V. A. Gavrilova* and I. N. Anisimova Genealogy of the Sunflower Lines Created on the Basis of Russian Varieties

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Abstract: America is the center of origin of the annual sunflower Helianthus annuus L. However, as an oil crop, sunflower was first cultivated in Russia. The seeds of first sunflower varieties contained 28-30% oil. The varieties with high oil content (47–51% oil in the seed), VNIIMK 6540 (k-1872), VNIIMK 8883 (k-1961), VNIIMK 8931 (k-1942), Armavirskii 1813 (k-1588), Armavirskii 3497 (k-1960), Armavirets (k-2116), and Peredovik (k-2051), were created based on the research work carried out by V.S. Pustovoyt, his associates, and followers. These and other domestic varieties have become basic for the lines created with the purpose of heterotic hybrid production on the basis of cytoplasmic male sterility (CMS) all over the world. The genealogy of lines is not always clear. At the same time, the material used by breeding institutions and companies is characterized by increasingly low genetic diversity. The knowledge on the genealogy of lines promotes fuller employment of the genetic potential of cultivated sunflower. The article reports the data on the lines created by the researchers of the N.I. Vavilov All-Russian Institute of Plant Genetic Resources (VIR) at the Kuban Experiment Station in the period from 1970 to 2015, as well as the data obtained by foreign researchers, which we are familiar with, on the lines obtained using Russian varieties. The lines were created by repeated self-pollination and progeny selection for morphological features, CMS, the ability to restore pollen fertility, the length of vegetation period, and resistance to downy mildew after each selfpollination event. As a rule, uniform lines were obtained 7–8 generations after self-pollination. The data on the genealogy of 38 lines created by the VIR researchers, 2 VNIIMK lines, and 16 foreign lines, as well as the information on the origin of the PET1 CMS source are presented.

Keywords: sunflower, lines, genealogy, domestic varieties, self-pollination

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Introduction

The cultivated sunflower originated from the wild-growing representatives of the annual species *Helianthus annuus L*. It seems likely that the ancestral *H. annuus* forms were tall with a single head (*i. e.* they had no branching) or with insignificant upper branching since this description is applicable to those plants whose seeds were found in archaeological excavations in a number of the American states, Mexico, and Canada. It is supposed that sunflower seeds were used as food by Indians. Iroquois extracted the oil and used it mainly to prepare paints for ritual ceremonies. The results of archeological digs suggest that the sunflower plant used by them had a large central head (7.5 cm in diameter) and several smaller ones. However, the evidence of the occurrence of unbranched forms exists as well (Gilmore, 1919). The sunflower was brought to Europe and placed in a botanic garden of Spain in 1510 after Columbus discovered America and became widespread as an ornamental plant. The cultivation of sunflower as an oilseed crop started in Russia after D.I. Bokarev performed his "industrial oil extraction" in 1861 in the Voronezh province. The first sunflower varieties Maslyanka (k-619), Uspenka (k-768) and others contained about 28–33% oil in the seed (Kuptsov, 1931). The first high oil varieties with 47–56 % oil in the seed were produced by V.S. Pustovoit and his followers (Morozov, 1964; Pustovoyt V.S., 1975). Domestic sunflower varieties, with high oil content, formed the basis of sunflower breeding world-wide, with the lines used in breeding for heterosis both in Russia and in other sunflower-growing countries where they were obtained. Some of the first varieties are maintained in the VIR collection. The development of sunflower lines by self-pollination of sunflower varieties started in our country in the 1930's (Plachek, 1930). The countries of the Eastern Europe and the USA (Friedt, 1992; Seiler and Rieseberg, 1997) became engaged in the development of sunflower lines based on Russian varieties upon the discovery of cytoplasmic male sterility (CMS) (Leclercq, 1966; Anashchenko, 1968) and the source of pollen fertility restoration (Kinman, 1970). Many lines were created at the VIR Kuban Experiment Station in the Krasnodar Territory by A.V. Anashchenko, V.T. Rozhkova, and F.K. Vilichku (Rozhkova and Anashchenko, 1977), and later by the authors of the current article.

The analysis of line genealogy makes it possible to assume what qualities a given genotype may possess, judging from the characteristics of the variety or the hybrid that was its ancestor. It is particularly important now that the breeders use the same source material when developing parental lines for hybrids which inevitably leads to the elimination of valuable alleles and loss of genetic diversity. It appears possible to recover the properties lost in the course of the line breeding if we go back to its origins. The present article reveals the genetic diversity of the lines whose ancestors were Russian sunflower varieties.

Materials and methods

To develop the lines, the domestic population varieties, available from the VIR collection whose genotypes contained the self-compatibility alleles revealed by self-pollination, were used. The disease-resistant varieties including Chernyanka 35 (k-1091), Sputnik (k-1755), and Progress (k-2233) were chosen. A series of lines was obtained using the high oil varieties with 47–55 % oil in the seed created in the All-Russian (former All-Union) Institute of Oil Crops (VNIIMK, Krasnodar), among which were VNIIMK 6540 (k-1872), VNIIMK 8883 (k-1961), VNIIMK 8931 (k-1942), as well as the varieties created at the VNIIMK Armavir Experimental Station including Armavirskii 1813 (k-1588), Armavirskii 3497 (k-1960), Armavirets (k-2116) and others. The high oil variety Peredovik (k-2051) created in VNIIMK which is characterized by disease resistance, increased seed yield, and increased plasticity, *i. e.* the ability to implement the hereditary potential under different conditions, was also used. All lines with high oleic acid content in the oil were obtained using the Pervenets variety (k-1798).

Inbred lines were obtained by repeated self-pollination of the population varieties. Genotypes capable of self-pollination were selected from the progeny obtained by self-pollination within the variety. The selection for the uniformity of morphological properties and the properties essential for breeding such as head inclination, head thickness, head attachment to the stem, and duration of the vegetation period, was performed after each self-pollination. At the first stage, self-fertile lines were obtained which did not segregate for morphological characters. Then the sterile analog of the line was obtained by repeated backcrossing with the PET1 CMS source provided by P. Leclercq. High inbred generation lines were studied using the techniques for the seed protein polymorphism analysis (Anisimova et al., 1991; Anisimova et al., 2003) and molecular genetic analysis. In particular, molecular markers specific for the aberrant mitochondrial gene orfH522 (Schnabel et al., 2008) and nuclear fertility restorer gene Rf1 (Horn et al., 2003) were used to define the cytoplasmon type (fertilesterile), as well as to detect the presence of the fertility restorer gene in the genotype of the line. Restorer lines were created by selecting the plants which carried the pollen fertility restorer genes through the use of pair crosses.

Results

It is possible to establish the genealogy of some domestic oilseed varieties with high oil content in the seed (Figure 1) through the analysis of the data for the years 1958–1961 provided by the State Commission for Testing Varieties. The plants characterized by increased oil content were found within the sunflower landraces growing in the former Mariupol District of the Ukrainian Soviet Socialist Republic. The repeated individual selection for oil content carried out in the former All-Union Research Institute of Oil and Essential Oil Crops brought to being the VNIIMK 1646 variety which was zoned in 1938. Oil content in the VNIIMK 1646 variety seed was as high as 40–46 % (Sunflower varieties, 1962). This variety served as basis for the development of many varieties with high oil content in the seed such as VNIIMK 1813 (Armavirskii 1813), VNIIMK 6540, and Armavirskii 3497 were bred from this



Figure 1: Genealogy of some domestic oilseed varieties with high oil content in the seed.

variety. The latter was used by P. Leclercq (Leclercq, 1966) in France as a pollinator in the crossing with *H. petiolaris* Nutt. Male sterility, which was later named CMS PET1 and which is up to date the main source of sterility for the production of industrial hybrids, was discovered in the BC4 generation of the cross performed in 1964 (Christov, 1999). VNIIMK 6540 was obtained as a result of the cross between VNIIMK 1646 and VNIIMK 1813, while, in turn, appeared to be the ancestor of the VNIIMK 8883 variety with the oil content in the seed ranging from 38 % to 45 %. The VNIIMK 8931 variety with the oil percentage of 43–48 % was produced by hybridization of the No. 5452 and VNIIMK 6540 varieties and the subsequent selection. The Peredovik variety with the oil percentage of 44–48 % was obtained from the hybrid VNIIMK 8931 × No. 6420.

Genealogy of some Russian sunflower varieties with high oil content in the seed

We can observe a gradual decrease in the plant height in the progeny obtained after self-pollination of the variety. Plant height remains stable after 5–8 generations of inbreeding, although the plants are much shorter than the original variety. The leaf size also decreases. The segregation of the forms that are phenotypically different from the original variety by branching, the color of leaf blade, leafstalk shape, etc. was observed in the second and the subsequent generations after self-pollination.

The Russian varieties Armavirskii 1813, Chernyanka, VNIIMK 6540, VNIIMK 8883, Yugo-Vostochnyi, Armavirskii 3497, Progress, etc., were used to create the first lines which were subsequently used in heterotic breeding, namely the American line HA113, from VNIIMK 1646, the VIR100, VIR101, VIR106 lines, from Armavirskii 1813, the HA62 line, from Armavirskii 3497, the VK lines (VK1, 3, 35, and 36), from VNIIMK 6540, etc. (Anashchenko *et al.*, 1992).

Lines obtained from the VNIIMK 8931 variety

The VNIIMK 8931 variety zoned in 1953 (Sunflower varieties, 1962) was resistant to the broomrape and sunflower moth races which prevailed at that time (97–100% armored achenes) and was characterized by 43–48% oil content in the seed, seed huskiness of 24–29%, and 1000 seed weight of 50–70g. Using the VNIIMK 8931 variety, Canadian crop breeders obtained a series of CM lines (CM303, 359, and 361) (Figure 2). Further in the United States, the CMS303 line was crossed with the PET CMS source to subsequently produce the CMS HA89 line which is today considered a standard in the sunflower breeding as well as in



Figure 2: Genealogy of the lines obtained on the basis of the VNIIMK 8931 sunflower variety.

the genetic studies (Friedt, 1992). A large number of CMS lines, valuable for breeding, were obtained on the basis of the HA89 line, with HA335, 336, 337, 338, and 339 being among them, while the fertility restorer lines on sterile basis (RHA340) were obtained via crossing HA89 with *H. argophyllus*.

In 1970 in the Institute of Oil Crops, Krasnodar, by treating VNIIMK 8931 seeds with nitrosomethylurea, K. I. Soldatov obtained a series of mutants which differed from the original variety in the plant height, duration of the vegetative period, and fatty acid composition of the oil (Soldatov, 1978). In the M3 generation, a mutant plant was identified by the oleic acid content which was 50.3%, with the oleic acid content in the VNIIMK 8931 variety being about 30 %. The oleic acid content in the progeny of this mutant averaged 72%. The first high-oleic variety, Pervenets, was created on the basis of this population (See Figures 1 and 2). Oleic acid prevents oil from oxidation, hence the oil obtained from high-oleic samples remains stable for a long time which is important for oil conservation and technical use, for the production of varnishes and paints. Pervenets is the only source of the high oleic character in the world (Fernandez-Martinez et al., 1979). The high-oleic CMS lines (HA341, 342, 343), fertility restorer lines (RHA344, 345, 346, 347, 348), and their derivatives were obtained on the basis of this variety in the United States (Friedt, 1992). The RHA345 line is the most often used as the male parent when producing industrial hybrids containing 70% oleic acid in the oil. The results of seed protein polymorphism and DNA polymorphisms analyses indicate that the RHA345 line possess the unique alleles of structural genes controlling synthesis of 11S globulin (helianthinin) and 2S albumin seed polypeptides. Lines with the changed morphological characters, for example, VIR125 (M24) and VIR708 were obtained on the basis of other mutant plants. The fatty acid composition of the oil from the seeds obtained from these lines shows no changes as compared with the original variety. The stem partition, in its middle part, into two halves with the formation of the two identical heads is a characteristic of the VIR125 line. This trait can be observed in 70 % of plants within the line. Plants with two heads appear in the progeny of both one-headed and two-headed plants. The trait expression depends on the environmental conditions: in rainy years, more plants with two heads are produced than in the droughty ones. The VIR708 line is distinguished by a very short leaf petiole so that the leaf seems sessile. All the traits discussed above are not characteristic of the VNIIMK 8931 plants and they appeared in different generations after the self-pollination of the plants treated with the mutagen. Both lines have the cytoplasmon of the fertile type, however the SCAR markers of the Rf1 gene restoring the PET1-type fertility were detected in the VIR125 line, while they are absent in the VIR708 line.

Lines obtained from the Peredovik variety

The lines obtained from the Peredovik variety differ in the plant height and duration of the vegetative period with little if any morphological differences.

The VIR lines, VIR310, 311, 312, and 313 (Gavrilova *et al.*, 2014), American CMS lines, HA277, 289, 300, 301, 302, 821, and 853, fertility restorer lines, RHA329, 330, 331, 332, 333, and 334, and Canadian CM607 and CM611 lines (Friedt, 1992) were obtained on the basis of Peredovik variety (Figure 3). All lines are stable and are characterized by good productivity. Interestingly, Peredovik turned out to be the source of the pollen fertility restorer genes for PET1 CMS. In this regard, it should be noted that high frequency of occurrence of the genotypes bearing the molecular markers of the *Rf1* gene (78%) was observed for the Peredovik variety population. Moreover, the Peredovik variety population examined was polymorphic for the allelic variants of helianthinin and methionine-rich 2S albumin SFA8.



Figure 3: Genealogy of the lines obtained on the basis of the Peredovik sunflower variety.

Lines obtained from the Armavirskii 1813 sunflower variety

The lines created on the basis of the Chernyanka, Progress, Sputnik, Armavirskii 1813 varieties (Figure 4) show significant differences in their morphological and economic properties. At the same time each line has its distinctive phenotypical traits (Anashchenko *et al.*, 1992). The lines differ in the size and shape of the leaf petiole (short, elongated, or erectoid) as well as in the size and color of the leaf blade. The VIR100 – VIR107 lines demonstrate no branching. By means of repeated crossings with the PET1 CMS source, the lines were made sterile, with the result of each of them being represented by the fertile and the sterile analogs. Individual lines (VIR104) are marked by polymorphic variants of seed proteins.



Figure 4: Genealogy of the lines obtained on the basis of the Armavirskii 1813 sunflower variety.

Lines obtained from the Progress sunflower variety

The Progress variety was created by G. V. Pustovoit on the basis of the interspecies hybrid obtained by crossing wild-growing *H. tuberosus* with cultivated sunflower (Pustovoyt G.V., 1975). Progress is resistant to many diseases affecting the sunflower including rust (namely to the pathogen races 2 and 5 according to the old classification of races) (Pustovoyt and Krokhin, 1978). The Progress variety is the ancestor of many VIR lines, VIR244, 245, 246, 247, 248, 249, 250, 285, 286, 287, 288, 365, 366, 411, 415, and 737) (Gavrilova *et al.*, 2014), as well as American lines, DM1, DM2, and DM3 (Friedt, 1992).

The genotype of the Progress variety represents such an abundant source of genetic variation that all the lines which were obtained from it by selfpollination differ phenotypically from each other (Figure 5). Many of them (but not all) are resistant to downy mildew, while VIR247, VIR249, VIR365, VIR415 show resistance not only to the old pathogen races (described before 1978), but also to the new ones (Nos. 300, 700, and 710), which appeared in the fields of the Russian Federation in the 1990s, alongside with the resistance to Phomopsis stem canker (Antonova et al., 2011). Lines differ by their ability to restore fertility of PET1 CMS pollen which indicates the differences in the allelic state of the *Rf1* locus. The presence of the dominant allele of the *Rf1* gene restoring the fertility of PET1 CMS plants was confirmed for certain lines using molecular markers. In particular, the sterile (PET1) cytoplasm type was described for the VIR249 and VIR365 lines using the orfH522 STS marker. Despite the absence of HRG01 and HRG02 SCAR markers closely linked to the *Rf1* gene in the genotype of the line, the presence of the dominant allele of this gene was confirmed by the results of test crossings and subsequent



Figure 5: Genealogy of the lines obtained on the basis of the Progress sunflower variety.

analysis of hybrids. At the same time, the VIR366 line, which maintained sterility in crosses, has the fertile type cytoplasmon with no *Rf1* gene markers present. The VIR249 line is the pollen fertility restorer as well, it is characterized by sterile cytoplasm and the presence of the SCAR markers of *Rf1* gene in its genotype. These facts indicate that, at least in the development of some lines, the methods of interlinear hybridization with the use of PET1 CMS lines as maternal forms were involved. The uniqueness of the lines obtained on the basis of Progress has also been confirmed by the data of the seed protein polymorphism analysis. For example, the VIR365 line bears a unique allele of the structural gene encoding the methionine-rich albumin SFA8 (Anisimova *et al.*, 2003).

Over the last years (2000-2015), new more aggressive races of the downy mildew pathogen and the broomrape (Orobanche cumana Wallr.), the floral plant parasitic for sunflower, have emerged. Hence, the search for sunflower genotype resistant to these races is a task of immediate importance. To achieve this goal, all available means should be exploited. The process of development of a breeding line should include the assessment of its resistance to diseases under the natural and artificial conditions, as well as using marker-assisted selection techniques. It is also necessary to screen the VIR collection. The analysis of the genealogy of breeding lines presented here will be also useful when addressing the resistance problem. In addition, it should be noted that plant breeders traditionally work with their own source material, being reluctant to use any new, creating new breeding lines by segregation of the hybrids already in use. This leads to the loss of genetic diversity (Sivolap et al., 1998; Popov *et al.*, 2002; Anisimova *et al.*, 2011) and the reduction of the adaptation potential of hybrids, and as a consequence to decreased resistance to diseases and adverse environmental factors. On the contrary, heterogeneity of the parental lines given high level of homozygosity in each of them contributes to the greatest possible levels of heterosis. The results of the seed proteins polymorphism analysis illustrate the genetic diversity of the lines created on the basis of domestic sunflower varieties. Modern varieties and breeding lines are characterized by the surprisingly monomorphic protein, while certain lines created on the basis of Russian varieties bear unique alleles of the helianthinin structural genes that do not occur in the breeding material. For example, unique alleles of the helianthinin coding genes were found in a number of lines (RHA340, RHA345, CMs44, VIR104, VIR130, VIR131, VIR302, and VIR369) including those mentioned in the present article (Anisimova *et al.*, 2004). Moreover, in some lines the unique alleles of the microsatellite (SSR) loci were revealed (Karabitsina et al., 2016).

Conclusions

The data on the genealogy of 46 Russian and foreign lines are provided. The origins of the following traits: high (over 50% oil in the seed) oil content, high oleic acid content in the oil, downy mildew resistance, and cytoplasmic male sterility were traced in the course of the analysis of the literature and authors' own data. For the first time, a plant with high oil content in the seed was found in the sunflower landraces growing in the former Mariupol District of the Ukrainian Soviet Socialist Republic, and the VNIIMK 1646 variety was obtained via repeated individual selection, which became the ancestor of the majority of Russian varieties, and further of the lines created all over the world. The only source of cytoplasmic male sterility which is intensively used for the production of industrial sunflower hybrids (PET1 CMS) was obtained by P. Leclercq in France by crossing *H. petiolaris* with the oilseed Armavirskii 3497 variety characterized by high oil content in the seed. The effective source of resistance to downy mildew is the Progress variety and the lines obtained on its basis. The source of high oleic acid content in oil (more than 70%), the Pervenets variety, was created on the basis of the mutant obtained from the VNIIMK 8883 variety characterized by high oil content in the seed. In practice, around the world, all high-oleic lines and hybrids have Pervenets in their genealogies.

Therefore, it should be assumed that Russian scientists and plant breeders have made a fundamental contribution to the development of heterotic breeding of sunflower.

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