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Inheritance of Leaf Tip Shape and Fringed Leaf Margin in Sunflower

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Abstract: After mutagenic treatment of sunflower seeds two traits of altered leaf shape were found – obtuse shape of leaf tip and strongly sinuate (fringed) leaf margin. To study the inheritance pattern of these traits, the mutant lines were crossed with the source lines and between themselves. It was shown that the traits of obtuse leaf and fringed leaf are recessive and inherited as monogenic traits when mating with the source line. In crossings of mutants, all F_1 plants had normal (acute leaf tip, fringe absence) phenotype. In F_2 generation, the 1/16 of plants with obtuse leaves and 1/4 of fringed plants were found. It was concluded that the shape of leaf tip is under the control of two genetic loci with duplicate genes interaction without cumulative effect. In that case obtuse shape of leaf tip is a double recessive homozygote. Fringed leaf is recessive in relation to the normal serrate leaf (absence of fringe) and inherited according to a single gene model. The chi-square test for goodness-of-fit indicated that the observed segregation pattern for the traits of leaf tip shape and leaf margin shape fit well to the expected ratio assuming that those two traits of leaf are inherited independently.

Keywords: sunflower, leaf shape, obtuse leaf tip, fringed leaf margin, inheritance pattern, duplicate genes

Introduction

Currently, cultivated sunflower varieties and hybrids are quite diverse in many qualitative and quantitative traits. However, they are surprisingly monotypic on the basis of traits of the leaf, including its shape.

At the same time, changing the shape of the leaf may be of interest in the following aspects. Firstly, the leaf shape largely determines the plant architecture, which in turn governs conduction of agrotechnical actions to gain an

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optimum yield per unit area. Secondly, the leaf is the main photosynthetic plant organ and characteristics of its structure are important for the flow of the process of photosynthesis. And finally, in the third, the shape of the leaf is a visually easily distinguishable trait that can be used in seed production as a marker to overcome the consequences of the mechanical and biological impurity.

In sunflower leaf shape, or rather its geometry, is genetically less investigated than its other characteristics, such as the leaf color or the leaf blade margin (Gavrilova and Anisimova, 2003; Vedmedeva and Soroka, 2016). This is partly due to a poor diversity of available geometric shapes for leaf blade, primarily due to the fact that not only the spontaneous but also induced mutations of this trait are rarely detected, although the frequency of morphoses affecting the leaf shape is fairly high (Jambhulkar and Joshua, 1999; Lyakh *et al.*, 2005; Lyakh et al., 2009; Soroka and Lyakh, 2009).

Mutations of the leaf shape in sunflower are often characterized by a high degree of pleiotropy, affecting both other traits of the leaf and other plant organs, from the cotyledon to the inflorescence. However, many changes in the shape of the leaf cause the depression in growth and development of plants, making them difficult to study and use (Fambrini *et al.*, 2010; Lyakh *et al.*, 2005; Soroka and Lyakh, 2009; Vedmedeva and Soroka, 2016).

The aim of this work was to study the inheritance patterns of two traits of leaf shape associated with the geometry of the leaf tip and the shape of the leaf blade margin when donors of these traits were crossed with the source lines as well as when crossed with each other.

Material and methods

Two traits of altered leaf shape in sunflower, namely obtuse shape of leaf tip and strongly sinuate (fringed) leaf margin were found in our experiments on induced mutagenesis. Fringed leaf was derived from ZL-169 line after treatment of immature seeds with a chemical mutagen ethyl methanesulphonate (Lyakh *et al.*, 2005) (Figure 1). The source line ZL-169 had the leaves with the typical serrate margin. Another trait (obtuse leaf tip) was discovered in ZL-95 line after treatment of immature embryos with the same mutagen (Soroka and Lyakh, 2009) (Figure 2). The shape of leaf tip of ZL-95 source line was acute, ordinary for sunflower.

Both mutant lines were characterized by normal manifestation of another trait under study. The mutant of ZL-169 line with fringed leaf margin possessed

Figure 1: Fringed leaf margin mutant (left) and the ZL-169 source line (right).

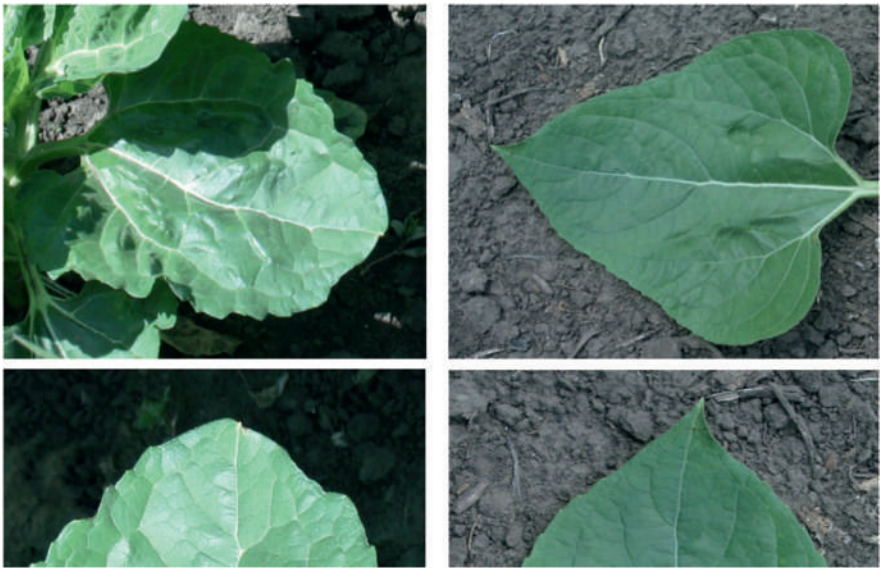

Figure 2: Obtuse leaf tip mutant (left) and the ZL-95 source line (right).

acute leaf tip, and the mutant of ZL-95 line with obtuse leaf tip had serrate leaf margin.

The mutant lines were crossed under field conditions with the source lines and between themselves. F_1 hybrids were self-pollinated to raise F_2 progeny where segregation ratios were scored for the given traits. The number of plants in F_2 population amounted to 200 plants for each of two replications in every cross combination.

The chi-square test for goodness-of-fit of the observed segregation pattern to the expected ratio for the traits of leaf tip shape and leaf fringe was used (Griffiths *et al.*, 1996).

Results and discussion

The F_1 hybrids after crossing the plants with fringed leaf margin with the source line ZL-169 (serrate leaf margin) were not different from the donor line ZL-169. Leaves of all F_1 plants were normally serrate. In F_2 generation, 68 plants out of 282 plants, i. e. approximately a quarter of them, had a fringed leaf. A segregation analysis performed has shown that the trait of fringed leaf was a recessive one and inherited as a monogenic trait when crossing to the source line (Table 1).

Table 1: Inheritance of traits "obtuse leaf tip" and "fringed leaf margin" when crossing mutant lines to the source lines.

Crossing	F_1 phenotype	Number of F_2 plants	F_2 phenotypes		Segregation ratio	χ^2
			normal trait	mutant trait		
Source line ZL-169 (normal) × mutant line (fringed)	Normal plants	282	214	68	3:1	0.12
	Normal plants	212	164	48	3:1	0.63
Source line ZL-95 (normal) × mutant line (obtuse tip)	Normal plants	209	165	44	3:1	1.64
	Normal plants	230	168	62	3:1	0.47

Note: χ^2_{05} (df = 1) = 3.84.

Crossing the mutant line with obtuse shape of leaf tip and the source line ZL-95 with acute leaf tip resulted in F_1 plants carrying normal (acute) shape of leaf tip.

In F_2 segregating population 44 plants with the obtuse tip contrasted to 165 plants with the acute leaf tip. Thus, F_2 plants were segregated in a 3:1 ratio (acute tip to obtuse tip). The data completely fit a single gene model for determination of leaf tip shape, with obtuse tip being the recessive allele (χ^2 = 1.64, p-value = 0.20) (Table 1). Similar inheritance patterns were observed within two other F_2 populations after crossing the plants with obtuse leaf tip and fringed plants with their source lines ZL-95 and ZL-169 respectively.

Table 2 presents the data on the inheritance of modified leaf tip and fringed leaf margin in crossings the mutants with each other. F_1 plants had normal phenotype, i. e. normal (acute) leaf tip shape and normal (serrate) leaf margin. In the second generation the segregation into four phenotypic classes was revealed: 156 normal tip, normal margin: 47 normal tip, fringed margin: 12 obtuse tip, normal margin: 4 obtuse tip, fringed margin in the first F_2 population, and 148: 40: 10: 3 in the second F_2 population respectively.

Table 2: Inheritance of traits “obtuse leaf tip” and “fringed leaf margin” when crossing mutant lines between themselves.

Population number	F_2 observed				F_2 expected				χ^2/p-value
	Acute tip		Obtuse tip		Acute tip		Obtuse tip		
	Normal margin	Fringed margin	Normal margin	Fringed margin	Normal margin	Fringed margin	Normal margin	Fringed margin	
1	156	47	12	4	154	51	10	4	0.77/0.86
2	148	40	10	3	142	47	9	3	1.42/0.70

Note: χ^2_{05} (df = 3) = 7.84.

The observed segregation data in each of the F_2 populations show a 15: 1 ratio of acute leaf tip to obtuse leaf tip. This ratio suggests a modification of the dihybrid 9: 3: 3: 1 Mendelian phenotypic ratio produced by interaction of two genes. These two non-allelic genes appear to be identical in function. Acute shape of leaf tip results from the presence in genotype of at least one dominant allele of either gene while obtuse leaf tip is a double recessive homozygote. So, shape of leaf tip is under the control of two-locus genetic system but not one pair of genes as it could be concluded after crossing the source line with its mutant analogue. We supposed that the ZL-95 source line and obtuse leaf mutant differed in one locus – A1A1a2a2 (ZL-95) x a1a1a2a2 (obtuse leaf mutant). Thus, the F_2 segregation ratio in this case would be as 3:1. Crossing the obtuse leaf mutant with the fringed leaf mutant (15:1 F_2 segregation ratio) evidenced that the components of that crossing differed in two loci – A1A1A2A2 (fringed leaf mutant) x a1a1a2a2 (obtuse leaf mutant).

The trait of the shape of leaf margin demonstrates 3: 1 segregation ratio both at crossing the source line with respective mutant and at crossing the mutants with fringed and obtuse leaves between themselves. Those data indicate that the trait of fringed leaf margin is recessive and inherited according to monogenic model.

While joint inheritance of obtuse leaf tip and fringed leaf margin is estimated, the F_2 shows a 45: 15: 3: 1 = (15: 1)(3: 1) model ratio. That is a modified phenotypic ratio of the threehybrid 27: 9: 9: 9: 3: 3: 3: 1 Mendelian ratio when one of the two traits is defined by duplicate genes without cumulative effect. The chi-square test for goodness-of-fit indicated that the segregation pattern for the traits of leaf tip shape and leaf margin shape fit well to the expected ratio assuming that those two traits of leaf are inherited independently.

The classic example of duplicate genes is the genes that control fruit shape in the plant called Shepherd's purse, *Capsella bursa-pastoris* Medic (Griffiths *et al.*, 1996). Two-locus genetic control of leaf shape was also found by Toker *et al.* (2012) in chickpea. The authors, however, stated 9: 3: 4 segregation patterns in F_2, instead of 15: 1, demonstrating non-allelic gene interaction in a recessive epistasis mode.

In its turn another genetic system is known to control leaf shape. Thus, Elmore (1986) revealed a single gene model for determination of leaf shape in *Ipomoea hederacea* (L.) Jacq where ivy leaf being the dominant allele to entire leaf allele. Similar results were demonstrated in *Vigna unguiculata* L. Walp by Nwofia (2014), who exhibited that leaflet shape to be monogenically controlled, with the lanceolate leaflet shape dominant over the ovoid leaflets. Zhanao Deng and Harbaugh (2006) studied inheritance of leaf shape in caladium. The observed segregation data confirmed that the three leaf shapes were controlled by two co-dominant alleles at one locus.

The shape of leaf blade is largely determined by the type of leaf venation. As stated in a monograph by Gavrilova and Anisimova (2003), Skoric *et al.* found that enhanced venation of the leaf blade is controlled by two recessive duplicate genes *vd1* and *vd2*. According to Demurin and Tolmachov (1986), fan-shaped venation of the leaf blade was defined by a single recessive gene *vs*. In our studies it was found the existence of at least two types of fan-shaped leaf venation controlled by recessive genes *vf1* and *vf2* with a complementary type of interaction and its own phenotypic manifestation for a double recessive homozygote (Soroka and Lyakh, 2015).

In the studies of Gavrilova and Anisimova (2003) it was shown that the normal shape of the leaf blade, as compared to the asymmetrical one, was caused by the interaction of two complementary dominant genes *As1* and *As2*. The same type of genetic control was demonstrated by the same authors for the tuberculate surface of the leaf blade.

It is interesting to note that mutation of the shape of the leaf tip was characterized by significant pleiotropic effect, affecting not only the true leaves, but also the cotyledonary leaves as well as stem, inflorescence, and even seeds. This mutant, unlike the plant with normal elongated leaf, had a rounded leaf tip and generally shorter oval leaf, oval cotyledons, truncated ray flowers and internodes, and almost rounded seeds with a length to width ratio close to 1.0 (Soroka and Lyakh, 2009). This may indicate that the above mentioned traits are probably under the control of the same genetic system, i. e. duplicate genes. In accordance with our findings Fambrini *et al.* (2010) also revealed the pleiotropic nature of the dominant Basilicum Leaf mutation altering the geometry of sunflower mature leaves. This mutation affected other leaf traits such as pigment content, vein architecture, margin type, stomatal density, and photosynthetic pigment content as well.

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