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Towards sweet cherry (*Prunus avium* L.) breeding: phenotyping evaluation of newly developed hybrids

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Abstract An increasing demand for cherry production (*Prunus avium* L.) in Greece led to the development of new high quality sweet cherry cultivars. Self-incompatibility in cherry is one of the most challenging issues for the species' cultivation and top breeding priority. The present study focuses on the development of new hybrids with improved traits such as productivity, fruit size, organoleptic characteristics and self-compatibility. For this purpose, thirty different cultivars were crossed and produced hybrids that were evaluated according to 34 morpho-physiological characteristics. The results were analyzed using the XLSTAT (version 2014.1) software and a dendrogram was constructed using the agglomerative hierarchical clustering method. Optimal hybrid clustering was achieved when characteristics of great economic importance such as fruit shape and size, growth habit and days to blooming were included in the analysis. Based on the results, new sweet cherry hybrids with the special character of self-compatibility were developed. Our findings provide crucial new information for sweet cherry future breeding programs and cultivation.

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Introduction

Cherry trees (*Prunus avium* L.) are cultivated either for their fruit consumption by humans (sweet cherry trees), or for their wood (wild cherry trees, also called mazzards). The centre of origin for cherry trees is considered to be the Caucasus area, through years they have expanded across mainland Europe and western Asia (Webster 1996). Sweet cherries were probably first cultivated in Greece (Hedrick 1915; Marshall 1954) and today, cherries are the most popular fruit tree crops in Greece (Ganopoulos et al. 2015). The high commercial demand for sweet cherries together with their highly competitive prices resulted in the rival of their cultivation in the cultivation centers and also in many new cultivation regions.

Traditional breeding programs apply two crossing strategies in order to develop new sweet cherry cultivars; primarily, hybridization (intra- and inter-specific) and also open pollination (Carrasco et al. 2013). Although most of the sweet cherry genotypes have been characterized as self-incompatible, self-compatible sweet cherry cultivars (e.g. cv. 'Stella') have been also reported (Lapins 1971; Cachi and Wünsch 2014). Pollen management and seedling production (conventional seed germination and embryo rescue) have been described as hurdles in the development of a breeding program. Sweet cherries produce a limited number of seedlings because of the difficulties in their sexual propagation (Carrasco et al. 2013; Iezzoni 2008). Lloyd (1992) confirmed the hypothesis that the selection of self-fertilized cultivars is very significant, especially under conditions where cross-pollination is inadequate, making self-pollination paramount in order to ensure their reproduction. Hence, breeding self-compatible cultivars in order to reduce labor and artificial pollination costs has been one of the important sweet cherry breeding goals at the Institute of Pomology, Naousa, Greece (ELGO-DEMETER) (Ganopoulos et al. 2011).

Multivariate data analysis is an effective approach for analyzing large data set of phenotypic characteristics. Multivariate data analysis is the preferable method for the identification of patterns and relationships in a given data set, presenting powerful statistical techniques, such as principal coordinate analysis (PCA) and cluster analysis (CA). Thus, this approach is usually implemented on large data sets of sweet cherry concerning quantitative and qualitative phenotypic characteristics (de Oliveira et al. 2012; Furones-Pérez and Fernández-López 2009; Ganopoulos et al. 2015; Mehmood et al. 2014). Particularly, cluster analysis which uses both quantitative and qualitative data can exploit all the available information for each sample, and each entry of the data set, as cluster analysis treats data sets uniformly and equally to each other, compared to other clustering methods (Peeters and Martinelli 1989).

The aim of this study was the phenotypic characterization of newly derived hybrids presenting improved quality characteristics and particularly, hybrids that are more productive, with larger fruit size, better organoleptic characteristics, self-compatibility and great importance for the country's market. Furthermore, based on this characterization, selection of best cultivars for sweet cherry orchards in Greece can be enhanced for Greece.

Materials and methods

The breeding program for self-compatibility started in 1993 at the experimental fields of the Institute of Pomology, Naousa, Greece (ELGO-DEMETER). F_1 progenies were developed in Greece from parents of breeding significance, presenting different phenotypes. A total of 30 progeny seedlings from intra-specific crosses were used in this study (Fig. 1), originating from the following crosses: 16 seedlings were from 'Burlat' × 'Stella' (B × S), 1 from 'Chinook' × 'Stella' (Ch × S), 8 from 'Hardy Giant' × 'Stella' (HG × S) and 5 from 'Germesdorfer' × 'Tragana Edessis' (G × T). Cultivars 'Burlat', 'Tsolakeiko' and 'Larian' was used as the control.

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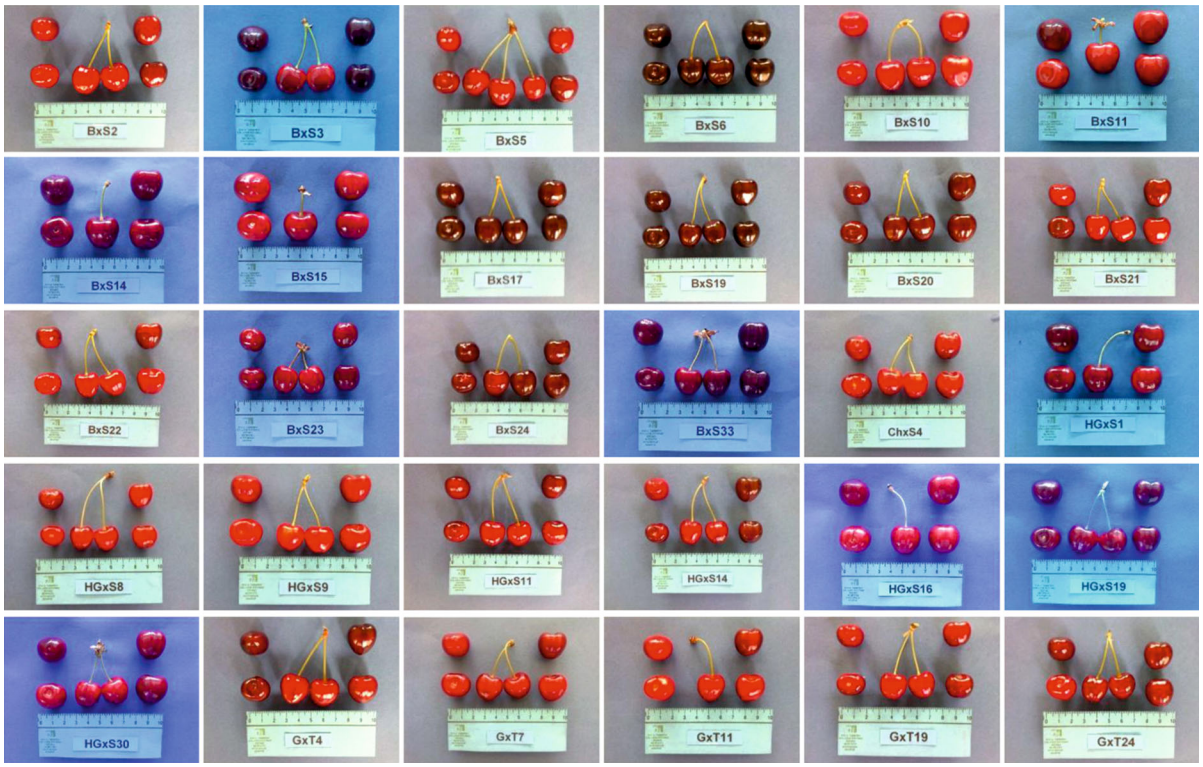

Fig. 1 Fruit features of the sweet cherry hybrids used in this study

'Stella' combines characteristics such as desirable appearance and higher quality, thus it was selected as the optimum variety that meets the needs both of producers (aiming to reduce labor costs) and of consumers (seeking high fruit quality). Thirty-four variables were examined on the basis of cherry descriptors (IPGRI 1985; UPOV 1976) at the experimental collection for 10 consecutive years (2002–2011). Standard horticultural practices, including fertilizer, irrigation and other cultural activities, were applied annually.

Analysis of morpho-physiological traits

Seven mature leaves were collected per year at the end of July from each progeny and the parameters referred below were measured using a digital caliper with a sensitivity of ± 0.01 mm. The flowers were collected at full bloom; ten flowers were collected from each plant per year. Cherry fruits were collected at maturity stage based on colour. A sample of a total of 115 cherry fruits was harvested per tree, per year. One hundred of them were used to determine the mean fruit weight. Fifteen fruits were used to study a series of quantitative and qualitative descriptors described below.

The 22 quantitative traits measured, included: yield [YL], blade length [BL], blade width [BW], ratio blade length/blade width [rBL/BW], petiole length [PtL], petiole thickness [PT], ratio blade length/petiole length [rBL/PtL], leaf: number of nectaries [NN], fruit length [FL], fruit width [FWi], fruit thickness [FT], fruit weight [FW], soluble solids content [SS], titratable acidity [TA], ratio titratable acidity/soluble solids [rTA/SS], pedicel length [PdL], stone length [SL], stone width [SW], stone thickness [ST], stone volume [SV], ratio weight stone/weight fruit [rWS/WF], ratio volume stone/volume fruit [rVS/VF].

The 12 qualitative traits evaluated included: tree vigour [TV], tree branching [TB], density [DN], lamella shape [LASH], lamella size [LAS], petal shape [PSH], fruit shape [FSH], fruit size [FS], fruit skin colour [FSC], firmness of flesh [FF], stone shape [SSH] and stone size [STS].

These traits and their scoring system were selected from the descriptors of the International Union for the

Protection of New Cultivars of Plants proposed for sweet cherry (IPGRI 1985; UPOV 1976).

Data scoring and analysis

Data were analyzed by the XLSTAT software (version 2014.1). 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 median mode in the latter. In both cases the correlation matrix was used, as standardization was necessary since the variables were measured in different units (Pimentel 1979). In each case, 2D plots were constructed, with regards to the two most important principal components, to facilitate the visualization of the results. Moreover, PCA was conducted on the correlation matrix since this PCA separates size from shape more effectively than the analysis of the covariance matrix (Aravanopoulos 2010a, b). 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 aggregation approaches for agglomerative hierarchical clustering (AHC), were based on Euclidean and Ward distances, using the combined data from both quantitative and qualitative traits for dendrogram construction.

Cross-compatibility validation using field and molecular approaches

Cross compatibility with var. 'Stella' has been evaluated for 3 years using a total of 100 flowers in each plant. Two flowers per flower cluster at the balloon stage were retained and each flower was emasculated by hand and covered with a paper bag until pollination. Fruit set was determined 30 days after the full bloom stage. Validation of cross-compatibility was done using molecular markers for detecting the self-compatible *S4′* haplotype according to (Zhu et al. 2004).

Results and discussion

Results from artificial cross-pollination

Crosses between 'Burlat' and 'Stella' (B × S), 'Chinook' and 'Stella' (Ch × S), and 'Germersorfer' and 'Tragana Edessis' (G × T) produced 16, 1 and 5 hybrids, respectively.

Blooming time

The stage of first bloom in all hybrids was accomplished between 26th March (Hybrid B × S19) and 16th April (HG × S16 and G × T7), with the majority however being within 28th March and 13th April. Full bloom initiated within 2–4 days after first bloom (Fig. 2). Flowering period for these hybrids lasted approximately 11–18 days. This is normal as the flowering time might vary depending on the weather conditions. The estimation of flowering time is extremely important for the establishment of successful fruit development. Therefore, an early flowering cultivar is not recommended as a pollinator for late flowering cultivars (Moghadam et al. 2009). Furthermore cherries are characterized both by the self-incompatibility and the cultivars incompatibility as a result of differences in flower physiology, thus a reasonable overlapping pollination period is highly important for fertilization (Moghadam et al. 2009). This overlapping pollination period is recommended to be at least 3 days regarding stone fruit trees (Nyéki et al. 2008). Yet, Moghadam et al. (2009) reported that in cherry the optimum blooming duration should be 10–14 days, with a minimum overlapping period between 4 and 6 days in order to achieve efficient pollination and fertilization. As early as 1975 Humphry-Baker (1975) proposed that sweet cherry growers should make the right combinations among pollinators and the pollinating cultivars for optimal pollination to take place and achieve both high quality and quantity. Furthermore, this study indicates that there is a direct relation between blooming time and flowering density, in agreement to Bassi et al. (1995) who suggested that in apricots early blooming cultivars have higher blooming density than late blooming cultivars.

Based on the blooming season the hybrids used in this study were classified into the following three groups (Fig. 2):

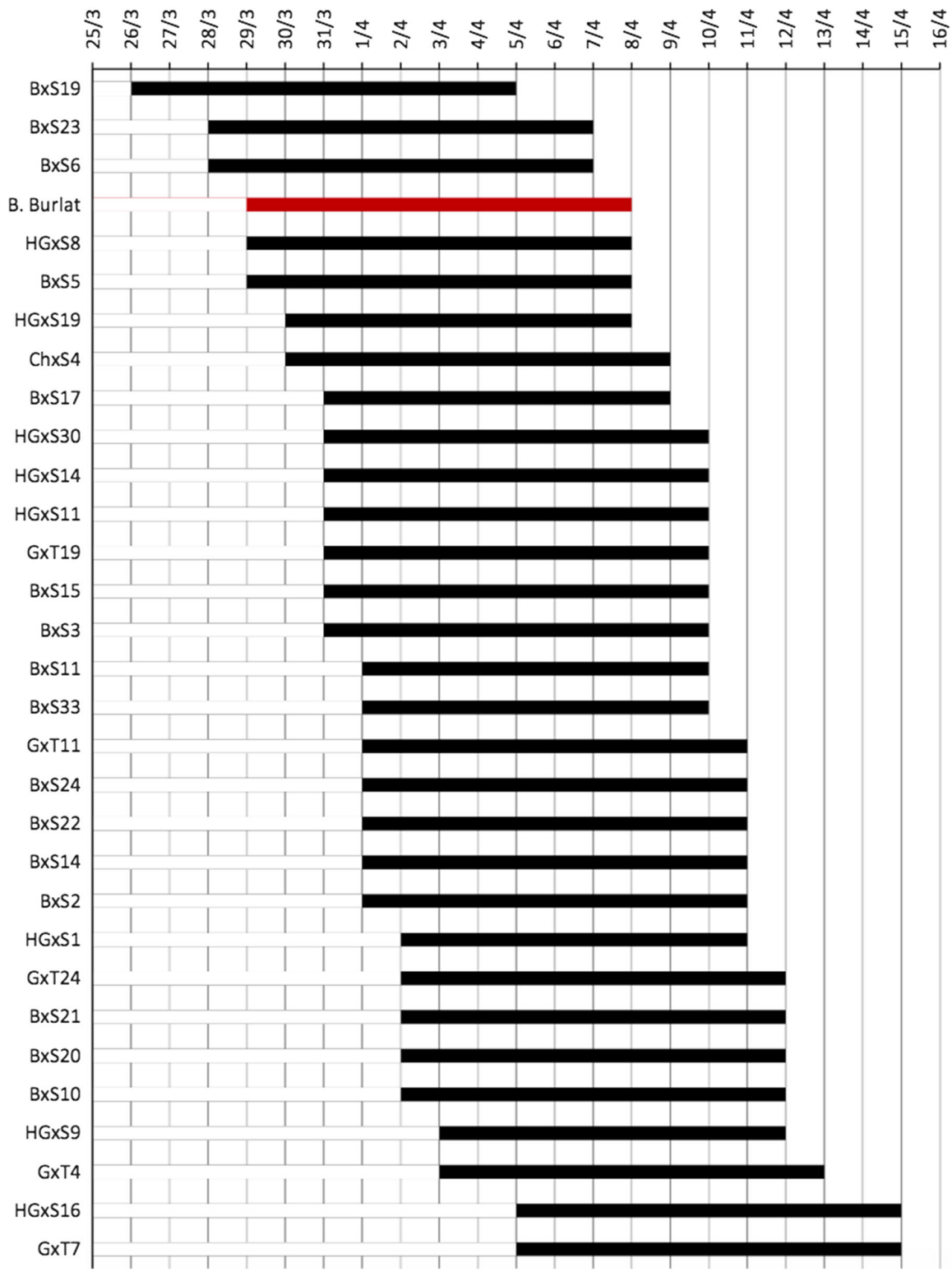

Fig. 2 Different stages of blooming time in 30 sweet cherry hybrids analyzed

First group: *early blooming* includes B × S19, B × S6, B × S23, HG × S8 and B × S5 hybrids.

Second group: *mean blooming* includes HG × S19, Ch × S4, B × S17, HG × S30, HG × S14, HG × S11, G × T19, B × S15, B × S3, B × S11, B × S33, G × T11, B × S24, B × S22, B × S14 and B × S2 hybrids.

Third group: *late blooming* includes HG × S1, G × T24, B × S21, B × S20, B × S10, HG × S9, G × T4, HG × S16 and G × T7 hybrids.

Based on our results, it is suggested that early and late blooming cultivars among mean blooming cultivars should be combined correctly, so as to have maximum overlapping period during full bloom stage. If these cultivars are compatible, an increase in fertilization will be observed.

Table 1 Descriptive statistics for 22 quantitative traits in 30 sweet cherry hybrids

Variable	Minimum	Maximum	Mean	SD	CV (%)
YL	0.010	0.160	0.100	0.038	38
BL	10.600	16.400	13.519	1.534	11.346
BW	4.430	7.460	5.864	0.797	13.591
rBL/BW	1.818	2.857	2.328	0.238	10.223
PtL	3.060	4.760	3.692	0.432	11.700
PT	0.120	0.200	0.158	0.024	15.189
rBL/PL	0.198	0.345	0.276	0.041	14.855
NN	2.000	4.000	2.161	0.454	21.008
FL	17.750	26.100	22.447	1.877	8.361
FWi	19.300	27.100	23.902	2.064	8.635
FT	16.000	23.600	19.848	1.738	8.756
FW	3.900	10.700	7.469	1.611	21.569
SS	9.000	17.400	13.526	2.288	16.915
TA	4.400	15.000	8.194	2.506	30.583
rTA/SS	0.370	1.000	0.606	0.136	22.442
PdL	2.600	4.900	3.719	0.584	15.703
SL	9.100	12.400	11.123	0.780	7.012
SW	7.800	9.900	9.026	0.560	6.204
ST	6.000	8.600	7.168	0.554	7.728
SV	0.330	0.750	0.454	0.100	22.026
rWS/WF	0.041	0.093	0.062	0.011	17.741
rVS/VF	0.045	0.094	0.069	0.012	17.391

Descriptive statistics and correlations for the quantitative variables

Twenty-two quantitative traits were measured and the descriptive statistics of minima, maxima, means, standard deviations and coefficients of variation (CV) are shown in Table 1. The results revealed extensive morphological variability. Some traits displayed high CV. These included: YL (38.28%), TA (30.58%) and SV (22.06%). In a recent study in *Prunus scoparia*, Khadivi-Khub et al. (2016) reported significantly different values for phenotypic characters presenting also high CV values that reflect a high level of phenotypic variation. The highest values had a range of 77.9–55.03% for different morphological traits. Strong positive, linear correlations were observed among all 22 quantitative traits (Table S1). The highest significant, positive correlation was between FT and FW (0.961). Significant positive correlations were also observed between FWi and FL (0.930), FWi and FW (0.959), FWi and FT (0.942), FL and FT (0.885), FWi and FL (0.876), and PT and BL (0.813). On the other hand, there were no highly significant, negative correlations between quantitative traits (Table S1). These results are partially in agreement with Ganopoulos et al. (2015) where most of the 22 quantitative traits studied, had shown strong positive correlations. For instance, significant positive correlations were observed between FWi and FW (0.843), but negative correlations were found between rTA/SS and TA (− 0.714).

Principal component analysis of quantitative variables

The distribution of hybrids, based on the PC-1 and PC-2, depicts the phenotypic variation among them and how widely dispersed they are along both axes (Fig. 3). Using Kaiser's criterion ("Eigenvalue" > 1) (Kaiser 1958), the dimension implied was reduced by the 22 quantitative traits to 6 significant components that explained 84.58% of the total variation (Table 2). The first component, which accounted for 34.39% of the total variation, included fruit length [FL], fruit width [FWi], fruit thickness [FT] and fruit weight [FW]. The second component, which explained 16.38% of the total variation, was mainly characterized by higher loadings of the ratio blade length/petiole length [rBL/PL], petiole length [PtL], ratio

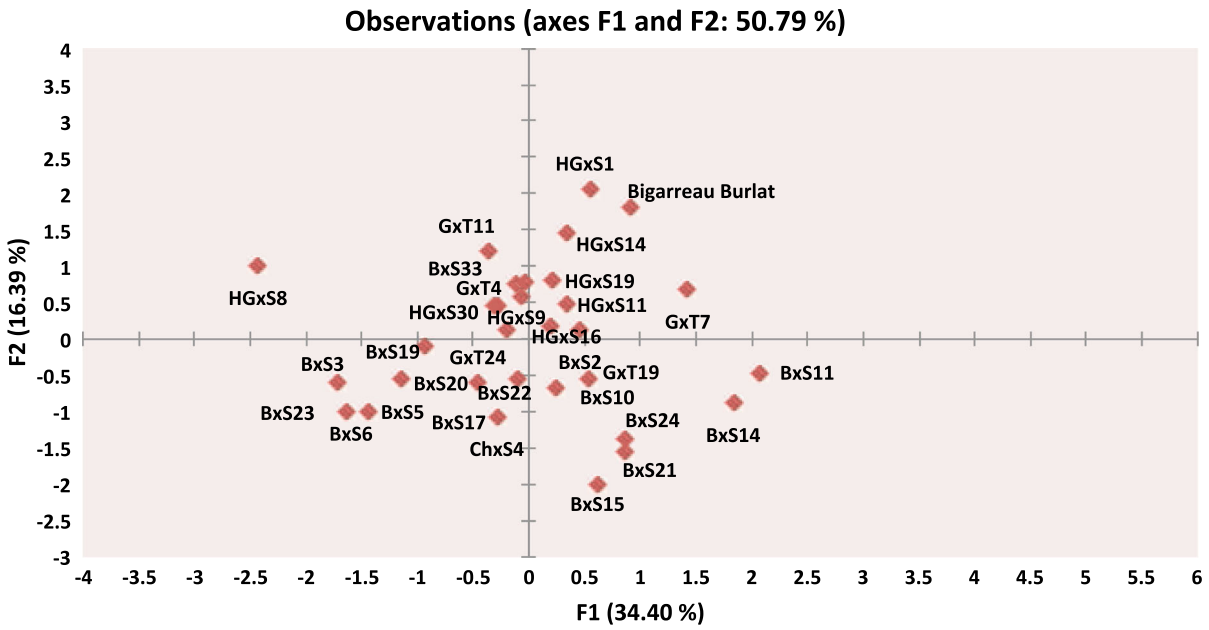

Fig. 3 Two-dimensional PCA plot of the 30 sweet cherry hybrids with regard to the first two principal components according to quantitative data analyzed. Variability explained: F1 (34.40%), F2 (16.39%)

blade length/blade width [rBL/BW] and pedicel length [PdL] (Table 2). The percentage of variation explained and the contribution of each variable to the first 6 components is presented in Table 2. Furthermore, a PCA scatter plot was constructed based on the first 2 components (Fig. 3) which explain almost 51% of the total variation. Those morphological traits were important for sweet cherry characterization, not only in Greek sweet cherries cultivars (Ganopoulos et al. 2011, 2015), but also for Italian traditional cultivars (Petruccelli et al. 2013), indicating that the diversity patterns around the Mediterranean region are closely related.

PCA has been widely used to track pairwise correlations among different traits in *P. avium* (Ganopoulos et al. 2015) and other *Prunus* species (Khadivi-Khub 2014; Khadivi-Khub et al. 2016; Nikolić et al. 2010; Rakonjac et al. 2010; Ruiz and Egea 2008; Sorkheh et al. 2009) and to evaluate sweet cherry germplasm (Beyer et al. 2002; Ganopoulos et al. 2011).

Principal component analysis (PCA) of qualitative variables

Using Kaiser's criterion ("Eigenvalue" > 1) (Kaiser 1958), we obtained 6 significant components, which explained 71.74% of the total variation (Table 3). The first component, which accounted for 19.92% of the total variation, included fruit size [FS], lamella size [LAS] and density [DN]. The second component, which explained 12.99% of the total variation, was determined by lamella size [LAS], petal shape [PSH] and fruit shape [FSH] (Table 3). The percentage of variation explained and the contribution of each variable to the first 6 components is presented in Table 3. Recently, Ganopoulos et al. (2015) evaluated 146 sweet cherry cultivars using phenotypic descriptors and found similar significant components. Fruit shape was also included in the first component, which indicates that it is an important qualitative variable for sweet cherry germplasm characterization. A PCA scatter plot was constructed based on the first 2 components that explain about 33% of the total variation (Fig. 4).

Table 2 First 6 components from the PCA analysis of 22 quantitative traits in 30 sweet cherry hybrids

	F1	F2	F3	F4	F5	F6
YL	− 0.140	− 0.176	− 0.182	0.344	− 0.023	− 0.739
BL	0.631	− 0.280	0.223	− 0.493	0.309	− 0.291
BW	0.682	0.251	0.191	− 0.458	− 0.102	− 0.226
rBL/BW	− 0.212	− 0.611	− 0.015	0.061	0.431	− 0.023
PtL	0.171	0.649	− 0.162	− 0.475	− 0.334	− 0.062
PT	0.706	0.031	0.267	− 0.509	0.243	− 0.095
rBL/PL	− 0.342	0.676	− 0.286	0.015	− 0.508	0.170
NN	0.182	0.116	0.468	− 0.261	0.307	0.562
FL	0.894	− 0.266	− 0.138	0.112	− 0.094	0.078
FWi	0.931	− 0.099	− 0.105	0.151	− 0.180	− 0.001
FT	0.935	− 0.124	− 0.121	0.124	− 0.151	0.069
FW	0.918	− 0.237	− 0.153	0.157	− 0.164	0.082
SS	0.214	0.285	0.562	− 0.015	− 0.285	− 0.198
TA	0.284	0.395	0.723	0.433	− 0.028	− 0.134
rTA/SS	0.173	0.340	0.498	0.582	0.199	− 0.045
PdL	0.206	0.793	0.219	− 0.059	0.159	− 0.208
SL	0.651	− 0.036	− 0.406	− 0.030	0.201	− 0.225
SW	0.644	0.491	− 0.256	0.380	0.162	0.091
ST	0.806	0.185	− 0.047	0.265	0.299	0.231
SV	0.675	0.352	− 0.570	0.035	0.171	0.048
rWS/WF	− 0.335	0.637	− 0.470	− 0.129	0.397	− 0.099
rVS/VF	− 0.567	0.475	− 0.122	0.064	0.519	− 0.037
Eigenvalue	7.567	3.605	2.512	1.971	1.666	1.287
Variability (%)	34.397	16.388	11.419	8.959	7.572	5.849
Cumulative %	34.397	50.785	62.204	71.164	78.736	84.585

Trait abbreviations: *YL* yield, *BL* blade length, *BW* blade width, *rBL/BW* ratio blade length/blade width, *PtL* petiole length, *PT* petiole thickness, *rBL/PtL* ratio blade length/petiole length, *NN* leaf: number of nectaries, *FL* fruit length, FWi fruit width, *FT* fruit thickness, *FW* fruit weight, *SS* soluble solids, *TA* titratable acidity, *rTA/SS* ratio titratable acidity/soluble solids, *PdL* pedicel length, *SL* stone length, *SW* Stone width, *ST* stone thickness, *SV* stone volume, *rWS/WF* ratio weight stone/weight fruit, *rVS/VF* ratio volume stone/volume fruit

Table 3 First 6 components from the PCA analysis of 12 qualitative traits in 30 sweet cherry hybrids

	F1	F2	F3	F4	F5	F6
TV	− 0.136	− 0.210	0.436	0.499	0.000	0.000
TB	0.129	0.860	− 0.218	0.001	0.000	0.000
DN	− 0.523	0.290	− 0.139	0.282	0.000	0.000
LASH	0.334	0.166	− 0.064	0.660	0.000	0.000
LAS	0.603	− 0.498	− 0.020	0.354	0.000	0.000
PSH	− 0.147	0.414	0.650	− 0.183	0.000	0.000
FSH	− 0.301	− 0.414	− 0.490	− 0.372	0.000	0.000
FS	0.851	0.052	0.048	− 0.232	0.000	0.000
FSC	0.000	0.000	0.000	0.000	0.330	0.944
FF	− 0.374	0.111	− 0.626	0.318	0.000	0.000
SSH	0.000	0.000	0.000	0.000	0.944	− 0.330
STS	0.793	0.240	− 0.351	− 0.052	0.000	0.000
Eigenvalue	2.390	1.559	1.441	1.219	1.000	1.000
Variability (%)	19.920	12.995	12.007	10.156	8.333	8.333
Cumulative %	19.920	32.915	44.922	55.078	63.411	71.745

Trait abbreviations: *TV* tree vigour, *TB* tree branching, *DN* density, *LASH* lamella shape, *LAS* lamella size, *PSH* petal shape, *FSH* fruit shape, *FS* fruit size, *FSC* fruit skin colour, *FF* firmness of flesh, *SSH* stone shape, *STS* stone size

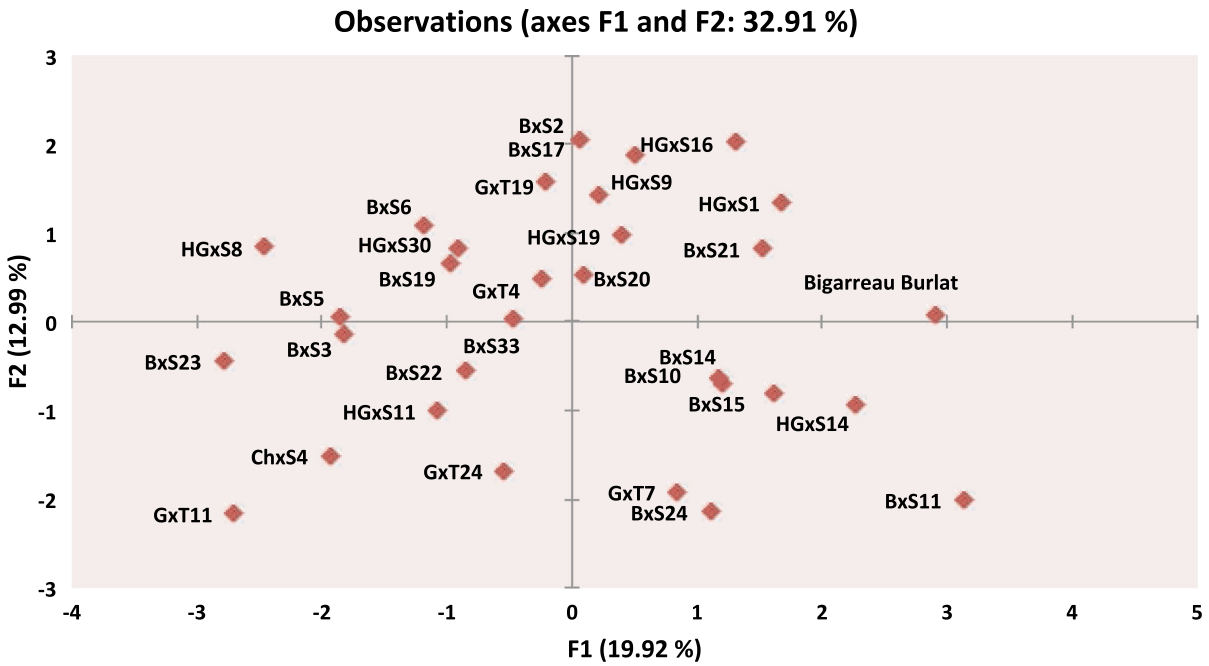

Fig. 4 Two-dimensional PCA plot of the 30 sweet cherry hybrids with regard to the first two principal components according to qualitative data analyzed. Variability explained: F1 (19.92%), F2 (12.99%)

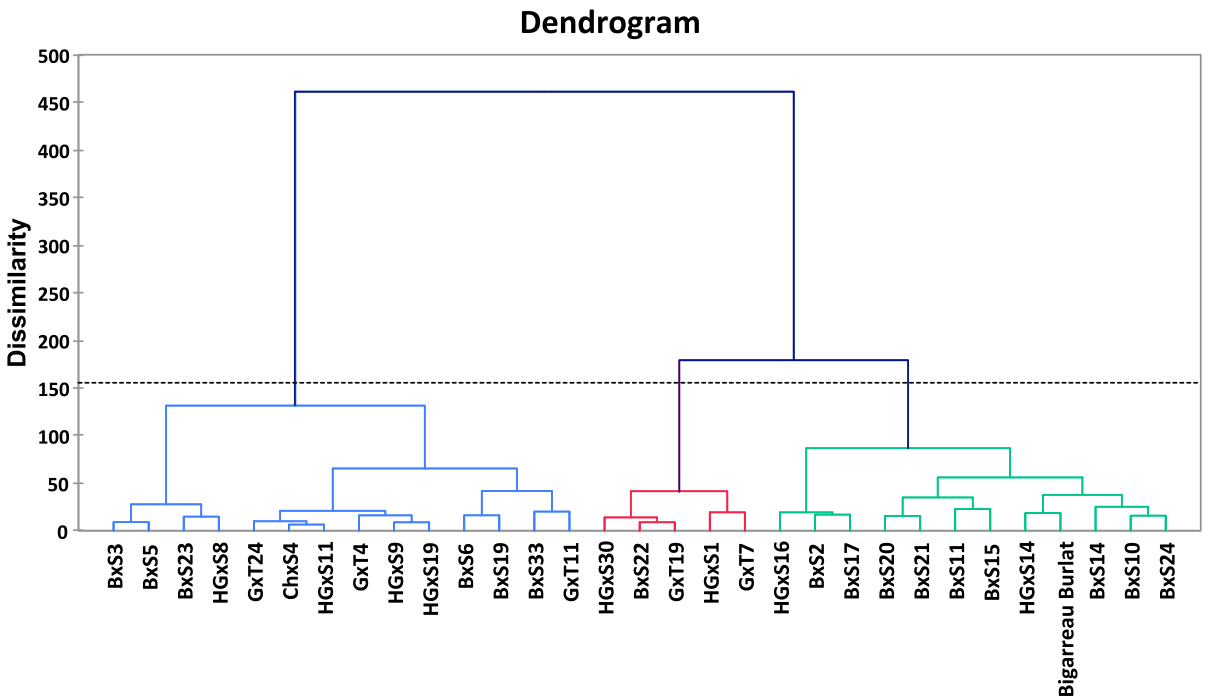

Fig. 5 Dendrogram using agglomerative hierarchical clustering (AHC) for 30 sweet cherry hybrids based on 22 quantitative and 12 qualitative traits

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 dissimilarity of the 30 sweet cherry hybrids, based on the combined quantitative and qualitative data, and the Ward's method was used for the agglomeration. The dendrogram (Fig. 5) pointed out the distinctness of the different hybrid genotypes, while several clusters are identified. The dendrogram revealed 3 distinctive groups. C1 contained 11 hybrids, C2 incorporated 14 hybrids, and finally C3 included 5 hybrids. The highest genetic distance exists between C2 and C3 (8.60), followed by C1 and C2 (8.31) and C1 and C3 (7.10). The Ward dendrogram and the scatter plot confirmed the variation between the sweet cherry hybrids. The inconsistency observed here between cluster and scatter plots may be attributed to the different levels of variation depicted by the various analyses. The cluster analysis was based on all morphological data and took into account the total variation observed, while the cumulative variance explained by the first two principal components, creating the scatter plot, was relatively low (50.79 and 32.91% for the quantitative and qualitative data, respectively).

Table 4 Cross-compatibility validation of sweet cherry hybrids analysed in this study using field and molecular approaches

Cultivar/hybrid	Field test	S4′ haplotype
Larian	✖	✖
Tsolakeiko	✖	✖
Tragana Edessis	✖	✖
B × S14	✔	✔
B × S23	✔	✔
HG × S30	✔	✖
HG × S11	✔	✔
B × S22	✔	✔
B × S33	✔	✔
B × S5	✔	✖
B × S19	✔	✔
B × S17	✔	✖

Cross-compatibility validation using field and molecular approaches

The self-compatibility test on the field has revealed that 7 B × S hybrids (B × S14, B × S23, B × S22, B × S33, B × S5, B × S19 and B × S17) and 2 HG × S hybrids (HG × S30 and HG × S11) were self-compatible or partly self-compatible. The field results have been compared by detecting the self-compatible *S4′* haplotype where only one HG × S hybrid (HG × S11) and 5 B × S hybrids (B × S14, B × S23, B × S22, B × S33, and B × S19) have been characterized as self-compatible (Table 4). Data from the morphological characterization of the 6 self-compatible hybrids identified show differences from cv. 'Stella', providing an alternative source for self-compatibility breeding in sweet cherry. The identification of these 6 self-compatible genotypes, in addition to the earlier described cultivar 'Stella', confirms the presence of a unique group of self-compatible plant material in Greek sweet cherry germplasm collection that requires conservation and further investigation in order to be used in breeding and research.

Conclusion

Fruit tree breeding such as cherry is a time-consuming job. In the hybridization program, results from 10 consecutive years showed the creation of new self-compatible sweet cherry hybrids, which were grouped in various clusters based on morpho-physiological traits of high economic importance. In addition, three hybrids displayed earliness in blooming time. Hence, the reported results could readily be employed in planning future sweet cherry breeding strategies.

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