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GENOME CONSTRUCTING OPENS NEW OPPORTUNITIES IN APPLE (*Malus domestica* Borkh.) BREEDING FOR SCAB IMMUNITY, FRUIT QALITY AND EASY ORCHARDING

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Abstract

Varieties which are immune to scab, triploid, with the fruits of high commercial quality, columnar, for growing in superintensive gardens, are a priority in apple (*Malus domestica* Borkh.) breeding. In addition, the varieties must have a high adaptiveness and productivity in specific agro climatic conditions. Scab caused by *Venturia inaequalis* (Cke. Wint.) is one of the most harmful diseases of apple (*Malus domestica* Borkh.) trees. This publication gives brief results of apple breeding for scab resistance. All-Russian Research Institute of Breeding Fruit Crops (RIBFC) is a pioneer in the development of scab immune home apple cultivars the development of which has been conducted since 1977. A list and origin of the best ten immune cultivars from twenty-two ones developed at the institute are given. The development of the digenic cultivars ($V_f + V_r$, $V_f + V_m$) with more prolonged and stable resistance to scab in contrast to monogenic cultivars is undertaken. Genetic and immunologic principles of breeding have been developed along with practice approaches. Methods of the artificial scab infection have been improved. Scientific recommendations on the intensification and acceleration of breeding have been worked out on a new genetic basis, i.e. with the donors having V_f gene. Another priority in breeding is the development of triploid apple cultivars, which are characterized by less year periodicity of fruit-bearing, higher fruit marketability and heightened autogamy. Using donors of diploid gametes, which have been cytoembryologically studied, for the first time in Russia and in the world a series of triploid cultivars have been developed at the RIBFC, nine of which are zoned (Avgusta, Bezhin Lug, Alexandr Boiko, Darena, Maslovskoe, Orlovskii Partizan, Osipovskoe, Patriot and Yablochnyi Spas). It has been found that 2×/4× and 4×/2× are the most promising crossing combinations for obtaining triploid seedlings. Breeding for columnar apple cultivars have been conducted at the RIBFC since 1984. Eight columnar apple cultivars developed at the RIBFC allow establishing super intensive orchards. Combining scab immunity (V_f), triploidy (3×) and columnar habit (C_o) in one genotype is difficult task. This problem can be solved by hybridization of columnar cultivars with V_f + 4× donor 30-47-88 (Liberty × 13-6-106) which has been obtained due to pre-breeding. A pattern of obtaining seedlings which combine scab immunity (V_f), columnar type (C_o) and triploid set of chromosomes, is given.

Keywords: apple, breeding, varieties, scab immunity, triploidy, columnar tree habit

Cultivars that are immune to scab, triploid, with the fruits of high commercial quality, columnar (for growing in superintensive gardens) remain a priority in apple (*Malus domestica* Borkh.) breeding. In addition, the varieties must have high adaptiveness and productivity in specific agroclimatic conditions.

Scab caused by *Venturia inaequalis* (Cke. Wint.) is one of the most harmful diseases of apple trees. Reducing apple harvest due to scab infection is not less than 40 % in Central Russia. Breeding apple cultivars for scab resistance started in the USA [1]. Currently, it is held in the Czech Republic [2-4], Germany [5, 6], Poland [7, 8], Italy [9], Belarus [10, 11], Ukraine [12], and other countries.

Targeted large-scale program for breeding scab resistant apple varieties has been conducted in the All-Russian Research Institute of Breeding Fruit

Crops (RIBFC) since 1977. In this, hybridization in 2.4 million flowers has been performed including 2307 combinations, 452.9 thousand breeding seedlings have been studied at artificial infection, and 60.5 thousand seedlings have been transferred to breeding gardens in 1977-2014 [13, 14].

Triploid apple cultivars are characterized by less year periodicity of fruit-bearing, higher fruit weight and marketability, higher autogamy and scab resistance. Swedish scientists were the initiators of polyploid apple tree breeding. Back in the late 1930, H. Nilsson-Ehle [15, 16] was inspired by the idea of breeding triploid cultivars using hybridization of widespread diploid cultivars with tetraploid ones. It was noted that this approach should be considered as the entry into a new era of apple breeding [17, 18]. Later, however, these activities have not been well developed in Sweden.

History of the origin of columnar apple varieties is as follows. In 1964, in Canada, in the village of Kelowna (British Columbia), the owner of apple garden found an unusual thick branch in a 50-year-old McIntosh tree which resembled a stick as it had almost no lateral branches and was covered with spurs and hastulas with a large number of apples. As agreed with the owner, this unusual spontaneous mutation was duplicated and named Wijeik [19]. This natural mutation did not have the required productivity and could not be widespread as a commercial variety but became the initial form for breeding. Stunting, short internodes, and abundant foliage are typical for McIntosh Wijeik. Columnar apple varieties are currently bred in the UK, Russia, and many other countries [20, 21]. Columnar type of apples trees is controlled by gene *Co*. Further studies of columnar apple trees biology and economic value are of great interest. There are reports about using columnar apple tree fruits to produce concentrated juice [22].

We are the first in Russia to set an objective of developing genetic basis for breeding considering regional climatic conditions and improving the pre-existing approaches to develop genotypes that combine scab immunity (V_f), columnar type (*Co*), and triploidy.

The purpose of this study was to obtain and assess local immune, triploid, and columnar apple varieties to breed the forms to be used in superintensive gardens.

Technique. We used original apple tree forms with identified genes encoding resistance to scab (V_f, V_r, V_m), columnar type (*Co*), and diploid gamete donors (4×). The research was based on breeding and variety testing programs and methods which included recommendations for statistical processing [23, 24]. To develop new varieties, pre-breeding (preliminary estimation of the original varieties and forms, using the best sources and donors) was extensively used. Genetic diversity of the samples was achieved by hybridization, including distant and repeated hybridization. Culling was carried out in greenhouses with artificial infectious background and in nursery gardens.

Staining and examination of samples for cytoembryological study were performed by standard methods [25] using Nikon-50i microscope (Nikon Co., Japan).

Results. Improving the breeding technology for apple varieties, development and use of the methods for breeding intensification in the research made it possible to reduce the period of apple varieties breeding from 30-35 years to 20 or even 18 years.

S c a b i m m u n i t y. RIBFC is a pioneer in the development of scab immune apple cultivars in Russia. A total of 22 scab immune varieties have been developed in the Institute and included in the State register of breeding achievements. The best 10 of them are summery Maslovskoe (variety Redfree × variety tetraploid Papirovka), Yubilyar (from the seedling 814, open pollination), fall variety Solnyshko (from the seedling 814, open pollination) and winter varieties Afrodita (from the seedling 814, open pollination), Bolotovskoe (variety Skryzhapel × 1924), Ven'yaminovskoe (from the seedling 814, open pollination), Imrus (variety Antonovka obyknovennaya × OR18T13), Kandil orlovsky (from the seedling 1924, open pollination), Rozhdestvenskoe (Welsey × BM 41497), Svezhest (variety Antonovka krasnobochka × PR12T67). Their use makes it possible to say that the lag behind other countries in developing own immune apple varieties has been eliminated in Russia, although the breeding was initiated in our country 30-40 years later. The use of the developed immune varieties with gene V_f and the immunity donors with gene V_r and other genes is planned for further breeding along with obtaining the forms homozygous for genes V_f and V_r for their subsequent use as the donor to increase the resulting number of immune seedlings and immunity. In addition, a gene pool on the digenic immunity basis is being formed ($V_f + V_r$, $V_f + V_m$, $V_r + V_m$). Seedlings of this type have already been developed [26] and are currently under the breeding assessment.

Development of triploid varieties. Apple breeding for polyploidy was started in Russia at RIBFC in 1970. In this, 454 cross combinations (657.9 thousand pollinated flowers) have been performed and 42.4 seedlings have been grown over the period of 1970-2014. A total of 14.7 thousand seedlings have been transferred to breeding gardens after repeated culling in greenhouses against artificial infectious background and in nursery gardens.

Over the entire period, crosses with the following polyploid forms have been performed: (4×) × (4×); (4×) × (3×); (4×) × (2×); (3×) × (2×); (3×) × (4×); (3×) × (3×); (3×) × (2×); (2×) × (4×); (2×) × (3×). (2×) × (4×), and (4×) × (2×) were found to be the most valuable combinations for obtaining triploid seedlings. The greatest number of triploid seedlings were obtained as a result of (2×) × (4×) crossbreeding. This is due to the fact that when choosing a hybridization maternal parent (4×) as the diploid gamete donor, many tetraploid seedlings uninteresting for breeding and a small number of triploid forms are obtained due to its usually increased self-fertility. In case the (4×) × (2×) crossings are required for obtaining triploid seedlings, flower castration in a tetraploid maternal parent is needed.

Cytoembryological studies of the reproductive features in polyploid apple forms when assessing their suitability for heteroploid crosses helped to plan the crossing combinations and sample size, and predict the results.

Cytoembryological control in the polyploids' breeding has been conducted in RIBFC since 1976, and crossing forms of different ploidy started since 1970. Over this period, 455 crossing combinations have been tested, 659,615 flowers have been pollinated, 47,927 seedlings have been obtained, and 13,202 seedlings have been planted in the garden after culling. Ploidity has been determined in a total of 14.5 thousand plants of 430 hybrid families [27-29].

Meiosis in microsporogenesis was studied in 18 tetraploid forms, of which 5 were periclinal chimeras of the type 1 and 13 were homogeneous tetraploids. These 18 forms were divided into three groups according to the number of disturbances (Table 1).

In group I, the rate of disturbances in the microsporogenesis stages studied was 10-20 %, whereas in group II it was the greatest making up 20 to 40 %. In the smallest group III, the forms were characterized by the highest rate of disturbancies (40-60 %). Meiosis was most close to normal in the 25-37-45 (4×), Papirovka (4×), and 20-9-27 (4×) forms. In them, microsporocytes with proper division prevailed at all meiotic stages (Fig. 1).

1. Type and number of meiotic disturbances at successive meiotic stages in triploid apple (*Malus domestica* Borkh.) hybrids (All-Russian Research Institute of Breeding Fruit Crops)

Form	Number of meiotic disturbances by stages, %							Average number of meiotic disturbances in all stages, %
	MI	AI	TI	MII	AII	TII	tetrads	
Group I								
25-37-45 (4×)	19.8	10.5	3.8	8.4	5.3	11.8	15.2	10.7
Variety Papirovka (2-4-4-4×)	35.5	30.5	4.9	9.0	14.5	3.3	1.7	14.2
25-35-144 (4×)	11.6	34.9	7.2	38.9	15.7	8.2	5.1	17.4
25-37-47 (4×)	17.5	34.1	9.1	22.5	22.5	8.7	10.9	17.9
25-35-121 (4×)	22.4	19.9	5.2	11.9	23.1	12.0	31.7	18.0
20-9-27 (4×)	22.0	36.1	6.2	33.1	28.8	7.7	7.2	20.2
Group II								
Variety Spartan (4×)	24.4	32.9	11.6	29.0	24.2	14.2	28.0	23.5
13-6-106 (4×)	18.5	36.4	22.8	54.6	24.2	22.4	8.7	26.8
25-37-40 (4×)	31.7	33.0	18.3	43.6	45.8	23.5	11.7	29.7
25-35-120 (4×)	29.8	51.6	23.4	29.8	30.3	21.7	21.2	29.7
25-37-35 (4×)	37.7	31.9	14.4	43.0	22.9	27.9	44.4	31.7
Variety Walsey-M (2-4-4-4×)	72.0	33.1	22.4	43.4	28.6	6.4	18.6	32.1
Variety Giant Spy (2-4-4-4×)	63.4	34.0	13.8	53.9	39.3	16.2	7.5	32.6
Variety Melba (4×)	38.5	40.7	16.0	38.3	46.9	17.0	30.7	32.6
Variety Antonovka ploskaya (2-4-4-4×)	61.2	41.1	33.9	45.3	44.7	22.4	6.4	36.4
30-47-88 (4×)	38.7	44.1	19.9	51.3	32.5	27.2	46.4	37.2
Group III								
Variety Walsey-F (2-4-4-4×)	76.0	66.7	20.0	51.8	44.3	34.5	28.0	45.9
Variety McIntosh (4×)	75.5	51.3	25.6	51.2	55.6	72.0	96.7	61.1

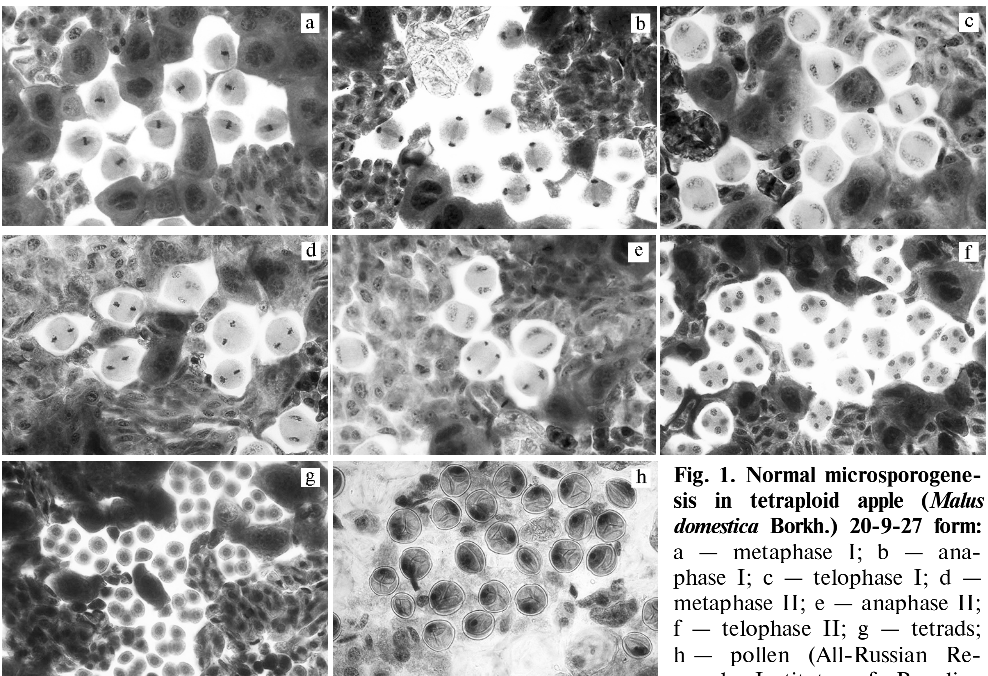
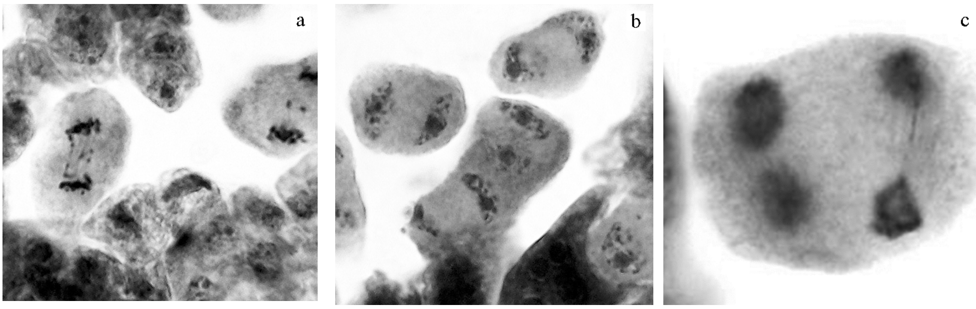


Fig. 1. Normal microsporogenesis in tetraploid apple (*Malus domestica* Borkh.) 20-9-27 form: a — metaphase I; b — anaphase I; c — telophase I; d — metaphase II; e — anaphase II; f — telophase II; g — tetrads; h — pollen (All-Russian Research Institute of Breeding Fruit Crops, staining according to S.G. Kaptar', 1967; microscope Nikon-50i, Nikon Co., Japan).

Figure 2 demonstrates several most typical disturbances in microsporogenesis in various tetraploid forms.

As a result of specific disturbances, a part of the pollen would contain a haploid number of chromosomes in some tetraploid forms. This should be considered when planning crossings with such forms.

In (2×) × (4×) crosses, 25-37-47, Welsey-F, Papirovka (2-4-4-4×), Giant Spy, and 30-47-88 forms were characterized by the highest resulting number of triploids in hybrid offspring. The fraction of triploid seedlings reached 84.0, 81.0, 77.6, 78.8, and 68.1 % in hybrid families with their involvement.



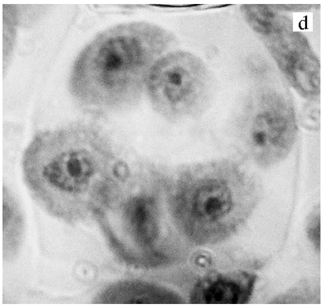


Fig. 2. Examples of typical disturbances at the stages of microsporogenesis in tetraploid apple (*Malus domestica* Borkh.) forms: a — anaphase I, retarded (30-47-88, 4×); b — telophase I, microsporocyte larger than normal (Melba, 4×); c — anaphase II, bridge (25-37-47, 4×); d — hexad (Spartan, 4×) (All-Russian Research Institute of Breeding Fruit Crops, staining according to S.G. Kaptar', 1967; microscope Nikon-50i, Nikon Co., Japan).

The new 30-47-88 (Liberty × 13-6-106) form is of the most interest. Seedling 13-6-106 was obtained from the seeds of a diploid variety Suvorovets planted in 1971. One of the 1957 seedlings was a homogeneous tetraploid. In 1994, the seedling was selected as a diploid gamete donor and used a father parent in crossing Liberty (gene V_f) × 13-6-106. Of the 31 seedlings obtained, a tetraploid seedling 30-47-88, the integrated complex donor of diploid gametes and scab immunity gene, was selected. The weight of its fruits is 180 g, appearance and taste are estimated at 4.3 points. The example described demonstrates that breeding some donors (pre-breeding) may require more time than obtaining variety itself.

Using this donor of diploid gametes and columnar forms, hybrid seedlings which combine columnar type (*Co*), scab immunity (V_f), and triploid set of chromosomes have been produced. Such seedlings have not been known in breeding practice until now. In the future, new varieties suitable for growing in the gardens of intensive type should be selected from this hybrid material.

A series of 15 triploid varieties, nine of which have already been included in the State Register of Selection Achievements (zoned) has been created in Russia and in the world for the first time using diploid gamete donors in different chromosome crossing of varieties and forms: Avgusta (Orlik × Papirovka tetraploidnaya), Aleksandr Boiko (Prima × Welsey tetraploidnyi), Bezhin lug (Severnyi sinap × Welsey tetraploidnyi), Daryona (Melba × Papirovka tetraploidnaya), Maslovskoe (Redfree × Papirovka tetraploidnaya), Orlovskii partizan (Orlik × 13-6-106), Osipovskoe (Mantet × Papirovka tetraploidnaya), Patriot [(Antonovka krasnobochka × SR 0523) × 13-6-106 (Suvorovets seedling)], Yablochnyi Spas (Redfree × Papirovka tetraploidnaya). Three of the nine triploid zoned varieties (Aleksandr Boiko, Maslovskoye and Yablochnyi Spas) have a triple set of chromosomes (3×) and scab immunity (V_f).

In some cases, it is possible to obtain scab immune triploid varieties from two diploid varieties, if one of the parents has the immunity gene (e.g., V_f) and the same or another parent tends to form unreduced gametes. Two scab immune triploid varieties, the Rozhdestvenskoe (Welsey × BM 41497 (V_f) and Yubilyar [(814 (V_f), open pollination] created by us and included in the State Register of Selection Achievements may be an example.

Columnar varieties. In recent years, gardeners have become familiar with columnar apple varieties belonging to a new plant biological form. Columnal varieties have a number of advantages. They make it possible to greatly intensify all the processes for fruit production, obtain large harvest as early as 3-5 year after garden foundation, significantly reduce and simplify manual labor for garden care, in particular to reduce or eliminate the work on cutting and forming tree crowns, and allow to greatly reduce the pesticide load in the garden and its surroundings.

Targeted breeding to create columnar varieties was started at RIBFC in 1984 [30, 31]. Over the entire period (1984-2014), 145 cross combinations has been tested, 184.8 flowers have been pollinated, 87.3 thousand normally developed seeds have been obtained, 33.1 thousand seedlings have been grown. Asa a result, 2717 seedlings have been transferred to breeding gardens after repeated culling. To date, two (Priokskoe and Sozvezdie) of the eight developed varieties (Vostorg, Girlyanda, Eseniya, Zelenyi shum, Pamyati Blynskogo, Poesiya, Priokskoe, and Sozvezdie) have been included in the State Register of Selection Achievements (zoned). Varieties Vostorg and Sozvezdie are passing state testing, the rest ones are under initial variety testing.

The best ones of the above columnar varieties may be donors for further breeding. In the future, it is attractive to obtain columnar triploid varieties genetically resistant to scab. A complex donor 30-47-88 (Liberty × 13-6-106), as both diploid gamete and scab immunity donor, is of particular value for this purpose.

Obtaining combined columnar, scab immune, and triploid apple forms

Columnar varieties and forms (with gene *Co*):
Valyuta, Priokskoe, Poesiya, Sozvezdie,
Stela, Strela, etc.

×

30-47-88 (gene V_f + 4×)
[Liberty (V_f) × 13-6-106 (4×)]

↓

Seedlings combing columnar type (*Co*), scab immunity (V_f), and triploidy (3×)

Due to such crossbreeding, 8885 seeds were produced and 2152 seedlings grown in 2010-2014.

Therefore, innovative approach in apple breeding made it possible to obtain triploid forms (3×) with scab immunity (V_f), columnar type (*Co* gene), and one form which is a diploid gamete and scab immunity donor at the same time. Using them in cross combinations would allow to create fundamentally new varieties with high competitive ability and a complex of essential commercial features that are not inferior to foreign varieties in the fruit yield, technological and consumer qualities, but are much superior in their adaptability.

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