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PRE-BREEDING STUDY FOR THE ENHANCEMENT OF BIOACTIVE FRUIT ATTRIBUTES IN STRAWBERRY (*Fragaria* × *ananassa* Duch.)

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ABSTRACT

The strawberry fruit market increasingly demands new cultivars producing berries with enhanced bioactive attributes. In this research the suitability of twelve strawberry cultivars for effective breeding aimed at the enhancement of bioactive fruit attributes was studied. The group of genotypes with different pedigree was examined in respect of variation, breeding parameters, correlation between the content of bioactive compounds and the antioxidant capacity of fruit. Pre-breeding research showed the highly significant differences between genotypes regarding all traits analysed. Relationship between the bioactive phytochemicals content and antioxidant capacity of fruit were mostly positive and significant. Only correlation between vitamin C and flavonoids was negative (−0.482). Path analysis exhibited the highest positive direct effect of total phenolic content on antioxidant capacity (0.609). Heritability of traits was very high, reaching values above 0.90. The highest genetic advance was observed for flavonoids. Results suggested that the genotype plays the main role in shaping of fruit antioxidant potential. This study indicated that the efficient strawberry breeding focused on obtaining the forms with enhanced bioactive berry properties could be highly possible.

Key words: antiradical capacity, polyphenols, vitamin C, polygenic traits, breeding parameters, correlation

INTRODUCTION

Berries, fruits especially members of Rosaceae families (strawberry, raspberry, blackberry), belong to the best dietary sources of bioactive compounds. To the bioactive compounds in berries belong antioxidants such as polyphenols or vitamins. Bioactive phytochemicals, either individually or combined, are responsible for various health benefits of berries, such as prevention of inflammation disorders, cardiovascular diseases, or protective effects to lower the risk of various cancers [Skrovankova et al. 2015, Afrin et al. 2016, Holt et al. 2020]. Concurrently with the increasing popularity with respect to nutrition and human health the interest in bioactive compounds has also increased among plant scientists [Perin et al. 2019, Nunes et al. 2020, Saridaş 2021]. Also, consumers are increasingly becoming aware of the potential benefits resulting from diets rich in fruits for maintaining a good health and preventing diseases. This has stimulated a growing demand for fruits with enhanced contents in bioactive compounds. It can be achieved by breeding based on genetic divergence of strawberry germplasm. However studies on the basis of genetic differentiation of bioactive compounds in strawberry are scanty. Breeding programs have mostly concen-

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trated on yield improvement, resistance to diseases, tolerance to abiotic stresses, longer shelf life, early or late production, adapted to the local conditions, and varietal diversification [Mezzetti et al. 2018, Zeist and Resende 2019]. Knowledge of gene action and breeding parameters value, correlation between bioactive phytochemicals in conjunction with their path effects helps in identifying suitable selection criteria for improving bioactive fruit attributes through breeding [Lal and Singh 2016, Pott et al. 2020]. In consequence, increasing bioactive phytochemicals content in berries may have an impact in other traits of interest, like tolerance to biotic and abiotic stress or flavor that should be taken into account in breeding new strawberry varieties [Kaushik et al. 2015, Sirijan et al. 2020]. Thus, this study was undertaken to assess the genetic discrepancy between strawberry genotypes for different bioactive compounds, their inter-relations and contribution to the total antioxidant potential of fruits. Results of the present pre-breeding research concerning the antioxidant capacity and content of bioactive phytochemicals in different strawberry genotypes may be useful for breeders, processors and general consumer of fruit.

MATERIAL AND METHODS

Plant material

The field experiment was set up in August 2016 at the Experimental Station, University of Life Sciences in Lublin (51°13'59" φN, 22°34'0" λE, elevation: 225.48 m) located in the south-east region of Poland. All tested plants cultivars: 'Bogota', 'Cabrillo', 'Filon', 'Kent', 'Korona', 'Merton Dawn', 'Pegasus', 'Selva', 'San Andreas', 'Teresa', 'Victoria' and breeding clone 74MS were planted in the field in three replications according to a randomized block experimental scheme. Twenty plants were planted in each replicate in spacing 80 × 30 cm in two rows, 10 plants per row. Typical agronomic procedures recommended for strawberry plantations were applied [Żurawicz et al. 2005]. In 2017 flowers were removed and the fruit samples were evaluated in the first year of full yielding *i.e.* in 2018. Samples of 500 g of full-ripened strawberry fruit of the cultivars tested were hand-harvested once in June for each repetition per cultivar. After harvest the fruit samples were delivered to the laboratory where they were manually washed and carefully sorted to remove any damaged ones. The level

Table 1. The origin of genotypes studied

Genotype	Pedigree	Type of bearing	Country of origin/date
74 MS	Advanced breeding clone (male sterile)	June-bearing	Poland/1988
Bogota	(Climax × Deutsch Evern) × (Tago 26.53.116 × Tago)	June-bearing	Netherlands/1978
Cabrillo	Advanced selections Cal.3.149-8 × Cal.5.206-5	Day-neutral	U.S./2015
Filon	Seal × Selva	June-bearing	Poland/2001
Kent	(Redgauntlet × Tioga) × Raritan	June-bearing	Canada/1981
Korona	Tamella × Induka	June-bearing	Netherlands/1978
Merton Dawn	Cambridge Favourite × Merton Princess	June-bearing	UK/1972
Pegasus	Redgauntlet × Gorella	June-bearing	UK/1990
San Andreas	Albion × Cal.97.86-1 (advanced selections)	Day-neutral	U.S./2008
Selva	CA.70.3-177(sister of Brighton) × CA71.98-605 (Tuftus × 63.7-10)	Day-neutral	U.S./1983
Teresa	Redgauntlet S_1 × Senga Sengana S_1	June-bearing	Poland/1998
Victoria	Local variety	June-bearing	Victoria's Yara Valley, Australia

of phytochemical compounds such as vitamin C, flavonoids expressed as quercetin and total phenolic expressed as gallic acid equivalent as well as antiradical capacity was measured in fruits extract. The research covered a genetically diversified genotypes − 11 cultivars and 1 breeding clone listed in Table 1. They were bred in various countries, during the implementation of breeding programs conducted in research units involved in the breeding of this species. They constitute a cross-section of genotypes obtained from the 70s of the twentieth century up to the present.

Analysis of vitamin C content

Fruit extract preparation for vitamin C content determination. From the homogenized fresh fruits of each cultivar was prepared a 5 g sample (in triplicate) which was mixed with 40 mL of 3% metaphosphoric acid. The mixtures were stirred for 30 min at room temperature. Solution was filtered through a filter paper (Whatman no. 42). The filtrate was diluted to 50 mL in volumetric flask with 3% metaphosphoric acid and the obtained extract was used for determination of vitamin C content.

Vitamin C content was determined with the spectrofluorometric method specified by Wu et al. [2003] with necessary modifications. 3% metaphosphoric acid, 7 M HCl, 0.1 M $Na_2S_2O_3$, and 0.005 M H_2SO_4 were used in the experiment. The oxidising solution was prepared by dissolving 1.3 g I_2 in 10 mL 40% KI and addition of 0.1 mL 7 M HCl and distilled water to a volume of 100 mL. The 0.1 M $Na_2S_2O_3$ solution was made by solving 1.25 g of the reagent and 0.01 g Na_2CO_3 in 50 mL of water. The derivatisation reagent was 10 mg OPDA (o-phenylenediamine) dissolved in 10 mL of 0.005 M H_2SO_4.

Fruit extract (2 mL, in duplicate) was added to 100 mL volumetric flasks. Next, 0.3 mL portions of a 0.005 M solution of iodine in potassium iodide were added, vortexed for 1 minute, and supplemented with 0.3 mL portions of 0.01 M $Na_2S_2O_3$. The pH of the samples was adjusted to approx. 6.0 by adding 0.3 mL of 2 M NaOH and the derivatisation was carried out by addition of 0.3 mL portions of the OPDA solution. The solutions were stirred for 30 minutes at the maximum stirred force. After that solution was diluted to 100 mL with distilled deionized water. The determinations were conducted on Cary Eclipse (Varian, Palo Alto, CA, USA) spectrofluorimeter at an excitation wavelength λ = 365 nm and an emission wavelength λ = 425 nm. The vitamin C content was calculated based on a standard curve obtaining using an aqueous solution of L-ascorbic acid standard in concentrations of 10, 20, 50 and 100 mg L^{-1}. The vitamin C content in the strawberry extract was expressed as mg L-ascorbic acid · 100 g^{-1} fresh sample.

Analysis of total phenolic and flavonoid content

Fruit extract preparation for total phenolic and flavonoid content as well as antioxidant capacity determination.

Before the experiment, whole fresh fruit samples for each cultivar were homogenised with a blender (PHILIPS). The appropriate fruit material (5 g, in triplicate) was extracted with 40 mL of 80% (v/v) methanol. The mixtures were stirred for 30 min (Multi-Rotator RS-60 (bioSan) at room temperature. Solution was filtered through a filter paper (Whatman no. 42) using a Büchner funnel. The filtrate was diluted to 50 mL in volumetric flask with 80% (v/v) methanol and the obtained extract was used for determination of total phenolic and flavonoids content as well as DPPH (2,2-diphenyl-1-picryldrazyl) assay.

Total phenolic content of fruit extracts was measured by Folin–Ciocalteu's phenol reagent [Kim et al. 2003]. First, 200 µl of appropriately diluted sample (in duplicate) was added to 2.6 ml of distilled deionized water. Then, 200 µl of 10% Folin–Ciocalteu's reagent (v/v) was added at time zero and mixed. After 6 min, 2 ml of 7% (w/v) Na_2CO_3 solution was added and mixed. Absorbance was measured after sample incubation for 90 min at room temperature. Absorbance was measured spectrophotometrically at a wavelength of λ = 750 nm on Cary 50 (Varian, Palo Alto, CA, USA) spectrophotometer versus a prepared blank sample. The blank consisted of 200 µl 50% (v/v) methanol instead of sample. Gallic acid in 50% (v/v) methanol solution at a range of concentrations (10–100 mg L^{-1}) was used as a standard and a calibration curve was drawn. The content of total phenolics was expressed as mg gallic acid equivalent (GAE) 100 g^{-1} of fresh weight.

Total flavonoids content of fruit extracts was determined using a spectrophotometric method [Floegel et al. 2011]. 500 µl of sample or standard (quercetin) (in duplicate) were mixed with 3.2 ml of distilled de-

ionized water. At time zero, 150 µl of 5% (w/v) sodium nitrite ($NaNO_2$) solution were added and mixed. After 5 min, 150 µl of 10% (w/v) aluminum chloride ($AlCl_3$) was added and mixed. After 6 min, 1 ml of 1 M sodium hydroxide (NaOH) was added and mixed. Absorbance of the colored flavonoid–aluminum complex was measured immediately at 510 nm on Cary 50 (Varian, Palo Alto, CA, USA) spectrophotometer versus a blank. The blank consisted of 500 µl of 50% (v/v) methanol instead of sample. For standard quercetin was dissolved in 50% (v/v) methanol to concentrations at a range 10–100 mg L^{-1} and a calibration curve was drawn. Total flavonoid content in the strawberry extract was expressed as mg quercetin equivalent (QE) 100 g^{-1} fresh sample.

Analysis of antioxidant capacity. Antioxidant capacity was determined by the DPPH (2,2-diphenyl-1-picryldrazyl) radical – scavenging method developed by Brand – Wiliams et al. [1995] slightly modified by Kim et al. [2002]. To 100 µl of the strawberry extract was added 3 ml of 0.1 mM DPPH. Samples were mixed and allowed to stand for 30 minutes in darkness at room temperature. After this time the absorbance was measured spectrophotometrically at a wavelength of λ = 517 nm on Cary 50 (Varian, Palo Alto, CA, USA) spectrophotometer. A control was prepared by diluting 100 µl methanol (analytical grade) in 3 ml of 0.1 mM DPPH. Fruit extracts were analysed in duplicates.

Antioxidant capacity was calculated according to the formula:

$$AA = (1 - (A_p / A_k)) \cdot 100\%$$

where: AA – antioxidant capacity, A_p – absorbance of the sample, A_k – absorbance of the control sample.

Antioxidant capacity was expressed as the percent of DPPH radical inhibition.

Data analysis. Results were statistically evaluated by analysis of variance (ANOVA), significance of differences between means was established by the Tukey test at $p \leq 0.05$ in Statistica 13.1 (2017), SAS Enterprise Guide 3.0 and Excel 3.0 statistical software. The coefficient of variation was calculated using the formula of Burton and DeVane [1953]. Breeding parameters like: heritability (H) and genetic advance (GA) were calculated according to Allard [1960] in an Excel spreadsheet. Heritability (H) is a parameter used for the evaluation of genetic determination of quantitatively inherited traits, like the content of bioactive fruit compounds and antioxidant capacity. High values of heritability indicate a greater possibility of genetic advance (GA) obtained with the selection. Genetic advance (GA) expected from the selection is a precise indicator of the improvement of features in genotypic value for the new breeding population compared with the base population under one cycle of selection at a given selection intensity. Genetic advance can be estimated in population by multiplication of the values of heritability (H), phenotypical standard deviation of traits (∂_p) in population and selection intensity coefficient (k), which values depends on the percent of selection intensity (in our research 20%, k = 1.4), so that GA = $k \cdot H \cdot \partial_p$. Relationships between fruit characteristics were estimating using Pearson's correlation coefficient (r). Analyses of correlation coefficient were computed using SAS System for Windows [SAS 1999]. Separating each total correlation coefficient into direct and indirect effects was achieved by path coefficient analysis as described by Wright [1921] and Dewey and Lu [1959]. Path coefficients were estimated on the basis of the relationships calculated in a correlated result-causation system [Kempthorne 1957], in which the vitamin C, flavonoids and total phenolic content were defined as the causation variables and the antioxidant capacity expressed as % of DPPH radical inhibition was defined as the result variables. Standard path coefficients, which are standardized partial regression coefficients, were obtained by solving a set of simultaneous equation using the "Doolitle Technique", as described by Graybill [1961].

RESULTS AND DISCUSSION

Analysis of variance for bioactive fruit attributes. Data from the analysis of variance (ANOVA) are presented in Table 2. The results indicated significant difference for all analysed features among genotypes studied. Moreover these analysis revealed the presence of genetic variability between genotypes used in the experiment. This is consistent with the reports on the significant differentiation of antioxidant capacity, the content of phenols, flavonoids and vitamin C between strawberry [Chel et al. 2007, Capocasa et al.

Table 2. Mean squares from analysis of variance (ANOVA) of studied characters in strawberry genotypes

Source of variation	df	Flavonoids[1]	Total phenolic content[2]	Vitamin C[3]	Antioxidant capacity[4]
Genotypes	11	39291.33**	18540.41**	622.96**	301.58**
Error	60	166.63	129.39	1.21	1.55

**significant at $p \leq 0.01$; [1] mg quercetin equivalent (QE) 100 g^{-1} FW; [2] mg gallic acid equivalent (GAE) 100 g^{-1} FW; [3] mg L-ascorbic acid 100 g^{-1} FW; [4] % of DPPH radical inhibition

Table 3. Mean performance of strawberry cultivars for characters studied

Genotype	Flavonoids[1]	Total phenolic content [2]	Vitamin C[3]	Antioxidant capacity[4]
74 MS	196.75^{f*}	202.26^{g}	71.16^{c}	78.18^{g}
Bogota	342.37^{e}	382.35^{a}	77.81^{b}	89.35^{ab}
Cabrillo	333.91^{e}	207.84^{g}	76.25^{b}	69.44^{i}
Filon	417.08^{c}	256.28^{f}	61.18^{e}	86.53^{cd}
Kent	328.99^{e}	347.06^{b}	66.34^{d}	83.99^{e}
Korona	338.94^{e}	292.09^{de}	55.98^{g}	81.13^{f}
Merton Dawn	394.30^{cd}	334.91^{b}	58.15^{f}	91.67^{a}
Pegasus	337.91^{e}	337.26^{b}	87.75^{a}	84.24^{de}
San Andreas	329.92^{e}	259.00^{f}	66.15^{d}	72.11^{h}
Selva	523.06^{a}	312.29^{cd}	51.50^{h}	88.11^{bc}
Teresa	380.30^{d}	288.80^{e}	71.51^{c}	90.50^{ab}
Victoria	462.52^{b}	325.60^{bc}	66.66^{d}	86.74^{c}

*means of the same column followed by the same letter do not differ significantly at 5% level of probability; [1] mg quercetin equivalent (QE) 100 g^{-1} FW; [2] mg gallic acid equivalent (GAE) 100 g^{-1} FW; [3] mg L-ascorbic acid·100 g^{-1} FW; [4] % of DPPH radical inhibition

2008, Singh et al. 2011]. Besides, due to the insignificant variance of error related to environmental factors, it can be stated that the environment does not affect the tested features in this study. This confirms the decisive role of genotype in shaping the bioactive fruit properties.

Mean performance of bioactive fruit traits. Mean values of the characters measured in 12 genotypes are given in Table 3. They allow to indicate those genotypes which due to the high level of analysed properties can be considered as very valuable. The highest antioxidant capacity was characterized by the cv. 'Merton Dawn', while the highest content of vitamin C was recorded for the cv. 'Pegasus'. However, the 'Bogota' cultivar should be considered the best, as it was the first in terms of phenolic content, the second in terms of vitamin C content and the third in terms of antioxidant capacity. The very high concentration of biologically active compounds in a cv. 'Bogota' indicates the value of breeding work. However, cv. 'Bogota' belongs to the group of june-bearing cultivars, and this mean that its fruits may be available on the market for a limited period of time. Therefore, it is desirable to obtain a high content of biologically active compounds in fruit day-neutral cultivars, which fruit supply period may be significantly longer. Among the examined in this work, day-neutral cvs.: 'Cabrillo', 'San Andreas' and 'Selva', only the last was characterized by a very high content of flavonoids and high antioxidant capacity. Nevertheless, cultivars 'Cabrillo' and 'San Andreas' are more popular among fruit producers due to the higher fruit attractiveness and yield

potential. Therefore, the availability of valuable fruit for consumers can be achieved as a result of breeding new cultivars in which nutraceutical fruit value will be combined with agronomic traits, resistance to diseases and pest and the entire set of other properties together determining the genotype value. However, this may appear as a time-consuming process due to the negative correlation between the size of the fruit and the antioxidant capacity which was observed by Capocasa et al. [2008]. Many authors have evaluated the chemical composition of strawberry fruit from the latest varieties to bred decades ago [Pincemail et al. 2012, Aaby et al. 2012, Lal et al. 2013, Ariza et al. 2015, Żebrowska et al. 2016, Kaczmarska et al. 2017, Capocasa et al. 2018]. The above-mentioned authors have analysed a total of over 100 genotypes, sometimes resulting in divergent results. As the authors conclude, they may result from the diversity of the examined genotypes, environmental conditions, the cultivation system, degree of maturity, harvest date and post-harvest storage.

Estimates of breeding parameters. In the group of antioxidant properties considered (Tab. 4), the coefficient of variation (CV) was the highest for flavonoids, while its lower level was observed successively for total phenolic, vitamin C content and antioxidant capacity. The heritability of the analysed features reached very high values. The highest value of heritability was observed for vitamin C content, the lowest for total phenolic. The values of genetic advance (GA) and genetic advance as percent of mean (GA%) were also differentiated. The highest values of GA were evaluated for flavonoids and total phenolic content, lower values were recorded for vitamin C and the lowest for antioxidant capacity. The lowest genetic advance as percent of mean was evaluated for antioxidant capacity, about twice higher was recorded for vitamin C, while the highest values were noticed for flavonoids and total phenolic content. Singh et al. [2011] showed that the amount of dietary antioxidants was significantly different among strawberry genotypes. In the case of ascorbic acid, the range was from 68.32 to 102.22 mg 100 g^{-1} FW. Thus, in this study the range of the analysed feature was slightly lower, while similar to the results obtained by Mishra et al. [2015]; in the range of 62.67–73.60 mg 100 g^{-1} FW. According to the above-mentioned authors, the estimates of the vitamin C heritability coefficient were at 87.87 and 76.70%, respectively, and were lower compared to the result obtained in this work. Selection can be more effective if as indicated by Singh et al. [2011] the heritability parameter and genetic gain as percentage of mean will be considered together than each of them separately. This is due to the fact that high values of GA are indicative for additive gene action whereas low values indicate prevalence non-additive gene action [Singh and Narayanan 1993]. In this study high heritability together with the highest GA% was observed for flavonoids content which may result from the additive gene action. This indicates the possibility of improving this property by hybridization and selection. Positive effects of selection towards a higher amount of various phytochemical compounds have been presented by Mezzetti et al. [2016]. In the case of antioxidant capacity, high heritability was associated with low GA%, therefore it should be considered as determined by non-additive gene action. With such a genetic control of traits, Singh et al. [2011] suggest the use of heterosis breeding. According to Kaczmars-

Table 4. Estimates of some breeding parameters for bioactive compounds traits in strawberry cultivars

Traits	Range	Mean ±SE	CV (%)	H	GA	GA% of mean
Flavonoids[1]	186.48–554.11	365.49 58.93	22.14	0.995	112.81	26.32
Total phenolic[2]	191.13–387.99	295.48 44.39	18.81	0.993	77.28	25.93
Vitamin C[3]	51.15–89.63	67.54 7.78	15.08	0.998	14.24	23.55
Antioxidant capacity[4]	65.70–91.81	83.50 5.55	8.49	0.994	9.87	11.07

CV − coefficient of variation, H – heritability, GA − genetic advance; [1] mg quercetin equivalent (QE) 100 g^{-1} FW; [2] mg gallic acid equivalent 100 g^{-1} FW; [3] mg L-ascorbic acid 100 g^{-1} FW; [4] % of DPPH radical inhibition

Table 5. Correlations matrix between antioxidants and antioxidant capacity in strawberry genotypes

Traits	Flavonoids[1]	Total phenolic[2]	Vitamin C[3]
Total phenolic content[2]	0.379*		
Vitamin C[3]	– 0.482*	0.031[ns]	
Antioxidant capacity[4]	0.493*	0.681*	– 0.206[ns]

*significant at $p \leq 0.05$; ns-not significant; [1] mg quercetin equivalent(QE) 100 g^{-1} FW; [2] mg gallic acid equivalent (GAE) 100 g^{-1} FW; [3] mg L-ascorbic acid 100 g^{-1} FW; [4] % of DPPH radical inhibition

Table 6. Correlation coefficients (r_{xy}) separated into direct (bold-on the diagonal) and indirect (unbold- above or below the diagonal) path effects of different bioactive compounds (X) on the antioxidant capacity of fruit (Y) in strawberry genotypes

	Path effects			r_{xy}
Traits (X)	Flavonoids (X_1)	Total phenolic content (X_2)	Vitamin C (X_3)	(Y)
X_1	**0.200**	0.231	0.062	0.493*
X_2	0.076	**0.609**	–0.004	0.681*
X_3	–0.097	0.019	**–0.128**	–0.206[ns]

*significant at $p \leq 0.05$, ns – not significant

ka et al. [2016, 2017] research, the effects of heterosis on the chemical composition of fruits were observed in the offspring of some inbred lines.

Relationship between fruit bioactive compounds and antioxidant capacity. Table 5 shows the values of correlations between fruit bioactive compound and antioxidant capacity. The highest significantly positive correlations was observed between antioxidant capacity and total phenolic content expressed as GAE. The significant high positive correlation was found between antioxidant capacity and flavonoids content (QE). Also between total phenolic and flavonoids content was observed a high significant positive correlation (0,379). In contrast, the highest significantly negative correlation was evaluated between vitamin C and flavonoids content. The influence of phenolic compounds on the oxidative activity expressed by the correlation coefficient was also reported by Cheel et al. [2007], Lal et al. [2013], Tulipani et al. [2008] in strawberry and by Moyer et al. [2002] in other berry species. Studies conducted by some authors indicate that ascorbic acid has a very important contribution to the antioxidant capacity [Aaby et al. 2012, Ariza et al. 2015]. But our results revealing the negative correlation between vitamin C and antioxidant capacity are consistent with the study conducted by Pincemail et al. [2012] and Lal et al. [2013].

Antioxidant effects on fruit antiradical capacity (path analysis). Values of path effect towards antiradical capacity in twelve strawberry genotypes are given in Table 6. These values were calculated on the basis of correlation coefficients (r_{xy}) between bioactive compounds content (causal variables *X*) and fruit antioxidant capacity (result variable *Y*). Separating each total correlation (r_{xy}) into direct and indirect effects (path coefficients) we measure the relative importance of the causal factors involved. Results of path analysis allowed to identified antioxidants that may be useful as selection criteria to improve fruit antioxidant capacity. Antioxidants showing the highest direct influence on the antioxidant capacity are good indicators in the selection aimed at increasing the fruit antioxidant potential.

The highest positive direct effect was exhibited *via* total phenolic (GAE) (0.609), followed by flavonoids (QE) (0.200). Vitamin C showed the negative direct effect on antiradical capacity (–0.128). The highest positive indirect influence of flavonoids on antiradical capacity was observed through total phenolic (0.231). Path coefficient analysis showed different contribution of bioactive compounds towards antiradical capacity in twelve strawberry cultivars. In general, total phenolic and flavonoid expressed as GAE and QE respectively, affected directly and positively the antiradical potential, but vitamin C showed the negative influence on this trait. In studies conducted by Lal and Singh [2016] vitamin C exhibited the low direct positive contribution towards free radicals scavenging (0.012), but among polyphenols only total phenolic expressed as GAE showed the highest positive effect on antiradical capacity with the value of 0.937. Differences in the antiradical capacity observed between various antioxidant in the present study and results obtained by others [Zeliou et al. 2002, Lal and Singh 2016] confirmed the statement that a genotype is an important factor in shaping antioxidant properties in strawberry. Besides, on the basis of our and previous investigations it can be stated that a total phenolic expressed as GAE plays the most important role in the antiradical capacity in many strawberry genotypes [Zeliou et al. 2002, Van de Velde et al. 2013]. Lal et al. [2013] postulated that phenols could be serving as a biochemical marker to identify productive genotypes with higher amounts of dietary antioxidants. This is also suggested by results of our pre-breading research because the positive direct effect on fruit antiradical capacity was the highest for total phenolic content which is also close to its correlation coefficient with the antioxidant capacity.

Phytochemical similarity of genotypes (cluster analysis). Figure 1 shows a cluster analysis based on the values of the bioactive compounds content and

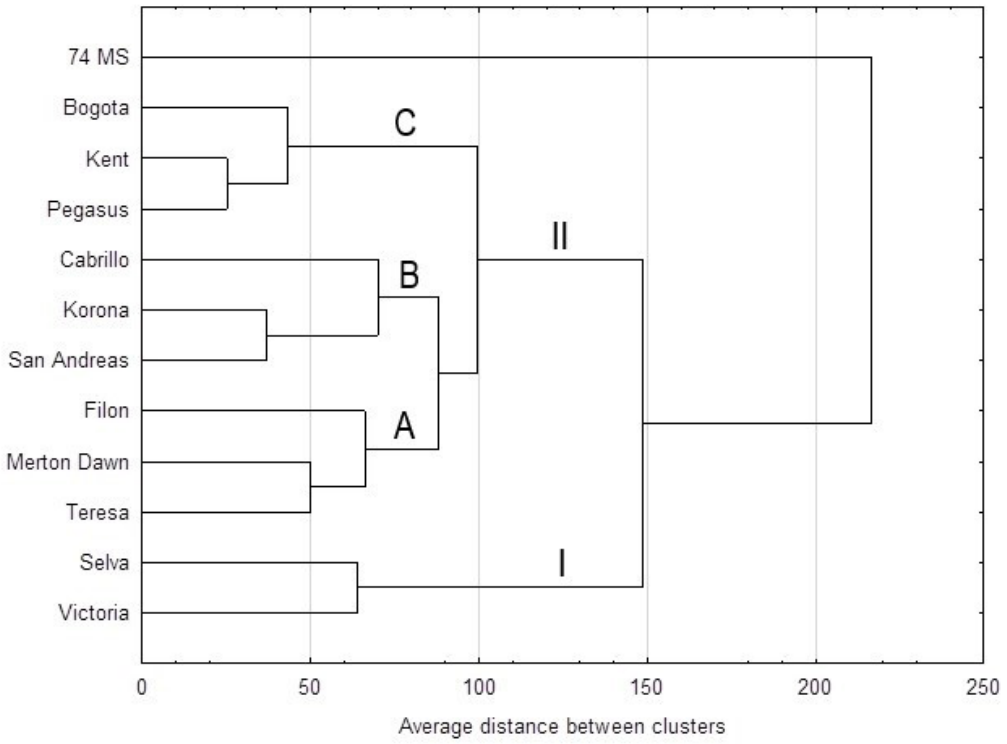

Fig. 1. UPGMA dendrogram of Euclidean distance revealing the relationship among the 12 strawberry genotypes based on analysed characteristics

antioxidant capacity of fruit in genotypes tested. This analysis presents separate homogeneous clusters of genotypes grouped on the basis of similarity in terms of the content of bioactive phytochemicals and antioxidant capacity of the fruit.

The cluster analysis grouped the 12 cultivars into two main clusters: I and II, while clone 74MS remained separate from both due to its significantly lowest content of flavonoids in fruits (Tab. 3). It is revealed by the longest distance of this clone from other genotypes in cluster analysis. The cultivars 'Selva' and 'Victoria' clustered into a separate clade I which represented similar potential for flavonoids, total phenolic content and antioxidant capacity. Cluster II consisted of 9 cultivars and could be divided into three subgroups: A, B and C, each consisted of three accessions. Subgroup A includes two cultivars of Polish origin and the English cultivar 'Merton Dawn'. In the B subgroup there were two day-neutral cultivars 'San Andreas' and 'Cabrillo' and 'Korona'. Within the C subgroup, the first cluster formed the 'Pegasus' and 'Kent' cultivars, to which the 'Bogota' cultivar later joined. The use of cluster analysis can be very useful in the case of the separation of distinct genotypes whose features we intend to combine in the process of hybridization. But it is not possible to directly compare the grouping of the analysed cultivars with the reports of other authors due to a different set of genotypes tested. Nevertheless, as emphasized by Lal et al. [2013] the use of cluster analysis reveals groups of cultivars with similar biochemical pathways. In addition, genotypes from distant clusters can be used to increase the content of specific bioactive compounds by hybridization and breeding.

CONCLUSIONS

Results of the present pre-breeding study exhibited the sufficient genetic gain estimated for total phenolic, flavonoids, vitamin C content and antioxidant capacity in twelve cultivars cultivated in a biennial production system. Hence the enhancement of antioxidant potential in strawberry fruits *via* a recombinant breeding is possible and can be highly effective. Total phenolic and flavonoid showed the higher antiradical capacity than vitamin C. Total phenolic content expressed as gallic acid equivalent (GAE) exhibited the highest capacity in free radicals scavenging. That's why this group of compounds can be regarded as an useful direct biochemical marker to identify strawberry genotypes with sufficient antiradical potential of fruits.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

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