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Sunflower Genetic Collection at the Vavilov Institute of Plant Industry

Abstract: The results of a long-term program aimed at discovering the hidden potential and genetic variation of sunflower accessions in a germplasm collection and the creation of a set of homozygous lines are presented. A genetic collection has different levels of development:

Level 1–homozygous lines with morphological characters are created;

Level 2–genetic control of the characters and segregation visually estimated under field conditions are studied;

Level 3–selection of lines homozygous for genes controlling biochemical characters; and

Level 4–the identification of characters using DNA markers is envisaged.

It is especially important that the VIR collection be actively maintained and used for genetic studies, gene mapping, as well as for creating cultivars and breeding lines to diversify the genetic base of cultivated sunflower.

Keywords: genetic collection, sunflower, genetic control, morphological characters, DNA markers

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Introduction

N.I. Vavilov recommended the use of self-pollination for revealing the hidden potential of variation in a wide range of diverse crops. He regarded inbreeding as an efficient method for analyzing polymorphism in cross-pollinated plants.

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Inbreeding in rye has helped to find new forms that had never been discovered, even in the major centers of rye diversity in Southwestern Asia (Vavilov, 1931). This method was used quite successfully during Vavilov' lifetime by Antropov and Antropova (1929) to reveal recessive mutations in rye. In the 1930s, a series of lines with modified morphological characters were produced by Plachek (1936) through self-pollination of a sunflower cultivar. Unfortunately, the majority of these lines have not survived. During the 1970s, the creation of a collection of sunflower inbred lines was started at VIR under the guidance of Anashchenko, A.V.

Production of homozygous inbred lines with certain characters has led to the establishment of genetic collections. According to Smirnov, V.G.: "A genetic collection is a collection of forms of the studied species which stably differ from the standard (wild) type in terms of manifestation of one or several characters" (Smirnov, 2005).

Intensive development of molecular-genetic mapping has demonstrated the importance of homozygous lines with characters of interest and their genetic control, adding to their value. A comparison of the data from crosses and analysis of segregation in F_2 hybrids (classical genetic analysis) with the data on molecular identification of genes yields additional information about the genetic control of a character. On the other hand, it is difficult and sometimes impossible to interpret results of sequencing or of molecular mapping when genetically uncharacterized material is used.

Genetic collections are not identical to character collections. The latter are composed of forms with continuous variation which is determined by genes for quantitative traits (Merezhko, 1994). The collection of 62 sunflower lines with tolerance to *Phomopsis helianthi* can be regarded only as a character collection at the present time due to the state of our knowledge. The existing assumptions concerning the mechanisms of resistance include, for instance, those that influence such factors as thickening of the cell wall, or pubescence of the stem and petiole (Antonova, 1999), or the influence of the duration of the vegetation period (Fick and Miller, 1997). Obviously, resistance to *Phomopsis* is controlled by several genetic systems and also depends on weather conditions during the period of infection spread and development.

The major method that we have employed in developing genetic characters has been by self-pollination of individuals. We have not used mutagens to develop any genetic characters. Sunflower accessions were screened for combining ability and self-fertility with the desired morphological and economically important characteristics. Individuals were self-pollinated no less than six generations. After that, both line multiplication and further inbreeding were performed. It was established that some seemingly homozygous lines can suddenly segregate new forms in the 5th, 6th, or even the 18th inbred generation. *cms* lines were produced by means of repeatedly backcrossing seven or eight times to a *cms* source received directly from Leclerq

(1969). Landrace-based self-pollinated lines were used as pollinators. Fertility restorer lines were produced in three different ways:

(1) introducing *Rf* genes into self-fertile lines;
(2) extracting lines from commercial hybrids by self-pollination; and
(3) selections of progenies from self-pollination of interspecific hybrids (Rozhkova and Anashchenko, 1977; Gavrilova et al., 2000).

Besides the materials resulting from own research, the genetic collection of VIR includes sunflower lines with information about genetic control of characters (supplied by Tolmachev, V.V. from the Ukrainian Institute of Oil Crops), as well as recombinant inbred lines (RILs) resulting from interspecific hybrids produced in France (INRA) and sent to VIR in the framework of GRESO (Groupe de recherche Est-Ouest sur le Tournesol; EWERG –East-West Research Group on Sunflower).

Lines with uniform morphological characters

The sunflower collection at VIR totals 2,780 accessions that include 2,230 accessions of cultivated (*Helianthus annuus* L.) and 550 of wild sunflower accessions belonging to 24 species (5 annual and 19 perennial).

The cultivated sunflower collection is represented by local varieties, landraces, and cultivars of national and foreign breeding programs, as well as populations collected during explorations. It also includes the first *cms*-based hybrids which are preserved as hybrid populations and lines. The collection is composed of lines developed as a result of studying interspecific hybrids, varietal diversity, and repeated self-pollination, or interspecific hybridization. The genetic collection includes 189 lines with different morphological characters; 120 fertility restorer lines; 20 *cms* lines and their fertile analogs; 46 lines with genes for resistance to races 330, 710, and 730 of downy mildew (*Plasmopara halstedii* (Farl.) Berl. and de Toni); and 90 of the 362 lines have seed storage protein markers for polymorphic variants.

Lines that show no segregation of morphological characters in several subsequent generations

We have obtained a series of natural mutants with different expressions of one and the same character. For example, some lines differ in terms of intensity of anthocyanin coloration of vegetative and generative organs exhibited by only one line, VIR 364. The most numerous is the group of lines with upper branching. Branches form in the upper third of the stem and may be short like in VIR 397, or long as in VIR 721. Lines also exist that branch throughout the stem

which may be compact (VIR 636) or spreading (VIR 702). Although genetic control of branching has been repeatedly investigated by several researchers (Putt, 1964; Kovačik and Škaloud, 1986; Miller and Fick, 1997; Gavrilova and Anisimova, 2003; Gavrilova *et al*., 2005), there does not seem to be a consensus of the gene control for the different branching types. This character is important in restoration lines for restoring pollen fertility in *cms* forms, as branching lines flower longer and produce more pollen, thus facilitating a longer period of *cms* pollination for commercial hybrid seed production. Some lines have very short petioles (VIR 708), or a very long reflected petiole (VIR 746), or no petiole at all (KG 49). Both plant habit and cultivation techniques depend on the petiole shape, especially at early stages of cultivar or hybrid development. Data on both recessive and dominant inheritance of Mendelian genes for the erect leaf character have been discussed by Gavrilova and Anisimova (2003).

The practical use of morphological characters with known genetic control such as the dark-green (*Gr*) and pale-green (*gr*) leaf color, white seed color, indentation and strong venation (*vs*) of the leaf blade, leaf blade knobbiness and asymmetry (*As*), erect petiole (*Er*), anthocyanin color (*A*), lemon (*l*) and orange (*la*) ray flower color are used as markers in heterotic breeding and for controlling line purity when they are maintained and multiplied for seed increase or during seed production (Gavrilova and Anisimova, 2003). The heterotic effects in commercial hybrid sunflower express itself in both seed yield and plant height. In order to produce hybrids with the optimal plant height (150–180 cm), dwarf lines can be used. A genetic analysis of the material obtained by crossing dwarf lines between themselves and a tall cv. Peredovik has identified three types of dwarfness. The first type is VIR 272 that acts in such a way that plant height is reduced due to the significant shortening of internodes, as well as increased internode number, hence extending the vegetation period (Table 1).

Table 1: Dwarf lines from the VIR collection at the Kuban Experiment Station of VIR, Krasnodar Territory, 1995

Line	Plant height (cm)	Internode length (cm)	Vegetation period duration (days)
VIR 501	60 ± 0.6	2.9 ± 0.03	80
VIR 272	62 ± 1.8	1.4 ± 0.04	108
VIR 648	66 ± 0.7	2.8 ± 0.03	82
VIR 328	68 ± 0.9	2.4 ± 0.04	90
VIR 319	75 ± 1.4	2.9 ± 0.07	90
VIR 253	82 ± 0.7	2.9 ± 0.03	88
VIR 500	105 ± 1.0	3.4 ± 0.05	90
VIR 649	106 ± 1.3	5.7 ± 0.07	92
Peredovik	200 ± 2.0	6.3 ± 0.07	94

Dwarfness in VIR 272 is determined by the *dw1 dw* 2 genes with intermediate inheritance and recessive epistatic interaction (Yesaev, 1998; Gavrilova *et al.*, 1999). A decreased internode length is due to the reduced cell size (Yakovleva, 2006). The average number of leaves in VIR 272 is 41 for about a 60-cm tall plant. The same number of leaves has been recorded for the tall cv. Gigant with plant height over 3.5 m. The second type of dwarfness is determined by the additive interaction of alleles of no less than three short stem genes (*sht1 sht2 sht3*) in VIR 319 and VIR 328. The third type of dwarfness is illustrated in VIR 253, VIR 500, VIR 501, and VIR 648. Control of this character is based on the polygenic action of no less than three semi-dwarf genes with intermediate inheritance (*sd1 sd2 sd3*). Triple and even larger stem shortening in the standard cv. Peredovik occurs at the expense of significant internode shortening. In this case, the number of leaves may be reduced to 15–17 (compare with 35–37 in cv. Peredovik) (Yesaev, 1998; Gavrilova *et al.*, 1999). The number of leaves and hence the number of internodes determines the duration of the "germination-to-flowering" phenophase, since formation of a larger number of leaves requires more time. Therefore, the lines with a large number of leaves have a longer vegetation period. All dwarf lines have smaller achene and root system than those in cv. Peredovik. However, the head size is not related to dwarfness, since the lines studied included VIR 319, VIR 328, and VIR 648 with a fairly large head (18–20 cm in diameter). The smallest head recorded was 8–10 cm for VIR 501.

The genetic collection of more advanced generation germplasm includes sunflower lines from the 5th–27th generation with all possible mutations of all morphological characters. As a result of our genetic analysis, 33 genes have been identified (Table 2) with genetic control of 16 morphological characters discovered in 18 lines from the collection (Gavrilova and Anisimova, 2003; Gavrilova *et al.*, 2005).

In order to determine the degree of resistance to new downy mildew races in sunflower lines in the VIR collection, genotypes resistant to this pathogen have been selected in the field based on observations performed over several years. They were tested in the immunity laboratory of the Pustovoit All-Russian Research Institute of Oil Crops (VNIIMK) for resistance to races 330, 710, and 730 which have spread in the Krasnodar Territory and Rostov Province in recent years (Antonova *et al.*, 2011). Forty-three lines were found to be resistant to race 330, 13 lines showed simultaneous resistance to two races, and 12 lines demonstrated resistance to three downy mildew races (Table 3). In addition to resistance to three downy mildew races and Phomopsis, VIR 249 can also restore fertility of the *cms* PET1 cytoplasm as a male parent when used for breeding commercial sunflower hybrids.

Table 2: Sunflower lines with genes identified by classical genetic analysis

No.	Line	VIR Catalog No.	Genotype
1	VIR 272	3515	*dw1 dw2*
2	VIR 319	3417	*sht1 sht2 sht3*
3	VIR 328	3475	*sht1 sht2 sht3*
4	VIR 253	3315	*sd1 sd2 sd3*
5	VIR 501	3508	*sd1 sd2 sd3 Wr1 Wr2 Gr3*
6	VIR 648	3420	*sd1 sd2 sd3 Gr1 Gr2 as1 as2*
7	VIR 340	3513	*gr1 gr2 gr3 a1 a2 p Vs*
8	VIR 130	2530	*P Br4Br5Br6 f1 f2 f3 vs*
9	VIR 448	3487	*A1 A2 ll pl*
10	VIR 536	–	*bl*
11	VIR 546	–	*a1 a2 LL LaLa*
12	VIR 729	3509	*Rfr1 Rfr2 Rfr3*
13	VIR 160	3220	*Rf1 Rf2 Rf3*
14	VIR 104	2504	*Hel*C_a
15	SM 144	2299	*HelCb*
16	VIR 131	2536	*HelAb*
17	VIR 302	3325	*HelAb*
18	VIR 708	3494	*Er*

Table 3: Sunflower geneticlines with resistance to downy mildew races 330, 710, and 730

No.	Catalog No.	Line	Origin
1	2793	VIR 172	Russia
2	3314	VIR 247	Russia
3	3338	VIR 387	Russia
4	3362	HA R4	Australia
5	3381	VIR 581	Russia
6	3497	VIR 702	Russia
7	3532	HA 89	USA
8	3623	RHA 278	USA
9	3635	VIR 632	Russia
10	3467	VIR 435	Russia
11	3570	VIR 800	Russia
12	3469	VIR 249	Russia

Source: Antonova *et al*. (2011).

Lines with certain uniform biochemical characters showing no segregation for several generations

The sunflower seed protein fraction includes two main components, namely the salt-soluble protein 11S globulin (helianthinin) and the water-soluble 2S albumins which differ by their molecular mass, amino acid composition, and physicochemical properties. The majority of lines in the genetic collection have been analyzed using 11S globulin enzyme electrophoretic banding pattern (Anisimova *et al.*, 1991; Anashchenko *et al.*, 1992; Anisimova *et al.*, 2004). Polymorphism of seed 2S albumins has been thoroughly studied in seven lines using a complex of biochemical methods (Anisimova *et al.*, 1995), polymorphism of SFA7 and SFA8 proteins, the main methionine-rich components of the albumin fraction investigated in 100 lines (Anisimova *et al.*, 2003), and polymorphism of *proteolytic enzyme* inhibitors studied in 70 lines (Konarev *et al.*, 2000).

The analysis of segregation in F_2 and F_a (F_a is $F_1 \times \text{Parents} = F_b$) populations from crosses of inbred lines differing in helianthinin composition has shown that polymorphism is determined by the allelic variation in at least three loci*: HelA*, *HelB*, and *HelC* (Table 2). The hybridological analysis identified polymorphic alleles in each of these loci (Table 4). It has been shown by dihybrid crosses that the HelA locus is inherited independently from *HelB* and *HelC*, while *HelB* and *HelC* demonstrated a linkage: the frequency of recombination in F_2 from two different cross combinations did not exceed 24%, while in the test cross [(VIR 130 × VIR 104) × VIR 130], the value was 19%. It confirms the localization of both genes in the same linkage group. In many cases, the presence of these alleles was associated with the line origin (Table 2). For instance, the presence of $HelC_c$ or

Table 4: Polymorphism of storage proteins in lines from the sunflower genetic collection

Line	Presence of polymorphic protein polypeptides[a]								
	9	11	12	29	30	33	34	$SEA8_n$	$SFA8_v$
VIR 104	+	−	−	−	+	+	−	+	−
VIR 122	−	−	+	−	+	+	−	+	−
VIR 130	−	−	+	+	−	+	−	−	+
VIR 131	−	−	+	−	+	−	+	+	−
VIR 302	−	−	+	−	+	−	+	+	−
VIR 369	−	−	+	−	+	+	−	+	−
SM44	−	+	−	−	+	+	−	+	−
i-469802	−	−	−	−	+	+	−	+	−

Source: Anisimova *et al.* (2004).
Note: [a]+, Polypeptide present; −, Polypeptide absent.

HelC$_b$ alleles indicated, as a rule, the presence of genetic material from wild forms. Such lines include VIR 104, HA61, RHA273, and RHA274. The *HelB*$_b$ allele, which is characteristic of helianthinin from the accession k-2266, was also found in the inbred lines created from it (Anisimova *et al.*, 2004).

Ninety inbred lines out of 188 analyzed possessed the characteristic helianthinin polypeptides. Besides the above allelic helianthinin variants, other electrophoretic variants were observed. Line VIR 387 lacked the major polypeptide band 10, while lines VIR 130, VIR 649, and VIR 302 lacked variant 4 in the helianthinin banding patterns.

The electrophoretic variant of the SFA8 protein was found in five lines (VIR 130, VIR 365, VIR 666, VIR 676, and VIR 262) which differed from the variants present in all other lines by its PAG mobility (Tris–Tricine–SDS electrophoretic buffer, pH 8.8) and isoelectric point mobility (Anisimova *et al.*, 2003). Codominant inheritance was observed in the F_1 from the cross VIR 130 × VIR 104, which was characterized by different variants of SFA8, while segregation in the F_2 showed that the normal and variant SFA8 were encoded by alleles at the same locus (Table 4).

The mutation that led to the appearance of the SFA8 protein variant has been identified. A comparative analysis of SFA encoding nucleotide sequences revealed that the protein is encoded by a small multigenic family. A single nucleotide substitution in the encoding region of the gene was found in the lines possessing the SFA8 variant. Such a substitution changes the molecule conformation, isoelectric point value, and, consequently, electrophoretic PAG mobility (Anisimova *et al.*, 2010).

Two types of inhibitors, trypsin (TI), and bifunctional inhibitors of trypsin/subtilisin (T/Sl) have been found in sunflower seeds (Konarev *et al.*, 2000). Inhibitor bands were found to be polymorphic in different inbred lines. The F_2 from a cross between VIR 670 × VIR 648 differed by the presence/absence of three different variants (a–c) of trypsin/subtilisin inhibitors (based on isoelectric focusing) has been analyzed regarding segregation at their encoding loci, i.e. *T/Sla*, *T/Slb*, and *T/Slc*. According to the results of hybridological analysis, all three loci are localized in one linkage group. The distance between *T/Sla* and *T/Slb* loci was 32% (in recombination units), while 23% between *T/Slb* and *T/Slc*.

Lines homozygous for phenotypical manifestation of certain characters and molecularly marked lines

Seventeen VIR *cms* lines have been produced from hybrids between a single source, *H. petiolaris* L. (*cms* PET1) obtained by Leclercq, P. in 1968 (Leclercq, 1969), and an unknown pedigree, *H. annuus* L. (Table 5). For instance, VIR 114

Table 5: Characteristics of fertile analogs of *cms* lines in the VIR collection, Kuban Station of VIR, Krasnodar Territory, 2012

Line	Catalog No.	Pedigree	Sprouting-to-flowering (days)	Sprouting-to-ripening (days)	Phomopsis infestation (%)	Productivity (g/plant)
Master (control)	3553	VNIIMK, Russia	60	101	8	71
VIR100	3443	cv. Armavirsky 1813, Russia	54	100	0	19
VIR101	3445	cv. Armavirsky 1813, Russia	57	99	0	30
VIR106	3447	cv. Armavirsky 1813, Russia	56	91	0	20
VIR109	2509	hybrid HS 52, Romania	62	110	69	19
VIR110	3449	hybrid HS 52, Romania	57	93	10	23
VIR111	3451	VNIIMK No 309	53	91	12	30
VIR114	3453	cv. Sputnik, Russia	48	84	0	–
VIR116	3455	cv. Vympel, Russia	55	90	8	26
VIR117	2517	cv. G-22, Russia	51	96	0	53
VIR129	2310	ZhS-17, Russia	54	91	0	54
VIR130	3595	k-2266, Germany	58	97	0	40
VIR137	3459	cv. Sputnik, Russia	52	92	0	–
VIR138	3461	cv. Vympel, Russia	51	90	44	16
VIR151	3463	k-2184, South Africa	55	96	0	16
VIR172	2793	k-705, Uzbekistan	50	88	16	30
VIR205	3465	Open pollinated population	57	97	0	38
VIR215	3295	VIR 111 × k-2266, Russia	56	95	7	10
VIR229	3304	hybrid VPBS-211, Yugoslavia	46	84	11	31
VIR340	3513	k-1933, Hungary	62	109	33	30
VIR434	3515	HA 378, USA	63	110	0	–
VIR435	3467	HA 335, USA	56	97	0	32
VIR436	3653	HA 336,USA	61	102	0	30
HA-89	2396	USA	60	96	0	17
VK-2086	3657	VNIIMK, Russia	60	98	5	19

was produced by crossing VIR 104 (*cms* PET) with cv. Sputnik (bred at the Armavir Station). Parental forms of VIR 104 are the *cms* source from Leclercq and cv. Armavirsky 1813. VIR 116 was created from an American line *cms* HA 234, which in turn had been obtained from a hybrid between a *cms* source from a wild sunflower from Texas and cv. Smena (bred at VNIIMK). The Vympel cultivar was used as the paternal parent for VIR 116. Pedigrees of both lines feature a *cms* source obtained from an interspecific hybrid between the wild annual and cultivated sunflower and also a Russian variety. The HA 89 line was from the USA, while VK-2086 was from VNIIMK.

The VIR genetic collection includes two other types of *cms*, RIG0 obtained from a perennial wild species, *H. pauciflorus* Nutt. (*rigidus*) (Cass.) Desf., and PEF based on *H. petiolaris* Nutt. *ssp. fallax* Heiser. Sterile analogs of VIR109 and VIR151 have been created based on *cms* PET1 and RIG0 (Gavrilova *et al.*, 2005).

The VIR collection contains 120 fertility restorer lines obtained using three different methods. In the first, lines were obtained through backcrossing self-fertile lines with a source of *Rf* genes and subsequent control of the progeny using paired crossings. The second and most common way of producing such lines is by extraction from commercial hybrids (Rozhkova and Anashchenko, 1997). Since commercial hybrids are produced based on *cms*, all fertile progeny have *Rf* genes and sterile cytoplasms (Table 6). The third method is from interspecific hybrid lines. As a general rule, interspecific hybrids are produced using sterile maternal lines of cultivated sunflower (Table 7). Therefore, that fertility restorer lines from interspecific hybrids also have a sterile cytoplasm.

The collection at the fourth level is represented by lines with *cms* and pollen fertility restoration genes (Table 8). The *atp9* and *orfH522* molecular markers (Schnabel *et al.*, 2008), which are specific for the mitochondrial genes associated with *cms* PET1, have been used to differentiate between the lines with fertile and sterile cytoplasm, as well as the difference between lines with *cms* PET1 (e.g. VIR 109, VIR 114, VIR 116, VIR 151, etc.) from *cms* of other types (Anisimova *et al.*, 2011). It has been established that many pollen fertility restorer lines with *Rf* genes have sterile cytoplasm (VIR 364, VIR 365, and VIR 558, Table 8), since they have been produced through self-pollination of commercial F_1 hybrids (Table 8). Sterile cytoplasm makes it possible to control the *Rf* genes when reproducing the line without additional crosses. Pollen fertility restorer lines, based on self-fertile lines (VIR 740 and others) through backcrossing with the restorer lines and the further testing of *Rf* genes in paired crosses, have fertile cytoplasm (Anisimova *et al.*, 2011).

Most lines restoring pollen fertility of *cms* have molecular markers (Horn *et al.*, 2003) for the Rf_1 gene. However, two lines (VIR364 and VIR 365), which restore pollen fertility in F_1 hybrids quite well, have been found during field

Table 6: *cms* PET1 pollen fertility restorer lines in the VIR collection

No.	Line	Catalog No.	Origin	Branching
1	VIR 183	3280	i-473670, Argentina	No
2	VIR 185	3285	k-2222, Armenia (local variety)	No
3	VIR 196	3286	SL 3376, Bulgaria	No
4	VIR 218	3297	VIR 113 (VIR 113 from Sputnik), Russia	No
5	VIR 220	3299	Yugoslavia, VPBS-211 (1981)	Lower
6	VIR 249	3469	Progress, Russia	No
7	VIR 260	3318	VIR 113, Russia	No
8	VIR 343	3477	ZhS-17 M, Russia	No
9	VIR 349	3503	VIR 113, Russia	Upper
10	VIR 358	3504	ZhS-17, Russia	Entire stem
11	VIR 364	3480	VIR 161, Russia	From mid-stem
12	VIR 365	3326	Progress x k-2699, Russia	No
13	VIR 376	3331	VIR 104, from Armavirsky 1813, Russia	Lower arched
14	VIR 377	3332	VIR 104 (from Armavirsky 1813), Russia	Lower
15	VIR 378	3333	VIR 104 (from Armavirsky 1813), Russia	Entire stem, compact
16	VIR 394	3481	Unknown	No
17	VIR 449	3527	Romania, possibly a hybrid	No
18	VIR 450	3434	RHA-271, USA	Entire stem, compact
19	VIR 558	3504	i-459886, Australia	Entire stem
20	VIR 580	3380	i-546502, hybrid	Lower
21	VIR 581	3381	RHA 278, USA	Entire stem, compact
22	VIR 582	3382	i-546513 hybrid, USA	Entire stem
23	VIR 583	3383	i-545789 RHA 340. USA	No
24	VIR 584	3384	i-548680, CM 611, Canada	Entire stem, compact
25	VIR 630	3437	SUNBREOL 265 hybrid, France	Entire stem

(*continued*)

Table 6: (*Continued*)

No.	Line	Catalog No.	Origin	Branching
26	VIR 631	3440	SUNBREOL 265 hybrid, France	Entire stem, compact
27	VIR 636	3441	SW509 × W 637 hybrid, France	Entire stem, compact, thin
28	VIR 641	3419	VIR 160, Russia	Upper
29	VIR 650	3385	i-535131, VIDEO hybrid, France	Entire stem, compact
30	VIR 651	3386	i-546504, USA	Upper
31	VIR 652		USA, RHA 265, i-546501	Entire stem, compact
32	VIR 653	3388	USA, i-545794	Entire stem
33	VIR 655	3422	SW509 × W 637 hybrid, France	Upper
34	VIR 656	3423	i-473692, Argentina	Entire stem
35	VIR 658	3424	i-441354 (from a wild population) BC3, USA	Entire stem
36	VIR 681	3505	VIR 160 × к 2266, Russia	Entire stem
37	VIR 699	3506	i-467063, USA	Entire stem, compact thin
38	VIR 700	3507	SW536 × W 635 hybrid, France	Upper
49	VIR 740	3528	Unknown	Upper
40	VIR 800	3570	k-3411, Finland, hybrid	Entire stem
41	VIR 801	3571	k-1039, Italy	Entire stem, spreading

Table 7: Pollen fertility restorer lines from the VIR collection obtained by interspecific hybridization

No.	Line	Catalog No.	Origin	Branching
1	VIR 756	3566	HA 232 × *H.* × *laetiflorus*	Upper
2	VIR 758	3554	VIR 129 × *H. floridanus*	Entire stem, compact
3	VIR 759	3555	HA 232 × *H. maximiliani*	Entire stem, compact
4		3557	HA 232 × *H. strumosus*	Upper
5	VIR 767	3560	VIR 151 × *H. trachelifolius*	Lower
6		3561	HA 232 × *H. mollis*	Upper
7	VIR 768	3568	VIR 151 × *H. maximiliani*	Entire stem, compact
8	VIR 770	3564	VIR 114 × *H. tomentosus*	No
9	VIR 777	3557	HA 232 × *H. strumosus*	No
10	VIR 800	3570	VIR 114 × *H. giganteus*	Upper
11	RIL 80	3598	83 HR4 × RHA345	Entire stem, spreading
12	RIL 130	3599	83 HR4 × RHA345	Entire stem, spreading

Table 8: Sunflower lines characterized using DNA markers associated with *cms-Rf* genetic system

No.	Line	VIR Catalog No.	Capacity to restore pollen fertility	*Rf1* gene markers (Horn *et al.*, 2003)[a]		Cytoplasm
				HRG01_454	HRG02_740	
1	VIR 101 PET1	3444	No	–	–	PET1
2	VIR 109 PET1	2509	No	–	–	PET1
3	VIR 109 RIG0	3648	No	–	–	X
4	VIR 114 PET1	3452	No	–	–	PET1
5	VIR 116 PET1	3454	No	–	–	PET1
6	VIR 151 PET1	3462	No	–	–	PET1
7	VIR 151 RIG0	3632	No	–	–	X
8	VIR 196	3286	Yes	+	+	PET1
9	VIR 249	3469	Yes	+	+	PET1
10	VIR 364	3480	Yes	–	–	PET1
11	VIR 365	3326	Yes	–	–	PET1
12	VIR 394	3481	Yes	+	+	PET1
13	VIR 558	3504	Yes	+	+	PET1
14	VIR 581	3381	Yes	+	+	PET1
15	VIR 637	3490	Yes	+	+	PET1
16	VIR 700	3507	Yes	+	+	PET1
17	VIR 740	3528	Yes	+	+	X or N

Source: Anisimova *et al*. (2011).
Note: [a]+, Marker present; –, Marker absent.

testing that do not have molecular markers for Rf_1. The absence of markers for these lines and the molecular-genetic analysis data on other Rf_1 donor lines allows one to speculate that other genes control pollen fertility restoration in genotypes of VIR 364 and VIR 365.

It has been observed that of the 38 analyzed Rf_1 gene donors, 4 are fertile based, 22 based on PET1, and 13 have sterile cytoplasm differing from PET1 (Table 8). All the mentioned lines restore pollen fertility in F_1 of hybrids with *cms* PET.

Conclusions

The aim of the present work was to create a genetic collection of sunflower that would include lines with identified inherited genes controlling a variety of phenotypic traits. Such a collection could be used as a reference when identifying the newly discovered genes. It is especially important that the VIR collection be actively maintained and used for genetic studies, gene mapping, as well as for creating cultivars and breeding lines to diversify the genetic base of cultivated sunflower.

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References

Anashchenko, A.V., Gavrilova, V.A., Anisimova, I.N., Rozhkova, V.T., Smirnova, N.G., 1992. Self-Pollinated Marked Sunflower Lines, Catalogue of the World Collection of VIR, St. Petersburg, pp. 1–26.

Anisimova, I.N., Alpatyeva, N.V., Gavrilova, V.A., 2010. Genotypic variation of the unique, methionine-rich protein SFA8 in sunflower. Genetics and biotechnology at the turn of millennia. *In*: Proc of the International Science Conference, October 25–29, Minsk, Belorussia, p. 6.

Anisimova, I.N., Fido, R.J., Tatham, A.S., Shewry, P.R., 1995. Genotypic variation and polymorphism of 2S albumins of sunflower. Euphytica 83(1): 15–23.

Anisimova, I.N., Gavriljuk, I.P., Konarev, V.G., 1991. Identification of sunflower lines and varieties by helianthinin electrophoresis. Plant Varieties and Seeds 4: 133–141.

Anisimova, I.N., Gavrilova, V.A., Alpatyeva, N.V., Ryabova, D.N., Rozhkova, V.T., 2011. Polymorphism of the *PPR-PFL*-genes homologous sequences in sunflower genome. Bulletin of Applied Botany, Genetics and Breeding 168: 64–79.

Anisimova, I.N., Gavrilova, V.A., Loskutov, A.V., Rozhkova, V.T., Tolmachev, V.V., 2004. Polymorphism and inheritance of seed storage protein in sunflower. Russian Journal of Genetics 40(9): 1215–1223.

Anisimova, I.N., Konarev, A.V., Gavrilova, V.A., Rozhkova, V.T., Fido, R.J., Tatham, A.S., Shewry, P.R., 2003. Polymorphism and inheritance of methionine-rich 2S albumins in sunflower. Euphytica 129(1): 99–107.

Antonova, T.S., 1999. Peculiarities of evaluation and selection of material for breeding for resistance to the main pathogens depending on the sunflower defense response. Senior doctoral dissertation (Biol.) author's abstract, Krasnodar, pp. 1–51.

Antonova, T.S., Ivebor, M.V., Rozhkova, V.T., Araslanova, N.P., Gavrilova, V.A., 2011. Results of evaluation of accessions from the VIR sunflower collection for resistance to downy mildew strains spread in the Krasnodar Territory. Bulletin of Applied Botany, Genetics and Breeding 167: 90–95.

Antropov, V.I., Antropova, V.F., 1929. Rye in the USSR and neighbouring countries. Appendix No. 36 to the Bulletin of Applied Botany, Genetics and Breeding, pp. 1–365.

Fick, G.N., Miller, J.F., 1997. Sunflower Breeding. Sunflower Technology and Production. ASA-CSSA-SSSA, Madison, USA, pp. 395–440.

Gavrilova, V.A., Anisimova, I.N., 2003. Genetics of Cultivated Plants. Sunflower, VIR, St. Petersburg, pp. 1–197.

Gavrilova V.A., Nizova, N.K., Tolstaya, T.T., Achtulova, A.M., Slyusar, E.L., 2000. Production, study and utilization of sunflower interspecific hybrid. *In*: Proc 15th International Sunflower Conference, Toulouse, France, Vol. 2, pp. 7–12.

Gavrilova, V.A., Rozhkova, V.T., Yesaev, A.L., 2005. Genetic Collection of Major Agricultural Crops. Sunflower. Identified Plant Diversity and Breeding, VIR, St. Petersburg, pp. 873–880.

Gavrilova, V.A., Yesaev, A.L., Rozhkova, V.T., 1999. Dwarf lines in the VIR collection. Bulletin of Applied Botany, Genetics and Breeding 156: 14–25.

Horn, R., Kusterer, B., Lazarescu, E., Prufe, M., Friedt, W., 2003. Molecular mapping of the *Rf1* gene restoring fertility in PET 1-based F_1 hybrids in sunflower (*Helianthus annuus* L.). Theoretical and Applied Genetics 106: 599–606.

Konarev, A.V., Anisimova, I.N., Gavrilova, V.A., Shewry, P.R., 2000. Novel proteinase inhibitors in seeds of sunflower (*Helianthus annuus* L.): Polymorphism, inheritance and properties. Theoretical and Applied Genetics 100(1): 82–88.

Kovačik, A., Škaloud, V., 1986. Collection of sunflower marker genes available for genetic studies. Helia 3: 27–28.

Leclerq, P., 1969. Une sterilite cytoplasmique chez le tournesol. Annales de Amelioration de Plantes 19: 99–106.

Merezhko, A.F., 1994. The Problem of Donors in Plant Breeding, VIR, St. Petersburg, pp. 1–128.

Miller, J.F., Fick, G.N., 1997. Genetics of Sunflower, Sunflower Technology and Production. ASA-CSSA-SSSA, Madison, USA, pp. 441–495.

Plachek, E.M., 1936. Sunflower breeding. Plant Breeding and Seed Production 8: 12–22.

Putt, E.D., 1964. Recessive branching in sunflower. Crop Science 4: 444–445.

Rozhkova, V.T., Anashchenko, A.V., 1977. Creation of self-pollinated lines and heterotic hybrids of sunflower on the basis of the global collection. Bulletin of Applied Botany, Genetics and Breeding 69: 53–55.

Schnabel, U., Engelmann, U., Horn, R., 2008. Development of markers for the use of the PEF1 cytoplasm in sunflower hybrid breeding. Plant Breeding 6: 541–652.

Smirnov, V.G., 2005. Importance of Genetic Collections for Fundamental Research and Genetic Programs. Identified Plant Diversity and Breeding, VIR, St. Petersburg, pp. 783–806.

Vavilov, N.I., 1931. Linnaean species as a system. Moscow-Leningrad, Selkolkhozizdat, pp. 1–32.

Yakovleva, E.A., 2006. Morpho-anatomical peculiarities of stem in dwarf sunflower forms (*Helianthus annuus* L.). Ph.D. dissertation author's abstract, VIR, St. Petersburg, pp. 1–19.

Yesaev, A.L., 1998. Genetic control of short stem character in some inbred lines of cultivated sunflower: Ph.D. dissertation author's abstract, VIR, St. Petersburg, pp. 1–21.