

## Research Article

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# Inheritance of the number of ray flowers in sunflower

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**Abstract:** The number, shape, size and color of the sunflower ray flowers are widely used as marker traits in hybrid seed production, for identification of genotypes, in ornamental floriculture. However, there is not enough information about the genetic control of these traits. The inheritance of the number of ray flowers and their absence on inflorescences was studied in cultivated sunflower. In the first case, two inbred lines of mutant origin with a contrasting manifestation of the studied trait were crossed. The  $F_1$  hybrid held an intermediate position between the parents, but approached the parental line with a large number of ray flowers. The average value of the number of petals in  $F_2$  was close to  $F_1$ . The study of segregation in  $F_2$  showed that the trait “number of ray flowers” is inherited polygenically and controlled by three pairs of non-allelic genes with additive effects. The inheritance of the absence of ray flowers was studied by crossing a line with petals and an apetalous accession.  $F_1$  hybrid showed ray flowers, and in  $F_2$ , segregation close to 42 (with ray flowers): 22 (without ray flowers) was observed. This implies participation of three non-allelic genes in the control of this trait, one of which in a recessive state suppresses the action of two other recessive genes. The revealed patterns of inheritance expand the information in the field of private genetics of sunflower and allow performing more purposeful breeding of this crop.

**Keywords:** apetalous character; epistasis; inheritance; number of ray flowers; sunflower; three-locus control.

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## Introduction

Sunflower (*Helianthus annuus* L.) is an important oilseed crop that is widespread throughout the world and occupies a leading place among oilseeds in the Ukraine. This crop is the main export of the country's oilseeds. In recent years, the area of sunflower cultivation in the Ukraine has been more than six million hectares.

The multi-flowered sunflower head, which is characteristic of many representatives of the *Asteraceae* family, has two types of flowers – tubular and ray or ligulate. First, the ray flowers start blooming, and then the tubular ones.

The ray flowers are important in the life of this plant. All members of the genus *Helianthus* have one or another number of petals, but none of them is petalless (Rogers et al. 1982). It is believed that the transformation of a wild sunflower into a cultivated plant contributed to an increase in the number of ray flowers, the size of flowers and the diameter of the inflorescence (Radanovic et al. 2018). As a cross-pollinating plant, the sunflower needs insects for pollination, the main of which are bees. And various traits of ray flowers can significantly affect the trophic behavior of these insects.

The number, shape, size and color of ray flowers are used as marker traits in hybrid seed production. Various traits of ray flowers are also important for identifying sunflower genotypes (Hladni et al. 2017; Vedmedeva and Soroka 2016).

It is known that sunflower is widely used as an ornamental plant. From the point of view of ornamental value, color, shape and number of ray flowers are the most significant traits. The diversity of these traits greatly expands the ornamental sunflower market (Divita et al. 2012).

While the inheritance of a number of ray flower traits in sunflower, such as, for example, coloration, has been studied well enough (Tolmachev et al. 1998; Zhang et al. 2008), there is very little information regarding their quantity or apetality.

The aim of our research is to study the inheritance of the number of ray flowers, as well as their absence on inflorescences in cultivated sunflower.

## Materials and methods

The research was carried out in 2019–2020. The material used were three mutant sunflower lines of Zaporozhye breeding (ZL1679-M, ZL95-M and ZL9-M) with a contrasting manifestation of the trait “number of ray flowers”, as well as  $F_1$  and  $F_2$  hybrids obtained from crossing these lines. The above-mentioned lines were selected as a result of the treatment of immature embryos or seeds with the chemical mutagen ethyl methanesulfonate. The indicated lines made up two pairs of crossing – ZL95-M  $\times$  ZL9-M and ZL95-M  $\times$  ZL1679-M.

The ZL1679-M line was developed on the basis of the ZL1679 line. It was characterized by the complete absence of ray flowers, although in some cases from one to four ray flowers on the inflorescence were observed. The ZL95-M line with a reduced number of ray flowers was obtained after mutagenic treatment of immature embryos of the ZL95 line. It also carried a number of other altered traits, such as leaf shape and plant height (Soroka and Lyakh 2009). The ZL9-M line significantly exceeded the ZL95-M line in the number of ray flowers. In addition, it had a mutant trait of leaf venation (Soroka and Lyakh 2015).

The seeds of the mutant lines and  $F_1$  and  $F_2$  hybrids were simultaneously sown in the field, and during the flowering period the number of ray flowers on each plant was counted. In total, 10 plants of each line and  $F_1$  hybrids and two segregating  $F_2$  populations of each cross combination were analyzed.

The correspondence between the actual segregation ratio in  $F_2$  populations and the theoretically expected segregation was determined by the  $\chi^2$  method (Griffiths et al. 2004).

## Results and discussion

### Inheritance of the trait “number of ray flowers”

The inheritance of the number of ray flowers was studied in a ZL95-M  $\times$  ZL9-M crossing combination with a contrasting manifestation of this trait in the parents (Table 1, Figure 1).

As can be seen from Table 1, in the ZL95-M line, the number of ray flowers varied from 21 to 32 with an average value of 25.2. The other parental line had 44–54 petals. In  $F_1$  hybrid plants, the trait varied from 38 to 45.

Differences in the average number of ray flowers between the parental lines were significant. Considerable differences were also observed between the  $F_1$  hybrid and both parental lines. At the same time, the  $F_1$  hybrid occupied an intermediate position between the parents, although it approached the parental line with a larger number of ray flowers. The  $F_2$  mean was close to  $F_1$  and intermediate

**Table 1:** The number of ray flowers in two inbred lines with a contrasting manifestation of the trait, and in  $F_1$  and  $F_2$  plants, pcs.

Genotype	Number of plants	Minimum	Maximum	Mean value
ZL95-M	10	21	32	$25.2 \pm 3.04^{\#}$
ZL9-M	10	44	54	$48.3 \pm 3.43$
$F_1$	10	38	45	$40.1 \pm 1.21^*$
$F_2$	413	7	70	$41.3 \pm 0.17$
$F_2$	212	10	68	$41.0 \pm 0.35$

\*Differences from ZL 95-M and ZL 9-M lines are significant at  $P \leq 0.001$  and  $0.05$ , respectively;  $^{\#}$ Differences between the lines are significant at  $P \leq 0.001$ .



**Figure 1:** Inbred lines of sunflower with a contrasting number of ray flowers and  $F_1$  hybrid (from left to right): ZL95-M,  $F_1$  ZL95-M  $\times$  ZL9-M, ZL9-M.

between the parents. This is in line with expectations in the case of polygenic inheritance where several genes with an additive effect are involved.

In the  $F_2$  segregating population of 413 plants, the number of ray flowers varied from 7 to 70, which significantly exceeded the variability of both parental lines and the  $F_1$  hybrid. In that case, plants appeared with both a smaller number of ray flowers than in the ZL95-M line and with a larger number of ray flowers than in the ZL9-M line (Table 1). In addition, the distribution curve for the  $F_2$  hybrid plants by the number of petals was bell shaped with one peak.

It was assumed that the trait “the number of ray flowers” is controlled polygenically with the genes acting in an additive fashion, and the parents in this crossing combination differ in three pairs of genes. Then this crossing can be written down as  $a1a1a2a2A3A3 \times A1A1A2A2a3a3$ . In this case, the expected segregation ratio in  $F_2$  will be 1:6:15:20:15:6:1. Table 2 presents the empirically derived phenotypic frequencies in two segregating populations resulted after self-pollination of two different  $F_1$  plants.

**Table 2:** Inheritance of the number of ray flowers in sunflower.

Total $F_2$ plants	F <sub>2</sub> phenotypes, number of ray flowers							Segregation ratio tested	$\chi^2$ (P-value)
	0–11	12–22	23–33	34–44	45–55	56–66	67–77		
Population 1									
413	3	25	90	145	120	27	3	1:6:15:20:15:6:1	19.67 (0.003)
Population 2									
212	2	13	41	81	60	14	1	1:6:15:20:15:6:1	12.91 (0.05)

$$\chi^2_{05} (df 6) = 12.59; \chi^2_{001} (df 6) = 22.46.$$

According to the hypothesis put forward, in  $F_2$  sample of 413 plants, the following ratio of phenotypic classes should be expected – 6:39:97:129:97:39:6. The resulting ratio is 3:25:90:145:120:27:3. The calculated chi-square value is 19.67, which is less than the acceptable chi-square value for six degrees of freedom and 0.1% significance level (22.45).

Since the obtained  $\chi^2$  value is less than its permissible value, it can be assumed that this segregation corresponds to the 1:6:15:20:15:6:1 model and the trait is under the control of three pairs of non-allelic genes that interact additively.

The actual ratio of phenotypic classes in the second  $F_2$  segregating population also corresponded to the theoretically expected ratio ( $\chi^2 = 12.91$ ) and did not contradict the accepted hypothesis about the control of the trait “number of ray flowers” by three genes with additive effects. The difference in segregation between the expected and obtained data in two analyzed populations is explained by both random reasons and, possibly, the presence of non-additive interactions between genes. The existence of non-additive interaction can be grounded by the fact that the  $F_1$  hybrids were closer to the parent with a greater number of ray flowers than with less number.

## Inheritance of the trait “absence of ray flowers”

The inheritance of the “absence of ray flowers” trait was studied in a ZL95-M  $\times$  ZL1679-M crossing combination where the maternal line was characterized by the presence of ray flowers, and the paternal line was characterized by their absence.  $F_1$  hybrids showed ray flowers ( $36.4 \pm 0.86$  on average), but in a much larger number than the maternal line (Figure 2). On the one hand, this indicated the recessive nature of the trait “absence of ray flowers”; on the other hand, it indicated the existence of some interaction between genes with respect to the number of ray flowers.



**Figure 2:** Inbred sunflower lines with the presence and absence of ray flowers and  $F_1$  hybrid (from left to right): ZL95-M,  $F_1$  ZL95-M  $\times$  ZL1679-M, ZL1679-M.

In the first  $F_2$  segregating population, the proportion of plants with a complete absence of ray flowers, as well as plants with 1–4 flowers, was rather high and amounted to 41.6%, which is close to the ratio 42 (with ray flowers): 22 (without ray flowers). This is confirmed by the calculated chi-square value (3.24), which does not exceed the standard value of 3.84 (Table 3). Hence, apparently three pairs of genes with the epistatic action for one of them are involved in the determination of this trait.

It can be assumed that the absence of ray flowers in the ZL1679-M line is due to two recessive genes with independent action. Moreover, the third gene present in this line does not suppress the action of the other two. The third gene, which is capable of suppressing the manifestation of those recessive genes, is obviously present (in a recessive state) in another parent component. Then this crossing can be written as follows:

$$\text{♀AABBcc} \times \text{♂aabbCC}$$

$$F_1 \text{ AaBbCc}$$

In  $F_2$  all plants with gene A or/and B in a recessive state and at least one dominant allele of gene C will have the same phenotype, which is expressed in the absence of ray flowers on the plant. Recessive homozygotes for gene C, regardless of the state of genes A and B (both A-B-cc and aaB-cc or A-bbcc), will form inflorescences with ray flowers. And only in the case of a double recessive homozygote for genes A and B (aabbcc), gene C in a recessive state, apparently, cannot suppress the action of the first two genes, which will lead to the formation of petals on the inflorescence.

A similar ratio of phenotypes, close to 42:22, was found in the second  $F_2$  segregating population, in which 97 plants out of 353 had the phenotype of a petalless parent.

Thus, according to our data, the trait “number of ray flowers” is inherited polygenically and controlled by at least three pairs of non-allelic genes with an additive effect. At the same time, not only additive, but also dominant effects are

**Table 3:** Inheritance of petallessness in sunflower.

Total $F_2$ plants	$F_2$ phenotypes		Segregation ratio tested	$\chi^2$ (P-value)
	With ray flowers	Without ray flowers		
Population 1 137	80	57	42:22	3.24 (0.072)
Population 2 353	256	97	42:22	7.24 (0.007)

$$\chi^2_{05} (\text{df } 1) = 3.84; \chi^2_{001} (\text{df } 1) = 10.83.$$

noticeable. As for the trait “absence of ray flowers”, three pairs of genes with an epistatic effect for one of them take part in its inheritance.

Petalless plants have different origins. They are found both in nature and under experimental conditions when crossing between related species or after induced mutagenesis.

The inheritance of petallessness depends on the plant species and genotype. A wide variety of systems for the genetic control of this trait was found in *Brassica napus*. According to Buzza (1983), petalless rapeseed flowers were controlled by two recessive genes, while Lü and Fu (1990) indicated four recessive genes involved in the inheritance of this trait. In turn, Kelly et al. (1995) reported that the apetal phenotype in one winter rapeseed genotype is controlled by an epistatic interaction between recessive alleles at a pair of homeological loci, and in another, by the interaction between alleles at three loci. According to Zhao and Wang (2004), a lack of petals in the mutant isolated from the hybrid progeny of *B. napus* was under the control of one locus. At the same time, incomplete dominance for the presence of petals over their absence was observed, as well as the absence of cytoplasmic effects. However, in another case it was shown on the same object that the trait of petal absence was regulated by the interaction of cytoplasmic and nuclear genes (Jiang and Becker 2003).

In sunflowers, the lack of ray flowers on the inflorescence is also known. According to Skaloud and Kovacik (1978), in cultivated sunflower, the absence of ray flowers is a recessive trait and is controlled by three genes – Fd 1, Fd 2, and Fd 3, the first of which suppresses the action of the other two. Our data on the inheritance of the absence of ray flowers also indicate the presence of at least three genes involved in the control of this trait, and the presence of an epistatic effect. The inheritance of petallessness in the plant isolated by M. Sujatha (2008) in the offspring of the *H. annuus* × *H. divaricatus* interspecific hybrid of sunflower proceeded differently. The study of inheritance showed that the trait is under monogenic recessive control.

The genetic control of the number of ray flowers and their size was studied by molecular methods. Thus, in studies conducted by J. M. Burke et al. (2002), there was reported a different number of QTL loci that were responsible for the above traits in sunflower.

It is believed that the balance of endogenous plant hormones is important in the process of initiation and differentiation of flower organs. At the same time, the use of exogenous hormones, especially cytokinins, can disrupt the natural balance of endogenous hormones and alter the development of flower organs (Venglat and Sawhney 1996). It can be assumed that the genes for apetalous sunflower are also involved in the regulation of the hormonal balance of its reproductive organs. Jiang and Becker (2001) also suggested that the expression of certain

characteristics in plants with apetal gene/genes was influenced by external conditions as well. The influence of environmental conditions on the expression of the petalless trait is also evidenced by our data showing that in some years plants with apetal genes develop sporadic ray flowers.

The data we have obtained expand the information in the field of private genetics of sunflower and allow more targeted breeding work with this crop.

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