DE GRUYTER HELIA 2019; 42(71): 203–212

K.V. Vedmedeva*

Inheritance of Top Branching in Sunflower (*Helianthus Annuus* L.) Collection Samples

https://doi.org/10.1515/helia-2019-0001
Received January 07, 2019; accepted February 18, 2019

Abstract: Aim of our research was to study the genetic diversity and establish the inheritance of top branching trait in the collection of 34 sunflower lines of the Institute of Oilseed Crops of the NAAS.

Experiments were carried out in 2005–2016 according to classical cultivation methods, using manual castration, crossings, forced self-pollinating, isolation and visual assessment of the first and second generation of obtained descendants. The statistical reliability of the obtained ratio was confirmed by calculating the Pearson's chi-squared test.

Presence of two loci determining the inheritance of the branching trait in sunflower was established. In one locus, recessive alleles are responsible for manifestation of the branching trait. In the second locus, dominant alleles are responsible for the manifestation of the branching trait.

In 23 lines of sunflower, it was established that a recessive homozygote for one gene causes phenotypical top and full branching.

In 8 lines of the collection, full branching trait is due to the dominant allele of the gene. In the lines InK235, APS49, the presence of two genes was established, the dominant alleles of which determine full branching trait. In the APS56 line, full branching is controlled by the dominant alleles of three genes.

Keywords: gene, line, mutant, sunflower, trait, inheritance

Introduction

Sunflower is one of the most established crops in Europe. Its branching trait is used in the creation of hybrids and decorative varieties of sunflower. Knowledge of the genetics and diversity of this trait in collections provides predictable result of its use in breeding. Study of the genetic basis of this trait began in the last century. Hockett and Knowles (1970) in their studies

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***Corresponding author: K.V. Vedmedeva,** Laboratory of genetic resources, Institute of Oilseed Crops of the National Academy of Agricultural Sciences, Institutskaya Str., 1, settl. Solnechny, Zaporozhye region, 70417, Zaporozhye, Ukraine, E-mail: vedmedeva.katerina@gmail.com

described four types of branching: basal branching, top branching, full branching with without a central head, full branching without a central head. Basal branching was singled out by us in a separate type and the results of the study were published (Vedmedeva, 2018). Remaining three types of branching turned out to be more difficult to separate from each other. Hockett and Knowles (1970) reported on the presence of two dominant genes. The Br2 gene caused top type of branching, and when the Br3 gene was added, branching was observed along the entire stem. Škaloud and Kovačik (1978) confirmed the existence of two complementary dominant genes that cause full branching: one gene caused the presence of short branches along the full stem, and the joint action of two genes produced long shoots along the full stem.

Full branching due to the recessive allele of one gene was discovered by Putt (1964). In the research by Hockett and Knowles (1970) expression of two genes was established, the recessive allele b2 caused the expression of top branching, and with the combined effect of the recessive alleles of the two genes b2 and b3, branching was observed throughout the stem.

Škaloud and Kovačik (1978) in the study of branching characterized by a large central head found that it is caused by the recessive alleles of two genes, each of which does not have its own expression. Studying another source of branching, Kovačik and Škaloud (1990) reported the presence of two complementary genes, whose recessive alleles separately and together caused branching.

Sandu (Sandu *et al.*, 1999) studied several sources of branching caused by recessive alleles and reported on the discovery of a series of 7 recessive alleles of one gene causing branching, the manifestation of which differed in the number, length and angle of position on the stem of the lateral shoots.

Presently the sunflower genome has been sequenced, but known genes of morphological characters have been applied insufficiently to its genetic map. For the branching trait, it is only one gene, the recessive allele of which br1 causes the formation of top branching (Solodenco *et al.*, 2015).

Summarizing all the above, it can be noted that the number of genes (loci) that determine the branching in the top and middle part of the stem, as well as along its entire length, has not yet been established. Although of course there is no doubt to the presence of multiple allelism for these genes.

The collection of the Institute of Oilseed Crops of NAAS has more than 100 sunflower lines with different types of branching. Branching trait manifests itself in them very diversely, from full branching with multi-order lateral shoots, to one or two shoots, which are located in all parts of the stem.

Aim of our research was to study the genetic diversity and establish the inheritance of the top and full branching in the collection of sunflower lines of the Institute Oilseed Crops of the NAAS.

Materials and methods

Collection of branching sunflower lines in the laboratory of Genetics and genetic resources of the Institute of Oilseed Crops of NAAS includes over 100 samples. Among them are lines with different types of branching. The most difficult part was to make a correct description of the types of branching. In the beginning, we used location of the branches in the top, middle and lower parts of the stem, simultaneously described the level of development of the lateral shoots, denoting them from 0 to 3 in each part of the stem, and counted the number of first-order shoots. Over the years of our research, experiments were carried out with different planting densities, up to 120 × 120 cm. The weather conditions of some years of research were favorable while others were extremely dry. As a result of the accumulated experience of describing the branching trait in sunflower, we were able to separate the class of basal (lower branching) separately. It was characterized by the obligatory presence of shoots in the axils of two pairs of lower leaves and for the inheritance of which the publication was already presented (Vedmedeva, 2018). We did not separate all other types of branching and included studying their inheritance in this publication. In our collection there has always been a central head, which visually was larger or at the level of the others. Selected group of lines with branching trait comprised of 34 lines.

Experiments were carried out in 2005–2016. The lines were sown manually on the experimental fields of the Institute of Oilseed Crops with a density of 40 thousand plants ha^{-1}. Cultivation was carried out according to the classical methods (Aksenov *et al.*, 2013). Crossing was performed using manual castration followed by pollination with pollen from another plant (Tronin, 2017). Plants of the parental lines and hybrids of the first generation were isolated. Expression of the trait in parental lines, hybrids of the first and second generations was studied visually. Statistical reliability of the obtained ratio was confirmed by calculating the Pearson’s chi-squared test (Gomez, 1984).

Results and discussion

Morphological description of the branching trait we carried out by us in all the years of study. Expression of the branching in the individual studied lines was simple to describe by the number of branches, but this does not give a real picture of the genotype. This trait, as it turned out, is strongly influenced by growing conditions. Long-term observations were carried out on the manifestation of the branching trait in selected lines. Results of the observations are presented in Table 1. Descriptions of lines over 9 years under different growing conditions showed the presence of a significant variation in most of the lines characterized by branching. Table shows the number of side branches of the first order and the number of leaves on the main stem, and the branching formula, in which the degree of development of side shoots in the top, lower and middle parts of the stem is presented in points. Indicator 0 denotes their absence, 1 – weak shoots, 2 – moderate development of the lateral branches, 3 – strong development of the lateral branches, the size of the lateral heads is comparable with the central head.

The average number of side branches in the studied collection ranged from 4.4 (L3138) to 21.2 (VK580). Each of the lines has its own growth season and a different number of leaves. All the lateral branches of the sunflower that we observed came from the axillary leaf buds. Therefore, the number of leaves is just as important to assess branching. Span of its average was between 17.7 (AH70029Rf) and 31.1 (VK516) leaves. Lines with more or fewer leaves than those studied, are very rare in the collection. In the studied collection, correlation ratio between the number of side branches and the maximum, minimum and average number of leaves were calculated. The highest of them was the correlation between the minimum values of 0.35, which is explained by the limitation of the resources for plant development. The rest showed a complete lack of correlation. Variability span for each of the lines was very large, and the variation in the number of side shoots in different lines ranged from 31 to 72 %. And this is despite the fact that observations on plants for 9 years were included in the experiment, which had the same sowing density and its optimal duration. With such a variety in trait expression, it is clear that the number of side branches cannot be a reliable characteristic of the genotype.

According to the presented observations on the location of stems, in the top part of the plant the last two leaf axils were allocated, and the rest, except for two or three lower ones, were attributed to the median branching. This definition becomes clear when we consider also the number of branches and the number of leaves in any line. For example, take I4RHA274. In the most favorable conditions of 9 years of observation, it had 32 leaves and 25 branches. There were no branches for 7 axils, of which 2 were at the top and 5 were at the bottom. In the year with the harshest weather conditions, only 4 branches were observed with 14 leaves. In this case, the absence of branches in the 2 upper axils and lower 8 axils was also observed. If we

Table 1: Variability of number of side branches and leaves in branching lines (2009–2017).

Line	Number of side branches			variability	Number of leaves			variability	Branching formula		
	Max	min	average		Max	min	average		top	medial	lower
APS42	20.0	6.0	12.9	33.6	28.4	11.0	19.3	42.0	0–2	3	0
APS49	14.4	5.6	9.1	72.8	30.0	13.8	24.4	50.6	0	3	0
APS56	16.0	3.0	9.3	34.8	34.0	17.4	26.0	18.9	0–1	2–3	0
I2K439	24.0	4.0	13.6	31.6	31.0	19.0	26.0	12.3	0–1	2–3	0
InK235	14.0	4.0	8.3	65.5	31.0	13.4	21.6	39.8	0–1	2–3	0
K-103	15.8	2.0	7.6	38.2	31.0	17.0	24.6	14.8	0–1	2–3	0
AH70029Rf	15.0	2.0	7.9	44.5	23.0	10.0	17.7	18.4	0	1–3	0
HAR7	16.0	1.0	8.4	50.6	30.0	14.0	21.8	17.7	0	2–3	0
I2K87	24.8	4.0	15.1	37.3	33.0	15.0	25.4	18.7	0	2	0
I4RHA274	25.0	4.0	15.7	41.6	32.0	14.0	22.7	20.4	0	1–3	0
In7034	17.0	4.0	11.6	30.1	27.0	14.0	19.6	21.4	0	1–3	0
InK1724	15.0	1.0	6.1	57.9	38.0	15.0	25.5	22.7	0	1–3	0
InK630	18.4	4.0	10.8	37.5	35.0	13.0	22.7	19.0	0	2–3	0
KLV80/1	28.0	1.0	13.1	55.4	30.0	15.0	22.5	16.7	0	2–3	0
L3138	11.0	1.0	4.4	61.3	32.0	15.6	23.8	19.5	0	1–3	0
M19	25.0	3.0	16.1	35.1	30.0	15.0	25.4	16.0	0	2–3	0
M23	26.0	4.0	16.2	41.5	35.0	21.0	26.3	13.3	0	3	0
M17/1	32.0	3.0	19.2	33.0	35.0	17.0	26.3	14.1	0	2–3	0
355,114 r2-4	11.2	9.0	10.0	9.0	22.0	20.8	21.3	2.3	0	2–3	0
VIR369	23.8	10.4	16.8	27.8	27.8	19.6	23.0	13.7	0	3	0
VK516	21.0	4.0	9.6	48.2	37.0	18.6	31.1	13.7	0	2–3	0
VK580	26.0	4.0	21.0	22.3	33.0	18.5	26.6	16.3	0	2–3	0
ZL7034V	23.0	5.0	12.2	43.5	31.0	11.0	22.5	34.6	0	3	0

(*continued*)

Table 1: (*continued*)

Line	Number of side branches			variability	Number of leaves			variability	Branching formula		
	Max	min	average		Max	min	average		top	medial	lower
KG49	22.0	5.0	12.1	47.6	31.6	9.0	19.0	35.7	0	2–3	0
ZL2554V	23.5	5.0	14.0	42.9	25.2	17.0	22.0	12.9	0	2–3	0
HA298	11.0	4.0	6.9	27.1	30.0	15.2	21.6	21.3	0	3	0
RHA297	24.0	9.0	18.5	27.3	28.0	22.0	25.3	8.2	0	2–3	0

also consider the previous studies with this line with different plant stand density (Vedmedeva, 2007), we see a clear increase in the number of branches, but in all cases the two top and the two lower axils did not participate in the branching in the presence of a normally developing central head. When this line was thickened to 25,000 plants per hectare, a decrease in the number of branches located in the lower, more shaded part of the stem was observed. A similar picture was observed in many other lines. The exception was a few lines, in which the upper two axils of the leaves under the head, and sometimes on it had branches. The manifestation of this trait was not regular and was always observed against the background of strong branching in the rest of the middle part of the stem. These were the lines: APS42, APS56, I2K439, InK235.

Summarizing the obtained observations of these 34 lines, we can say that branching trait found in them can be described as medial, but researchers Škaloud and Kovačik (1978) still denoted it as top branching, probably due to studying it in more favorable conditions than ours, and we will also continue to denote this branching as top.

All 34 lines were included in crosses with lines without lateral shoots to establish the type of inheritance of this branching. Several lines were used as the latter ones: Temr1254, VA1, KR2, ZL169, ZL22, LVO7, KP11. The first generation hybrids were divided according to the phenotype into branching and non-branching. In each combination, at least 10 hybrid plants were studied. Four of them were isolated and self-pollinated to obtain a second generation. According to the type of hybrids obtained, the branching lines were divided into two groups. The first consisted of 23 lines, for which hybrids of the first generation had no branches; and the second consisted of 8 lines, for which hybrids of first generation had branches.

Further study of the second generation hybrids in the first group showed ratio between two classes of 3 non-branching to 1 branching. In total, in the first group, 35 combinations of crosses with branching lines M30, In7034, M19, ZL678B, RHA274, L2563, RHA297, InK87, InK1724, APS35, ZL2554, L2094-13, InK561-2, L2544, KG49, VIR369, KLV80/1, VK580, NAR7, HA298, L3138, M23, I2K670. Number of descendants of the second generation for each combination ranged from 77 to 368 plants. Pearson’s chi-squared test results for these combinations ranged from 0.001 to 2.46 while the table value of the Pearson’s chi-squared test for the two classes is $\chi^2_{0.5(k=1)} = 3{,}84$.

Next, genetic identification of the 23 lines mentioned above with recessive monogenic control between them was carried out. It consisted of crossing lines from this group with each other. Due to the mismatch of the timing of flowering, only 45 out of all the possible combinations were obtained. They included three main maternal lines that were crossed with each other. All hybrids of the first generation had the branching trait.

Other part of the first generation hybrids from crosses of non-branched lines with 11 branching lines, which had the branching trait, resulted in different second generation ratios. Results of these 16 crosses are presented in Table 2.

The results in the Table 2. showed that most of the second-generation cross combinations had ratio of 1 part non-branching to 3 parts branching. These are the lines: APS24, APS30, BK516, InK103, APS42, InK439, In355111, InK630. The second generation from crossing lines InK235, APS49, APS56 showed a smaller ratio of non-branching offspring, which was 1/15 or 1/63.

Table 2: Inheritance of top and full branching (2009–2017).

Cross combination (trait of parent line)	F_2 phenotypic classes		Total	Expected ratio	χ^2
	no branching	branching			
ZL169 × APS24 (branching)	64	185	249	1:3	0.07
ZL169 × APS30 (branching)	57	175	232	1:3	0.02
ZL169 × BK516 (branching)	52	177	229	1:3	0.64
MB4 × InK103 (branching)	32	109	141	1:3	0.40
APS42 (branching) × VIT4	67	183	250	1:3	0.43
ZR169 × APS42 (branching)	76	220	296	1:3	0.07
InK439 (branching) × InK2218	45	120	165	1:3	0.45
InK439 (branching) × KR1	29	63	92	1:3	2.08
LVO7 × In355111 (branching)	53	187	240	1:3	1.09
InK630 (branching) × Temr1254	42	114	156	1:3	0.31
Temr1254 × InK630 (branching)	33	97	130	1:3	0.01
InK630 (branching) × MV4	39	115	154	1:3	0.01
Temr1254 × InK235 (branching)	35	100	135	1:3	0.06
I2K2003-1 × InK235 (branching)	7	137	144	1:15	0.47
LVO7 × APS49 (branching)	2	60	62	1:15	0.97
10,362 × APS56 (branching)	2	163	165	1:63	0.13

Note:$X^2_{0.5\ (k=1)} = 3.84$

Paired crosses were made between the lines with the dominant branching type: InK439, AH70029Rf, InK235, APS 56, InK103. As a result, there were no plants in the progeny of the second generation without branching in a sample of at least 160 plants. This indicates the identity of the gene that causes branching.

In order to clarify the inheritance of the branching trait between two groups of lines, crosses were made between the lines, in which the branching is due to the dominant and recessive alleles of the genes. In the first generation, all plants had branching. In the second generation of InK630 x In7034, ratio between branching 172 plants and non-branching 48 plants was observed. Pearson's chi-squared test for the 13:3 ratio was 1.36, and for the 3:1 ratio – 1.19 while table value for the two classes is $\chi^2_{0.5(k=1)=}3{,}84$. Control of plant crossing was carried out using morphological markers. The line In7034 has a recessive trait of a band-shaped ray flowers. Number of descendants of the second generation in other crossings was not sufficient.

Considering the small number of descendants and cross combinations with branching lines which are controlled by more than one dominant allele, we cannot assert the exact number of dominant alleles or genes. The results obtained can only indicate presence of at least two different loci whose alleles cause the manifestation of the trait of the top and full branching. In one locus, the branching trait is due to dominant, and in the other locus due to recessive alleles.

First locus, the recessive alleles of which cause expression of top and full branching was found in a large group of lines M30, In7034, M19, ZL678B, RHA274, L2563, RHA297, InK87, InK1724, APS35, ZL2554, L2094-13, InK561-2, L2544, KG49, VIR369, KLV80/1, VK580, HAR7, HA298, L3138, M23, I2K670. This list is updated annually and most likely it is possible to include most, if not all, branching paternal forms used in modern hybrids.

In second locus, the dominant alleles caused the appearance of branching trait and it was found in lines: APS24, APS30, InK103, VK516, APS42, InK439, In355111, InK630. Digenic and trigenic control of dominant alleles was determined for the InK235 and APS56 lines. But a detailed study of such a large number of dominant genes for our samples was not possible.

Results of the study of branching inheritance confirm Putt's (1964) data on the presence of a single gene whose recessive allele causes full stem branching but mostly in the upper part of the plant. We could not single out second gene in the studied collection, recessive allele of which participates in full stem branching, reported by Hockett and Knowles (1970). Two dominant genes we identified that control total branching, confirm data by Škaloud and Kovačik (1978). In the APS56 line, ratio indicated presence of three genes, the dominant alleles of which cause full stem branching. This fact does not contradict, but complements the information on genetic control of the branching trait in sunflower.

Conclusions

The sign of branching is determined by one gene in a recessive state or genes in a dominant state in an amount of from one to three.

In 23 lines of sunflower, it was established that a recessive homozygote for one gene causes expression of top and full branching.

In 8 lines of the collection, the trait of full branching is due to the dominant allele of the gene. In the lines InK235, APS49, presence of two genes was established, the dominant alleles of which determine full branching trait. In the APS56 line, the full branching trait is controlled by the dominant alleles of three genes.

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