

STUDIUL UNOR SELECȚII NOI OBȚINUTE ÎN PROGRAMUL DE AMELIORARE GENETICĂ A CĂPȘUNULUI

STUDY OF NEW STRAWBERRY SELECTIONS FROM THE ROMANIAN STRAWBERRY BREEDING PROGRAMME

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Abstract

Since 1995, the genetic improvement of strawberry (*Fragaria x ananassa* Duch.), in Romania, was developed mainly at RIFG Pitesti, within The Small Fruits Laboratory, which accumulated an experience in the field of more than 35 years (the first hybrids were obtained in 1978). As in the past, the classic selection scheme was followed. The choice of the parents was based on the productivity, quality and diseases tolerance of some Romanian and foreign varieties studied over a long period of time. The hybridizations were done in the field in individual bags and the selection of the obtained hybrids was carried out according to the known phases: the vegetative propagation of the selected plants, the organization of contest trial (linear) and contest microcultures (randomized) with number of plants and replicates specific to strawberry culture. The evaluated hybrids with a high percentage of selected plants in the hybrid fields were: `Queen Elisa x Mira`, `Queen Elisa x Marmolada x (Addie x Dana)`, `Queen Elisa x Real`, `Mira x Real` and `Mira x Queen Elisa`. The paper presents the behavior of eight strawberry selections and three parental varieties in field conditions during 2015-2017. The characteristics studied are: plant yield (g/plant), average fruit weight (g), firmness (N), shape, color, chemical content and tolerance to diseases. As a result of the researches carried out on `08-19-9` (`Queen Elisa x Real`), it was registered at ISTIS Bucharest in 2016 for promotion as a new variety in 2019.

Cuvinte cheie: *Fragaria x ananassa* Dutch., productivitate, caracteristici de calitate, toleranța la boli
Key words: *Fragaria x ananassa* Dutch., productivity, quality traits, diseases tolerance.

1. Introduction

The breeding program at Research Institute for Fruit Growing Pitesti (RIFG) develops strawberry cultivars (*Fragaria x ananassa* Dutch.) adapted for spring fruit production in Romania and follows the classical selection scheme of parental selection from germplasm, directed crosses, seedling screening, evaluation of new selections, and production of biological material for nurseries. The main breeding goals are combining high yield with fruit quality, stable fruit set and tolerance to biotic/abiotic stress. The RIFG has released 6 varieties since 1980: `Premial` (1989), `Coral` (1993), `Real` (1998) `Magic` (1998), `Floral` (2004), `Sarom` (2018), Branîște et al., 2007. In recently years, the most-used varieties were `Premial` and `Magic`, which occupied 70% of planted surface.

The varieties used in new strawberry fields must be well adapted to climatic and soil conditions specific to each growing area and to display a high resistance to pests and diseases (Barneche and Bonow, 2012; Sturzeanu and Temocico, 2018).

Therefore, the need to establish national programs aiming at strawberry variety improvement is evident. For plant assortment breeding, production assessment is fundamental, and fruit quality is essential to consumer acceptance of the product. The quality of strawberry fruit for fresh consumption is given by: taste, appearance, nutritional value, firmness and total yield and commercial production (Azodanlou et al., 2003; Diamanti et al., 2012; Temocico et al., 2017). Taste is one of the most important components of quality and is determined by the balance between sugar and acidity (Mezzetti, 2013; Reis et al., 2013). For varieties designed to processing the main evaluated traits are: dry mater content (° Brix), acidity, productivity and anthocyanin content (Schwieterman et al., 2014; Vieira et al., 2017).

Due to the strawberry octoploid nature and variability, in genetic improvement programs, selection based on one or more characteristics may have an unfavorable outcome due to negative genetic correlations between them (Hancock et al., 2008; Vieira et al., 2017). Therefore, it is necessary to use methodologies to allow selection based on a set of variables that includes different characteristics of economic interest (Cruz et al., 2014). The selection indicators, which were originally proposed by Smith (1936) and Hazel (1943), are the simultaneous choice of several characteristics, which increases the effectiveness of promising genotypes selection. In strawberry breeding programs using the clues of simultaneous selection for fruit quality (size, shape, taste, aroma, color) and production attributes (Vilarinho et al., 2003) is highly important. The objective of this study was to evaluate the relevant

attributes of fruits quality and production of eight elites and three parental varieties. Elites selected till now may be suitable for commercial cultivation and are used in current strawberry breeding activities.

2. Material and methods

The research was conducted in 2015-2017 period, in an experimental plot of the Research Institute for Fruit Growing Pitesti, in randomized blocks with three repetitions (ten plants per each replicate) and were studied the strawberry genotypes : `08-8-4`, `08-14-5`, `08-14-9`, `08-15-16`, `08-18-3`, `08-19-9`, `08-19-11`, `08-23-55`, `Mira`, `Queen Elisa`, `Real`. The indicators studied were recorded at the optimal time for harvesting the fruits, on a sample of 20 strawberry fruits. The plant yield was determined by weighing ripened fruits at each harvest and then summing all harvests. The average weight of the fruit was determined by weighing using the HL-400 digital balance. The length and diameter of the fruit were determined by measuring this using digital caliper. The size index was calculated by the formula: $H + D + d / 3$, where H = the height of the fruit, D, d = the large and the small diameters (Botu and Botu, 1997). The firmness of the fruit was determined for each sample with a Bareiss HPE II Fff penetrometer, a non-destructive test.

The external fruit color was determined with a colorimeter Konica Minolta CR 400, based on system Huntel L*, a*, b* on both sides of the fruit (L* corresponds to brightness, a* and b* chromaticity coordinates from green to red and from blue to yellow, respectively). Chroma index was determined by the formula $C = (a^{*2} + b^{*2})^{1/2}$ and hue angle by the formula $h^{\circ} = \arctangent(b^*/a^*)$, where 0°= red-purple, 90°= yellow, 180°= bluish-green and 270°=blue (McGuire, 1992). Low values of colour indicators L, a, b, h, C indicate the dark colour of the fruit (Zorrilla - Fontanesi et al., 2011).

The biochemical characteristics of the fruit were determined in a sample of approximately 200 g per repetition. The total dry mater was determined by the gravimetric method by measuring the water loss at 105 ° C heating. The content of organic acids, expressed as g/L total acidity, was analyzed by the titrimetric method using 0.1 N sodium hydroxide. The total sugars content (%) was determined using the Fehling-Soxlet method (1964). The Vitamin C content expressed in mg/100 g of fresh fruit was determined by the titrimetric method after the extraction with 2% hydrochloric acid. Determination of total anthocyanins was done by the spectrophotometric method (Fuleki and Francis, 1968).

Observations and assessments were done on the disease incidence (DISINC%) and the attack severity (DISSEV%) in the field, under natural conditions of infection for five diseases affecting frequently the strawberries: leaf spot (*Mycosphaerella fragariae*) and leaf scorch (*Diplocarpon earliana*), red stele (*Phytophthora fragariae*), anthracnose (*Colletotrichum acutatum*) and gray mold fruit rot (*Botrytis cinerea*). The damage degrees of the pathogens were calculated according to the formula: $DD\% = DISINC\% \times DISSEV\% / 100$. Evaluation of behavior in strawberry-specific diseases was performed upon a modified scale (from 0 = no symptom to 6 = very strong attack), Delhomez et al. (1995).

The statistical analysis of the data was performed using the SPSS 14.0 software and the Duncan comparison test was used to determine the difference between variants, with an error probability of ≤ 0.05 .

3. Results and discussions

From an agronomic point of view, a competitive variety of strawberries can be characterized by several important features: plant yield (over 600 g), medium or large fruit (over 20 g), uniform in shape and color, taste and aroma, highlights a consistency of complete and complex biochemical composition, good behavior during the transport and handling of fruits designed for fresh consumption (Temocico et al., 2017; Sturzeanu and Temocico, 2018).

In our study, the highest value of production/plant was recorded on elite `08-19-9` and statistically significantly differentiated from two of the three parental varieties (witness) and three of the eight elites studied (Table 1).

Average fruit weight, over 20 grams, was recorded at the most of the genotypes studied, except for the `Real` witness variety and two more elites from the `Mira` x `Real` combination (`08-15-16` and `08-23-55`). Elite `08-19-9` had the highest average fruit weight of 25.67 g (Table 1).

The values recorded for the fruits firmness were higher to the control varieties for all the studied elites, which is particularly important, because this character influences the resistance to the transport and handling of the fresh fruits.

By measuring the height and diameter of the fruits, the fruits size index (Table 2) was calculated to identify the genotype differences without error. Long fruits were recorded at `Mira` genotype (length 53.02 and 40.02 mm diameter), `08-8-4` (length 52.71 and 39.46 mm diameter), `08-19-9` (length 51.90 and 35.46 mm diameter) and `08-15-16` (length 50.00 and 35.33 mm diameter).

The fruit size index recorded values of more than 40 units in six of the eight elites studied, the elite `08-19-9` recorded the highest value (44.63) (Table 2).

Color is one of the most important characteristics that influences the choice of fruits by the consumers, the shine bright-red fruits being preferred (Tiwari et al., 2009). The fruit color, the color range CIE L* a* b* is a ladder currently used for evaluation (Sturzeanu et al., 2015; Sturzeanu et al., 2016), the differences between the graphs represented in the color space corresponds to the visual differences between the graphically represented colors. The CIE L* a* b* color space is organized as a cube, the L* axis represents brightness, the maximum value 100 is the white color, and the minimum value 0 is the black color. Axes a* and b* do not have specific numerical limits. Positive values to a* show red and negative green. The positive values for b* show the yellow color, and the negative ones the blue color. Low values of color indicators L*, a*, b*, h°, C* generally indicate the darker color of the fruits (Zorrilla-Fontanesi et al., 2011). Fruits color, the CIE L* a* b* color range recorded positive values for all genotypes of strawberry fruit (Table 3).

The results of the color parameters revealed statistically significant differences between genotypes.

L* (brightness) is the absolute attribute for color perception and refers to how an area appears to be bright; the mean values of this attribute were grouped into four homogeneous classes of statistical significance and varied between 26.11 (08-14-9) and 31.01 (08-18-3) (Table 3).

The total dry mater content of the studied genotypes ranged from 10.06 for the `Mira` variety to 10.87 for elite `08-23-55`, with no statistically assured differences (Table 4).

In seven of the eight elites studied, the total titratable acidity expressed as citric acid did not show statistical differences compared to the control varieties. Significant differences are between `08-14-5` elite and the other genotypes studied.

Organic acid content ranged from 0.60 on `Mira` and 0.96 on elite `08-14-5` (Table 4).

As regard the total sugar content, the highest sugar value was recorded on genotype `08-14-5` (9.82%), while the lowest total sugar content was specific to the genotype `08-8-4` with 4.31% sugar. Statistical differences are shown in Table 4.

Vitamin C analyzed on the 11 genotypes ranges from 71.25 mg/100 g on `Real` to 95.80 mg/100 g on the elite `08-19-9`. Significant differences were observed in case of elites `08-14-5` and `08-19-9` versus all other genotypes analyzed in Table 4.

The anthocyanins pigments, the substances that confer the red color of the fruits, ranged from 7.16 mg/100 g on elite `08-23-55` and 19.45 mg/100 g on `Queen Elisa` variety. Statistical differences are between `Queen Elisa` and all other genotypes presented in Table 4.

Determination in field conditions of the attack degree, revealed the genotypes behavior on the specific fungal pathogens attack (Table 5).

Regarding the behavior to the *Mycosphaerella fragariae* attack, `Queen Elisa` was the most attacked variety. The best behavior had the elites `08-19-9` and `08-14-9` (Table 5).

The evaluation of the response to *Diplocarpon earliana* attack showed that among the studied genotypes, the only one who showed symptoms was the elite `08-23-55` (Table 5).

Among the evaluated genotypes, the most sensitive to *Phytophthora fragariae* attack of the was `Mira` (Table 5).

The genotypes studied had good behavior on *Colletotrichum acutatum* attack, except the elite `08-19-11` (Table 5).

The fruits analysis highlighted that elites `08-19-9`, `08-8-4`, `08-14-9` and `08-18-3` showed tolerance to *Botrytis cinerea* attack (Table 5).

4. Conclusions

Elite `08-19-9` recorded the highest values in terms of production/plant and average fruit weight, while elite `08-8-4` recorded the highest value of fruit firmness.

As regard the biochemical quality of the fruits we can see that the elite `08-19-9` has the highest content of vitamin C, the elite `08-14-5` has the highest value content of sugar and organic acids, and the `Queen Elisa` variety is the richest in anthocyanins and also the most attractive colored.

Based on the data obtained from the eight evaluated elites, it was proposed to register at the ISTIS (State Institute for Testing and Registration of Varieties) of the elite `08-19-9`, and to be registered in 2019 as new variety in the Official Catalog of Varieties.

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References

1. Azondanlou R., Darbellay C., Luisier J., Villettaz J., Amado R., 2003. Quality assessment of strawberry (*Fragaria* species). *Journal of Agricultural and Food Chemistry*, vol.51: 715-721.
2. Barneche ACDO and Bonow S., 2012. Novos desafios para o melhoramento genético da cultura do morangueiro no Brasil. EPAMIG, Belo Horizonte.
3. Branîşte N., Budan S., Butac M., Militaru M., 2007. Soiuri de pomi, arbuşti fructiferi şi căpşuni create în România, Ed. Paralela 45.
4. Botu I., M. Botu, 1997. Metode şi tehnici de cercetare în pomicultură, Ed. Conphys, 240-257.
5. Cruz CD, Carneiro PCS and Regazzi AJ, 2014. Modelos biométricos aplicados ao melhoramento genético. 3rd ed. revista ampl. UFV, Viçosa.
6. Delhomez N., O. Carisse, M. Lareau, S. Khanizadeh, 1995. Susceptibility of strawberry cultivars and advanced selections to leaf spot caused by *Mycosphaerella fragariae* *HortScience* 30(3) 592-595. <http://hortsci.ashspublications.org/content/30/3/592.full.pdf>.
7. Diamanti J., Capocasa F., Balducci F., Battino M., Hancock M., Mezzetti B., 2012. Increasing strawberry fruit sensorial and nutritional quality using wild and cultivated germplasm. *PLoS ONE* 7(10): e46470. <https://doi.org/10.1371/journal.pone.0046470>.
8. Faedi W., Baruzzi G., Lovati F., Sbringhi P., Lucchi P., 2002. Monografia di cultivar di fragola. Ed. ALSIA;
9. Fuleki, T., Francis F. J. 1968. Extraction and determination of total anthocyanin in cranberries. *Journal of Food Science*, Chicago, v. 33, n. 1, p. 72-77.
10. Hancock J.F., Sjulín T.M., Lobos G.A., 2008. Strawberries, Temperate Fruit Crop Breeding, p. 393-437.
11. Hazel LN., 1943. The genetic basis for constructing selection indices. *Genetics* 28: 476-490.
12. Mezzetti B., 2013. Breeding and biotechnology for improving the nutritional quality of strawberry. *Journal of Berry Research*, Vol. 3: 127-133.
13. Reis RC, Viana ES, Jesus JL, Dantas JLL, et al., 2013. Caracterização físico-química de frutos de novos híbridos e linhagens de mamoeiro. *Pesq. Agr. Bras.* 50: 210-217. <http://dx.doi.org/10.1590/S0100-204X2015000300004>.
14. Smith HF., 1936. A discriminant function for plant selection. *Ann. Eugen.* 7: 240-250. <http://dx.doi.org/10.1111/j.1469-1809.1936.tb02143.x>.
15. Schwieterman M. L., Colquhoun T. A., Jaworski E. A., Bartoshuk L. M., Gilbert J. L., Tieman D. M., Odabasi A. Z., Moskowitz H. R., Folta K. M., Klee H.J., Sims C. A., Whitaker V. M., Clark D. G., 2014. Strawberry Flavor: Diverse chemical composition, a seasonal influence and effect on sensory Perception, <http://dx.doi.org/10.1371/journal.pone.0088446>.
16. Sturzeanu M., Ancu I., Chitu E., 2015. The evaluation of fruits quality in some strawberry cultivars (*Fragaria x ananassa* L. Duch.). *Fruit growing research* vol. XXXI, 2015, 23-29. ISSN 2286-0304, ISSN-L; 2286-0304 <http://publications.icdp.ro/publicatii/lucrari%202015/03.Monica%20Sturzeanu.pdf>.
17. Sturzeanu M., Ancu I., Chitu E., Stanciu C., 2016. Some fruit qualities characteristics of strawberry selection from Queen Elisa x Premial. *Fruit growing research* vol. XXXII, 2016, 44-47. ISSN 2286-0304, ISSN-L 2286-0304. <http://publications.icdp.ro/publicatii/lucrari%202016/07.Lucrari%20Sturzeanu%20Monica.pdf>
18. Sturzeanu M., Temocico G., 2018. Soiuri de căpşun cultivate în România. ISBN 978-606-764-038-0;
19. Temocico G., Sturzeanu M., Ion V., 2017. Choice of strawberry varieties by the Romanian growers. *Scientific Papers Series Management, Economic Engineering in Agriculture and Rural.*
20. Tiwari S.K., Venkatakishnan P., Gosain S., Joshi J., 2009. Effect of Polarimetric Noise on the Estimation of Twist and Magnetic Energy of Force-Free Fields, *ApJ*, 700, 199-208, doi:10.1088/0004-637X/700/1/199.
21. Vieira S.D., Souza D.C., Martins I.A., Ribeiro G.H.M.R., Resende L.V., Ferraz A.K.L., Galvão A.G., Resende J.T.V., 2017. Selection of experimental strawberry (*Fragaria x ananassa*) hybrids based on selection indices *Genet. Mol. Res.* 16 (1): gmr16019052 DOI <http://dx.doi.org/10.4238/gmr16019052>.
22. Vilarinho AA, Viana JMS, Santos JF and Câmara TMM, 2003. Eficiência da seleção de progênies S1 e S2 de milho-pipoca, visando à produção de linhagens. *Bragantia* 62: 9-17. <http://dx.doi.org/10.1590/S0006-87052003000100002>.
23. Zorilla-Fontanesi Y., Cabeza A., Domínguez P., Medina J. J., Valpuesta V., Denoyes-Rothan B., Sánchez Sevilla J. F., Amaya I., 2011. Quantitative trait loci and underlying candidate genes controlling agronomical and fruit quality traits in octoploid strawberry (*Fragaria x ananassa*) *Theoretical and Applied Genetics* 123, 755-778.

Tables

Table 1

Genotypes	Plant yield (g)	Average fruit weight (g)	Firmness (N)
Mira	638 a*	25,50 a	9,71 c
Queen Elisa	494 b	21,16 abc	10,92 c
Real	347 c	13,67 e	10,71 c
08-8-4	536 b	22,67 abc	34,38 a
08-14-5	555 b	21,17 bcd	29,64 ab
08-14-9	658 a	24,50 ab	25,19 b
08-15-16	620 a	19,83 cd	24,54 b
08-18-3	661 a	20,00 cd	29,33 ab
08-19-9	683 a	25,67 a	29,63 ab
08-19-11	556 b	20,83 bcd	26,72 b
08-23-55	623 a	17,00 de	29,37 ab

* The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($P \leq 0.05$)

Table 2

Genotypes	Diameter (mm)	Length (mm)	Size index
Mira	40,02 abc*	53,02 a	44,36 ab
Queen Elisa	38,53 abc	40,51 c	39,19 de
Real	38,09 abc	34,28 d	36,83 e
08-8-4	39,46 abc	52,71 ab	43,88 abc
08-14-5	37,19 abc	48,46 ab	40,95 cd
08-14-9	41,68 ab	48,97 ab	44,11 ab
08-15-16	35,33 bc	50,00 ab	40,22 de
08-18-3	39,00 abc	41,09 c	39,70 de
08-19-9	35,46 bc	51,90 ab	40,95 cd
08-19-11	43,32 a	47,24 b	44,63 a
08-23-55	34,38 c	41,22 c	36,66 f

* The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($P \leq 0.05$)

Table 3

Genotypes	Brightness (L*)	Chromaticity a*- axis (red-green)	Chromaticity b*- axis (yellow-blue)	Hue angle (h°)	Chroma Index (C*)
Mira	29,08 abc*	23,25 bcd	10,07 bcd	27,65 b	34,05 b
Queen Elisa	26,94 bcd	21,72 d	10,19 abcd	23,67 cd	29,65 bc
Real	26,48 cd	22,78 cd	8,27 d	31,62 a	38,45 a
08-8-4	29,06 abc	26,64 abc	11,66 ab	23,39 cd	29,11 cd
08-14-5	29,69 ab	25,35 abcd	11,59 ab	24,04 cd	27,92 cd
08-14-9	26,11 d	23,02 bcd	8,91 cd	21,13 d	24,72 d
08-15-16	28,28 abcd	24,84 abcd	11,22 abc	24,54 c	27,34 cd
08-18-3	31,01 a	27,98 a	12,51 a	24,05 cd	30,70 bc
08-19-9	29,85 a	27,45 ab	12,47 a	24,49 c	30,20 bc
08-19-11	28,91 abc	26,84 abc	11,16 abc	22,42 cd	29,11 cd
08-23-55	28,25 abcd	25,67 abcd	10,90 abc	22,91 cd	27,92 cd

* The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($P \leq 0.05$)

Table 4

Genotypes	Total dry mater (%)	Total acidity (%)	Total sugars (%)	Vitamin C (mg/100g)	Total anthocyanins (mg/100 g)
Mira	10,6 a*	0,60 c	9,07 b	88,60 d	11,28 e
Queen Elisa	9,89 a	0,68 bc	5,58 d	90,05 c	19,45 a
Real	10,15 a	0,71 b	8,99 b	71,25 f	17,40 c
08-8-4	10,12 a	0,69 bc	4,31 e	93,95 b	8,17 g
08-14-5	10,21 a	0,96 a	9,88 a	94,75 ab	11,07 e
08-14-9	9,95 a	0,74 b	8,74 b	79,05 e	14,73 d
08-15-16	10,00 a	0,65 bc	7,86 c	79,50 e	11,54 e
08-18-3	10,12 a	0,66 bc	8,22 c	79,25 e	10,11 f
08-19-9	10,11 a	0,66 bc	8,00 c	95,80 a	18,63 b
08-19-11	10,54 a	0,67 bc	9,82 a	94,50 ab	8,32 g
08-23-55	10,87 a	0,66 bc	8,20 c	79,30 e	7,16 h

* The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($P \leq 0.05$)

Table 5

Genotype	<i>Mycosphaerella fragariae</i> (DD%)	<i>Diplocarpon earliana</i> (DD %)	<i>Phytophthora fragariae</i> (DD %)	<i>Colletotrichum acutatum</i> (DD %)	<i>Botrytis cinerea</i> (DD %)
Mira	0,83 b*	0,00 b	0,07 a	0,00 b	0,33 bc
Queen Elisa	3,33 a	0,00 b	0,00 b	0,00 b	0,12 cd
Real	0,58 b	0,00 b	0,00 b	0,00 b	0,05 d
08-8-4	0,33 b	0,00 b	0,00 b	0,00 b	0,00 d
08-14-5	0,33 b	0,00 b	0,00 b	0,00 b	0,67 cd
08-14-9	0,17 b	0,00 b	0,00 b	0,00 b	0,00 d
08-15-16	0,25 b	0,00 b	0,00 b	0,00 b	0,53 ab
08-18-3	0,95 b	0,00 b	0,00 b	0,00 b	0,00 d
08-19-9	0,20 b	0,00 b	0,00 b	0,00 b	0,00 d
08-19-11	0,48 b	0,00 b	0,00 b	0,07 a	0,68 a
08-23-55	0,50 b	0,18 a	0,00 b	0,00 b	0,33 bc

* The values in the table that do not have common letters differ significantly for a statistical assurance level of 5% ($P \leq 0.05$)