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PROGRAMUL DE AMELIORARE LA SPECIA MĂR ÎN EVOLUȚIE LA STAȚIUNEA DE CERCETARE-DEZVOLTARE PENTRU POMICULTURĂ VOINEȘTI

APPLE BREEDING PROGRAM IN PROGESS AT RESEARCH STATION FOR FRUIT GROWING VOINEŞTI

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Abstract

At the Research Station for Fruit Growing Voinești, the apple breeding program started after 1950, the year the unit was founded, when the first hybrid combinations were made. Over time and accumulated experience, cultivars or genotypes with a high level of genetic resources have been used in hybrid combinations, especially to obtain apple varieties with genetic resistance to diseases, an objective amplified after 1970, when the 'Prima' variety was registered. The high volume of hybridization works, carried out almost annually, was materialized by the large number of hybrid seedlings resulting from the thousands of seeds obtained and the transplanting of the seedlings in the selection orchard. The research undertaken aimed at obtaining apple varieties with genetic resistance to diseases, identifying potential genitors and valuable elites from selection fields and contest micro cultures, being evaluated and proposed for new hybrid combinations or testing and homologation. The studies being focused on the period 2016 - 2020, mention the obtaining of 915 genotypes, from 13 hybrid combinations, in which valuable parents were used in terms of genetic resistance to *Venturia inaequalis* (owners of the *Vf* gene), productivity and fruit quality. The number of existing hybrids in the selection field, resulted in a percentage of 53.8% compared to the number of pollinated flowers and 80.9% compared to 1,131 hybrid seeds obtained from 1,765 pollinated flowers, in a percentage of 64.1%. From the hybrid combinations previously carried out, there were identified genotypes that possess the gene for resistance to scab (*Venturia inaequalis)* and increased resistance to powdery mildew (*Podosphaera leucotricha)*, productivity and fruits quality, some of which are candidates for obtaining new varieties, respectively, the elites: H 14/311-05, H 4/38-04, H 1/59-04, H 3/37-04, and elites H 4/17-04; H 19/6-04, H 2/3-04 are registered at ISTIS. Obtaining new apple varieties with genetic resistance to diseases is the main factor in the establishment of new ecological orchards, environmentally friendly, according to the consumer preferences.

Cuvinte cheie: combinații hibride, genotipuri rezistente la *Venturia* spp., elite de măr.
Key words: hybrid combinations, *Venturia spp*. resistant genotypes, apple elites.

1. Introduction

At the Research Station for Fruit Growing Voinești, the apple breeding program started after 1950, the year the unit was founded, when the first hybrid combinations were carried out and continued until today, with a specific program for improving the assortment. Over time and accumulated experience, varieties or genotypes with a high level of genetic resources have been used in hybrid combinations, especially to obtain apple varieties with genetic resistance to diseases, an objective amplified after 1970, when the 'Prima' cv. was registered.

In extensive inter- and intraspecific hybridization work and repeated crosses, maternal and paternal parents, a series of apple cultivars or genotypes with genetic resistance to diseases, with valuable fruit productivity and quality characteristics were used.

The high volume of hybridization works, carried out almost annually, was realized by the large number of hybrid seedlings resulting from the thousands of seeds obtained and the transplanting of the seedlings in the selection orchard. The research undertaken led to the approach of some improved methods, both to obtain hybrid progeny, but also their selection in the seedling phase in the fortification field or in the selection fields and the transfer in field trials, for selection of the elites in order to be test by the State Institute for Testing and Registration of Varieties (ISTIS) for obtaining of new varieties.

2. Material and methods

The complex genetic basis existing at Research Station for Fruit Growing (RSFG) Voineşti, composed of selection fields, the nursery hybrids and field trials, constituted and constitutes the main

source for an own apple breeding program, for obtaining apple varieties with genetic resistance to diseases. In this paper, the results obtained in the period 2016-2020 are evaluated; the hybrid combinations performed are presented, accompanied by the number of pollinated flowers and seeds obtained, the resulting hybrids and the ones resistant to *Venturia spp*., existing in the selection fields, evaluated until 2022.

In the working stages, potential parents and valuable elites existing in contest micro cultures and selection fields were identified, being evaluated and proposed for new hybrid combinations, or testing and registration. From the previously performed hybrid combinations, the elites: H 14/311–05, H 4/38–05, H 1/59–04, H 3/37–04, H 4/17–04, H 2/3–04, H 19/6–04, which possess the gene for resistance to *Venturia spp* (*Vf*) and increased resistance to powdery mildew, productivity and fruit quality, are part of the competition micro cultures and are taken into account to apply for obtaining new varieties.

Also, presented are the schemes for shortening the post-ripening period of apple seeds and the period for obtaining varieties with genetic resistance to diseases, used in the evolving breeding program from RSFG Voineşti, carried out until 2022.

3. Results and discussions

The breeding process being continuous, new selection bases composed of hybrid offspring with a complex genetic variability were created annually, being involved as maternal and paternal parents, varieties or elites in such a way as to result in new valuable forms, corresponding to the objectives proposed over time as short as possible.

Success in breeding is largely conditioned by the clarity of the proposed objectives, but at the same time it is dependent on the existence and knowledge of genetic resources, in order to obtain a great variability and genetic diversity.

The use of parents that possess the gene of resistance and productivity imprints in the offspring a higher rate of transmission of valuable characters, making the work of the breeder easier in a certain way. The other features added to the essential conditions are highlighted only on the basis of further research.

Evaluation of the apple hybridization program during the period 2016 - 2020

Regardless of the breeding method used in the creation of varieties, it is mandatory to obtain initial material, selected according to the desired characters, with the potential chosen parents being crossed with each other, to obtain hybrid seeds and plants, which are tested and selected according to the principles of apple improvement.

In the study years 2016-2020, there were used in the hybrid combinations, valuable maternal and paternal parents in terms of genetic resistance to scab, having the *Vf* gene, among them we list the apple varieties: 'Inedit', 'Opal', 'Luna', 'Iris', 'Ariwa', 'Goldrush', Sirius', 'Topaz', 'Valery', 'Cezar', 'Florina' and some elites: H 8/86, H 1/59, H 1/16, H 1/8, H 4/17, H 1/12 and H 14/1.

From the data presented in table 1, it follows that in the years 2016, 2017, 2018 and 2020, there were pollinated 1,765 flowers from 13 hybrid combinations, from which a number of 1,131 hybrid seeds were obtained being stratified and sown in jiffy pots. After a first selection for resistance to the *Venturia spp*., a number of 915 hybrids were transplanted into the fortification field, representing a percentage of 51.8% compared to the pollinated flowers and 80.9% compared to the number of hybrid seeds sown.

In the fortification field, the apple hybrids vegetated properly, annually being analyzed according to the criterion of resistance to the attack of the main diseases and according to visible morphological characters.

Apple seedlings with disease resistance, obtained from apple hybrid combinations, complete the existing selection base in the hybrid nursery, some of them being selected, grafted in the nursery on 'M9' rootstock and transferred to competition micro cultures.

Following the observations made over the years, a number of 428 scab resistant hybrids remained in the selection fields in a percentage of 45.3% compared to the number of hybrids obtained initially. The number of scab resistant hybrids, compared to the number of pollinated flowers, represents a percentage of 24.2% and 37.8% compared to the number of hybrid seeds.

The apple hybrid combinations 'Goldrush x H 1/59', 'Sirius x H 1/59' released in 2017 and 'Topaz x H 1/16', 'Valery x Cezar' released in 2018, obtained the highest percentage of scab resistant hybrids from all resulting seedlings (more than 50%).

Vegetative growth of apple seedlings in the fortification field

The apple seedlings from the 2018 hybrid series, existing in the fortification field, were analyzed regarding the vegetative growth, achieved in the first year after planting (Table 2).

The vegetative growth expressed by the height of the seedlings (cm) in the hybrid nursery, after one year of vegetation, is as follows: out of 306 analyzed hybrids, obtained from 3 hybrid combinations, 94 hybrids had a height between 20-50 cm (30.7%), 123 hybrids between 50 - 80 cm (40.2%) and 89 with

a height over 80 cm (29.1%). The lowest growth vigour was recorded at the hybrids resulting from the 'Valery x Cezar' cross combination, respectively 40.4% with a height between 20 - 50 cm.

With the transplanting of the apple hybrids obtained in jiffy pots, in the fortification field, it is found that after one year, the vegetative growth reaches values of 20-80 cm, year in which, after a prior positive mass selection, considering the criterion of disease resistance, vigour and other characteristics, one can harvest a branch and graft 2 – 3 eyes onto the rootstock M.9. Grafted nursery trees are planted in competition microcultures and further selected after at least 3-4 years for disease resistance, nice fruit appearance and taste, fruiting potential, etc.

If the selection is not carried out according to the vegetative characteristics, the hybrids on their own roots are left in the fortification field, becoming a selection field, until the first fruits are obtained, the selection being carried out according to the resistance to diseases and the quality of the fruits. There are situations when apple hybrids obtained from certain combinations, being still on their own roots, bear fruit as early as the 3rd year after planting.

Methods of shortening the time period for obtaining some varieties of apple

After 2000, the methodology regarding hybridization works and obtaining initial selection material were improved, in the sense of shortening the periods of both the post-ripening period of apple seeds and shortening the duration of creating apple varieties with resistance genetics in diseases.

Steps on shortening the post-ripening period of seeds

After establishing the genetic resources employed in the hybridization schemes, the seeds are obtained, which before being sown, must go through the post-ripening process, which by the classic method, the specific duration is 90 days.

RSFG Voinești improves the method of obtaining post-ripened seeds, used in the process of creating new apple varieties.

A first method of shortening the post-ripening period of apple seeds, used at Voineşti, consists in stratifying the seeds starting from the first decade of February.

The dry seeds, treated with a phytopharmaceutical solution, are mixed with 3 parts of sand sterilized by burning, well moistened, put in boxes (if there are more), or plastic cups, buckets with holes so as not to stagnate the irrigation water, being stored in the refrigerator or cold storage, where the temperature is kept constant between 0-2°C.

After about 20 days, they are removed from the refrigerator or cold storage and transferred to a cellar or storage where the temperature is kept constant between 4-6°C. After 40-45 days the seeds sprout and are suitable for sowing in pots or jiffy tablets.

The post-ripening period is reduced to 40-45 days and the degree of uniformity of seed germination is certain.

A second method consists in keeping the hybrid fruits under natural cooling conditions, and in the last decade of January or the first decade of February; the seeds are removed, washed with tap water and treated with a phytopharmaceutical solution. The moistened seeds are placed with the sterilized sand in boxes, glasses or perforated trays, being transferred to a cool cellar or basement and in no more than 40-45 days, they emit roots and when they are 0.5-1cm they can be sown in the field, in pots or jiffy tablets. This second method applies only to apple varieties that bear fruit with winter storage.

Methodology for shortening the duration of creation of apple varieties

Obtaining apple varieties is a long-term and particularly complex activity, especially when considering the creation of varieties with genetic resistance to diseases, regardless of the research method used.

After the establishment of the genetic resources employed in the hybridization schemes and the performance of the hybrid combinations, the seeds and seedlings are obtained, which are selected after a prior infection with virulent strains of *Venturia spp*., being continued in the fortification field, until they enter the fruit. During this time, mass positive selection takes place through direct observation and marking of the hybrids selected according to the selection criteria (disease resistance, pleasant appearance and taste of the fruits, fruiting potential, etc.), their grafting in the nursery on weak vigour rootstock ('M9') planted permanently, in contest micro cultures.

From the research performed at Voineşti, the apple hybrid seedlings on their own roots left in the fortification field, becoming a selection field, bear fruit from the 3rd - 4th year after planting. In this way, a feature acquired by some hybrid combinations, of early fruit entry, is capitalized on, which is reflected by reducing the duration by an appreciable number of years and the expenses, but also the reduction of the area of land necessary to obtain and test the hybrid material until approval and the promotion of new varieties in culture.

Hybrids selected according to their growth characteristics, fruit quality and genetic resistance to diseases, passed in competition micro cultures, can register at ISTIS for testing with the purpose of registration, a period lasting 2-3 years, during which time their authenticity, varietal purity, agronomic and

use value are checked and confirmed. The introduction into culture of the new approved varieties takes place only after they have been entered in the Official Catalogue of varieties.

The method of shortening the period of obtaining apple varieties with genetic resistance to diseases, materialized with the registered in 2016 of 3 apple varieties ('Valery', 'Cezar' and 'Revidar'), obtained from the 2004 hybrid series. From obtaining the hybrid series to approval, 12 years have been recorded, compared to obtaining apple varieties by conventional methods, which took or have been taking more than 25 years.

Promoting the methodology of shortening the acquisition of apple varieties with genetic resistance to diseases stimulates the scientific substantiation of the proposed solutions to increase competence, in accordance with the new guidelines from scientific research in European countries with advanced fruit growing.

Prospective genotypes useful for the breeding program

From the apple selection fields, resulting from the hybrid combinations carried out previously, some genotypes with increased genetic variability were identified, possessing the gene for resistance to scab and powdery mildew, productivity and fruit quality, being grafted on the rootstock 'M9' and use for the establishment of competition micro cultures.

Following the observations and determinations made, valuable genotypes capable of competing in obtaining new apple varieties or using them as maternal and paternal parents in breeding work were appreciated. From the selected apple genotypes, a series of elites obtained at RSFG Voineşti, can apply to obtain new varieties, 7 of which are presented below.

Characterization of apple elites

Elite H 14/311–05. Obtained from the cross combination 'Goldenspur x Florina'. The tree has a medium vigour, precocious, fruiting on short formations. Flowering is medium and overlaps with most cultivars. The fruit weight has over 165 g, spherical in shape, yellow-green skin colour over all surfaces. The flesh is white-yellowish, crunchy, very good taste, similar to 'Golden delicious' cv. The solid soluble content is over 14%. Harvesting maturity is at the beginning of October, and consumption is extended until March 1st. Elite is resistant to attack by *Venturia inaequalis* şi *Podosphaera leucotricha*. It stands out for its precocity, resistance to the attack of the main diseases, special quality of the fruits (Fig. 1).

Elite H 4/38–05. Obtained from the cross combination 'Goldenspur x Florina'. The tree is medium vigour, precocious, fruiting on short formations. Flowering is mid to late. The fruit weight has 160 g, conical, yellow on the entire surface. The flesh is yellowish, crunchy with a very good taste. Harvest maturity begins with the first decade of October, and the consumption period extends until April 1st. Elite is resistant to attack by *Venturia inaequalis* şi *Podosphaera leucotricha*. It stands out for its precocity, resistance to the attack of the main diseases, excellent fruit quality and good storage capacity over the winter (Fig. 2).

Elite H 1/59–04. Obtained from the cross combination 'Florina x H 1/12'. The tree is medium vigour, fruiting on short formations. Flowering is early to mid. The fruit weight has 165 g, spherical, yellow-green skin on the entire surface. The flesh is yellowish-white, crunchy with a good taste, slightly acidic. Harvest maturity begins with the first decade of October, and the consumption period extends until March. Elite is resistant to attack by *Venturia inaequalis* şi *Podosphaera leucotricha*. It stands out for its precocity, resistance to the attack of the main diseases, excellent fruit quality and good storage capacity over the winter (Fig. 3).

Elite H 3/37–04. Obtained from the cross combination 'Florina x Idared'. The tree is of medium vigour, very precocious, fruiting on standard formations. The flowering is medium. The fruit weight is over 160 g, yellow skin, covered with red on 2/3 of the surface. The flesh is yellowish, crunchy with good taste. Harvest maturity begins in the first decade of October, and the consumption period extends until the end of April. Elite is resistant to attack by *Venturia inaequalis* and lightly attacked by *Podosphaera leucotricha*. It stands out for its precocity, resistance to the attack of the main diseases and ability to keep the fruits over the winter (Fig. 4).

Elite H 4/17–04. Obtained from the cross combination 'Goldenspur x Florina'. The tree is of low-medium vigour, precocious, fruiting on short formations. Flowering is mid to late. The fruit weight is 165 g, conical, yellow on the entire surface. The flesh is yellowish, crunchy, with a very good taste. Harvest maturity begins with the first decade of October, and the consumption period extends until March 1st. Elite is resistant to attack by *Venturia inaequalis* şi *Podosphaera leucotricha*. It stands out for its precocity, resistance to the attack of the main diseases, fruiting on short formations, excellent fruit quality and good storage capacity over the winter (Fig. 5).

Elite H 2/3–04. Obtained from the cross combination 'Florina x Idared'. The tree is low-medium vigour, very precocious, fruiting on short and long branches. The flowering is medium, the flower bud is light pink. The fruit weight is over 165 g, with skin colored in yellow-green, covered with red on 3/4 of the surface. Flesh white - yellowish, crunchy with good taste. Harvest maturity begins in the last decade of

September, and the consumption period lasts until January. Elite is resistant to attack by *Venturia inaequalis* and by *Podosphaera leucotricha*. It stands out for its precocity, resistance to the attack of the main diseases, fruit quality, with storage until January (Fig. 6).

Elite H 19/6–04. Obtained from the cross combination 'Florina x Idared'. The tree is low-medium vigour, spread out, fruiting on short and long branches. Flowering is late, the flower bud is dark pink, the petals are overlapping. The fruit weight is over 160 g, skin colored in dark red, on ¾ of the surface. The fruit shape is obloid. The flesh is yellowish-white, crunchy with good taste. The harvest maturity is late, with consumption maturity by March. Elite is resistant to attack by *Venturia inaequalis* and *Podosphaera leucotricha*. It stands out for its resistance to the main diseases, quality of the fruits, with storage over the winter (Fig. 7).

Elites H 4/17–04, H 2/3–04 and **H 19/6 – 04** are registered to ISTIS for testing, the others are further evaluated both as potential partners in further hybrid combinations or proposals for approval testing. Apple varieties with disease resistance, approved and returned to growers, have different ripening periods that cover a large period of consumption, they were and are the main factor in the establishment of new plantations that produce ecological, environmentally friendly fruits and in accordance with consumer preferences.

4. Conclusions

Research Station for Fruit Growing Voineşti started own apple breeding program since its foundation (1950), in order to released apple varieties with genetic resistance to diseases.

During 2016 – 2020 period, the following apple varieties: 'Inedit', 'Opal', 'Luna', 'Iris', 'Ariwa', 'Goldrush', 'Sirius', 'Topaz', 'Valery', 'Cezar', 'Florina' and elites (H 8/86, H 1/59, H 1/16, H 1/8, H 4/7, H 1/12 and H 14/1) were used in the hybrid combinations, as maternal and paternal parents, valuable in terms of genetic resistance to scab. From 13 hybrid combinations made with disease-resistant apple cultivars and elites, a total of 1,131 seeds from 1,765 pollinated flowers resulted, which were stratified and sown in jiffy tablets. Following the first selection after the resistance to scab, a number of 915 hybrids were transplanted into the fortification field, representing a percentage of 51.8% compared to the pollinated flowers and 80.9% compared to the sown hybrid seeds, their number decreasing to 428 (46.8%) in subsequent selection years.

The studies and research carried out in previous years have highlighted a number of valuable elites, of which seven have been nominated as promising candidates for obtaining new disease-resistant apple varieties or using them as maternal and paternal parents in improvement works: H 14/311-05, H 4/38-05, H 1/59-04, H 3/37-04, H 4/17-04, H 2/3-04, H 19/6-04.

The methodology regarding hybridization matters and obtaining initial selection material have been improved, in the sense of shortening both the post-ripening period of apple seeds and shortening the duration of creating disease-resistant apple varieties.

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Tables and Figures

Table 1. Evaluation of disease resistance in seedlings from the apple breeding program, in the period 2016 – 2020, at RSFG Voineşti

No.	Year / Cross combination	Pollinated flowers (number)	Hybrid seeds (number)	Seedlings (number)	Scab resistant hybrids in the selection field	
					No	%
	2016					
1	Inedit x Opal	186	100	77	32	41.6
2	Luna x Iris	127	83	68	29	42.6
3	Ariwa x H 8/86	110	89	75	36	48.0
	Total	423	272	220	97	44.1
	2017					
1	Golsrush x H 1/59	72	26	15	8	53.3
2	Goldrush x Inedit	92	29	17	7	41.1
3	Sirius x H 1/59	80	17	10	6	60.0
4	Sirius x Inedit	160	59	36	16	44. 4
	Total	404	131	78	37	47.4
	2018					
1	Topaz x H 1/16	306	195	164	82	50.0
2	Valery x Cezar	164	130	94	50	53.2
3	Goldrush x H 1/8	102	69	48	21	43.8
	Total	572	394	306	153	50.0
	2020					
1	Goldrush x H 4/17	230	151	119	56	47.1
2	Florina x H 1/12	165	122	86	41	47.7
3	Goldrush x H 14/1	215	133	116	44	37.9
4	Total	610	406	311	141	45.3
	GENERAL TOTAL	**1,765**	**1,131**	**915**	**428**	**46.8**
% reported to the number of flowers			64.1	51.8	428	24.2
% reported to the number of hybrid seeds				80.9	428	37.8

Table 2. Vegetative growth of seedlings in the hybrid nursery, in the first year of vegetation (2018 serie)

No.	Hybrid combination	Analyzed seedlings	Seedlings height – cm -					
			20 - 50		50 – 80		> 80	
			No.	%	No.	%	No.	%
1	Topaz x H 1/16	164	49	29.9	68	41.4	47	28.7
2	Valery x Cezar	94	38	40.4	29	30.8	27	28.8
3	Goldrush x H 1/8	48	7	14.6	26	54.1	15	31.3
	Total	306	94	30.7	123	40.2	89	29.1

Fig. 1. Elite H 14/311–05

Fig. 2. Elite H 4/38 – 05

Fig. 3. Elite H 1/59 – 04

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Fig. 4. Elite H 3/37 – 04

Fig. 5. Elite H 4/17 – 04

Fig. 6. Elite H 2/3 – 04

Fig. 7. Elite H 19/6 – 04