Scientia Horticulturae xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Diversity of morpho-physiological traits in worldwide sweet cherry cultivars of GeneBank collection using multivariate analysis

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ARTICLE INFO

Article history:
Received 23 June 2015
Received in revised form 25 August 2015
Accepted 30 September 2015
Available online xxx

Keywords:
Phenotypic diversity
Prunus avium
Germplasm collection
Hierarchical cluster analysis

ABSTRACT

This study presents an assessment of morpho-physiological diversity for one hundred and forty-six sweet cherry (*Prunus avium*) cultivars, originating from different countries and maintained in an *ex situ* GeneBank collection. Data of thirty-five traits, describing phenology, plant morphology, yield and fruit quality were recorded over three years and analyzed using principal component analysis (PCA). An unsupervised hierarchical cluster analysis was performed the different cultivars using the Euclidean distance metric and the Ward's agglomeration method. Significant positive and negative correlations were detected among the different morpho-physiological traits. The sweet cherry cultivars were classified into three main clusters, suggesting that the characterized sweet cherry collection has high potential for specific breeding goals. Correlations among the traits, which will be useful for breeding in fruit size and quality, are discussed. Sweet cherry cultivars, which were classified in diverse clusters, could be potential parents for hybridization and new genotypes could be created with a combination of desirable traits that complement one another. These new genotypes could have a high heterotic behavior and thus could substantially contribute to existent sweet cherry breeding programs.

1. Introduction

Cherries *(Prunus avium* L.) include sweet cherry trees cultivated for human consumption and wild cherry trees, also called mazzards, cultivated for their wood. Cherries are thought to have originated in the Caucasus area, whereas at present they are found across mainland Europe and western Asia (Webster, 1996). Undoubtedly, cherries were an early food source for primitive inhabitants of Europe, as pits have been recovered from cave dwellings that date back to 4000–5000 B.C. Sweet cherries were probably first cultivated in Greece (Hedrick, 1915; Marshall, 1954). Today, cherry cultivation is one of the most popular fruit tree crop in Greece (Koukourojiannis, 1996). Commercial demand for sweet cherry resulted in the contemporary increased agricultural production.

Greek growers, in order to follow current market demands, have changed their sweet cherry production from local cultivars, mainly from 'Van' and 'Burlat' groups (Ganopoulos et al., 2011), to the non-native sweet cherry cultivars. The replacement of local, well adapted to the local climate conditions, by commercial sweet cherry cultivars led to a dramatic loss of genetic diversity in terms of adaptability, tolerance to diseases and fruit quality. Therefore, it is of utmost importance the need for preservation of endangered fruit germplasm and establishment of programs that target the conservation of genetic resources in Greece (Ganopoulos et al., 2011). Keulemans (1993) underlined the contribution of local cultivars to variability and better adaptability. The Experimental Station of Institute of Pomology in Greece, which was established in 1970s, created the first Greek germplasm collection with local and international sweet cherry cultivars (Kazantzis, 2013).

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http://dx.doi.org/10.1016/j.scienta.2015.09.061
0304-4238/

One of the most important factors influencing plant breeding is the existence of genetic diversity. Therefore, the identification and estimation of the genetic diversity as well as its nature are of paramount importance for a successful breeding program. Furthermore, it is crucial to have not only the information on the genetic diversity but also the information on plant germplasm and the physical presence of the germplasm in order to preserve it, exploit it and sustainable use it in plant breeding and in agriculture or even for other uses (Khadivi-Khub et al., 2014). To the best of our knowledge, this is the first study describing the complete process of morpho-physiological characterization of a worldwide sweet cherry germplasm collection. Furthermore, similar research in fruit crops has been conducted in a worldwide olive GeneBank collection by using morphological markers (Díez et al., 2012; Trujillo et al., 2014).

In order to identify and analyze the genetic diversity of the various sweet cherry cultivars used, one can rely solely on the phenotypic traits (IPGRI, 1985; UPOV, 1976). Morphological analysis is a quick and commonly used method to identify and characterize the germplasm through phenotyping.

To discover phenotypic traits that mostly contribute to the total diversity in a germplasm collection and characterize the levels of similarity/dissimilarity among the cultivars, a characterization of phenotypic diversity and structure is needed (de Oliveira et al., 2012; Furones-Pérez and Fernández-López, 2009; Mehmood et al., 2014). Multivariate data analysis includes powerful statistical techniques for analyzing data with many variables simultaneously to identify patterns and relationships. Since information obtained from morphological characterization is derived from a large data set consisting of qualitative and quantitative traits, the use of multivariate analysis is particularly preferred (de Oliveira et al., 2012; Furones-Pérez and Fernández-López, 2009; Mehmood et al., 2014). Furthermore, multivariate analysis has been used for genetic variability estimation. The most suitable multivariate techniques for morphological characterization of genotypes are principal component analysis (PCA) and cluster analysis (Mohammadi and Prasanna, 2003; Peeters and Martinelli, 1989). The combination of these statistical methods could provide comprehensive information of characteristics that crucially contribute to genetic diversity in plants (Khodadadi et al., 2011).

The aims of this study were (i) to evaluate the phenotypic diversity in 146 worldwide sweet 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

Morpho-physiological genetic diversity was assessed in 146 worldwide sweet cherry (*P. avium* L.) cultivars. These cultivars are part of the *ex situ* Greek Fruit GeneBank collection in Naoussa (Table 1) and represent the total diversity of Greek sweet cherry cultivars and a large part of international cultivars. Thirty-five variables were examined on the basis of the cherry descriptors at the experimental collection for three consecutive years (2010–2012). Different horticultural practices including fertilizer, application spraying and irrigation and other cultural practices were made on regular intervals each year. At the beginning of the study (2010), the trees were mature (8 years old) and also healthy, and in cropping condition.

2.2. Analysis of morpho-physiological traits

Mature leaves were collected, approximately at the end of July. From each of the four trees studied per cultivar, seven leaves were sampled per year, and the following parameters were measured using a digital caliper with a sensitivity of ±0.01 mm. The flowers were collected at full bloom; ten flowers were taken from each of the four trees studied per cultivar and year. Fruits were collected at maturity. Cherry fruits maturity was determined based on the color characteristics of each cultivar, taking into account information provided by growers and from personal experience and observation. A sample of a total of 106 cherry fruits was taken from each of the four trees studied per cultivar and year. One hundred of them were used to determine the mean fruit weight. The remaining six cherries were used to study a series of quantitative and qualitative descriptors.

The 21 quantitative traits evaluated, included Stone volume [StV], Yield [YL], Ratio volume stone/volume fruit [VSt/VFr], Ratio weight stone/weight fruit [WeSt/WeFr], Petiole length [PL], Petiole width [PeWi], Ratio petiole length/width [PL/Wi], Blade length [BlLe], Blade width [BlWi], Ratio blade length/petiole length [BlLe/PL], Stone length [StLe], Stone width [StWi], Stone thickness [StTh], Soluble solids [SoSo], Titratable acidity [TiAc], ratio titratable acidity/soluble solids [TiAc/ SoSo], Pedicel length [PeLe], Fruit polar diameter [PoDi], Fruit equatorial diameter [EqDi], Fruit width [FrWi] and Fruit weight [FrWe].

The 14 qualitative traits investigated included tree vigour [TrVi], tree habit [TrHa], tree branching [TrBr], lenticels size [LeSi], lamella shape [LaSh], number of nectaries [NuNe], petal shape [PeSh], fruit shape [FrSh], fruit size [FrSi], fruit skin colour [FrSCo], firmness of flesh [FiFl], stone shape [StSh] and stone size [StSi].

These traits were selected from the International Union for the Protection of New Cultivars of Plants descriptors proposed for sweet cherry (UPOV, 1976; IPGRI, 1985). The scoring system is the same as used by UPOV and IPGRI. When possible, all measurements of a trait were made on the same date to avoid differences in the environment or developmental stages of the tree.

2.3. Data scoring and analysis

Data for the 146 sweet cherry cultivars, involving 35 traits, were analyzed via XLSTAT software (version 2014.1). The Principal Components Analysis (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, the main reason being that standardization was necessary since the variables were measured in different units. Within PCA, factor loadings >0.55 were regarded as significant since the number of observations was 146 (see also Mehmood et al., 2014). In each case, 3D plots were constructed, with regard to the three most important principal components, to facilitate the visualization of the results. In the 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 used for the agglomerative hierarchical clustering (AHC).

3. Results

3.1. Descriptive statistics and correlations for the quantitative variables

The 21 quantitative traits were measured and the descriptive statistics of minima, maxima, means, standard deviations and coefficients of variation (CV) are shown in Table 2. The results revealed extensive morphological variability. Some traits displayed high CV. These included YL (43.97%), StV (30.75%), TiAc/SoSo (30.49%), TiAc (28.64%) and VSt/VFr (28.12%).

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

Number (No)	Cultivar	Origin	Fruit skin colour	Flesh firmness	Fruit size
1	2e 48-28	Canada	Dark red	Strong	Very small
2	Adriana	Italy	Dark red	Strong	Large
3	Angela	Canada	Dark red	Weak	Small
4	B. Producta Delbard	France	Orange red	Medium	Very large
5	Bargioni I-137	Italy	Orange red	Medium	Very large
6	Bargioni I-37	Italy	Dark red	Strong	Small
7	Bargioni I-38	Italy	Dark red	Strong	Medium
8	Bargioni I-62	Italy	Dark red	Strong	Small
9	Bargioni I-63	Italy	Dark red	Strong	Very large
10	Bargioni M-47	Italy	Dark red	Strong	Medium
11	Belle Magnifique	France	Light red	Strong	Very large
12	Bianca Di Verona	Italy	Orange red	Strong	Large
13	Bigarreau Burlat	France	Dark red	Medium	Very large
14	Bigarreau Burlat S-370	France	Dark red	Medium	Very large
15	Bigarreau Geant D' Hedelfingen	Germany	Darkish	Medium	Medium
16	Bigarreau Goeur De Pigeon	France	Darkish	Strong	Medium
17	Bigarreau Marmotte	France	Dark red	Strong	Large
18	Bigarreau Moreau	France	Dark red	Strong	Medium
19	Bigarreau Napoleon	Germany	Orange red	Strong	Small
20	Bigarreau Reverchon	Italy	Dark red	Strong	Large
21	Bigarreau Stark Hardy Giant	USA	Dark red	Strong	Very large
22	Bigarreau Tigre	France	Darkish	Strong	Large
23	Bing	USA	Darkish	Strong	Very large
24	Black Russian	USA	Darkish	Medium	Medium
25	Black Tartarian	Europe	Darkish	Weak	Small
26	Blanka Kukleitska	Bulgary	Dark red	Strong	Medium
27	Brooks	USA	Dark red	Medium	Large
28	Burlat e1	Italy	Dark red	Strong	Very large
29	Canada Giant	Canada	Dark red	Strong	Very large
30	Chinook	USA	Red	Strong	Very large
31	Ciliegio If Roma BB2	Italy	Brown red	Medium	Very large
32	Ciliegio If Roma T-57	Italy	Red	Strong	Small
33	Compact Stella	New Ziland	Brown red	Strong	Small
34	Corniola	Italy	Dark red	Strong	Very small
35	Cristalina	Canada	Dark red	Strong	Medium
36	Cuglyeva Acacra	Bulgary	Dark red	Strong	Large
37	Della Marka Modenese	Italy	Orange red	Weak	Small
38	Di Mauria	unknown	Yellow	Weak	Small
39	Droganova Zuta	Bulgary	Yellow	Strong	Very large
40	Durona Di Cesena	Italy	Dark red	Strong	Large
41	Durone Di Vignola	Italy	Darkish	Medium	Very large
42	Durone Di Vignola II	Italy	Darkish	Strong	Large
43	Early Rivers	United Kingdom	Dark red	Weak	Very small
44	Empereur Francis	Germany	Orange red	Strong	Very large
45	Fercer (Arcina)	France	Dark red	Strong	Very large
46	Ferrovia	Italy	Dark red	Strong	Very large
47	Ferrovia spur	Italy	Dark red	Strong	Very large
48	Germersdorfer	Hungary	Light red	Strong	Very large
49	Giorgia	Italy	Dark red	Strong	Very large
50	Glorius Stark Gold	USA	Yellow	Weak	Small
51	Grossa Di Pistoia	Italy	Darkish	Strong	Large
52	Grossa Rossa	Italy	Orange red	Strong	Large
53	Guillaume	France	Dark red	Strong	Large
54	Hative De Bale	Switcherland	Darkish	Weak	Very small
55	Hative De Berny	France	Darkish	Weak	Very small
56	Hebros	Bulgary	Dark red	Strong	Very large
57	Hedelfingen V 18775 × 20	Germany	Darkish	Medium	Large
58	Hudson	USA	Light red	Strong	Small
59	Jaboulay	France	Dark red	Strong	Small
60	Jubilee	USA	Light red	Medium	Large
61	Kordia	Czech Republic	Dark red	Strong	Large
62	Kustendilska Hrustjalka	Bulgary	Dark red	Medium	Small
63	Lambert ÒΨimη	USA	Darkish	Medium	Very large
64	Lapins	Canada	Red	Strong	Very large
65	Larian	USA	Brown red	Strong	Very large
66	Merton Bigarreau	United Kingdom	Light red	Strong	Very small
67	Monnembegi	Romania	Dark red	Strong	Very small
68	Napoleon S-787	France	Orange red	Strong	Small
69	Negre Di Bistrita	Romania	Brown red	Strong	Very small
70	Nera Di Piemonde	Romania	Darkish	Weak	Small
71	Nero II clone 52 P3	Italy	Dark red	Strong	Very large
72	Nero II clone 78 P2	Italy	Dark red	Strong	Medium
73	Nero II e1 8	Italy	Dark red	Strong	Large
74	New Star 26-3-7.	Canada	Dark red	Strong	Large

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Table 1 (*Continued*)

Number (No)	Cultivar	Origin	Fruit skin colour	Flesh firmness	Fruit size
75	New York 1143 (NY 1143)	USA	Orange red	Strong	Large
76	New York T-27	USA	Orange red	Strong	Medium
77	Noble	United Kingdom	Dark red	Strong	Very large
78	Northstar	Canada	Dark red	Medium	Very large
79	Pobeda	Bulgary	Dark red	Medium	Small
80	Precoce Bernard	France	Brown red	Medium	Small
81	Precoce Della Marca	Italy	Red	Weak	Small
82	Primavera	Germany	Dark red	Strong	Very small
83	Prime Giant (Giant Red)	USA	Dark red	Strong	Very large
84	R3 Daritska Beluide	Bulgary	Orange red	Weak	Very small
85	Rainier	USA	Orange red	Strong	Very large
86	Rana Cherne Edra	Bulgary	Orange red	Medium	Small
87	Regina	Germany	Dark red	Strong	Large
88	Rosii Di Bistrita	Romania	Dark red	Weak	Large
89	Sam	Canada	Dark red	Medium	Large
90	Schmidt's Bigarreau	Europe	Darkish	Medium	Large
91	Seneca	USA	Dark red	Weak	Very small
92	Solymári Gömbölyü	Hungary	Dark red	Strong	Very small
93	Staccato (Splendid)	Canada	Red	Strong	Medium
94	Stark Gold Bigarreau	USA	Yellow	Weak	Small
95	Starkrimson	USA	Dark red	Medium	Very large
96	Stella	Canada	Light red	Medium	Very large
97	Sue	Canada	Orange red	Medium	Very large
98	Summit	Canada	Light red	Medium	Very large
99	Sunburst	Canada	Light red	Medium	Very large
100	Ulster	USA	Dark red	Strong	Medium
101	V-1927	unknown	Dark red	Strong	Medium
102	Valera	Canada	Brown red	Medium	Small
103	Valerij Tschkalov	Hungary	Dark red	Strong	Very large
104	Van	Canada	Dark red	Strong	Medium
105	Vega	Canada	Orange red	Strong	Very large
106	Verdel Ferbolus	France	Red	Strong	Large
107	Victor	Canada	Orange red	Weak	Large
108	Vittoria	Italy	Dark red	Strong	Medium
109	Vogue	Canada	Dark red	Strong	Large
110	Windsor	Canada	Darkish	Medium	Medium
111	Ziraat	Turkey	Dark red	Medium	Very large
112	Agiorgitika Lilantiou	Greece	Dark red	Weak	Very small
113	Athinaika	Greece	Dark red	Medium	Small
114	Arkadias	Greece	Darkish	Strong	Medium
115	Basiliadi	Greece	Dark red	Medium	Very large
116	I.O.P. 1	Greece	Dark red	Strong	Very small
117	I.O.P. 2	Greece	Dark red	Strong	Very small
118	I.O.P. 3	Greece	Dark red	Strong	Very small
119	Karamela Lilantiou	Greece	Orange red	Strong	Small
120	Kapsiotika	Greece	Orange red	Weak	Small
121	Kifisias	Greece	Dark red	Weak	Very small
122	Kifisias Proimotero	Greece	Orange red	Medium	Large
123	Kokkina Anastasias	Greece	Dark red	Strong	Medium
124	Koromilokeraso Vitalou	Greece	Dark red	Strong	Small
125	Lemonidi	Greece	Dark red	Medium	Very large
126	Mavra Anastasias	Greece	Dark red	Strong	Large
127	Mavro Proimo Achaias	Greece	Dark red	Strong	Very large
128	Mavro Proimo Vitalou	Greece	Dark red	Medium	Small
129	Mavro Tripoleos	Greece	Dark red	Medium	Medium
130	Mesoproimo Tragano Evias	Greece	Dark red	Strong	Very small
131	Moschato Tragano Opsimo Evias	Greece	Orange red	Weak	Very small
132	Bakirtzeika	Greece	Light red	Strong	Very large
133	Napoleon Karamela	Greece	Orange red	Medium	Small
134	Opsimi Karamela Tripoleos	Greece	Orange red	Medium	Very small
135	Opsimo Tragano Komotinis	Greece	Dark red	Strong	Small
136	Petrokeraso Tragano Achaias	Greece	Orange red	Strong	Large
137	Proimo Kolindrou	Greece	Dark red	Weak	Very small
138	Proimo Tragano Komotinis	Greece	Dark red	Strong	Very small
139	Samou	Greece	Light red	Strong	Medium
140	Tourkika	Greece	Dark red	Strong	Very large
141	Tragana Edessis	Greece	Dark red	Strong	Large
142	Tragana Edessis-Naoussis	Greece	Dark red	Strong	Very large
143	Tragana Edessis-Sarakinon	Greece	Dark red	Strong	Very large
144	Tragano Komotinis	Greece	Dark red	Medium	Very small
145	Fraoula Volou	Greece	Yellow with blush	Medium	Large
146	Chalkidos Unknown	Greece	Dark red	Strong	Very large

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Table 2
Descriptive statistics for 21 quantitative traits in 146 sweet cherry cultivars.

Variable	Minimum	Maximum	Mean	Std. deviation	CV (%)
Stone volume	0.190	0.750	0.439	0.135	30.75
Yield	25.000	190.000	71.096	31.264	43.97
Ratio volume stone/volume fruit	7.700	35.300	16.931	4.762	28.12
Ratio weight stone/weight fruit	1.700	26.600	14.714	3.320	22.56
Petiole length	9.900	16.000	12.437	1.266	10.17
Petiole width	4.540	8.080	6.058	0.583	9.62
Ratio petiole length/width	0.400	0.600	0.487	0.040	8.21
Blade length	2.720	6.220	4.195	0.562	13.39
Blade width	0.110	0.220	0.158	0.019	12.02
Ratio blade length/petiole length	2.060	5.250	3.102	0.593	19.11
Stone length	8.400	18.000	11.120	1.099	9.88
Stone width	7.000	15.700	8.936	0.896	10.02
Stone thickness	5.400	11.700	7.102	0.720	10.13
Soluble solids	9.600	22.300	16.644	2.470	14.84
Titratable acidity	3.000	17.400	8.539	2.446	28.64
Ratio titratable acidity/soluble solids	0.900	7.100	2.112	0.644	30.49
Pedicel length	2.700	7.300	4.515	0.719	15.92
Fruit polar diameter	11.500	26.600	21.387	1.947	9.11
Fruit equatorial diameter	18.100	28.500	23.586	2.016	8.54
Fruit width	15.500	24.000	19.838	1.486	7.49
Fruit weight	3.100	12.100	6.896	1.502	21.78

Strong positive, linear correlations were observed among all the 21 quantitative traits (Table 3). The highest significant, positive correlation was between FrWe and EqDi (0.858). Significant positive correlations were also observed between EqDi and FrWi (0.844), PL and PeWi (0.709), StLe and StWi (0.686), VSt/VFr and WeSt/WeFr (0.646), PeWi and BlWi (0.565), EqDi and WeSt/WeFr (0.541), FrWe and WeSt/WeFr (0.495), and FrWi and WeSt/WeFr (0.458). On the other hand, there were also high, significant, negative correlations between some quantitative traits (Table 3). These included for instance, the TiAc/ SoSo and TiAc (-0.714) and VSt/VFr and StVo (−0.712) (Table 3). We reported significant correlations between traits contributing to fruit yield and quality, which is helpful for plant breeding. For example, to attain high yield and superior quality cultivars, cross combinations could be performed between cultivars with large fruit size ('Lemonidi', 'Mpakirtzeika', 'Starkrismson' and 'Ziraat'), low stone volume ('Brooks', 'Hative De Berny', 'Hative De Bale', 'Seneca', 'Merton Bigarreau' and 'Monnembegi') and high total soluble solids ('2e 48-28′, 'Monnembegi', 'Napoleon S-787′, 'R3 Daritska Beluide', 'Rainier', 'Rosii Di Bistrita', 'Sue', 'Arkadias', 'Kapsiotika' and 'Tragana Edessis-Naoussis').

3.2. Principal component analysis of quantitative variables

The distribution of cultivars, based on the PC-1, PC-2 and PC-3, shows the phenotypic variation among the cultivars and how widely dispersed they are along both axes (Fig. 1). Using Kaiser's criterion ("Eigenvalue" >1) (Kaiser, 1958), we reduce the dimension implied by the 21 quantitative traits to six significant components that explained 73.59% of the total variation (Table 4; Fig. 1). The first component, which accounted for 22.90% of the total variation, included stone volume, petiole length, blade width, fruit polar diameter, fruit equatorial diameter, fruit width and fruit weight. The second component, which explained 16.75% of the total variation, was mainly correlated to characters related to ratio of volume stone/volume fruit, ratio weight stone/weight fruit, stone length, stone width and stone thickness. The third component that explained 10.15% of the total variation included petiole length, petiole width and stone length. The fourth component, accounted for 9.55% of the total variation, was determined by titratable acidity and ratio of titratable acidity/soluble solids. The fifth component explained 7.71% of the total variation, and included blade length

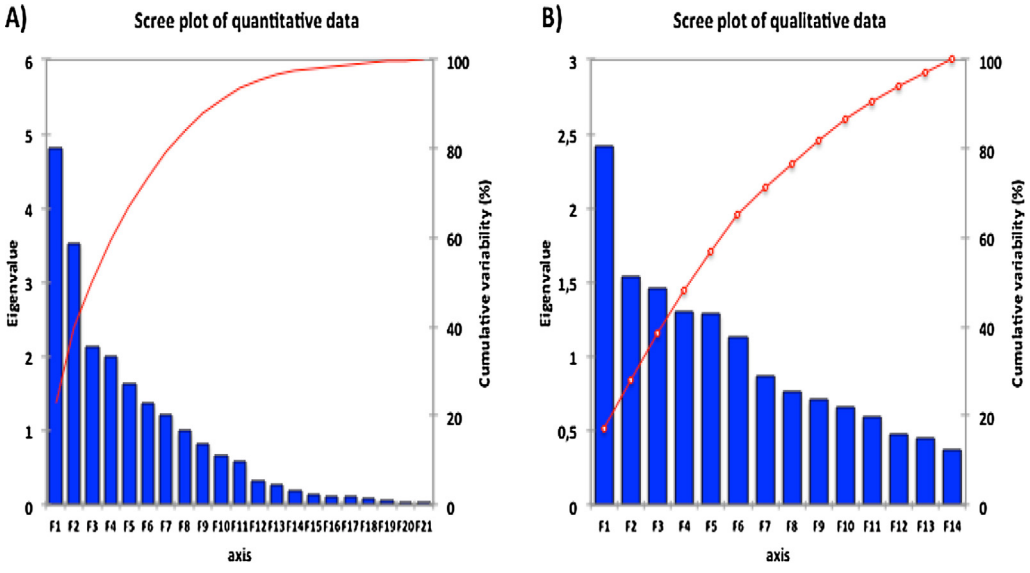

Fig. 1. 'Scree plot' of eigenvalues obtained from the PCA process for (A) quantitative and (B) qualitative traits.

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Table 3
Correlation coefficients (Pearson) among 21 quantitative traits in 146 sweet cherry cultivars.

Variables	StV	YL	VSt/VFr	WeSt/WeFr	PeLe	PeWi	PL/Wi	BlLe	BlWi	BlLe/PeLe	StLe	StWi	StTh	SoSo	TiAc	TiAc/SoSo	PeLe	PoDi	EqDi	FrWi	FrWe
StV	**1**																				
YL	**0.199**	**1**																			
VSt/VFr	**−0.712**	0.008	**1**																		
WeSt/WeFr	**−0.227**	−0.027	**0.646**	**1**																	
PeLe	0.130	0.115	0.050	0.100	**1**																
PeWi	0.158	0.095	−0.028	0.012	**0.709**	**1**															
PL/Wi	0.059	−0.041	−0.118	−0.094	**−0.389**	**0.316**	**1**														
BlLe	**−0.225**	**−0.244**	**0.200**	0.155	**0.167**	0.077	−0.118	**1**													
BlWi	**0.262**	**0.277**	−0.025	0.065	**0.671**	**0.565**	**−0.171**	0.141	**1**												
BlLe/ PeLe	0.085	**0.282**	−0.055	−0.131	**0.431**	**0.333**	**−0.259**	**−0.614**	**0.277**	**1**											
StLe	**0.353**	0.091	**−0.280**	**−0.471**	0.068	0.087	0.063	−0.156	−0.005	0.032	**1**										
StWi	**0.427**	0.142	**−0.284**	**−0.434**	0.126	**0.219**	0.123	**−0.176**	**0.182**	0.094	**0.686**	**1**									
StTh	**0.352**	**0.214**	**−0.243**	**−0.487**	**0.181**	**0.223**	0.054	**−0.216**	**0.228**	**0.263**	**0.542**	**0.790**	**1**								
SoSo	0.024	0.049	−0.013	0.066	0.007	0.058	0.060	−0.003	0.000	0.027	−0.123	−0.078	−0.108	**1**							
TiAc	**0.282**	−0.034	**−0.186**	0.096	0.134	0.150	0.020	−0.042	0.123	0.088	−0.080	−0.002	0.016	**0.460**	**1**						
TiAc/ SoSo	**−0.271**	0.150	**0.190**	−0.048	−0.104	−0.142	−0.052	0.050	−0.123	−0.067	0.049	−0.053	−0.099	0.135	**−0.714**	**1**					
PeLe	**0.212**	0.106	**−0.216**	**−0.272**	−0.027	0.058	0.096	0.150	0.096	−0.059	0.060	0.091	0.126	**0.214**	0.062	0.058	**1**				
PoDi	**0.206**	0.081	**0.302**	**0.457**	**0.287**	**0.195**	-0.114	−0.038	**0.216**	0.050	**0.335**	**0.192**	0.103	90.021	0.004	0.034	**−0.172**	**1**			
EqDi	**0.389**	0.070	**0.231**	**0.541**	**0.267**	**0.221**	-0.025	0.010	**0.312**	−0.030	0.118	**0.298**	**0.191**	−0.005	0.137	−0.133	−0.104	**0.595**	**1**		
FrWi	**0.460**	**0.245**	**0.169**	**0.458**	**0.310**	**0.258**	-0.038	−0.050	**0.360**	0.085	0.129	**0.320**	**0.313**	0.090	**0.256**	**−0.210**	−0.025	**0.660**	**0.844**	**1**	
FrWe	**0.334**	0.142	**0.330**	**0.495**	**0.328**	**0.257**	-0.070	0.041	**0.330**	0.020	0.161	**0.259**	**0.208**	0.021	0.142	−0.109	−0.046	**0.701**	**0.858**	**0.843**	**1**

Values in bold are different from 0 with a significance level alpha = 0.05. Trait abbreviation: Stone volume [StV], Yield [YL], Ratio volume stone/volume fruit [VSt/VFr], Ratio weight stone/weight fruit [WeSt/WeFr], Petiole length [PL], Petiole width [PeWi], Ratio petiole length/width [PL/Wi], Blade length [BlLe], Blade width [BlWi], Ratio blade length/petiole length [BlLe/ PL], Stone length [StLe], Stone width [StWi], Stone thickness [StTh], Soluble solids [SoSo], Titratable acidity [TiAc], ratio titratable acidity/soluble solids [TiAc/SoSo], Pedicel length [PeLe], Fruit polar diameter [PoDi], Fruit equatorial diameter [EqDi], Fruit width [FrWi] and Fruit weight [FrWe].

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Table 4
First 6 components from the PCA analysis of 21 quantitative traits in 146 sweet cherry cultivars.

Traits	F1	F2	F3	F4	F5	F6
StV	0257	−0.256	0.014	0.266	−0.037	0.043
YL	0.132	−0.078	0.016	−0.214	−0.196	0.431
VSt/Vfr	0.005	0.414	−0.128	−0.202	−0.019	0.054
WeSt/WeFr	0.102	0.483	−0.004	0.086	−0.112	0.037
PL	0.266	0.043	0.340	−0.329	0.188	−0.146
PeWi	0.240	−0.041	0.300	−0.167	0.261	−0.007
PL/Wi	−0.033	−0.105	−0.102	0.266	0.118	0.178
BlLe	−0.050	0.189	0.038	0.059	0.653	−0.093
BlWi	0.270	0.003	0.298	−0.225	0.225	0.007
BlLe/ PeLe	0.133	−0.125	0.275	−0.357	−0.440	0.021
StLe	0.165	−0.300	−0.318	−0.043	0.079	−0.091
StWi	0.242	−0.320	−0.243	−0.014	0.098	−0.058
StTh	0.228	−0.328	−0.144	−0.096	0.029	−0.054
SoSo	0.013	0.031	0.225	0.200	−0.022	0.567
TiAc	0.125	−0.015	0.395	0.438	−0.140	−0.029
TiAc/ SoSo	−0.114	0.043	−0.296	−0.377	0.125	0.445
PeLe	0.000	−0.161	0.100	0.101	0.314	0.447
PoDi	0.305	0.185	−0.232	−0.029	−0.052	−0.011
EqDi	0.360	0.189	−0.167	0.145	−0.007	0.001
FrWi	0.394	0.136	−0.096	0.145	−0.069	0.094
FrWe	0.373	0.200	−0.158	0.083	0.008	0.050
Cumulative%	22.906	39.661	49.815	59.374	67.083	73.590
Variability (%)	22.906	16.755	10.155	9.558	7.710	6.506

Trait abbreviation: Stone volume [StV], Yield [YL], Ratio volume stone/volume fruit [VSt/VFr], Ratio weight stone/weight fruit [WeSt/WeFr], Petiole length [PL], Petiole width [PeWi], Ratio petiole length/width [PL/Wi], Blade length [BlLe], Blade width [BlWi], Ratio blade length/petiole length [BlLe/ PL], Stone length [StLe], Stone width [StWi], Stone thickness [StTh], Soluble solids [SoSo], Titratable acidity [TiAc], ratio titratable acidity/soluble solids [TiAc/ SoSo], Pedicel length [PeLe], Fruit polar diameter [PoDi], Fruit equatorial diameter [EqDi], Fruit width [FrWi] and Fruit weight [FrWe].

and ratio of blade length/petiole length. The sixth component explained 6.50% of the total variation, included yield, soluble solids, pedicel length and ratio of titratable acidity/soluble solids. Furthermore, a PCA scatter plot was constructed based on the first three components (Fig. 2). The plot grouped the cultivars according to their phenotypic similarity and morphological traits. For instance, cultivars Bakirtzeika and Lemonidi with the highest fruit weight, largest fruit width and longest fruit length were placed closely in the upper right plane. These results demonstrate that fruit equatorial diameter, fruit weight and fruit width are highly positively correlated and as a result, these morphological traits led to the highest loading factors in this PCA analysis. Thus, PCA showed that some traits had the highest loadings in the first two components. These traits included stone volume, petiole length, blade width, fruit polar diameter, fruit equatorial diameter, and fruit width and fruit weight. These results indicate that such traits are suitable both for the assessment of genetic diversity and for phenotypic characterization of sweet cherry germplasm (Fig. 3).

3.3. Correlations for qualitative traits

There were more significant negative correlations than positive ones among the 14 qualitative traits (Table 5). Negative correlations were observed between the following traits: FiFl and TrBr (−0.297), StSi and TrBr (−0.293), FrSh and FiFl (−0.269), TrVi and FrSi (−0.262), FrSh and FrSi (−0.244), TrBr and TrVi (−0.175), and LaSh and TrVi (−0.174). Positive correlations included TrBr and NuNe (0.364), FrSi and StSi (0.342), FrSi and LaSi (0.283) LaSh and NuNe (0.280) and FiFl and FrSi (0.229).

3.4. Principal component analysis (PCA) of qualitative variables

Using Kaiser's criterion ("Eigenvalue" >1) (Kaiser, 1958), we obtained 6 significant components, which explained 65.11% of the total variation (Fig. 1). The first component, which accounted for 17.24% of the total variation, included tree branching, firmness of flesh, fruit size, number of nectaries and lamella size. The sec-

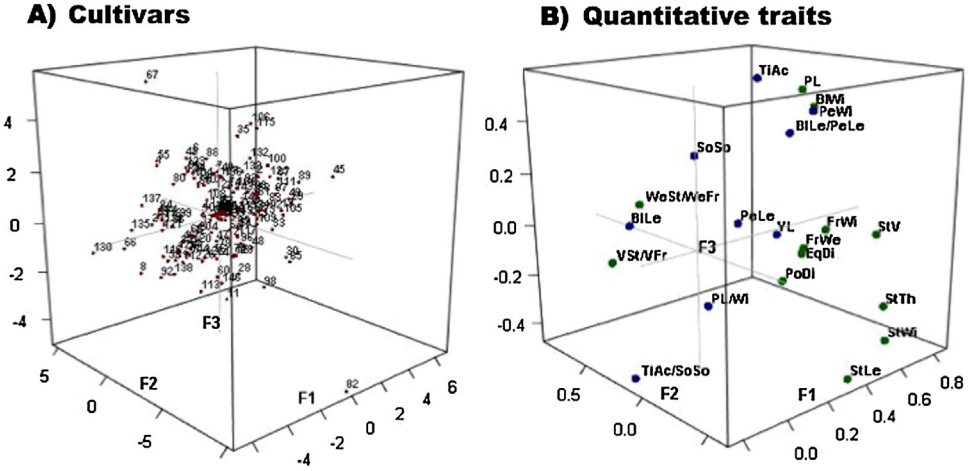

Fig. 2. (A) Three-dimensional PCA plot of the 146 sweet cherry cultivars with regard to the first three principal components. (B) Three-dimensional PCA plot of the 21 quantitative traits with regard to the first three principal components. Variability explained: F1 (22.91%), F2 (16.76%), F3 (10.16%).

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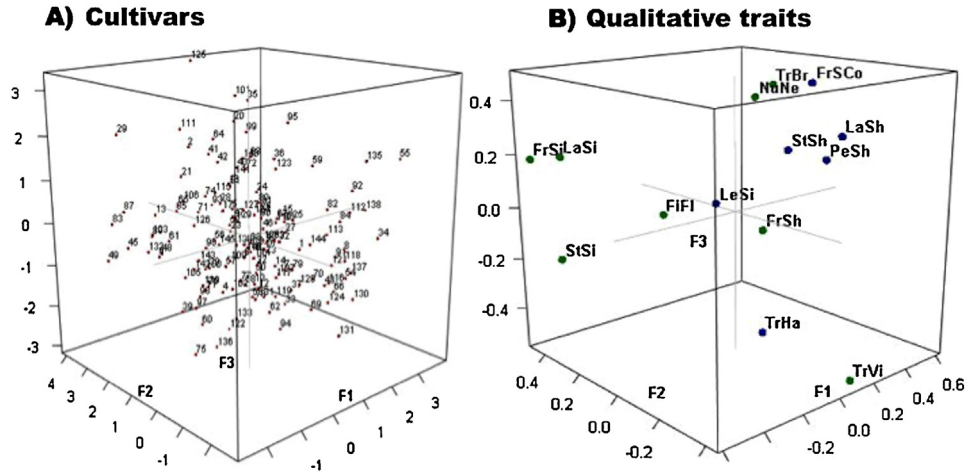

Fig. 3. (A) Three-dimensional PCA plot of the 146 sweet cherry cultivars with regard to the first three principal components. (B) Three-dimensional PCA plot of the 14 qualitative traits with regard to the first three principal components. Variability explained: F1 (17.25%), F2 (10.95%), F3 (10.43%).

Table 5
Correlation coefficients (Spearman) among 14 qualitative traits in 146 sweet cherry cultivars.

Variables	TrVi	TrHa	TrBr	LeSi	LaSh	LaSi	NuNe	PeSh	FrSh	FrSi	FrSCo	FiFl	StSh	StSi
TrVi	**1**													
TrHa	0.108	**1**												
TrBr	**−0.175**	−0.115	**1**											
LeSi	−0.042	**−0.172**	**−0.262**	**1**										
LaSh	0.012	−0.071	0.005	0.107	**1**									
LaSi	**−0.174**	−0.141	−0.112	−0.015	−0.160	**1**								
NuNe	−0.055	−0.025	**0.364**	−0.067	**0.280**	−0.119	**1**							
PeSh	0.076	−0.151	−0.021	−0.086	0.128	−0.029	0.008	**1**						
FrSh	0.017	−0.009	**0.251**	−0.016	0.043	−0.161	**0.217**	−0.087	**1**					
FrSi	**−0.262**	−0.051	−0.138	0.115	−0.145	**0.283**	−0.075	−0.105	**−0.244**	**1**				
FrSCo	0.007	0.099	0.127	−0.046	0.097	−0.031	**0.208**	0.122	**−0.166**	−0.014	**1**			
FiFl	−0.028	−0.065	**−0.297**	0.135	−0.075	**0.181**	**−0.259**	0.115	**−0.269**	**0.229**	0.069	**1**		
StSh	−0.114	−0.073	−0.105	0.160	−0.128	0.069	**−0.175**	−0.059	**−0.237**	−0.067	0.158	0.138	**1**	
StSi	0.037	0.035	**−0.293**	0.060	**−0.168**	**0.279**	0.051	−0.059	−0.084	**0.342**	−0.062	0.152	−0.143	**1**

Values in bold are different from 0 with a significance level alpha = 0.05. Trait abbreviation: Tree vigour [TrVi], tree habit [TrHa], tree branching [TrBr], lenticels size [LeSi], lamella shape [LaSh], number of nectaries [NuNe], petal shape [PeSh], fruit shape [FrSh], fruit size [FrSi], fruit skin colour [FrSCo], firmness of flesh [FiFl], stone shape [StSh] and stone size [StSi].

ond component, which explained 10.94% of the total variation, was determined by fruit size, stone size and stone shape. The third component, explained 10.43% of the total variation, and was mainly correlated to characters related to tree vigour, fruit skin colour and tree habit. The fourth component, accounted for 9.31% of the total variation included lamella shape and stone shape. The fifth component, explained 9.14% of the total variation, included tree habit, lenticels size and fruit skin colour. The sixth component, explained 8.03% of the total variation, and included petal shape, tree habit and lenticels size (Table 6).

Table 6
First 7 components from the PCA analysis of 14 qualitative traits in 146 sweet cherry cultivars.

	F1	F2	F3	F4	F5	F6	F7
TrVi	0.077	−0.329	−0.447	−0.290	−0.063	0.076	−0.458
TrHa	0.043	−0.057	−0.385	−0.010	−0.484	−0.411	0.316
TrBr	0.394	0.252	0.306	0.223	−0.155	0.147	−0.052
LeSi	−0.184	−0.128	0.094	−0.045	0.586	−0.415	−0.151
LaSh	0.218	−0.164	0.224	−0.431	0.287	−0.202	0.349
LaSi	−0.329	0.302	0.165	0.017	−0.076	0.224	−0.254
NuNe	0.341	0.272	0.267	−0.327	−0.046	−0.260	−0.244
PeSh	0.032	−0.275	0.207	−0.392	−0.045	0.559	0.056
FrSh	0.345	0.243	-0.204	0.073	0.287	0.034	−0.193
FrSi	−0.365	0.387	0.	−0.117	−0.049	−0.122	0.274
FrSCo	0.057	−0.204	0.406	−0.195	−0.460	−0.290	−0.246
FiFl	−0.389	−0.184	0.121	−0.	−0.019	0.103	0.119
StSh	−0.179	−0.352	0.285	0.440	−0.033	−0.197	−0.328
StSi	−0.301	0.363	−0.182	-0.380	−0.066	−0.099	−0.348
Cumulative%	17.249	28.196	38.627	47.937	57.086	65.117	71.329
Variability (%)	17.249	10.947	10.431	9/310	9.149	8.031	6.212

Trait abbreviation: Tree vigour [TrVi], tree habit [TrHa], tree branching [TrBr], lenticels size [LeSi], lamella shape [LaSh], number of nectaries [NuNe], petal shape [PeSh], fruit shape [FrSh], fruit size [FrSi], fruit skin colour [FrSCo], firmness of flesh [FiFl], stone shape [StSh] and stone size [StSi].

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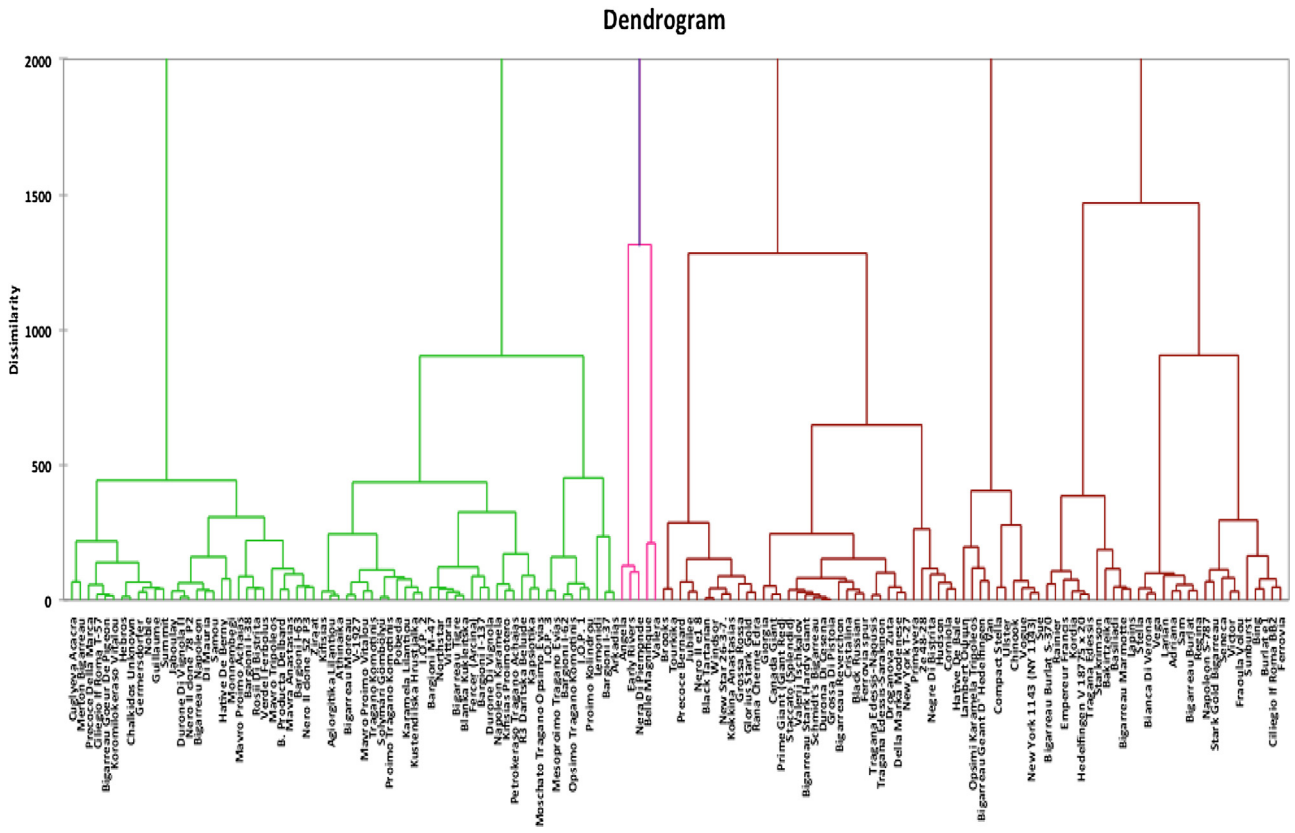

Fig. 4. Dendrogram using agglomerative hierarchical clustering (AHC) for 146 sweet cherry cultivars based on 21 quantitative and 14 qualitative traits.

3.5. Dendrogram using agglomerative hierarchical clustering (AHC)

Unsupervised agglomerative hierarchical cluster analysis was used in order to divide the available data up into groups of increasing dissimilarity. The Euclidean distance was used as a metric to measure the genetic dissimilarity of the 146 sweet cherry cultivars, based on the combined quantitative and qualitative data, and the Ward's method was used for the agglomeration. The dendrogram of Fig. 4 pointed out that, all sweet cherry cultivars were different from each other, and several clusters are identified. The dendrogram revealed three distinct groups. C1 contained 75 cultivars, C2 had 5 cultivars, and finally C3 included 66 cultivars. Among all 146 cultivars from different world regions, there were no specific clusters based on locality. The highest genetic distance exists between C2 and C3 (115.60), followed by C1 and C2 (71.14) and C1 and C3 (46.06).

4. Discussion

The main goal of germplasm management is to collect and to characterize diverse species or forms of the same species at national and regional level. To do so it is important to perform, as a first step, the evaluation of morphological and agronomic traits of interest. Plant breeders then routinely use this morphological characterization for the initial description and classification of the germplasm under consideration.

We estimated the morpho-physiological traits variation in sweet cherry cultivars established in the Greek GeneBank and provided basic knowledge on the range of variation of several morphological traits. The studied cultivars displayed significant variation in fruit weight traits as well as moderate variation for yield, stone volume, ratio of titratable acidity/soluble solids, titratable acidity, and ratio of volume stone/volume fruit (Pommer, 2012).

Herein, the largest variation among the fruit traits corresponded to fruit weight and yield as it has been previously reported (Christensen, 1974; Ganopoulos et al., 2011; Hjalmarsson and Ortiz, 2000; Petruccelli et al., 2013). In addition, these authors have also suggested that the fruit weight could represent the utmost significant trait for distinguishing sweet cherry cultivars. Furthermore, the fruit weight (fruit size) is a very crucial trait due to its economic importance: some local cultivars, e.g., 'Lemonidi' and 'Mpakirtzeika', have shown relative large fruits with considerably elevated weights and volumes. The morphological traits that were observed as crucial for sweet cherry cultivars characterization in this study were fruit weight and fruit size which were also found to be important in sweet cherry from Greece (Ganopoulos et al., 2011; Petruccelli et al., 2013), which indicates similar diversity patterns in the Mediterranean region.

The fruit weight showed positive correlation with stone weight ($r = 0.495$, $p < 0.001$) and also fruit equatorial diameter with fruit width ($r = 0.844$, $p < 0.001$) in agreement with (Khadivi-Khub, 2014). Our results also showed a very close correlation among fruit weight and fruit dimensions (length, width and diameter; $r = 0.82$, $r = 0.90$ and $r = 0.86$, respectively); therefore, these parameters could potentially be used to predict each other. A degree of correlation between fruit weight and fruit dimensions was also detected earlier in cherries (Khadivi-Khub, 2014; Rakonjac et al., 2010). All variables related to fruit size (fruit weight and dimensions) showed significant positive correlation with leaf traits (petiole length and petiole width) at $p = 0.05$, indicating a role of leaf in the increased fruit size (Demirsoy and Demirsoy, 2004). A positive correlation between the sweet cherry fruit size and leaf traits was also determined before by Rakonjac et al. (2010). Posi-

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tive correlations were also obtained between leaf traits with each other.

Most of the qualitative traits evaluated are considered crucial for species registration and discrimination in the test guidelines proposed by UPOV (UPOV, 1976). Notably, the fruit characteristics, such as skin color, flesh colour and firmness, are those that typically differentiate sweet cherry cultivars (Antonius et al., 2012; Hjalmarsson and Ortiz, 2000; Petruccelli et al., 2013). The skin color of the fruit is a very important quality characteristic, which also helps the assessment of the stage of fruit maturity as it has been shown when (Esti et al., 2002) evaluated the changes in color of sweet cherry and used this trait for monitoring pigment evolution. Moreover, fruit color has a significant impact on consumer perception of fruit quality, especially as regards the attractiveness of fruit (Ruiz and Egea, 2008). Consumers generally seem to prefer dark red cherries (Petruccelli et al., 2013). Another important characteristic is fruit firmness which is relevant to an assessment of the quality of fruit, fruit shelf life, and consumer acceptance. Fruit firmness is a combination of skin and flesh strength, and in general cultivars with the firmest fruit are preferred by consumers increasing as a result their value in the market (Kappel et al., 1996; Petruccelli et al., 2013).

Multivariate statistical methods such as PCA and cluster analysis could be valuable tools for monitoring cultivars, and characterizing and classifying plant germplasm in GenBank collections (Iezzoni and Pritts, 1991). Further, PCA is useful in defining the number of main factors, thus decreasing the number of efficient parameters to differentiate genotypes. Additionally, associations between characteristics emphasized by this method may correspond to genetic linkage between loci controlling traits with a pleiotropic effect (Iezzoni and Pritts, 1991; Rakonjac et al., 2010). Many studies, in agreement with our study, indicated that fruit and leaf traits are crucial factors in phenotyping and morphologically characterizing the diversity in sweet cherry breeding materials (Antonius et al., 2012; Ganopoulos et al., 2011; Hjalmarsson and Ortiz, 2000; Lacis et al., 2009; Rakonjac et al., 2010). Furthermore, PCA analysis has been widely used for evaluation of sweet cherry germplasm (Beyer et al., 2002; Christensen, 1974; Ganopoulos et al., 2011; Hjalmarsson and Ortiz, 2000; Khadivi-Khub, 2014; Lacis et al., 2009; Petruccelli et al., 2013; Rodrigues et al., 2008; Sánchez et al., 2008). We have used PCA for the identification of the most significant variables in the data set presented herein. For each factor, a principal component loading of more than 0.55 was considered significant, which indicated that seven components explained 73.59% of the total variance. The first three components, consisted of 21 quantitative variables, explained 49.81% of the total variability observed (Table 4), indicating that these attributes have the highest variation between the cultivars and had the greatest impact on separation of the cultivars (Iezzoni, 2008; Khadivi-Khub, 2014; Lacis et al., 2009). PCA analysis of the qualitative variables has shown similar results (Table 5). Further, PCA analysis achieved the characterization of cultivars groups by identifying highly discriminating variables like traits regarding the fruit. Thus, PCA analysis suggested that future evaluations can rely on a reduced number of traits with a minimum loss of information for the discrimination and characterization of the different varieties/cultivars resulted in a reduction in labour, time and cost.

Fig. 4 depicts the unsupervised hierarchical cluster analysis of 146 sweet cherry cultivars. The cultivars analyzed grouped into three main clusters. (Khadivi-Khub, 2014) characterized 41 sweet cherry genotypes with morphological descriptors and found that all genotypes were divided into five clusters. The dendrogram produced showed high diversity between sweet cherry cultivars indicating that analyzed germplasm collection could be assumed in breeding programs as a good gene pool for contrasting traits. For instance, sweet cherry cultivars 'Cuglyeva Acacra' from Bulgaria and 'Merton Bigarreau' from U.K. were highly distinguished from other cultivars.

5. Conclusions

PCA combined with unsupervised cluster analysis for agronomic, morphological and fruit quality traits revealed a wide diversity in sweet cherry maintained in Germplasm Bank at the Institute of Pomology (Greece). Understanding the phenotypic diversity among the sweet cherry cultivars is crucial for the conservation of traditional genetic material that is endangered. Furthermore, this information is very useful for registration of new sweet cherry cultivars carried out by EU-Community Plant Variety Office.

The study presented herein provides useful information on agronomic, morphological and fruit quality traits of sweet cherry cultivars grown under the climatic conditions of Imathia area in North Greece. Future breeding programs could take advantage of those genetic materials and use them as parental genotypes in order to achieve genetic recombination and improvement in agronomic, morphological and fruit quality traits. Thus, new strategies could be created for new breeding programs of sweet cherry cultivars with better adaptation to the limiting agro-climatic conditions of Greece.

When separate traits are analyzed, information of paramount importance about the internal similarities of cultivars and genetic structure is lost. Contrariwise, when combined application of PCA and cluster analysis is used, important traits for sweet cherry cultivars characterization are revealed and provide comprehensive information. Hence, in order to thoroughly analyze the diversity of a GeneBank collection, a multivariate statistics approach taking into account both the genetic and the phenotypic data is of utmost importance.

Conflict of interest

The authors declare that they have no conflict of interest.

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Please cite this article in press as: Ganopoulos, I., et al., Diversity of morpho-physiological traits in worldwide sweet cherry cultivars of GeneBank collection using multivariate analysis. Sci. Hortic. (2015), http://dx.doi.org/10.1016/j.scienta.2015.09.061