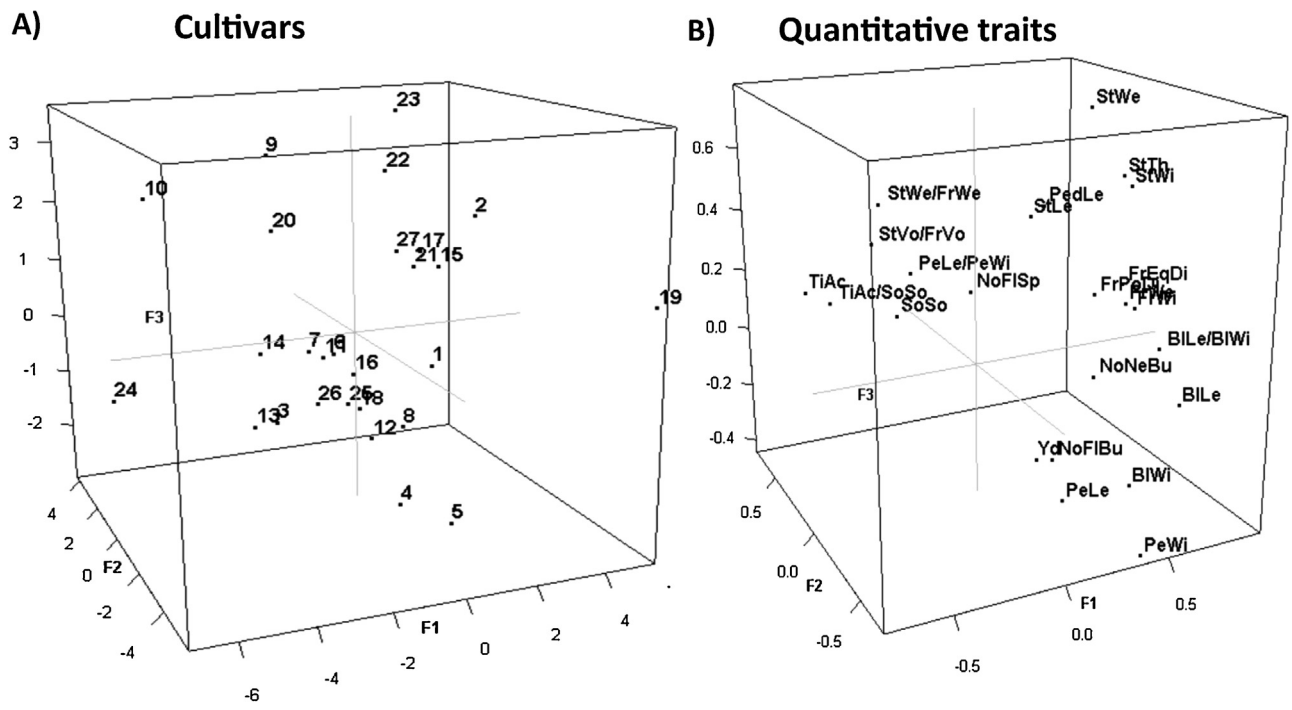

Fig. 2. A) Three-dimensional PCA plot of the 27 sour cherry cultivars with regard to the first three principal components. B) Three-dimensional PCA plot of the 21 quantitative traits with regard to the first three principal components. Variability explained: F1 (34.20%), F2 (16.22%), F3 (12.54%).

and significant positive relationship between soluble solids and titratable acidity, whilst no correlation was observed in Spanish apricot genotypes (Ruiz and Egea, 2008).

A high phenotypic variability (Fig. 1) with significant differences between cultivars was observed among the fruit quality tested traits. Certain cultivars depicted either higher or lower values regarding specific quality traits compared with the commercial cultivars. Regarding the fruit weight trait, most of the cultivars produced larger fruits compared to 'Rubin', 'Cerise Belle Magnifique', 'Marasca Moschata', 'Griotte De Provence', and 'Ministro Bodbieski' cultivars. Moreover, the cultivar "Kanaris" revealed a characteristic combination of soluble solids and titratable acidity. These characteristics, besides the superior appearance and quality, may be crucial parameters for global market (see also Grafe and Schuster, 2014).

Sour cherries are consumed in different forms and are processed towards different sour cherry products. We observed a high diversity regarding the fruit quality related attributes, which might allow developing cultivars with characteristics especially suited for special products, or even selecting them across the existing gene pool. Regarding the fresh consumption varieties, we have identified Greek cultivars, such as 'Kanaris' and 'Florinis', to share favorable levels of soluble solids and titratable acidity. Furthermore, the observed diversity suggests that there are cultivars which could meet certain dietary requirements of specific groups of consumers, such as sour cherries with low soluble sugars for diabetics (Grafe and Schuster, 2014).

3.2. Principal component analysis of quantitative variables

The most significant traits in the datasets were revealed by applying PCA, which is a procedure that lies within the framework of multivariate statistical analysis. PCA has been already employed to determine the genetic relationships among cultivars, to study correlations among tree traits and to evaluate cherries and sweet cherry (Beyer et al., 2002; Ganopoulos et al., 2015, 2011; Hjalmarsson and Ortiz, 2000), sour cherry (Hillig and Iezzoni, 1988; Höfer and Peil, 2015; Krahl et al., 1991; Rakonjac et al., 2010) and *Cerasus* (Khadivi-Khub et al., 2012; Shahi-Gharahlar et al., 2010).

The dispersal of cultivars, based on the PC-1 (first principal component), PC-2 and PC-3, represents the existing phenotypic variation among the cultivars, and also indicates how extensively they spread along axes (Fig. 2). Using the Kaiser's criterion ("Eigenvalue" >1) (Kaiser, 1958), it was possible to reduce the dimension of the 24 quantitative traits to only six components, which could explain 82.34% of the total variation (Table 4). The first component, which accounted for 34.20% of the total variation, was strongly correlated with fruit traits, such as fruit equatorial diameter, fruit width and fruit weight. The second component, which accounted for 16.22% of the total variation, was mainly correlated with the characters of petiole length, petiole width, ratio petiole length/width, soluble solids and number of flowers per spur. The third component, that explained 12.54% of the total variation, was associated with the traits pit weight, ratio weight pit/weight fruit and pit thickness. The fourth component, accounted for 8.50% of the total variation, was determined by the characters of number of nectary glands and pit length. The fifth component explained 6% of the total variation, and was associated with the ratio of blade per petiole length, the fruit polar diameter and the pit length. Finally, the sixth component was correlated with the characters of pedicel length and titratable acidity and explained 4.84% of the total variation. In addition, a PCA scatter plot was constructed, based on the first three components (Fig. 2). This plot grouped the cultivars according to their phenotypic resemblance and morphological characteristics. For example, the cultivars 'Randy Mecy' and 'Rekseler', which posed the highest fruit weight, the largest fruit width and the longest fruit length, were positioned closely in the upper right area. These results demonstrate the highly positive correlation of fruit equatorial diameter, fruit weight and fruit width, rendering these morphological traits the highest factors loadings in the PCA. Color characteristics, the fruit size and weight, and the traits related to the abscission between fruit and stalk were the most effective parameters to discriminate sour and duke cherries genotypes according to Höfer and Peil (2015). Additionally, our results

Table 4
First 6 components from the PCA of 24 quantitative traits in 27 sour cherry cultivars.

	F1	F2	F3	F4	F5	F6
Yd	0.361	1.325	2.312	4.002	7.115	1.044
NoFlBu	1.536	0.094	4.047	12.365	3.446	0.319
PeLe	0.012	10.518	1.654	9.270	16.642	0.732
PeWi	1.716	12.507	6.057	0.665	3.754	0.341
PeLe/PeWi	0.025	14.165	0.411	7.799	0.430	1.862
BlLe	5.145	8.040	0.067	4.867	3.281	3.401
BlWi	1.809	8.930	2.059	1.411	6.567	0.299
BlLe/BlWi	5.392	4.102	0.222	0.489	13.963	4.650
NoNeBu	2.722	1.028	0.055	20.198	0.084	0.300
NoFlSp	1.370	11.005	0.046	0.098	6.041	4.252
FrPoDi	7.399	1.456	0.228	3.869	10.690	3.459
FrEqDi	10.468	1.277	0.401	0.284	0.051	0.365
FrWi	11.016	0.658	0.043	0.009	0.004	0.256
FrWe	10.966	1.272	0.052	0.038	0.392	0.024
SoSo	0.017	13.951	0.038	8.021	2.234	3.330
TiAc	7.225	0.025	2.934	0.022	3.992	12.487
TiAc/SoSo	6.906	1.024	3.450	2.260	1.909	9.084
PedLe	0.799	0.491	8.381	0.036	1.751	39.596
StLe	0.277	1.005	7.894	15.649	11.837	3.495
StWi	7.035	0.072	8.456	3.890	0.588	0.000
StTh	6.095	0.123	9.893	3.667	2.522	2.019
StWe	2.495	0.854	20.356	0.037	0.672	3.547
StWe/FrWe	4.178	2.634	12.210	0.244	0.085	3.778
StVo/FrVo	5.035	3.444	8.734	0.810	1.950	1.358
Eigenvalue	8.210	3.893	3.011	2.042	1.442	1.163
Variability (%)	34.209	16.221	12.546	8.509	6.009	4.847
Cumulative%	34.209	50.429	62.975	71.484	77.493	82.340

Table 5
First 7 components from the PCA of 14 qualitative traits in 27 sour cherry cultivars.

	F1	F2	F3	F4	F5	F6	F7
FrTy	−0.054	0.846	0.138	0.113	0.060	−0.140	−0.023
TrVi	0.185	0.034	0.248	0.484	0.449	0.484	−0.396
TrSh	0.416	0.420	−0.350	0.156	−0.313	0.348	0.245
TrHa	−0.319	−0.004	0.664	0.142	0.142	−0.018	0.490
TrBr	0.248	0.330	−0.534	−0.370	0.272	0.278	0.053
LaSh	−0.559	0.165	−0.558	0.251	−0.301	−0.187	0.044
LaSi	0.049	0.590	0.377	0.073	−0.358	0.192	−0.200
PeSh	−0.255	−0.047	−0.445	−0.064	0.620	0.077	0.122
ArPe	0.290	0.642	0.103	−0.497	0.052	0.066	0.155
FrSh	−0.715	−0.016	0.131	−0.427	0.086	0.072	−0.079
SkCo	−0.169	−0.452	0.119	−0.098	−0.237	0.656	0.292
FrSi	0.793	−0.059	0.294	−0.168	0.247	−0.264	0.168
StSh	−0.358	0.424	−0.054	0.589	0.268	−0.016	0.281
StSi	0.704	−0.344	−0.230	0.390	−0.081	−0.093	0.134
Eigenvalue	2.629	2.299	1.764	1.468	1.206	1.049	0.758
Variability (%)	18.777	16.420	12.600	10.485	8.616	7.493	5.413

are in accordance with studies, which also confirmed that the fruit size and weight are useful parameters to discriminate cultivars (in sour cherry; Khadivi-Khub et al., 2013, 2012; Shahi-Gharahlar et al., 2010 and in sweet cherry; Ganopoulos et al., 2015).

PCA confirmed that some traits had the highest influence in the first three components. These traits included the fruit equatorial diameter, the fruit width and weight, the petiole length and width, the ratio of petiole length/width, the soluble solids, and the numbers of flowers per spur. These results suggested that such traits are suitable both for the estimation of genetic diversity and for the phenotypic characterization of the sour cherry genetic resources.

3.3. Principal component analysis (PCA) of qualitative variables

Using the Kaiser's criterion ("Eigenvalue" >1) (Kaiser, 1958), six significant components were obtained that explained 74.39% of the total variation (Table 5). The first component, which accounted for 18.77% of the total variation, was correlated with fruit size, fruit shape and lamella shape characters. The second component, which explained 16.42% of the total variation, was associated with the arrangement of petals and lamella size. The third component explained 12.60% of the total variation, and was mainly correlated to characters related to tree habit, tree branching, lamella shape and shape of petal. The fourth component accounted for 10.48% of the total variation and was correlated with the characters of lamella size, pit shape, the arrangements of petals and fruit shape. The fifth component explained 8.61% of the total variation and was correlated with the characters of shape of petal, tree vigour and lamella size. The sixth component explained 7.49% of the total variation and was determined by the characters of skin colour, tree vigour and tree shape (Table 5). A PCA scatter plot was constructed based on the first three components (Fig. 3). The results are in agreement with various other studies that reported the maximum contribution of fruit size, and fruit shape characters towards the genetic divergence in cherries (Ganopoulos et al., 2015; Khadivi-Khub et al., 2013, 2012). Farsad and Esna-Ashari (2016) suggested that PCA analysis of 27 qualitative and quantitative morphological traits explained over 86.59% of total variation in the first seven axes. In this study, using PCA, the number of the main factors that could be taken into account for the accurate discrimination of genotypes was adequately assigned. This method could also permit the correlation of the phenotypic traits with the genetic linkages between

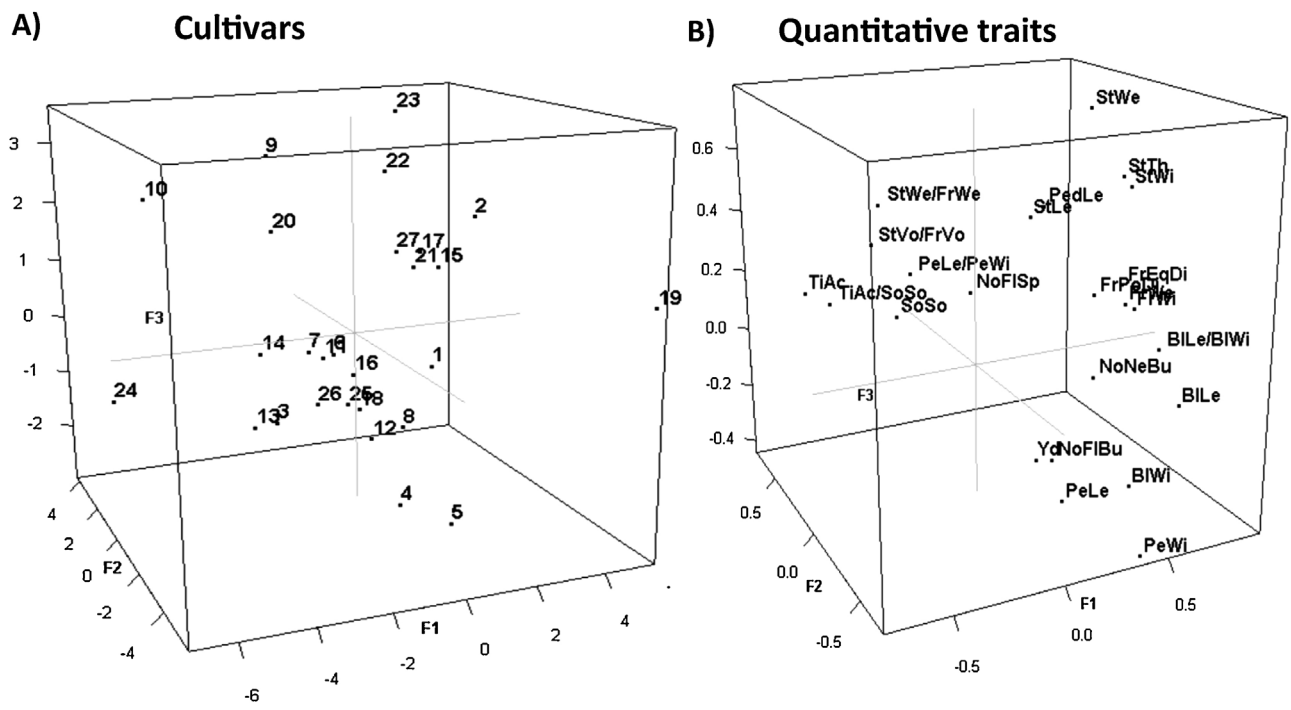

Fig. 3. A) Three-dimensional PCA plot of the 27 sour cherry cultivars with regard to the first three principal components. B) Three-dimensional PCA plot of the 14 qualitative traits with regard to the first three principal components. Variability explained: F1 (18.77%), F2 (16.42%), F3 (12.60%).

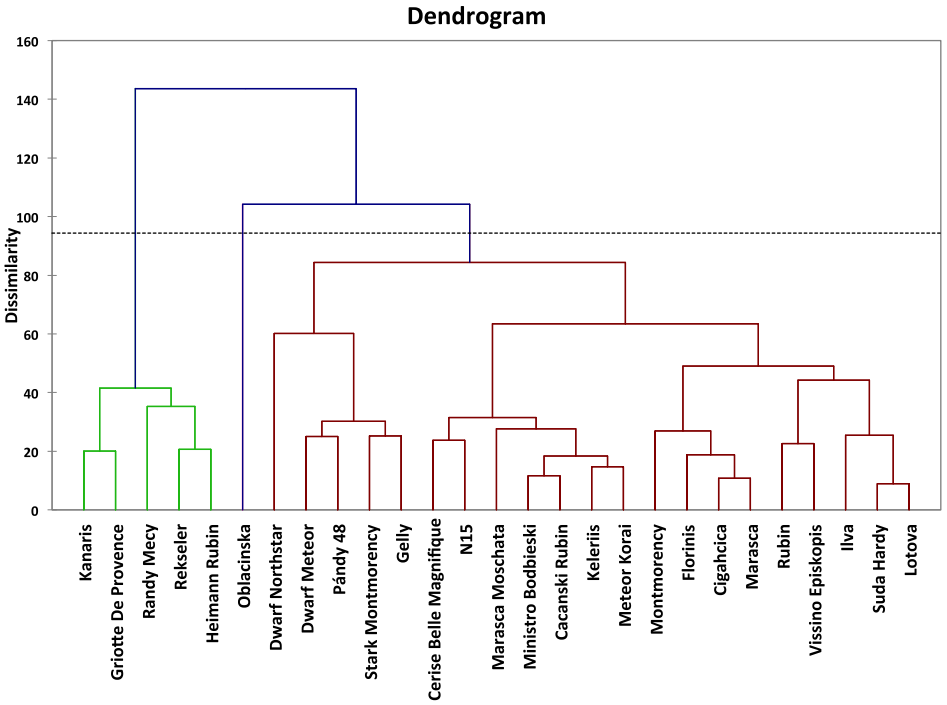

Fig. 4. Dendrogram using AHC for 27 sour cherry cultivars based on 24 quantitative and 14 qualitative traits.

the respective traits loci (Iezzoni and Pritts, 1991; Rakonjac et al., 2010).

3.4. Dendrogram using agglomerative hierarchical clustering

In this study, an unsupervised AHC analysis was used in order to separate the available data into groups of increasing dissimilarity. The Euclidean distance was used as a metric to measure this genetic dissimilarity among the 27 sour cherry cultivars, based on the combined quantitative and qualitative data; the Ward's method was used for agglomeration. The relative dendrogram (Fig. 4) revealed three distinct groups; the C1 is comprised of 21 cultivars, the C2 contained 5 cultivars, and finally the C3 included only the cultivar 'Oblacinska'. This result is consistent with the variation that has been initially observed in the data within the sour cherry cultivars. Moreover, among all the 27 sour cherry cultivars, there were no specific clusters based on the locality. The highest genetic dis-

tance was observed between C2 and C3 (26.74), followed from these between C1 and C3 (21.14), and between C1 and C2 (9.32).

According to Perez-Sanchez et al. (2008) such dendrograms, constructed using various morphological parameters, were able to reveal relationships among the cultivars of sweet, sour, and duke cherries. Shahi-Gharahlar et al. (2010) postulated that the sour cherry cultivars and genotypes were located within both the sweet cherry and improved rootstocks. Furthermore, Rodrigues et al. (2008) suggested that the dendrogram of morphological analyses separated sweet cherries from sour cherries. However, genotypes of duke cherries were grouped as a separate sub-cluster, although they were clustered with certain sour cherries (Khadivi-Khub et al., 2013). This is in accordance with the results of this study that exhibit high similarity between duke cherry and sour cherry. The division in the amarelle and in the morello cultivar groups through clustering analysis of phenotypical characteristics has been also previously reported (see Antonius et al., 2012). Finally, Höfer and Peil (2015) reported that the three duke cherry cultivars, which were grouped within one sub-cluster, were also grouped with sour cherry cultivars that had a characteristic juice, ranging in colour from cream-white fruit flesh and colourless to cream-yellow.

4. Conclusion

In this study, significant diversity has been revealed, regarding the morpho-physiological traits in the evaluated germplasm. This diversity allows the effective selection of parents in various breeding programs, referring to fruit quality and aiming at different aspects of postharvest utilization, besides high yield and resistance to diseases. Thus, this study emphasizes the importance of preservation of genetic resources for any fruit tree breeding program.

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