SCREENING FOR SUPPRESSOR GENOTYPES ON A HIGH OLEIC MUTATION IN SUNFLOWER

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Abstract

The individual plants of 96 accessions of the world germplasm collection of VIR (St. Petersburg) and the genetic collection of VNIIMK (Krasnodar) were crossed with four high oleic lines in the field in the summer of 2001, 2002 and 2003. HA 89A OL and VK876A OL were the female CMS high oleic testers. VK580 OL and LG26 OL were the male testers. Fatty acid composition of bulked or single seeds was determined by gas chromatography of methyl esters. The F1 populations in the crosses of 96 germplasm accessions with high oleic testers revealed the *Ol* mutation to be dominant in 59% (57/96) producing the high oleic class, incompletely dominant in 38% (36/96) producing the intermediate oleic class, and recessive in 3% (3/96) producing the low oleic class. Analysis of F1 individual seeds of eighty accessions selected as suppressor-carrying showed variation from 31 to 91% of oleic content. There was segregation for all of the F1 populations. The decrease of the F1 mean value from 77 to 63% was determined by the increase of a portion of normal seeds from 0.25 to 0.75. Two suppressor-carrying accessions, K 1587-2 and VIR 721-3, were crossed as females with two high oleic lines. The F1 single seeds of VK 580 OL demonstrated intermediate or low average oleic content with a high portion of normal seeds from 0.50 to 0.70. It was unexpected to the observe full dominance degree of the *Ol* mutation associated with absence of the F1 normal seeds in the crosses of these two suppressor-carrying accessions with a high oleic line LG 26 OL.

Introduction

The first step in fatty acid alteration of sunflower (*Helianthus annuus* L.) seed oil was done in VNIIMK, Russia with high oleic variety Pervenets resulting from DMS chemical mutagenesis on the seeds of variety VNIIMK 8931 (Soldatov, 1976). There are additional data about the high oleic character in another mutant line with 80% oleic acid content from Bulgaria (Ivanov and Ivanov, 1992) and a mutant line with 90% of oleic acid content from Italy (Andrich et al., 1992). Nevertheless, Pervenets was exclusively used as a donor of the high oleic trait in breeding programs throughot the world.

Research on genetic control of a high oleic mutation led to the hypotheses of one dominant gene *Ol* (Fick, 1984; Urie, 1985), major gene *Ol* and gene-modifier *Ml* (Miller et al., 1987), three complementary genes *Ol1*, *Ol2* and *Ol3* (Fernandez-Martinez et al., 1989), gene *Ol* with incomplete penetrance determined by genotypic epistatic factors of reversion (Demurin et al., 1996), five genes *Ol1*, *Ol2*, *Ol3, Ol4* and *Ol5* (Velasco et al., 2000), high oleic locus oleHOS and suppressor locus Sup (Lacombe et al., 2001). All of these hypotheses

try to explain the lack of mutant seeds from a monogenic number expected in the crosses of mutant and normal lines and in their progenies. In order to clarify the type of inheritance of a high oleic trait the suppressor genotypes for mutation *Ol* should be found in the world germplasm collection of sunflower.

Materials and Methods

The pollen from individual plants of 96 accessions of the world germplasm collection of VIR (St. Petersburg) and the genetic collection of VNIIMK (Krasnodar) was collected in the field in summer 2001, 2002 and 2003. HA 89A OL and VK876A OL were female CMS high oleic testers. VK580 OL and LG26 OL were male testers. Fatty acid composition of bulked seed or individual seeds was determined by gas chromatography of methyl esters.

Results and Discussion

The F1 seeds from the crosses of 57 accessions with the high oleic testers were high oleic in a bulked seed from 80 to 90% of oleic acid content (Table 1). These crosses showed the *Ol* mutation to be dominant. The average oleic content in 36 crosses was intermediate and varied from 60 to 80%. These data support the incomplete dominance in phenotypic expression of the mutation in the heterozygote. Finially, three F1 populations demonstrated reversal in dominance from 50 to 60% of average oleic content. It corresponds to the recessive type of inheritance.

Table 1. Average oleic acid content in oil of F1 seeds in the crosses of germplasm accessions with CMS high oleic testers HA89A OL and VK876A OL in the field at Krasnodar, 2001-2003.

Male parent, accession	F1 mean oleic acid content, %	Oleic class
K 3, 223, 370, 534, 537, 588, 912, 1091, 1459, 1464, 1483, 1506, 1531, 1594, 1675, 1687, 1970, 2068, 2462, 2479, 2667, 2776, 2997; VIR No**.** 577084, 577432, 577433; HA 413, 414; L 2595-2-4; AN 70029Rf; Sl 2349; VK 73	85 – 90	high
K 46, 103, 254, 437, 578, 590, 1232, 1691, 1721, 1810, 2141, 2645, 3079, 3350; RHA 356; Sl 2213; LG 11-3, 28; KG 24, 102, 113-2; VK 61, 98-1, 404; L 207	80 – 85	
K 513, 615, 1374, 1692, 2863, 2890; VIR No**.** 577083; Sl 2290, 2399-2, 2950; KG 28, 113; VK 268; Z 231	75 – 80	intermediate
K 79, 225-3; VIR 369, 721-1; RHA 274-1, 298; KG 21, 113-1; LG 27; VK 636; 83HR4; Tchernianka 66-2	70 – 75	
K 2039; VK 66-2-2, 400, 653; VIR 721-4; M 1046	65 – 70	
K 1587-2; VIR 721-2; RHA 416; VA 4	60 – 65	
K 824; VIR 721-3	55 – 60	low
K 235	50 – 55	

Analysis of F1 individual seeds of eighty accessions selected as suppressor-carrying showed variation from 31 to 91% of oleic content (Table 2). There was segregation in all of the F1 populations. The decrease of the F1 mean value from 77 to 63% was determined by the increase of a portion of normal seeds from 0.25 to 0.75. Normal seeds were classified as less than 70% of oleic content.

Table 2. Oleic acid content in 20 single seeds of F1 in the crosses of germplasm accessions with CMS high oleic tester VK876A OL in the field at Krasnodar, 2003.

Male parent, accession	F1 mean	Range		Portion of normal F1 seeds (<70% of oleic acid)
		min	max	
LG 28	77	53	91	0.25
83HR4	73	31	89	0.30
LG 27	73	48	91	0.45
K 1587-2	72	56	90	0.55
VIR 721-3	71	34	90	0.55
K 235	65	48	90	0.75
RHA 416	63	41	91	0.65
K 824	63	37	90	0.65

Two suppressor-carrying accessions, K 1587-2 and VIR 721-3, were crossed as female with two high oleic lines (Table 3). The F1 single seeds of VK 580 OL demonstrated intermediate or low average oleic content with a high portion of normal seeds from 0.50 to 0.70. It was unexpected to observe the full dominance degree of *Ol* mutation associated with absence of F1 normal seeds in the crosses of these two accessions with a high oleic line, LG 26 OL. All of the high oleic lines were stable in the character with about 88% of oleic content.

Table 3. Oleic acid content in 20 single seeds of F1 in the crosses of suppressor-carrying accessions with high oleic testers VK580 OL and LG26 OL in the field at Krasnodar with hand-emasculation, 2003.

Crosses	F1 mean	Range		Portion of normal F1 seeds (<70% of oleic acid)
		min	max	
K 1587-2 × VK 580 OL	71	47	91	0.50
VIR 721-3 × VK 580 OL	54	29	89	0.70
K 1587-2 × LG 26 OL	89	85	92	0.00
VIR 721-3 × LG 26 OL	86	70	91	0.00

Thus, the F1 populations in the crosses of 96 germplasm accessions with high oleic testers revealed the *Ol* mutation to be dominant in 59% (high oleic class, 57/96), incompletly dominant in 38% (intermediate oleic class, 36/96) and recessive in 3% (low oleic class, 3/96). Single seed segregation of F1 is determined probably by the heterozygote status of the most accessions. Nevertheless, the same variation was also observed for inbred lines. It might be explained by the absence of phenotypic expression of the suppressor without the *Ol* mutation.

Homozygote status of the suppressor can be detected in the *Ol* mutation background resulting in a low oleic phenotype (Lacombe et al., 2001).

On the other hand, the microsomal oleate desaturase activity is lost in developing embryos of the high oleic mutant at the beginning of the period of active lipid synthesis (Garces et al., 1991). It occurs because of the strong reduction in accumulation of Δ12-desaturase transcript (Lacombe et al., 2000). From the molecular genetics point of view the high oleic mutation interferes with the regulation of the transcription of the seed-specific FAD2 gene (Martinez-Rivas et al., 2001). Suppressor genotypes might influence this regulation.

Suppressor-carrying genotypes of normal lines lead to the reduction of oleic content of the F1 seeds in the crosses with the high oleic lines. Moreover, the high oleic genotype (LG26 Ol) might be resistant to reversion action of the suppressor.

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