Unstable expression of Ol gene for high oleic acid content in sunflower seeds

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Abstract
The inheritance of high oleic acid content in crosses of six high oleic lines with six normal lines was studied to coordinate different hypothesis about genetic control of this trait.
The results obtained agree with neither hypothesis based on three dominant complementary Ol_1,Ol_2 and Ol_3 genes nor hypothesis of one dominant *Ol* and one recessive modifier *Ml* genes.
It was shown that the penetrance of *Ol* gene in heterozygote ranges from 0 to 100% in different genetic backgrounds. For twenty one F_1 populations it averaged 66%, and for three F_2 populations - 57%.
Incomplete penetrance is determined by genotypic factors of normal inbred lines causing a phenotypic reversion. Particular epistatic genes have not been identified. These genotypic factors lead to the abnormal, non-Mendelian inheritance with appearance of mosaic in oleic acid content heterozygous seeds. About 35% of F_1 seeds of two crosses with complete range of variability in oleic acid content were mosaic with high oleic gemmule and normal cotyledons. All they belonged to the intermediate phenotypic class of whole seeds.
Ol gene is considered a locus with genetically unstable expression.

Key-words: incomplete penetrance, oleic acid, mosaic seeds

Introduction

The open-pollinated variety Pervenets with high oleic acid content in seed oil (6) has become a world-wide source of this trait in sunflower breeding programs. The development of sunflower hybrids with new oil quality has led to the genetic research on high oleic character (about 85% of oleic acid content in total fatty acid composition).

There are three principal hypotheses concerning the inheritance of high oleic mutation. All they admit the embryo control on seed fatty acid composition. The first I postulates a dominant gene *Ol* controlling this trait (2, 4, 5, 7). The second II is introduced to explain the regular lack of seed number in a mutant phenotypic class of F_2 and test-cross populations resulting in different types of segregation between high/intermediate/low oleic groups. The action of three non-allelic complementary genes Ol_1, Ol_2 and Ol_3 is exploited (1). At last, in order to elucidate incomplete dominance in F_1, the lack of mutant phenotypes in F_2 and the observation of high oleic F_3 seeds on selfed F_2 plants from an intermediate class, the third hypothesis III has been explicated (3). It says that high oleic phenotypes can be found in cool maturation environment when partially dominant gene *Ol* is combined with the recessive homozygote of a modifier gene *Ml*.

Moreover, several facts, such as deviation of F_1 seeds, reversal in dominance and abnormal segregations (7, Demurin, unpublished data), could not be explained by above hypotheses.

Our investigation on genetic control of high oleic trait in sunflower seeds was carried out in Novi Sad, Yugoslavia in 1994-1995 with the aim to coordinate the different models of inheritance. Some results are presented in this paper.

Materials and methods

A special set of twelve constant inbred lines with different fatty acid composition and approximately the same period of vegetation was composed:

- six high oleic (about 88% of $C_{18:1}$) LG26OL, VK66OL from Russia, VNIIMK; FARGO OL from the USA; HA89OL from Spain; JAPAN4OL and JAPAN34OL from Japan
- one with increased content of oleic acid (about 60%) LG27 from Russia
- four with common content of oleic acid (about 35%) VK66, VK678 and HS from Russia; HA89 from Yugoslavia
- one with decreased content of oleic acid (about 20%) from Russia.

In summer 1994 F_1 was obtained, in the late 1994 (greenhouse) - F_2 and BC_1 and in the early 1995 (greenhouse) - F_3. Only one-head-by-head crossing were made and individual marked seeds were analyzed and planted. Eight well-known stable genes *T* ,*T₁*, *P*, *Br*, *Vs*, *O*, *Rf* and *Tph1* were observed as control. Fatty acid composition was determined by gas chromatography of methyl esters.

Results

Parents and F_1 (Tab.1). Six high oleic lines have obtained *Ol* gene from variety Pervenets (data on gene identification are not presented) and were bred true.

Reciprocal F_1 seeds from crosses of high oleic lines with LG27 and LG28 indicated complete dominance of high oleic trait.

VK66 and VK678 in crosses with high oleic parents gave both uniform high oleic hybrid populations and several crosses segregating in mutant and normal seeds within individual heads. Division between high and normal oleic classes was made at 75% of oleic acid content. These deviated normal phenotypes led to the decreasing of dominance degree calculated for the mean of seed bulks.

Further, F_1 hybrid seeds from the crosses of high oleic lines with HA89 and HS varied from mutant to normal phenotypes within individual heads or were normal only. It resulted in the reversal of dominance.

Essentially that for all heads possessing the deviated seeds, the 1:1 ratio for high oleic/normal phenotypes was not observed to disagree with hypothesis III. This ratio could be possible if low oleic parents were *Mlml* heterozygote.

F_2 and test-cross (Tab.1). The segregation ratios of F_2 and test-crosses for LG27 and LG28 corresponded to the one dominant gene hypothesis I.

Data from F_2 and test-cross families of VK66 and VK678 parents, in the case of F_1 seeds had a mutant phenotype, showed high oleic/normal phenotypic segregations to fit the ratios of hypothesis I or hypothesis II. One exception was observed when F_2 population from VK678CMS×VK66OL cross corresponded neither to hypotheses I nor II because of the lack of high oleic seeds. Another F_1 seed from this cross with normal phenotype gave F_2 and test-cross distributions which did not fit the expected ratios for hypothesis III because of the lack of normal seeds.

Further, F_2 populations derived from both mutant and normal F_1 seeds in crosses of high oleic lines with HA89 and HS showed absence of the hypothesis I ratio. These distributions corresponded to hypotheses II or III and , in same cases, did not fit any of ratios expected. As a rule, the last was due to the lack of normal seed number for 3:13 mutant/normal seed ratio in accordance with the hypothesis III.

F_3 (Tab.2). Three F_2 populations were chosen to compare the genotypic and phenotypic segregations on the base of selfing of individual marked F_2 plants after half-seed determination of fatty acid composition. Two of them showed 9:7 ratio (hypothesis II) and one- 3:13 ratio (hypothesis III) in phenotypic segregations.

It was undoubtedly demonstrated that the first and the second F_2 populations had 3:1 ratio in genotypic segregation instead of 9:7 ratio in phenotypic segregation. About 50.0% and 33.3% of F_2 seeds with a normal phenotype contained 'silent' high oleic allele *Ol*, respectively. It resulted in decreasing of seed number in the high oleic phenotypic class. Thus, identification of all normal F_2 seeds obtained from high oleic F_1 plants as Ol_1-ol_2ol_2, $ol_1ol_1Ol_2$- or $ol_1ol_1ol_2ol_2$ genotypes is not correct to disagree with hypothesis II.

Meanwhile, hypothesis III allows several normal F_2 seeds obtained from normal F_1 plants to produce after selfing the segregating progenies with mutant phenotypes in F_3 (the third population). On the other hand, according to the hypothesis III high oleic F_2 seeds of *Ol- mlml* genotype can produce in F_3 either uniform high oleic families or 3:1 segregating families. Data on F_3 progenies obtained from three high oleic F_2 seeds did not fit any of these ratios.

Seed-cut-analysis (Tab.3). The F_1 seeds of two crosses with complete range of variability in oleic acid content were cut into two parts, i.e. gemmule and cotyledons, which were analyzed separately. An average weight proportion was 10% of gemmule and 90% of cotyledons. There were no large differences between these parts in oil content (41% and 43%, respectively) determined with methyl margarate as internal standard.

The comparison of fatty acid composition has revealed fully unexpected phenomenon. About 35% of F_1 seeds were mosaic in oleic acid content, while all seeds of inbred parent lines had no substantial differences in fatty acid composition.

Interesting to note that only 'high oleic gemmule-normal cotyledons' mosaic type was observed. Moreover, all mosaic F_1 seeds of both VK66OL×HA89 and VK678CMS×VK66OL crosses belonged to the intermediate phenotypic class of whole seeds from 45% to 65% of oleic acid content. Phenotypic coincidence (the portion of non-mosaic seeds) between gemmule and cotyledon parts was 0.80 and 0.50, respectively.

Discussion

In crosses with uniform high oleic F_1 seeds, hypothesis I is quite reliable. It should be stressed that appearance of deviated normal phenotypes in F_1 in several crosses is not artifacts or possible mistakes of hybridization. The reversal in dominance takes place regularly.

Segregations with deviated normal F_1 seeds or even complete reversal in dominance followed by the lack of seed number in the mutant high oleic phenotypic class of F_2 and test-crosses cannot be explained by hypothesis I. It is important that OL mutation has an ability to express itself in progenies of high, intermediate or low oleic phenotypic classes.

Hypotheses II and III may statistically fit expected ratios for phenotypic segregations in same cases. Nevertheless, the whole individual scheme of pedigree in the line from parents to F_1, F_2, test-cross and F_3 has shown these hypotheses not to be truthful.

Obviously, several normal inbred lines possess the genetic backgrounds with epistatic factors which cause unstable expression of *Ol* gene. This phenotypic reversion may be associated with mosaic in oleic acid content seeds. As result, a high oleic gemmule (germplasm tissue) along with normal cotyledons (somatic tissue) complicate the research on inheritance when a seed with normal phenotype in whole may produce a mutant plant.

In order to work out the theoretical model of the genetic control of high oleic mutation in sunflower, the *Ol* gene hypothesis I is believed to be modified with genetic instability approach.

In general, we have come to the following conclusions:

- The penetrance of *Ol* gene in heterozygote ranges from 0 to 100% in different genetic backgrounds ;
- Incomplete penetrance is determined by genotypic factors of normal inbred lines causing a phenotypic reversion. Particular epistatic genes have not been identified;
- These genotypic factors lead to the abnormal, non-Mendelian inheritance with appearance of mosaic in oleic acid content heterozygous seeds.

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Table1. Inheritance of high oleic mutation in F_1, F_2 and test-cross generations

cross	dominance degree	oleic acid content, %		segregation high/ normal	oleic acid of F_1 seed	segregation high/ normal	ratios for P>0.05
		mean	range				
	F_1				F_2 and test-cross*		
LG27×LG26OL	1.33	90	90-90	10/0	90	34/16	3:1,9:7
LG27×HA89OL	0.87	87	86-90	10/0	86	42/8	3:1
HA89OL×LG27	1.00	89	88-90	10/0	89	36/14	3:1
HA89CMSOL×LG27	1.00	89	85-90	10/0	90	21/29	1:1*
LG28×LG26OL	0.97	85	79-90	10/0	82	39/11	3:1
LG28×HA89OL	0.91	86	82-90	10/0	82	39/11	3:1
HA89OL×LG28	0.94	87	85-88	10/0	85	32/18	3:1,9:7
HA89CMSOL×LG28	1.00	89	88-91	10/0	88	22/28	1:1*
VK66×JAPAN4OL	0.93	87	85-90	10/0	85	33/17	3:1,9:7
VK66×VK66OL	0.93	86	74-88	9/1	88	21/29	27:37
VK66OL×VK66	0.83	83	53-88	9/1	85	31/19	9:7
					53	18/32	no(III)
VK678×LG26OL[1]	0.92	84	79-87	10/0	85	30/20	9:7
VK678×JAPAN4OL	0.92	87	86-89	10/0	86	30/20	9:7
VK678×JAPAN34OL	0.85	87	86-88	10/0	86	29/21	9:7
VK678×HA89OL	0.84	85	74-88	9/1	86	36/14	3:1
VK678CMS×VK66OL	0.16	67[3]	40-90	7/23	84	14/36	no(I,II)
						7/43	1:3,1:7*
					66	36/14	no(III)
						22/28	no(III)*
HA89×VK66OL	0.00	62	38-81	3/7	81	22/28	9:7,27:37
					57	9/41	3:13
VK66OL×HA89[1]	0.54[2]	48[3]	21-83	7/23	78	26/24	9:7
					23	6/44	3:13
HA89OL×HA89	0.21[2]	57	44-84	1/9	60	25/25	no(III)
FARGOOL×HA89	0.58[2]	47	36-62	0/10	62	20/30	no(III)
					38	11/39	3:13
HA89OL×HS	0.88[2]	43	25-65	0/10	55	20/30	no(III)

[1] - crosses used for F_3 , [2] - normal oleic acid content is dominant, [3] - heads used in seed-cut-analysis

Table 2. Pedigrees of three populations as progenies of individual plants analyzed, grown and selfed (h - high oleic, n - normal and hc - head containing high oleic seeds)

	populations		
crosses	VK678×LG26OL	VK66OL×HA89	
F_1 seeds	10h : 0n	7h :	23n
	↓	↓	↓
F_2 seeds	23h : 16n	17h : 21n	3h : 27n
	↓ ↓	↓ ↓	↓ ↓
F_3 heads (10 seeds analyzed per a head)	23hc 8hc : 8n	17hc 7hc : 14n	2hc :1n 9hc : 18n
Persantage of normal F_2 seeds containing Ol gene	50.0% (8/16)	33.3% (7/21)	33.3% (9/27)
Penetrance of Ol gene in F_2	74.2% (23/31)	70.8% (17/24)	25.0% (3/12)

Table 3. The number of seeds of different types in seed-cut-analysis (h - high oleic, n - normal)

line, cross	geno-type	phenotypes				germ/cotyledon phenotypic coincidence
		germ h/ cotyl. h	germ h/ cotyl. n	germ n/ cotyl. h	germ n/ cotyl. n	
LG26OL	OlOl	3	-	-	-	1.00
LG27	olol	-	-	-	3	1.00
LG28	olol	-	-	-	3	1.00
HA89	olol	-	-	-	3	1.00
F_1 VK66OL× HA89	Olol	4	4	-	12	0.80
F_1 VK678CMS× VK66OL	Olol	5	10	-	5	0.50