

Multivariate assessment of cultivars' biodiversity among the Polish strawberry core collection

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Abstract

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The diversity of parental genotypes has a major impact on the progress of plant breeding. This study assessed phenotypic biodiversity of 91 strawberry genotypes in the core collection of the Research Institute of Horticulture in Skierniewice, Poland, using multivariate statistical methods. The assessment was carried out from 2008 through 2010 for 13 traits describing plant growth vigour, flowering and fruit ripening times, fruit yield, fruit appearance and quality (position of the calyx in relation to the surface of the fruit, fruit size and firmness, skin and flesh colour, fruit glossiness, intensity of the fruit's aroma and sweetness), as well as the genotypes' capacity for repeat-fruiting during the summer-autumn period. A principal component analysis using a hierarchical cluster analysis reduced the number of dimensions of more than a dozen traits describing the diversity of the assessed cultivars and identified six homogeneous groups, each consisting of cultivars with similar traits; this will simplify mate selection in the hybridization program and may reduce the size of the core collection.

Keywords: *Fragaria × ananassa*; cluster analysis; parental forms; principal component analysis (PCA); strawberry breeding

The diversity of parental genotypes is an important factor determining the effectiveness of the work in plant breeding. Therefore, parental forms that differ in terms of the value of phenotypic traits are normally selected for cross-breeding programs. Cultivars, selection clones and wild species can all be used. This makes it possible to obtain a biologically diverse population of F_1 hybrids, from which individuals with new traits desired by the breeder are selected. Using closely-related parental forms in the crossing combinations results in the narrowing of the gene pool in the population of hybrid progeny and carries the risk of producing the effect of inbreeding (KURAS et al. 2005).

In assessing the suitability of parental genotypes for cross-breeding of fruit plants, a number of traits are taken into consideration, depending on the purpose of breeding, including their productivity, fruit quality, resistance to biotic and abiotic factors, and sometimes also other functional traits such as plant growth habit (in the case of cultivars intended for combine harvesting of fruit), or thornlessness in gooseberry, raspberry or blackberry. Particularly valuable in breeding are those parental forms that are characterized by many positive traits, i.e. a complex of traits important for the breeding direction pursued. Determining the diversity and its range based on one trait is fairly easy

to do. More problematic is the determination of the similarity or diversity of genotypes when attempts are made to analyse many traits simultaneously. Very often, these traits are more or less correlated, which causes additional difficulties in determining similarity. The knowledge of the diversity of genotypes among the genetic resources available to the breeder enables appropriate selection of the right parental forms for cross-breeding programs and helps avoid crossing varieties that are genetically related and biologically similar. Valuable assistance is provided here by multivariate statistical methods, which make it possible to estimate the degree of diversity of potential parental forms in terms of many traits (PLUTA et al. 2012). By conducting a principal component analysis, we can assess the direction and strength of the relationships between the traits being considered. This method also allows us to reduce the number of the traits describing a given cultivar to a few principal components with which those traits are correlated. Making inferences on the basis of the principal components enables us to recreate the contributions of all the variables involved in the analysis, by using the description of the relationship with the components.

In the analysis of similarity and assessment of the biodiversity of parental genotypes within a genus/species, multivariate methods of classification are also very helpful. Such methods have already been used to evaluate the genetic resources of blackcurrant (MAĐRY et al. 2010; PLUTA et al. 2012). Being able to identify homogeneous groups of genotypes on the basis of many traits is particularly desirable for the determination of their suitability for cross-breeding programs and the direction in which they can be further used in creative breeding. From a practical point of view, cross-breeding should involve the use of parental forms belonging to different groups. This allows us to obtain biologically and genetically diverse hybrids of the F_1 generation and to select relatively easily many valuable genotypes among them.

The aim of this study was to evaluate the usefulness of multivariate statistical methods for determining the variation and multi-trait similarity of genotypes, and to characterize the relationships between the traits that describe the essential functional features of strawberry genotypes, such as plants appearance, flowering, fruit yield and quality, and also the capacity of plants for repeat-fruiting over the summer-autumn period. The analysis was also aimed at reducing many dimensions (13 traits) describing the strawberry cul-

tivars to the first few principal components. Although such simplification is associated with a loss of some of the information about the total variation, it allows us to obtain an easier way of drawing conclusions based on the first principal components. It is also possible to make a graphical representation of the similarity of the cultivars in a coordinate system with a reduced number of dimensions.

MATERIAL AND METHODS

Plant material and experimental design. The research material consisted of plants of 91 strawberry genotypes (cultivars) growing in the core collection of the Fruit Breeding Department of the Research Institute of Horticulture in Skierniewice, Poland. By the decision of the curators of the European database of strawberry genetic resources, these genotypes had been selected from the Polish collection of more than 300 genotypes on the basis of their suitability for creative breeding (on the account of unique values of some of their traits) and their popularity for commercial cultivation in the European Union countries. The genotypes varied in terms of many phenotypic traits and important functional features. Each genotype was represented by 10 plants (one replication), growing at a spacing of 0.25×1.1 m. All maintenance treatments (fertilization, mulching, removal of runners, weeding, and soil loosening) were performed in accordance with the recommendations for production plantations. Protection of the plants against diseases, pests and weeds was carried out on the basis of the guidelines of the existing Programme for the Protection of Fruit Plants. During dry periods, the plants were watered every 3–5 days by means of a self-propelled sprinkling machine (IRTEC 40FBT/130; IRTEC, Castelvetro di Modena, Italy).

Traits assessed. The assessment of the genotypes was conducted in 2008–2010. It consisted in analysing thirteen traits (flowering time, bearing type, calyx insertion in relation to the surface of the fruit, skin colour, fruit firmness, flesh color, ripening time, plant vigour, yielding capacity, fruit size, intensity of aroma, intensity of sweetness and fruit glossiness; Table 1) which described the selected genotypes in terms of their production value and consumption qualities of their fruit. Most of the traits were of a quantitative nature, or were given points on a rating scale representing the quantita-

tive direction of the variation in the quality traits in the direction desired by farmers, producers, and consumers of strawberries. Depending on the trait being assessed, a three- or five-point scale was used, established earlier for the needs of the EU GenBerry project – AGRI GEN RES 036 (European Commission 2013), co-financed by the European Commission, on the basis of the existing methodologies of the International Union for the Protection of New Varieties of Plants (UPOV 2008) and COST Action 836 (MASNY, ŻURAWICZ 2004).

Statistical methods. The basic analyses were performed with the mean values of the traits assessed, which had been calculated on the basis of the observations of each genotype over a period of three years (2008–2010). The variability of the test cases was analysed in terms of outliers, which were not found in the analysed set. The methodology for this type of analyses, which includes three main stages employing univariate and multivariate methods, has been used in the work of many authors concerning fruit plants, such as strawberry, blackcurrant or heather plants (LOPES et al. 2012; PLUTA et al. 2012; PADULA et al. 2013).

In order to determine the relationship between the traits, a correlation analysis was performed, which resulted in a matrix of their coefficients, helpful for interpretation. The main method, which helped to reduce the number of variables, was the principal component analysis (PCA). In the analysis, we used a Varimax rotation, which allowed us to minimize the components to those that had high factor loadings. In the PCA (MORRISON 2005), we obtained a matrix of rotatable components relative to the original variables that had eigenvalues greater than 1. Another matrix, which was used in subsequent analyses to construct two-dimensional graphs, was a matrix of the correlations of the first principal components with the cultivars under assessment.

To identify homogeneous groups (clusters), we performed a hierarchical cluster analysis according to the Ward's method (CROSSA, FRANCO 2004). In that analysis, we created a matrix of distances between the cultivars on the basis of the 13 traits assessed, using the squared Euclidean distance. In the final stage, we constructed a dendrogram showing the multi-trait similarity of the cultivars being evaluated. The significance of the differences between the identified groups was tested by the analysis of variance (ANOVA), separately for each variable, with the group number used as a factor. The analy-

ses were performed separately for each trait, and, in the next step, we used Student's *t*-test to perform detailed comparisons between the groups identified by the hierarchical cluster analysis.

In order to better illustrate the results obtained, we constructed two-dimensional graphs with three main items of information. On the *x* and *y* axes, we placed the appropriate first main components. The items of information presented in this system were the correlations of the original traits with the components (marked with '+' sign); we also placed all the tested genotypes, indicating to which homogeneous group they belonged, as identified by the hierarchical cluster analysis (the legend shows group numbers from 1 to 6, and their markers).

RESULTS AND DISCUSSION

Within the gene pool analysed in the study, we observed very high variability in the traits assessed. The genotypes that stood out due to the extreme values of the traits (min. or max.) in all the years of study are presented in Table 1.

On the basis of the mean phenotypic values of the traits for the three-year observations, we calculated phenotypic correlation coefficients between those traits (Table 2). The highest correlation coefficient was estimated for the traits: intensity of fruit aroma and intensity of sweetness, which was 0.84. Another pair of the most strongly correlated traits was fruit size and fruit glossiness, for which the correlation coefficient was 0.61. Strongly correlated were also the following pairs of traits: fruit firmness and glossiness (0.59), fruit firmness and size (0.58), flowering time and fruit ripening time (0.52), and skin colour and flesh colour (0.52). Earlier studies conducted with strawberry plants also indicated strong relationships between these pairs of traits (UKALSKA et al. 2006). The existence of a strong phenotypic correlation for the traits: flowering time and fruit ripening time in strawberry, based on an analysis of F_1 progeny obtained in diallel and hierarchical designs, was also confirmed by the results of GAWROŃSKI (2011), and GAWROŃSKI and HORTYŃSKI (2011). For most of the listed pairs of traits (fruit firmness and glossiness, fruit firmness and size, skin colour and flesh colour) highly significant, positive values of the phenotypic correlation coefficients coincide with the values of genetic correlation coefficients, previously estimated for these pairs of traits on the basis of the

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Table 1. Variation within the gene pool of strawberry core collection grown in Skierniewice, Poland based on the phenotypic assessment of traits during 2008–2010

Traits assessed	Variation observed within the gene pool			
	min. trait value	found in genotypes	max. trait value	found in genotypes
Flowering time	1	Diamante, Medina, Paros, Premial, Sweet Charlie, Georg Soltwedel, Jucunda, Pink Panda, Gariguet, Maya, Redgauntlet, Charlotte, Cirano, Gaviota, Mara des Bois, Parker, Ventana, Kama	9	Bogota, Pandora, Sophie
Bearing type	1	most of the genotypes, e.g. Marmolada, Dukat, Elkat, Chandler, Honeoye, Kent	5	Diamante, Pink Panda, Tango, Capitola, Aromas, Charlotte, Cijosse, Cirano, Gaviota, Irvine, Lidka, Mara des Bois
Calyx insertion	1	Real, Cigoulette, Emily, Gariguet, Marmolada, Onda, Polka, Redgauntlet, Aromas, Cirano, Lidka, Grenadier	3	numerous genotypes, e.g. Ventana, Gorella, Seneca, Talisman, Diamante
Skin colour	3	Tango, Patty, Miss, Magic, Diamante	9	Dir. Paul Wallbaum
Fruit firmness	1	Allstar, Precosa	9	Madeleine, Magic, Camarosa
Flesh colour	1	Allstar, Pink Panda, Precosa, Tango	9	Dir. Paul Wallbaum
Ripening time	1	Honeoye, Sweet Charlie, Annapolis, Gaviota	9	Pink Panda, Bogota, Pandora, Sophie
Plant growth vigour	3	Favette, Pink Panda, Precosa, Tango, Aromas, Parker, Roreal	7	Chandler, Elkat, Roxana, Havelland, Dir. Paul Wallbaum, Salut, Cilady, Magic, Madeleine, Daroyal, Gerida, Karmen
Yielding capacity	1	Pink Panda, Precosa, Cirano, Gaviota, Lambada, Lidka, Parker	9	Dukat, Elkat, Heros, Salut
Fruit size	1	Jucunda, Pink Panda, Precosa	9	Vikat
Aroma intensity	3	Maya, Mme Moutot, Totem, Bogota, Daroyal, Capitola, Annapolis	7	numerous genotypes, e.g. Bounty, Cireine, Feng Xiang, Korona, Sweet Charlie, Favette, Precosa
Sweetness intensity	3	Pink Panda, Anapolis, Camarosa, Daroyal, Mme Moutot	7	numerous genotypes, e.g. Bounty, Cireine, Diamante, Korona, Madeleine, Miss, Sweet Charlie, Precosa
Fruit glossiness	3	numerous genotypes, e.g. Allstar, Cambridge Fav., Favette, Melody, Pink Panda, Precosa, Mme Moutot, Totem	7	numerous genotypes, e.g. Cifrance, Ciloe, Diamante, Chandler, Feng Xiang, Korona, Madeleine, Paros

ranking scale: flowering time (1 – very early, 3 – early, 5 – medium early, 7 – late, 9 – very late), bearing type (1 – no capacity for repeat-fruiting, 3 – some repeat-fruiting, 5 – everbearing), calyx insertion (in relation to the surface of the fruit) (1 – sunken, 2 – level with the surface, 3 – raised above the surface), skin colour (1 – white, 3 – orange/pink, 5 – red, 7 – dark red, 9 – very dark), fruit firmness (1 – very soft, 3 – soft, 5 – medium firm, 7 – firm, 9 – very firm), flesh colour (1 – white, 3 – light red, 5 – intense red, 7 – dark red, 9 – very dark), ripening time (1 – very early, 3 – early, 5 – medium early, 7 – late, 9 – very late), plant vigour (3 – low, 5 – moderate, 7 – high), yielding capacity (1 – very low, 3 – low, 5 – moderate, 7 – high, 9 – very high), fruit size (1 – very small, 3 – small, 5 – medium, 7 – large, 9 – very large), intensity of aroma (1 – none, 3 – weak, 5 – moderate, 7 – strong), intensity of sweetness (3 – weak, 5 – moderate, 7 – strong), fruit glossiness (3 – weak, 5 – moderate, 7 – strong)

results of an assessment of F_1 progeny of 32 hybrid families obtained by hybridization in a diallel system of eight strawberry varieties characterized by repeat-fruiting (MASNY et al. 2010). By comparison, SHAW (1991) and HASING et al. (2012) demon-

strated in their studies that phenotypic and genetic correlations between skin colour and flesh colour of strawberry fruit (as measured by chromatography) were low or moderate, but below the level of significance. For the other pairs of traits, the correlation

Table 2. Correlation coefficients for the traits assessed

	Flowering time	Bearing type	Calyx insertion	Fruit skin colour	Fruit firmness	Flesh colour	Ripening time	Plant vigour	Yielding capacity	Fruit size	Fruit aroma	Sweetness	Fruit glossiness
Flowering time	1.00	–0.30	0.01	0.13	–0.04	0.05	0.52	0.17	0.29	0.15	0.08	0.06	–0.23
Bearing type	–0.30	1.00	–0.11	–0.21	0.16	–0.20	–0.05	–0.37	–0.32	–0.13	0.00	0.06	0.03
Calyx insertion	0.01	–0.11	1.00	–0.11	–0.11	0.10	–0.08	0.12	0.01	–0.05	0.34	0.29	–0.11
Fruit skin colour	0.13	–0.21	–0.11	1.00	–0.31	0.52	0.12	0.12	0.05	–0.29	0.03	–0.01	–0.34
Fruit firmness	–0.04	0.16	–0.11	–0.31	1.00	0.00	–0.09	–0.09	0.04	0.58	–0.17	–0.12	0.59
Flesh colour	0.05	–0.20	0.10	0.52	0.00	1.00	0.00	0.19	0.20	0.03	–0.05	0.06	–0.06
Ripening time	0.52	–0.05	–0.08	0.12	–0.09	0.00	1.00	0.10	0.24	0.04	0.05	–0.06	–0.32
Plant vigour	0.17	–0.37	0.12	0.12	–0.09	0.19	0.10	1.00	0.49	0.19	–0.05	–0.08	0.05
Yielding capacity	0.29	–0.32	0.01	0.05	0.04	0.20	0.24	0.49	1.00	0.42	–0.04	–0.08	0.17
Fruit size	0.15	–0.13	–0.05	–0.29	0.58	0.03	0.04	0.19	0.42	1.00	–0.18	–0.15	0.61
Fruit aroma	0.08	0.00	0.34	0.03	–0.17	–0.05	0.05	–0.05	–0.04	–0.18	1.00	0.84	–0.22
Sweetness	0.06	0.06	0.29	–0.01	–0.12	0.06	–0.06	–0.08	–0.08	–0.15	0.84	1.00	–0.16
Fruit glossiness	–0.23	0.03	–0.11	–0.34	0.59	–0.06	–0.32	0.05	0.17	0.61	–0.22	–0.16	1.00

numbers in bold indicate statistically significant values of the correlation coefficient at $\alpha = 0.05$

coefficient was less than 0.5, and for a large majority of them was below the level of significance. A small number of relationships between traits indicate a high potential for biodiversity because the traits with a very low correlation coefficient are inherited independently of each other, which promotes the creation of rich and genetically diverse progeny populations. According to MICHALIK (2009), selection in a population in which there is no genetic variation will be ineffective despite the observed phenotypic differences between the plants and in spite of choosing the most favourable of them. On the other hand, the knowledge of the existence of a strong correlation between the important functional traits allows us to simplify the selection process because by looking for high values of one of two strongly correlated traits, we can expect with a high degree of probability high values of the other trait.

By conducting a PCAs with the use of a Varimax rotation, we reduced the number of dimensions that describe the strawberry cultivars in the core collection, by separating out the first five principal components (with factor loadings above 1.0), which accounted for 73.7% of the total variation (Table 3). The individual components explained similar propor-

tions (a dozen percent or so); the largest difference, of more than 2% of the total variation, was between the first and second component. The strongest correlation with the first component (PC1), explaining 18.1% of the total variance, was shown by traits such as fruit firmness, fruit size, and fruit glossiness. This component can therefore be interpreted as describing mainly the external quality of the fruit, which consists of the traits that determine the attractiveness and commercial value of strawberries. At the same time, the fact that these traits share the same main component indicates that large, highly glossy strawberries will also be very firm. Between these traits, which were the most strongly correlated with the first component, there were also strong relationships defined by the phenotypic correlation coefficient with values above 0.5.

The second principal component (PC2) explained 15.7% of the total variation and was most strongly associated with traits such as fruit aroma and sweetness, and (to a lesser extent) the position of the calyx in relation to fruit surface. Thus, for the most part, this component describes the taste quality of the strawberry fruit. Also in the case of this component, the pairs of traits correlated with

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Table 3. Eigenvalues and proportion of the total variance in 91 strawberry cultivars, as explained by the first five principal components for the original 13 traits and the correlation coefficients between these traits and the first five PCs

	Principal component				
	PC1	PC2	PC3	PC4	PC5
Flowering time	0.005	0.079	0.239	0.808	0.039
Bearing type	0.083	0.008	−0.710	−0.083	−0.146
Calyx insertion	−0.120	0.557	0.349	−0.220	−0.144
Skin colour	−0.353	−0.076	0.066	0.113	0.814
Firmness	0.850	−0.073	−0.202	−0.009	−0.020
Flesh colour	0.089	0.058	0.201	−0.055	0.874
Ripening time	−0.106	−0.056	0.035	0.867	−0.008
Plant vigour	0.018	−0.033	0.803	0.037	0.060
Yielding	0.310	−0.019	0.661	0.313	0.100
Fruit size	0.840	−0.082	0.267	0.144	−0.080
Aroma	−0.118	0.916	−0.067	0.109	−0.006
Sweetness	−0.039	0.920	−0.137	0.033	0.084
Fruit glossiness	0.804	−0.129	0.070	−0.329	−0.122
Eigenvalue of correlation matrix	2.35	2.05	1.95	1.72	1.51
Explained proportion of total variance (%)	18.09	15.73	15.00	13.21	11.64
Cumulative proportion of total variance (%)	18.09	33.82	48.82	62.03	73.67

values in bold indicate correlation coefficients with the value equal to or greater than 0.5 in absolute value

it were characterized by relatively high values of the correlation coefficient.

Plant vigour and yielding were most strongly correlated with the third principal component. The strong correlation of both these traits with the same principal component indicates that strawberry plants with high growth vigour will also be characterized by a high yielding capacity, which is additionally confirmed by the value of the phenotypic correlation coefficient (0.49) for this pair of traits. A trait that was strongly negatively correlated with the same component was the bearing type, which means that vigorously growing and abundantly fruiting strawberry plants do not typically have the capacity for repeat-fruiting in the summer-autumn period. The estimated negative values of the phenotypic correlation coefficients for the two pairs of traits, i.e. bearing type and plant vigour, and bearing type and yielding capacity, were quite high and statistically significant (−0.37 and −0.32, respectively), and confirm the existence of relationships between these traits. The loadings of the other variables are shown in Table 3, in which the max. value of the coefficient

of correlation between the variable and the principal component is highlighted in bold.

The PCA allowed us to describe proportions of the total variation by means of two-dimensional graphs depicting the distribution of the most important functional traits as determined by their correlation with the principal components (Figs 1 and 2). Correlating the traits with the individual principal components enabled us to make multidimensional deductions on a simplified basis of one dimension of a principal component representing several traits (dimensions). Presentation of the tested genotypes on two-dimensional PCA graphs in the company of the original traits makes it easy to determine the multi-trait position of those genotypes. This kind of graphical representation makes it easy to identify genotypes with extreme values of traits so that they can be used in further breeding work. On the basis of Fig. 1, showing the first and second principal components, it can be concluded, for example, that in the group of genotypes that are valuable on account of the important functional traits they possess, such as large fruit size, high fruit firmness and

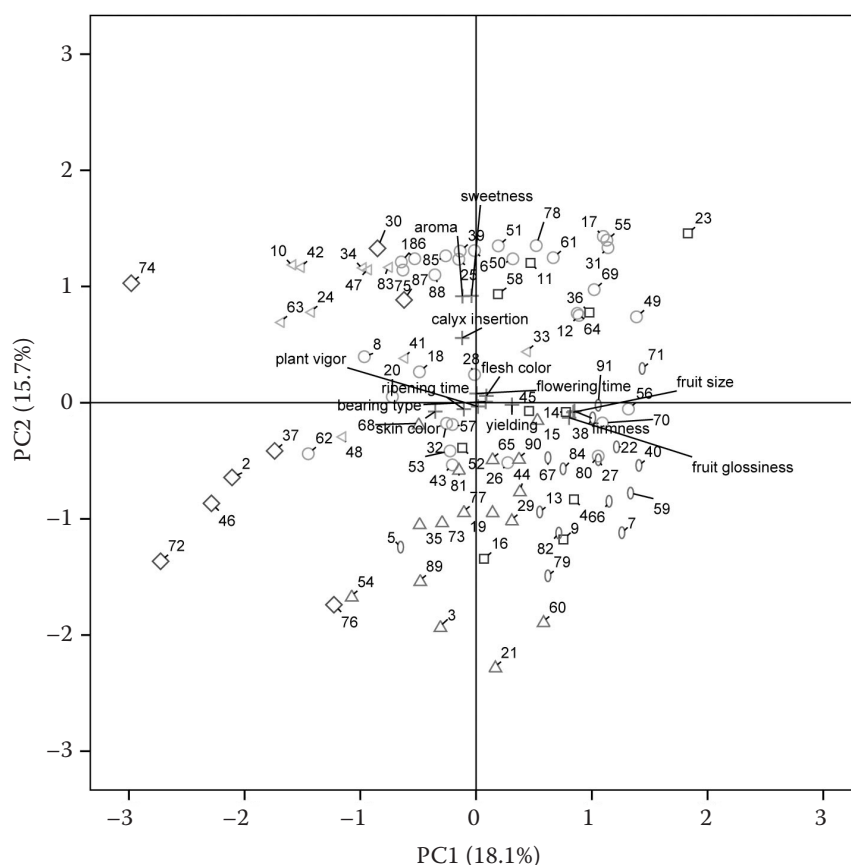


Fig. 1. Principal component analysis (PCA) for the first and second principal component for the traits assessed (marked "+"), together with the test objects divided into 6 groups using the Ward's method

Aga (1), Allstar (2), Annapolis (3), Aromas (4), Bogota (5), Bounty (6), Camarosa (7), Cambridge Fav. (8), Capitola (9), Catskill (10), Charlotte (11), Cifrance (12), Cigoulette (13), Cijossee (14), Ciloe (15), Cirano (16), Cireine (17), Coral (18), Cortina (19), Dana (20), Daroyal (21), Darselect (22), Diamante (23), Dir. Paul Wallbaum (24), Dukat (25), Elkat (26), Elsanta (27), Elvira (28), Emily (29), Favette (30), Feng Xiang (31), Filon (32), Florence (33), Fratina (34), Gariguette (35), Gaviota (36), Georg Soltwedel (37), Gerida (38), Gorella (39), Granda (40), Grenadier (41), Havelland (42), Heros (43), Honeoye (44), Irvine (45), Jucunda (46), Kama (47), Karmen (48), Kent (49), Korona (50), Lambada (51), Lidka (52), Luna (53), M-me Moutot (54), Madeleine (55), Magic (56), Majoral (57), Mara Des Bois (58), Marmolada (59), Maya (60), Medina (61), Melody (62), Mieke Schindler (63), Miss (64), Nadina (65), Onda (66), Pandora (67), Parker (68), Paros (69), Patty (70), Pegasus (71), Pink Panda (72), Polka (73), Precosa (74), Premial (75), Real (76), Redgauntlet (77), Roreal (78), Roxana (79), Salut (80), Seal (81), Seneca (82), S. Sengana (83), Sophie (84), Sweet Charlie (85), Talisman (86), Tango (87), Tenira (88), Totem (89), Ventana (90), Vikat (91)

high glossiness, and also strong aroma and sweet taste, there are Diamante, Cireine, Madeleine, Feng Xiang, Paros, Medina, Kent and Roreal. On the other hand, Pandora, Sophie, Florence, Vikat, Granda and Pegasus combine the traits of high-quality fruit (size, firmness, and glossiness) with late flowering and fruiting times (Fig. 2a, showing principal components 1 and 4). The genotypes with low values of these traits are Precosa, Jucunda and Georg Soltwedel (Fig. 2a). The genotypes Gaviota and Sweet Charlie are, apart from strong aroma and sweet taste of the fruit, characterized by early

flowering and fruit ripening times, whereas Favette, Roreal, Fratina, Dukat, Havelland, Tango and Dir. Paul Wallbaum combine high values of the traits of fruit taste and aroma with late flowering and fruit ripening times (Fig. 2b, showing principal components 2 and 4).

In the next stage of the study, we conducted a hierarchical cluster analysis using the Ward's method in order to assign the 91 genotypes to homogeneous groups (Fig. 3). The result was a dendrogram, which depicted multi-trait similarity of the test objects and hierarchical distances between the individual

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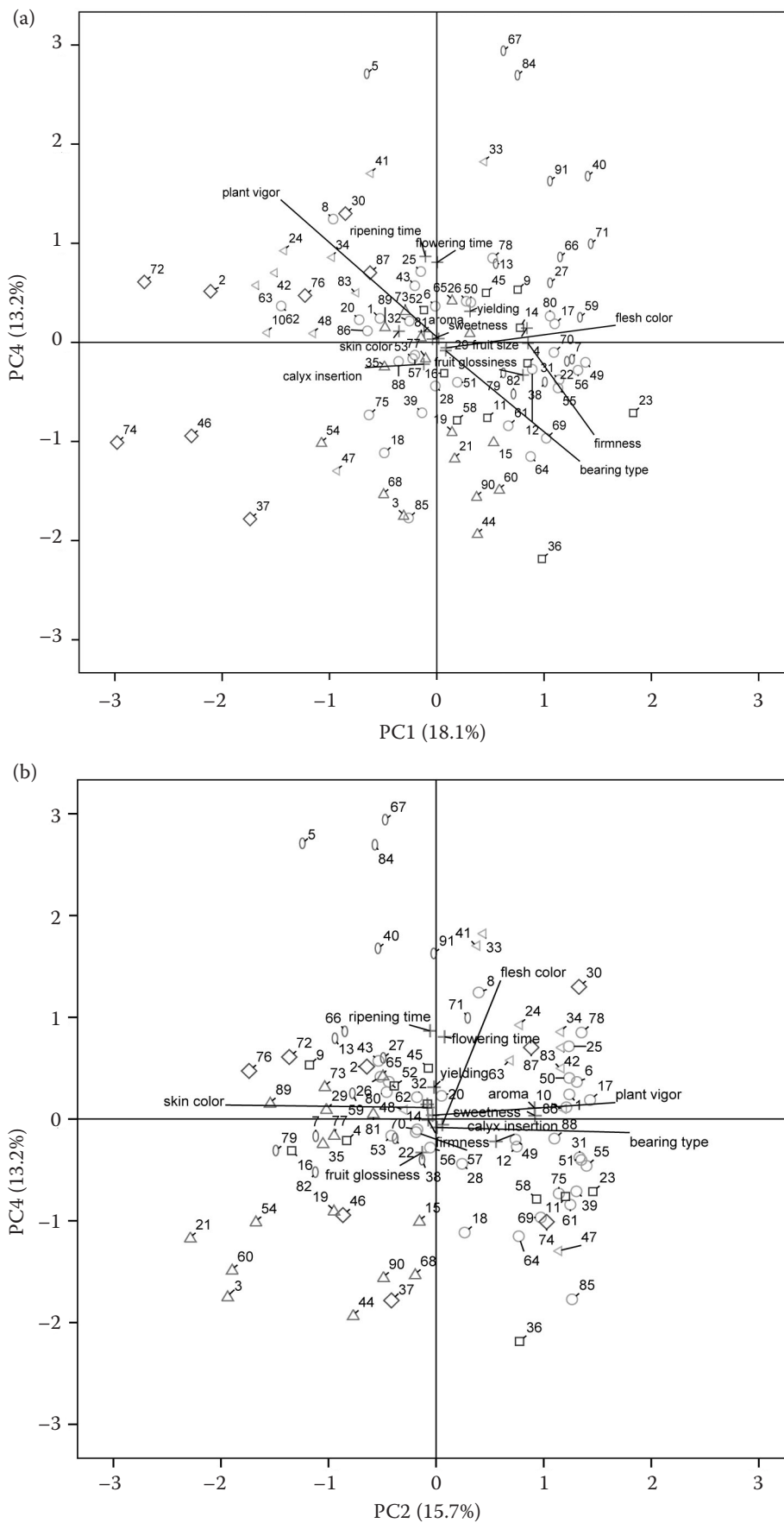


Fig. 2. Principal component analysis (PCA) for (a) the first and fourth and (b) second and fourth principal component for the traits assessed (marked "+"), together with the test objects divided into 6 groups using the Ward's method. Numerical designations of the objects are as in Fig. 1.

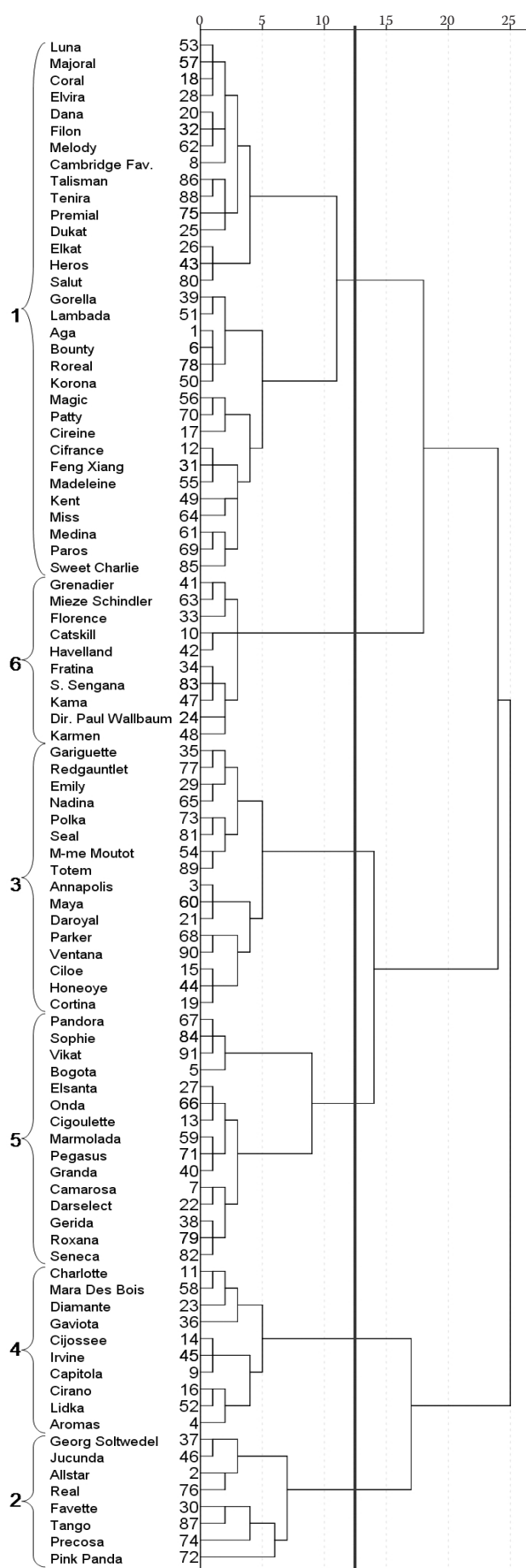


Fig. 3. Dendrogram showing the division of 91 strawberry genotypes into 6 groups using the Ward's method and the squared Euclidean distance. The genotypes within the individual groups show phenotypic similarity while genotypes belonging to different groups indicate significant biodiversity for a complex of the traits assessed

groups. The dendrogram was cut off at a level where there was a clear increase in the value of binding, which occurred at the rescaled distance of about 12, corresponding to 50% of the normalized distance. The division that is shown below identified six disjoint homogeneous groups characterized by the properties of the correlation of the objects with the principal components, and thus with the traits that are most strongly associated with them. The number in parenthesis after the name of the cultivar is its numerical designation used in the graphs.

Group 1: Aga (1), Bounty (6), Cambridge Fav. (8), Coral (18), Cifrance (12), Cireine (17), Dana (20), Dukat (25), Elkat (26), Elvira (28), Feng Xiang (31), Filon (32), Gorella (39), Heros (43), Kent (49), Korona (50), Lambada (51), Luna (53), Madeleine (55), Magic (56), Majoral (57), Medina (61), Melody (62), Miss (64), Paros (69), Patty (70), Premial (75), Roreal (78), Salut (80), Sweet Charlie (85), Talisman (86), Tenira (88).

Group 2: Allstar (2), Favette (30), Georg Soltwedel (37), Jucunda (46), Pink Panda (72), Precosa (74), Real (76), Tango (87).

Group 3: Annapolis (3), Ciloe (15), Cortina (19), Daroyal (21), Emily (29), Gariguet (35), Honeoye (44), Maya (60), M-me Moutot (54), Nadina (65), Parker (68), Polka (73), Redgauntlet (77), Seal (81), Totem (89), Ventana (90).

Group 4: Aromas (4), Capitola (9), Charlotte (11), Cijossee (14), Cirano (16), Diamante (23), Gaviota (36), Irvine (45), Lidka (52), Mara des Bois (58).

Group 5: Bogota (5), Camarosa (7), Cigoulette (13), Darselect (22), Elsanta (27), Gerida (38), Granda (40), Marmolada (59), Onda (66), Pandora (67), Pegasus (71), Roxana (79), Seneca (82), Sophie (84), Vikat (91).

Group 6: Catskill (10), Dir. Paul Wallbaum (24), Florence (33), Fratina (34), Grenadier (41), Havelland (42), Kama (47), Karmen (48), Mieke Schindler (63), S. Sengana (83).

By analysing the values of the phenotypic traits that describe the genotypes assigned to the different homogeneous groups, we can make general characterizations of the groups (Table 4). In Group 1 predominate genotypes that are characterized by the

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Table 4. Mean values of the traits for each group isolated by the hierarchical cluster analysis, with the min. and max. mean values (in bold) from the group for a given trait, and division into groups within a single trait produced by the analysis of variance (ANOVA)

Trait	Group						Overall mean	Min.	Max.
	1 (<i>n</i> = 32)	2 (<i>n</i> = 8)	3 (<i>n</i> = 16)	4 (<i>n</i> = 10)	5 (<i>n</i> = 15)	6 (<i>n</i> = 10)			
Flowering time	3.73 ^{ab}	3.50 ^{ab}	2.58^a	2.60 ^a	5.40^b	5.07 ^b	3.81	2.58	5.40
Bearing type	1.06 ^a	2.00 ^b	1.08 ^a	5.00^c	1.00^a	1.00^a	1.56	1.00	5.00
Calyx insertion	2.86^b	2.42 ^{ab}	2.00^a	2.17 ^{ab}	2.00^a	2.50 ^{ab}	2.41	2.00	2.86
Skin colour	4.79 ^{ab}	4.42^a	5.46 ^b	4.73 ^{ab}	4.96 ^{ab}	6.67^c	5.10	4.42	6.67
Firmness	5.60 ^b	3.08^a	5.83 ^b	6.73^b	6.56 ^b	3.80 ^a	5.51	3.08	6.73
Flesh colour	4.58 ^b	2.00^a	5.38 ^{bc}	4.47 ^b	4.56 ^b	6.53^c	4.69	2.00	6.53
Ripening time	4.48 ^{abc}	4.83 ^{abc}	3.46^a	3.87 ^{ab}	5.84^c	5.60 ^{bc}	4.61	3.46	5.84
Plant vigour	5.75^b	4.67 ^{ab}	4.71 ^{ab}	4.13^a	5.49 ^{ab}	5.60 ^b	5.23	4.13	5.75
Yielding capacity	5.42 ^b	2.75^a	3.75 ^{ab}	3.20 ^a	5.53^b	4.33 ^{ab}	4.55	2.75	5.53
Fruit size	5.42 ^b	2.75^a	5.00 ^b	5.00 ^b	6.87^c	3.73 ^a	5.12	2.75	6.87
Aroma	6.15 ^{cd}	5.50 ^{bc}	4.04^a	5.27 ^{bc}	4.73 ^{ab}	6.73^d	5.45	4.04	6.73
Sweetness	6.19 ^{bc}	5.50 ^{ab}	4.46^a	5.93 ^{bc}	4.73 ^a	6.73^c	5.62	4.46	6.73
Fruit glossiness	5.56 ^b	3.67^a	5.50 ^b	6.00 ^b	6.38^b	3.67^a	5.36	3.67	6.38

^{a–d} indicate significant variation between the mean values for a given trait in the different groups of cultivars as determined by Student's *t*-test; the numbers in bold are the mean values for the group that take on the min. or max. value

strongest growth, abundant yielding, and quite large, firm fruit with an intense red colour of the skin and flesh, and with the calyx raised above the surface of the fruit. The genotypes classified into Group 2 are those that are not very productive, with the smallest, least firm and most matte (without gloss) fruit, with the lightest skin and flesh. The genotypes in Group 3 are characterized by the earliest flowering and fruit ripening times, medium large, firm, but the least aromatic and least sweet fruit with intensely red skin and moderately high gloss, and the calyx deeply sunken into the fruit. In Group 4 there are genotypes with the capacity for repeat-fruiting in the summer-autumn period, characterized by the lowest plant vigour and low productivity, and their fruits are of medium size, with fairly light skin and flesh, but exceptionally firm. Group 5 represents mainly the traditional cultivars with late flowering and fruit ripening times, characterized by the highest yielding capacity and the largest, highly glossy fruit with a deeply sunken calyx. The genotypes classified into Group 6 are the traditionally maturing cultivars with the darkest (skin and flesh), matte (without gloss) and soft berries, but ones that are the sweetest and most aromatic.

CONCLUSION

Division of the available gene pool into homogeneous groups, which bring together genotypes with multi-trait similarity, is of great importance in breeding work. It facilitates selection of parental forms for cross-breeding programs in terms of many traits studied simultaneously. This allows the breeder, in the preliminary stage of developing the program of crosses, to select an isolated group containing genotypes with similar values of the traits, corresponding to the direction of the breeding work, and, in a later stage, to select from the genotypes classified into that group those that are the most appropriate for this purpose. The application of PCA and hierarchical cluster analysis made it possible to show in a complementary way the variation and multi-trait similarity of the genotypes gathered in the strawberry core collection. Each of the five first principal components explained from 12 to 18% of the total variation, accounting jointly for 74% of that variation. The hierarchical cluster analysis with the Ward's method allowed us to divide the analysed gene pool into homogeneous groups containing

genotypes with multi-trait similarity; as a result, we obtained 6 groups containing from 7 to 29 objects. The correlation analysis, on the other hand, allowed us to determine the relationships between the traits and their strength. The strongest positive correlation was found between the following traits: fruit aroma – fruit sweetness, fruit size – fruit glossiness, fruit firmness – fruit glossiness, fruit firmness – fruit size, flowering time – fruit ripening time, and fruit skin colour – fruit flesh colour.

References

- Crossa J., Franco J. (2004): Statistical methods for classifying genotypes. *Euphytica*, 153: 19–37.
- European Commission (2013): Preserving genetic resources in agriculture. Achievements of the 17 projects of the Community Programme 2006–2011: 96–101. Available at http://ec.europa.eu/agriculture/genetic-resources/publications/brochure-2013_en.pdf (accessed March 31, 2014).
- Gawroński J. (2011): Evaluation of the genetic control, heritability and correlations of some quantitative characters in strawberry (*Fragaria × ananassa* Duch). *Acta Scientiarum Polonorum Hortorum Cultus*, 10: 71–76.
- Gawroński J., Hortyński J. (2011): Hierarchic crossing design in estimation genetic control quantitative traits of strawberry (*Fragaria × ananassa* Duch.). *Acta Scientiarum Polonorum Hortorum Cultus*, 10: 77–82.
- Hasing T., Osorio L.F., Whitaker V.M. (2012): Estimation of genetic parameters and gains for color traits of strawberry. *Euphytica*, 186: 303–311.
- Kuras A., Korbin M., Danek J. (2005): Zróżnicowanie genetyczne wybranych odmian i klonów hodowlanych z rodzaju *Rubus* przy użyciu metod RAPD i ISSR. In: Michalik B., Żurawicz E. (eds): Zmienność genetyczna i jej wykorzystanie w hodowli roślin ogrodnich. Skierniewice, Instytut Sadownictwa i Kwiaciarnictwa: 377–381.
- Lopes L., Sa O., Pereira J.A., Baptista P. (2012): Genetic diversity of Portuguese *Arbutus unedo* L. populations using leaf traits and molecular markers: An approach for conservation purposes. *Scientia Horticulturae*, 142: 57–67.
- Masny A., Żurawicz E. (2004): Field performance of selected strawberry genotypes collected at the Research Institute of Pomology and Floriculture (RIPF), Skierniewice, Poland. *Acta Horticulturae* (ISHS), 649: 147–150.
- Masny A., Sieczko L., Żurawicz E., Mądry W. (2010): Zmienność i współzależność cech ilościowych u rodzin mieszańcowych truskawki powtarzającej owocowanie. Część I: Związki pomiędzy cechami. *Zeszyty Problemowe Postępów Nauk Rolniczych*, 555: 551–560.
- Mądry W., Pluta S., Sieczko L., Studnicki M. (2010): Phenotypic diversity in a sample of blackcurrant (*Ribes nigrum* L.) cultivars maintained in the Fruit Breeding Department at the Research Institute of Pomology and Floriculture in Skierniewice, Poland. *Journal of Fruit and Ornamental Plant Research*, 18: 23–37.
- Michalik B. (2009): Hodowla roślin. Poznań, Państwowe Wydawnictwo Rolnicze i Leśne.
- Morrison D.F. (2005): *Multivariate Statistical Methods*. Duxbury Advanced Series. Belmont, Duxbury, London, Thomson Learning.
- Padula M.C., Lepore L., Milella L., Ovesna J., Malafronte N., Martelli G., Tommasi N. (2013): Cultivar based selection and genetic analysis of strawberry fruits with high levels of health promoting compounds. *Food Chemistry*, 140: 639–646.
- Pluta S., Mądry W., Sieczko L. (2012): Phenotypic diversity for agronomic traits in a collection of blackcurrant (*Ribes nigrum* L.) cultivars evaluated in Poland. *Scientia Horticulturae*, 145: 136–144.
- Shaw D.V. (1991): Genetic variation for objective and subjective measures of fresh fruit color in strawberries. *Journal of the American Society for Horticultural Science*, 116: 894–898.
- Ukalska J., Mądry W., Ukalski K., Masny A., Żurawicz E. (2006): Patterns of variation and correlation among traits in a strawberry germplasm collection (*Fragaria × ananassa* Duch.). *Journal of Fruit and Ornamental Plant Research*, 14: 5–22.
- UPOV (2008): International Union for the Protection of New Varieties of Plants. Strawberry (*Fragaria* L.) Guidelines for Distinctness, Uniformity and Stability, TG/22/10 09-04-2008. Available at www.upov.int/en/publications/tg-rom/tg022/tg_22_10.pdf (accessed May 17, 2008).

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