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RESEARCH ON THE USE OF SOME APPLE GENITORS IN THE BREEDING PROCESS FOR GENETIC RESISTANCE TO DISEASE AND FRUIT QUALITY

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Abstract

Research conducted at the Research and Development Station for Fruit Growing Voineşti in the period 2012 - 2014, took into account the increase of genetic variability through the use of hybrid combinations that involved commercially valuable genotypes or selected elite in order to obtain desired characteristics: genetic resistance to disease, low vigor, constant production, firm pulp and good storage capacity. In apple breeding for genetic resistance at RDSG Voineşti were used as genitors, apple elites and recently created cultivars with genetic resistance to disease, which have embedded complex resistance genes: Iris, Inedit, Goldrush, Ariwa, Topaz, Golden Lasa or genotypes created in Voineşti: H1/22, H5/20, H2/75, H1/27, H1/7, H4/37 etc. Apple seedlings hybrids obtained in jiffy 7 in greenhouse were transplanted in the fortification field, after a previous mass selection, depending on objectives. After the selection of forms derived from hybrid seedlings planted in the fortification field, it resulted 40.3% to 96.2% seedlings resistant to scab, the following combinations: Inedit x Topaz (83.3%), Topaz x H1/27 (93.1%), Inedit x Ariwa (93.7%), Goldrush x H1/3 (96.2%) etc. Creating apple varieties with genetic resistance to disease is an ongoing process that must be done by annually hybrid combinations using high economic value genitors which enclose complex genetic resistance genes.

Key words*: genetic resistance to disease, genitors, apple hybrid seedlings.*

INTRODUCTION

Apple breeding is a long time process and has a special complexity regardless the research method used. In the programme dedicated for obtaining apple varieties with genetic resistance to disease, at RDSFG Voinesti annually were made various hybrid combinations using maternal and paternal material chose according to the purpose.

In order to create autochthonous apple cultivars, the basic work such as inter and intraspecific hybridizations remains together with the selection of the promising hybrids obtained from early juvenile stage or obtained in previous years (Cociu V. et all, 1999).

Increasing genetic variability is achieved in particular at hybrids combinations where genitors as apple elites or varieties with genetic resistance to disease recently created are used, hybrids which have embedded complex resistance genes (Petre V. and Petre Gh., 2014). In creating the apple varieties with genetic resistance at RDSFG Voinesti, it was used in crosses, resistant apple varieties, with high commercial value or apple elite with genetic resistance to disease, from the latest generation.

MATERIALS AND METHODS

Research conducted at Voineşti between 2012 - 2014 for obtaining apple varieties with genetic resistance to diseases were related to sexual hybridization or selection inside the promising hybrids of biological material obtained from juvenile phase. In hybridizations it was used paternal and maternal material such as new elite and varieties with genetic resistance to disease.

In the process of sexual hybridization, it were followed all steps specific for hybridization

process: choosing the parents, marking trees, flowers emasculation, harvesting and conditioning pollen, pollination, inventory pollinated flowers on combinations. From the hybrid fruits, the seeds were extracted and conditioned.
The specific duration of apple seeds post maturation is 90 days, so the layering of those was done in the last decade of January. The hybrid seeds were mixed with sterile sand well misted after fungicide disinfestations.
When the apple seeds begin to root in the stratification slot, these were sown in jiffy pots no 7 into the greenhouse. The apple hybrids at the 10-15 cm height and 8-10 leaves stage were moved out in the fortification field. All the hybrids with scab symptoms were eliminated before planting outside.
All the pollinated flowers were counted, fruits and obtained seeds the same. Meanwhile, in the fortification field it was evaluated the hybrids resistance to *Venturia inaequalis.*
Selected genotypes from the previous hybrid series which presented genetic resistance to diseases and fruits with high quality traits were grafted in the nursery. With these ones it was established in 2009 a trial micro-culture. It were made observations and determinations regarding the growth vigour of trees, ripening time of the fruits, productivity and the fruits quality in terms of size (weight) and soluble dry content (SDC).

RESULTS AND DISCUSSIONS

Aiming to a good performance in the fruit growing production, it has to promote cultivars with a genetic base that could assure an increase of yield potential and fruit quality, an improved resistance to diseases and pests in order to protect the environment and a good adaptation to economical and performing technology.
One important thing is to manage the cultural technology for keeping the production capacity and quality unaffected. In this way, the cultivars performance is emphasized.
Apple breeding is a long time process and has a special complexity regardless the research method used.

Apple genitors used for crossing
In the programme dedicated for obtaining apple varieties with genetic resistance to disease, at RDSFG Voinesti annually it were made various hybrid combinations using genitors chose according to several goals such as: scab resistance, low vigour, constant yielding, firm pulp of the fruits, a long shelf life.
Choosing the genitors is highly important for obtaining descents with economical and commercial value, good appearance of fruits and special tasting quality. When we use genitors with small fruits, the negative trait is dominant and is hard to attain a good size and tasting quality. Sometimes entire hybrid combinations could be removed due to the low biological value of the biological material. Therefore, a good decision is to use resistant cultivars with high economic value.
In order to gain faster disease resistance and in the same time productivity and quality traits, is necessary to cross maternal and also the paternal genitors that present resistance and proved excellence in the field.
For enlarging the apple variability at RDPS Voinesti it were used as genitors elites and new bred cultivars with genetic resistance: Iris, Inedit, Goldrush, Ariwa, Topaz, Florina, Golden Lasa or genotypes created in the Research Station: H 1/22, H 5/20, H 2/75, H 1/27, H 1/7, H 4/37, etc. All the genitors have the Vf gene.
Knowing the combinative value of the cultivars used in crossing process is a great concern of the breeder.
Breeding method
For creating autochthonous apple cultivars, the main method remains intra and interspecific hybridization followed by selection in the descendent biological material since the juvenile stage. After the genitors selection and setting the crossing combinations step, the hybridization start with emasculation of the maternal genitor, procedure which allow to not protecting the flowers by bags. This recommendation was made by Prof. J.R. Hough from the Rutgers University (USA) starting to 1970.
After crossing procedure, consequent operations are realised. First is related to obtaining hybrid fruits, extraction and post

maturation of the seeds. Next step is to saw the hybrid seeds in jiffy pots no 7. A special phase is about the artificial infection of the seedlings followed by selection of the resistant ones. The selected hybrid seedlings are plant in the fortification field. For quality test they are moved after in the selection field (test 1). Promoting the elites in the test 2 (DSO) and 3 (VAT) and registering to the ISTIS organization.

The results obtained in the period 2012-2014 are presented in the paper.

In 2012 were made 15 crossings, being emasculated and pollinated 2.531 flowers. It results 557 fruits set at 10.06.2012 representing 22% of the pollinated flowers (Table 1.).

The percentage of sett fruits at first inventory was between 0% at Florina x H1/22, Florina x Ariwa, Topaz x H 5/20, Topaz x H 2/75 and 69,5% at combination Goldrush x H 1/7. The low share of fruit set in 2012 was determinate by the climatic conditions in the blossoming time. Temperatures of 28 – 30^{0}C and a low humidity of the air (22 – 24%) conducted to the stigma dehydration.

At harvest time, 491 fruits were collected and 2.743 hybrid seed were extracted. From these, after stratification, only 1.449 post maturated seed were retained. In 2013, these hybrid seed were sown in jiffy pots (no 7) and have been obtained 1.066 hybrid seedlings. It was actually transplanted in the fortification field 73.5 % of the seeds.

Table 1. Apple hybrid seedlings obtained from crossings made at RDSFG Voinesti in 2012

No	Crossing	Pollinated flowers	Fruits		Hybrid Seeds	Sown seeds	Seedlings	
			No	%			No	%
1	Florina x H 1/22	222	0	0	0	0	0	0
2	Florina x Ariwa	195	0	0	0	0	0	0
3	Topaz x H 1/22	122	4	3,2	28	20	15	75,0
4	Topaz x H 5/20	224	0	0	0	0	0	0
5	Topaz x H 2/75	156	0	0	0	0	0	0
6	Topaz x H 1/27	128	7	5,4	38	43	29	67,4
7	Inedit x Topaz	161	2	1,2	12	9	6	66,6
8	Inedit x Ariwa	106	10	9,4	63	55	32	58,1
9	Goldrush x Golden Lasa	268	85	31,7	488	162	121	74,7
10	Goldrush x H 1/13	243	86	35,4	518	190	132	69,4
11	Goldrush x H 4/37	173	90	52,0	526	280	202	72,1
12	Goldrush x Inedit	256	78	30,4	413	275	208	75,6
13	Goldrush x Iris	102	23	22,5	98	90	60	66,6
14	Goldrush x H 5/20	93	49	52,6	261	160	128	80,0
15	Goldrush x H 1/7	82	57	69,5	298	165	133	80,6
	TOTAL	2531	491	19,4	2743	1449	1066	73,5

In 2013 were realised 22 hybrid combinations and a number of 4.008 flowers were emasculated and pollinated. Only 113 hybrid fruits were obtained (determination at 15.06.2013). This is only 3.5% of the total pollinated flowers (Table 2.).

The percentage was ranking from 0% at combinations Dalinbel x Inedit, Dalinbel x Generos, Dalinbel x H 3/123, Dalinet x H 4/131, Red Topaz x H 3/123, Rosana x Inedit, Opal x H 4/17 to 45% at Goldrush x H 4/40.

Similar to year 2012, the percentage of fruit set was extremely reduced by the climate conditions registered in the flowering period. Temperatures of 27 – 29^{0}C in the full blossom correlated to the low relative humidity of the air at noon (25 – 33%) produced a dehydration of the pollen and stigma.

Only 113 hybrid fruits were picked being extracted 496 seeeds. From the 453 post maturated and sown seeds resulted 351 seedlings (77.4%) which were planted in the fortification field.

Table 2. Apple hybrid seedlings obtained from crossings made at RDSFG Voinesti in 2013

No	Crossing	Pollinated flowers	Fruits		Hybrid Seeds	Sown seeds	Seedlings	
			No	%			No	%
1	Dalinbel x Inedit	120	0	0	0	0	0	0
2	Dalinbel x Generos	122	0	0	0	0	0	0
3	Dalinbel x H – 3/123	130	0	0	0	0	0	0
4	Dalinet x Inedit	106	2	1,8	7	6	4	66,6
5	Dalinet x Generos	341	10	2,9	61	58	45	77,5
6	Dalinet x H – 3/123	291	2	0,7	12	10	8	80,0
7	Dalinet x H - 4/131	185	0	0	0	0	0	0
8	Dalinred x H – 4/131	351	2	0,5	13	13	8	61,5
9	Dalinred x Inedit	210	0	0	0	0	0	0
10	Dalinred x H – 3/123	144	4	2,7	18	16	13	81,2
11	Dalinred x Generos	224	4	1,8	21	21	15	71,4
12	Goldrush x Crimson	120	23	19,2	98	94	68	72,3
13	Goldrush x H – 4/40	122	43	35,2	171	152	123	80,9
14	Luna x Inedit	175	9	5,1	19	4	9	81,8
15	Luna x H – 4/47	168	7	4,1	39	36	29	80,5
16	Red Topaz x H – 4/131	206	1	0,5	4	4	3	75,0
17	Red Topaz x H – 3/123	307	0	0	0	0	0	0,
18	Rosana x Inedit	157	0	0	0	0	0	0
19	Opal x H – 4/17	147	0	0	0	0	0	0
20	Opal x H – 4/47	124	1	0,8	6	6	6	100,0
21	Inedit x H – 1/132	83	2	2,4	8	7	6	85,7
22	Inedit x Generos	175	3	1,7	19	19	14	73,6
	T O T A L :	4.008	113	2,8	496	453	351	77,4

In 2014, as genitors were selected as follows: Inedit, Florina, Enterprise, H 1/46 and H 4/50, which present valuable traits especially a good storage capacity (Table 3).

Table 3. Hybrid combinations realised in 2014 at RDSFG Voinesti

No	Hybrid combination	Pollinated flowers	Hybrid fruits		Hybrid seeds	
			No	%	No	Average no/fruit
1	Florina x Inedit	126	114	90,4	463	4,06
2	Inedit x Enterprise	520	110	21,1	352	3,20
3	H 1/46 x H 4/50	370	36	9,7	105	2,92
	TOTAL	1016	260	25,6	920	3,54

From the three hybrid combinations 1016 flowers were pollinated and results 260 fruits picked up on 1.10.2014, representing 25.6% of total pollinated flowers.

The share of hybrid fruits different from 9.7% at combination H 1/46 x H 4/50 and 90.4% at Florina x Inedit cross.

From the 260 hybrid fruits were extracted 920 seeds with an average number of 3.54 seeds/fruit. It is notable the Florina x Inedit combination which record a percent of 90,4% harvested fruits. The average number of seeds/fruit is 4.06, bigger than previous combinations.

The evaluation of hybrids resistance to diseases in the fortification field

In the case of 2012 hybrids series, from 1.066 apple hybrids (Table 4) resulted by 11 hybrid combinations (genitors with Vf) it shows field scab resistance1.012 trees representing 95.8% from the total hybrids in the fortification field. The percent of scab resistant hybrids varied from 80% at Topaz x H 1/22 combination and 96.2% at Goldrush x H 1/13.

Table 4. Evaluation of the scab resistance hybrids in the field (series 2012)

No	Hybrid combination	Analysed hybrids	Scab resistant hybrids	
			Number	%
1	Topaz x H 1/22	15	12	80,0
2	Topaz x H 1/27	29	27	93,1
3	Inedit x Topaz	6	5	83,3
4	Inedit x Ariwa	32	30	93,7
5	Goldrush x Golden Lasa	121	114	94,2
6	Goldrush x H 1/13	132	127	96,2
7	Goldrush x H 4/37	202	191	94,5
8	Goldrush x Inedit	208	200	96,1
9	Goldrush x Iris	60	57	95,0
10	Goldrush x H 5/20	128	123	96,0
11	Goldrush x H 1/7	133	126	94,7
	TOTAL	1066	1012	95,8

The apple genotypes behaviour in the trial field

From the previous hybrids series, it were selected a couple of apple genotypes with genetic resistance to diseases, high quality and good appearance of fruits. These were grafted in the nursery on M9 rootstock and consist of a new micro culture trial in 2009. The traits monitored were: growth vigour, ripening time, productivity and fruit quality.

The trees vigour registered by the 20 apple genotypes/M9 from micro culture trial emphasized by the trunk diameter and growth increase shows that in the 6th leaf of the trees, the diameter of the trunk varied from 35.5 mm at H1/78 to 53.9 at H1/7 (Table 5). The annual growth increase was ranged between 4.1 mm (H 1/21, H 3/80) to 9.1mm (H 1/7).

Ripening time of the apple hybrids in the years 2012-2014 was from September 3rd to October 5th. Most of them are autumn-winter maturation. From the group of September maturation were remarked H 1/16, H 9/27, H 8/86, H 3/80. End of September – first decade of October group are represented by H 1/11, H 1/22, H 1/112, H 1/7, H 1/27, H 2/125, H 4/131, H 8/88, H 1/46.

The fruit production obtained in the 4th year varied from 0-3.5 kg/tree. With high yield are noted H-8/86, H-8/88, H-1/46, H-9/27, H-1/99, genotypes where was registered over 3.5 kg/tree. In the climatic conditions of the 2013 year, 18 from 20 studied genotypes was bearing fruits.

In the 5th leaf, production ranged from 0-5.5 kg/tree, high productivity shows H 1/16; H 8/86; H 1/78; H 8/88; H 1/63, where it was weighted more than 5 kg/tree.

Table 5. Growth vigour, ripening time, productivity and fruit quality of the apple genotypes in the micro culture trial (2012-2014)

No	Genotype	Trees vigour in the 6th year (2014)		Ripening date	Yield in the 6th leaf (kg/tree)	Average weight of the fruits (g) (2012 -2014)	Dry substance content (%) average of 2012 - 2014
		Ø trunk (mm)	Annual growth increase				
1	H 1/11	40,5	6,4	25-28.09	4,5	200	13,6
2	H 1/22	37,5	5,6	25-28.09	8,5	175	15,1
3	H 1/112	40,1	6,1	24-25.09	7,0	170	16,0
4	H 3/80	36,8	5,6	09-10.09	4,0	170	14,1
5	H 2/75	44,5	8,3	14-15.09	6,5	173	14,0
6	H 1/102	43,7	7,2	10-12.09	5,5	175	13,2
7	H 1/7	53,9	9,1	27-30.09	5,0	160	14,1
8	H 4/130	38,8	6,5	15-20.09	6,0	165	13,7
9	H 1/78	35,5	6,9	01-05.10	10,5	190	14,0
10	H 9/27	40,2	6,4	05-10.09	7,0	172	14,2
11	H 8/86	38,1	6,8	05-10.09	11,5	155	14,8

No	Genotype	Trees vigour in the 6th year (2014)		Ripening date	Yield in the 6th leaf (kg/tree)	Average weight of the fruits (g) (2012 -2014)	Dry substance content (%) average of 2012 - 2014
		Ø trunk (mm)	Annual growth increase				
12	H 1/16	38,6	6,3	03-05.09	9,5	160	13,0
13	H 1/27	36,6	5,7	20-25.09	8,0	160	14,4
14	H 1/99	43,1	6,3	10-15.09	12,0	150	14,0
15	H 2/125	46,3	8,2	20-25.09	8,0	180	15,1
16	H 1/63	38,5	5,7	10-11.09	9,0	200	14,4
17	H 4/131	45,9	8,3	20-27.09	5,0	197	14,8
18	H 8/88	44,5	8,4	01-05.10	8,0	155	14,0
19	H 1/46	35,7	5,6	25-29.09	7,5	182	15,5
20	H 1/21	40,2	6,0	15-20.09	11,0	145	14,6

The fruit production in the 6th year at all 20 studied genotypes (in the climatic conditions of 2014) ranged from 4 kg/tree (H 3/80) to 12 kg/tree (H 1/99). Most productive genotypes were H 1/99, H 8/86, H 1/16, H 2/125, H 1/21, H 1/78, H 1/63, H 8/88, H 1/27, H 1/22 with more than 8 kg/tree (22 t/ha), in a orchard density of 2.857 trees/ha.

The fruits quality was assessed taking into account the average wight of the fruits and the dry substance content (2012 – 2014). Concerning the fruits size, the weight ranged by 145 g (H 1/21) to 200 g (H 1/ 11, H 1/63). Most of the apple genotypes exceeded 170g. The dry substance content has the limits between 13% (H 1/16) and 16% (H 1/112), 17 from 20 apple genotypes recorded more than 14% DSC, as average value (2012 – 2014).

The best genotypes remarked by productivity and fruits quality were submitted to ISTIS for testing and obtaining the patent protection (H 1/16 and H 1/78).

CONCLUSIONS

The breeding programme developed at RDSFG Voinesti use as genitors only cultivars or elites with genetic resistance to scab (*Venturia inaequalis*).

Crossings made in the last three years conducted to a different number of seedlings according to the climate conditions specific in each year of hybridization.

The evaluation of 20 genotypes from the micro culture trial shows a variable vigour of the trees (trunk diameter 35.5-53.9 mm and annual growth increase of 4.1-9 mm).

Most of apple hybrid genotypes are autumn-winter type and has the ripening period in September of first days in October.

The highest productivity obtained in the 4th year was 4.8 kg/tree, in the 5th year 5.5 kg/tree and in the 6th year 12 kg/tree. The most productive apple genotypes were: H 1/99, H 8/86, H 1/16, H 2/125, H 1/21, H 1/78, H 1/63, H 8/88, H 1/27, H 1/22.

The fruits size is very good for most of the genotypes (over 170 g/fruit) and the dry substance content bigger than 14%.

The best genotypes H1/16 and H 1/78 were submitted for testing and patent obtaining at ISTIS.

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