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Inheritance of Rounded Seed Shape in Sunflower

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Abstract: Two mutant inbred lines of sunflower, F_1 and F_2 hybrids derived after reciprocal crossings between those mutant lines, were used to study seed shape inheritance pattern. Seed shape was evaluated as a length to width (L/W) ratio. One of the mutant lines had rounded shape of the seeds which length to width ratio was close to 1.0. Another line differed with elongated seeds with L/W ratio equal to 1.6–1.7. The length to width ratio of 1.4–1.5 in F_1 hybrids was intermediate as compared to its parents, hybrid seeds being still elongated and differing sharply from the rounded seeds. F_2 populations showed two phenotypic classes in the shape of seeds with elongated seeds (L/W ratio > 1.3) exceeding greatly rounded seeds (L/W ratio < 1.3) in number. The used chi-square test indicated that the observed segregation corresponded to the theoretically expected both 15: 1 and 63: 1 ratios. The revealed segregations may indicate the control of the seed shape by two or even three unidirectionally acting genes. This implies that rounded seed shape is a double or triple recessive homozygote.

Keywords: sunflower, seed shape, rounded seed, length to width ratio, inheritance

Introduction

The shape of the seed is an expression of the ratio of its linear dimensions (Hristoforov, 1973) and is of interest in several aspects. It is considered that the ratio of linear seed size for a particular genotype is a constant trait (Hossain *et*

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al., 2010). The average value of this ratio is taken as optimal. Deviation from the optimal shape, caused by the inharmonious development of any linear dimension, leads to seed deformation, which ultimately affects its sowing characteristics. To determine the level of deformity, a special parameter was developed – seed deformity index (Makrushin *et al.*, 1996). Based on this parameter, a technology for seed calibration has been developed for a number of crops (Pavlov, 2002). Being an important technological indicator, the shape of seed is also used to develop devices for seed cleaning and processing.

However, the change of seed shape can be not only the result of modification variability. The seed shape, changed in comparison with other genotypes, can be a qualitative characteristic of the accession, being a ratio of linear dimensions which are under other genetic control. In other words, the seed shape in this case acts as a marker trait, which is of interest both for genetic research and seed production (Sakai *et al.*, 1996). The shape of the seed can also serve as a visual characteristic of the genotype, allowing it to be more quickly and easily identified (Genchev, 1989).

The goal of the present work was to elucidate the genetic control of the found mutant trait of rounded seed shape in sunflower, which was not earlier studied.

Materials and methods

As material there were used two parental lines (tobacco-like plant and dwarf one) of sunflower, F_1 hybrids derived after reciprocal crossings between those lines, and two F_2 populations derived after self-pollination of the F_1 hybrids. The tobacco-like plant was selected after mutagene treatment of immature embryos of ZL-95 line (Soroka and Lyakh, 2009) and, in addition to changes in the shape of leaves and ray florets, was characterized by rounded seeds. That rounded seed shape was assessed by length to width ratio, which was close to 1.0 but not exceeded 1.3. The dwarf line was originated from ZL-169 line (Lyakh *et al.*, 2005) and had elongated seeds.

The parents, F_1, and F_2 were sowed simultaneously in one field plot. After maturing five randomly selected plants of both parents and F_1 hybrids, and all F_2 plants were taken for the analysis. 20 seeds from the middle part of each plant head were measured with a caliper to determine seed length and width. After that length to width ratio was calculated for each seed and then this index was averaging for every plant.

In each F_2 population the number of plants with seed length to width ratio equal to 1.30 or less (which was characteristic of the tobacco-like plants) and number of plants with ratio more than 1.30 was counted. To test if the observed frequencies of plants in F_2 populations correspond the expected ones a Chi-square test and Yates' chi-squared test were used (Ajala, 1984; Griffiths *et al.*, 2004).

Results and discussion

The linear dimensions of the tested seeds and indices of their shape (length to width ratio) in the F_1 hybrids and its parents are presented in Table 1 and Figure 1.

As can be seen from the Table 1 and the Figure 1, the seeds of plant with rounded seed differed significantly in length from the seeds of dwarf plant with elongated seed, whereas differences in the width of the achene between those lines were absent. Both lines in linear dimensions of the seed were noticeably inferior to the hybrid. In the line with rounded seed, the length to width ratio was within the range of 1.18–1.25, resulting in an average of 1.22. This ratio was significantly different from the length to width ratio of the dwarf line and F_1 reciprocal hybrids. Although L/W ratio in F_1 hybrids was intermediate as compared to its parents, the shape of the seeds of these samples remained elongated and still differed sharply from the line with rounded seed.

Table 1: Seed size and shape in rounded and elongated seed lines, and in F1 hybrids between them.

Genotype*	Seed length, mm	Seed width, mm	Length/width ratio
Rounded seed line	7.37	6.10	1.22
Elongated seed line	10.19	6.21	1.66
F_1 Rounded×elongated	11.41	7.81	1.48
F_1 Elongated×rounded	11.22	7.59	1.50
LSD_{05}	0.60	0.58	0.13

*The averaged data of 5 plants for both lines and F_1 hybrids are presented.

Bearing in mind some differences in L/W ratio between F_1 hybrids and the dwarf line, it was originally intended to see in the second plant generation more than two phenotypic classes. Those classes, for example, should be in the ratio of 1: 4: 6: 4: 1 in the case if this trait is controled by two pairs of polymeric genes, and in other ratios in the case of another number of genes. However, the analysis of the F_2 populations showed that only two phenotypic classes in the shape of seeds could be clearly distinguished. One of them was considerably smaller in number and was characterized by strongly rounded seeds. Another class, which

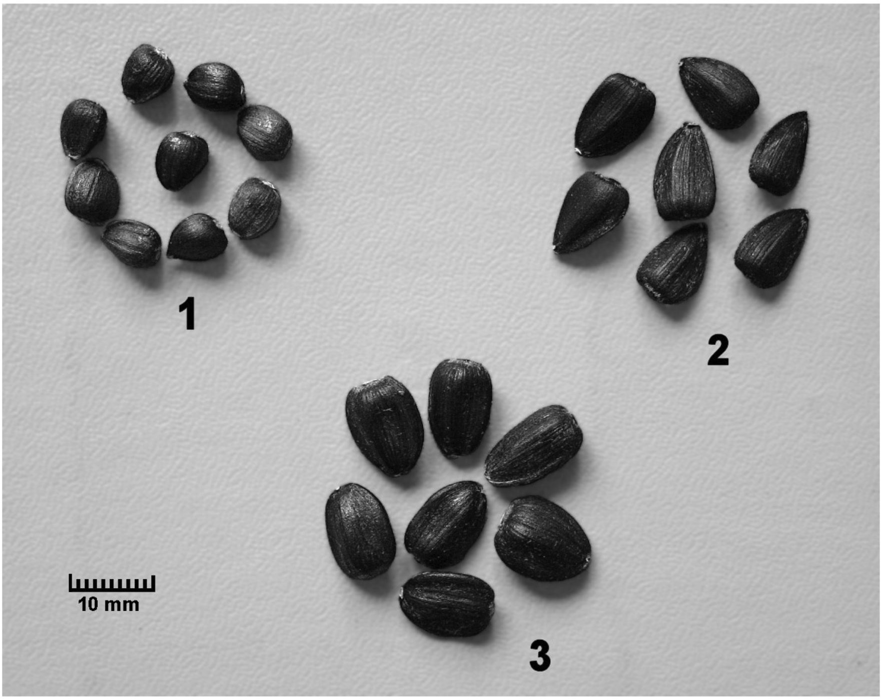

Figure 1: Seeds of the mutant parental lines and F_1 hybrid: (1) line with rounded seeds; (2) line with elongated seeds; (3) F_1 hybrid (rounded seeds´elongated seeds).

largely exceeded in number the first class, was characterized by the seeds of an elongated shape of a varying degree of manifestation (Table 2).

In the F_2 population (rounded seed x elongated seed cross combination), consisting of 220 plants, only 6 plants were isolated with the seeds where length to width ratio not exceeded 1.30, i. e. as in a tobacco-like plant with rounded seeds. Within this class of plants, the range of variation of length to width ratio was 1.06–1.28. Other plants of this population demonstrated that parameter in the range of 1.35 or higher. In the F_2 reciprocal segregating population of 146 plants, 4 plants only were characterized by length to width ratio of less than 1.30 in the range of 1.12 to 1.25.

The calculated chi-square value for the segregation in the first F_2 population in the two-locus control of uniquely acting genes determining the shape of the seeds indicates that observed segregation corresponds to the theoretically expected 15: 1 ratio. At the same time, this segregation does not contradict the 63: 1 model, when three non-allelic pairs of genes control the analyzed trait. For

Table 2: Inheritance of rounded shape of the seeds in sunflower.

F_1 phenotype	Total of F_2 plants	No. of F_2 plants		Segregation ratio tested	χ2 (P value)
		L/W[#] ratio≤1.30	L/W ratio>1.30		
L/W ratio 1.40	220	6	214	15: 1	4.88 (0.027)
(Rounded×elongated)				63: 1	3.04 (0.081)
L/W ratio 1.53	146	4	142	15: 1	2.27* (0.132)
(Elongated×rounded)				63: 1	1.27* (0.26)

χ^2_{05} (df 1) = 3.84; χ^2_{01} (df 1) = 6.6; *Chi-square is calculated taking into account Yates correction; [#]L/W ratio – seed length to seed width ratio.

the second F_2 population, the chi-square values were calculated with the Yates correction, since the number of plants in one of the classes was less than 5. The used chi-square test also revealed the correspondence of the actual segregation to the theoretically expected at all significance levels for both the two-locus and three-locus control of the seed shape.

Thus, the analysis of the segregation in both populations indicated that it matched both the 15: 1 and 63: 1 schemes, observed in the case of interaction of two or three non-allelic pairs of genes by the type of non-cumulative polymerism. Within those populations, only a small fraction of plants had seed shape characteristics of the line with rounded seed. The remaining plants in the populations exhibited an elongated seed shape of a varying degree of manifestation.

In general, the revealed segregations may indicate the control of the seed shape in sunflower by two or even three uniquely acting genes. At the same time the seed shape, characteristic of a plant with rounded seed, is a double or triple recessive homozygote.

Inheritance pattern of duplicate genes is intrinsic for genetic control of fruit shape in *Capsella bursa pastoris*. In this plant the presence of at least one dominant allele of any of two non-allelic genes causes heart-shaped fruit, while narrow fruit is the result of the combination of recessive alleles of those genes. Two-locus control of fruit shape is known for other plants as well, f.e. pumpkin, though, with different from shepherd's purse type of gene interaction (Griffiths *et al.*, 2004).

Hossain *et al.* (2010) analyzed segregation ratios for different seed shape among the two recombinant inbred line populations of *Cicer arietinum* L. and indicated control of seed shape under two genes. In its turn Genchev (1989) determined the number and type of genes controlling seed shape in *Phaseolus vulgaris* L. F_2 plants were divided into groups with elliptical seeds and seeds with a shape different from elliptical. It was found that elliptical seed shape is controlled by three polymeric genes.

As for sunflower, inheritance of seed shape in this crop was not elaborated. At the same time there were some attempts performed to elucidate genetic control of linear seed parameters (Jocic *et al.*, 2000). The authors have found the predominance of additive gene effects in the inheritance of seed length, width and thickness. That corresponds, in a sense, with our data, which demonstrated that seed shape is under the control of several pairs of genes.

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HERENCIA DE FORMA REDONDEADA DE SEMILLAS EN GIRASOL

Resumen

Se usaron dos líneas mutantes endogámicas de híbridos de girasol, F1 y F2 derivadas después de cruzamientos recíprocos entre esas líneas mutantes, para estudiar el patrón de herencia de forma de semilla. Se evaluó la forma de la semilla como una relación de longitud a anchura (L/W). Una de las líneas mutantes tenía una forma redondeada de las semillas cuya relación de longitud a anchura era cercana a 1,0. Otra línea difería con las semillas alargadas con relación L/W igual a 1,6-1,7. La relación de longitud a anchura de 1,4-1,5 en híbridos de F1 fue intermedia en comparación con sus padres, las semillas híbridas aún siendo alargadas y diferenciándose bruscamente de las semillas redondeadas. Las poblaciones F2 mostraron dos clases fenotípicas en forma de semillas con semillas alargadas (relación L/E > >1,3) que excedieron las semillas muy redondeadas (relación L/E < <1,3) en número. La prueba de chi cuadrado utilizada indicó que la segregación observada correspondía a las relaciones teóricamente esperadas 15: 1 y 63: 1. Las segregaciones reveladas pueden indicar el control de la forma de la semilla por dos o incluso tres genes que actúan unidireccionalmente. Esto implica que la forma redondeada de la semilla es un homocigoto recesivo doble o triple.

HÉRITAGE DE LA FORME ARRONDIE DE SEMENCES AU TOURNESOL

Résumé

Deux lignées consanguines mutantes de tournesol, les hybrides F1 et F2 dérivées après des passages réciproques entre ces lignes mutantes ont été utilisées pour étudier le modèle d’héritage de la forme des graines. La forme de la graine a été évaluée comme un rapport longueur/largeur (L/W). L’une des lignes mutantes avait une forme arrondie des graines dont le rapport longueur/largeur était proche de 1,0. Une autre ligne différait avec les graines allongées avec un rapport L/W égal à 1,6-1,7. Le rapport longueur/largeur de 1,4-1,5 dans les hybrides F1 était intermédiaire par rapport à ses parents, les graines hybrides étant encore allongées et diffèrent nettement des graines arrondies. Les populations de F2 ont montré deux classes phénotypiques sous forme de graines avec des graines allongées (rapport L/W > >1,3) dépassant les graines très arrondies (rapport L/W < <1,3) en nombre. Le test de chi carré utilisé a indiqué que la ségrégation observée correspondait aux

rapports théoriquement attendus à la fois 15: 1 et 63: 1. Les ségrégations révélées peuvent indiquer le contrôle de la forme de la graine par deux ou même trois gènes à action unidirectionnelle. Cela implique que la forme de graine arrondie est un homozygote récessif double ou triple.