Homo- and heterozygous longitudinal gradient of oleic acid content in sunflower seeds

Yakov Demurin, Oxana Borisenko, Nikolay Bochkarev
All-Russia Research Institute of Oil Crops (VNIIMK), Filatova 17, Krasnodar 350038, Russia
E-mail: yakdemurin@yandex.ru

ABSTRACT

The results of the research on a seed oleic acid gradient present both in inbred lines and hybrids of a high oleic genotype with suppressor-carrying normal lines of sunflower are presented. Seven inbred lines were used: high oleic VK508, VK876; increased oleic LG27; low oleic RIL100, LG28, RHA416 and K824. The oil obtained from cotyledons of the line LG27 had a higher content of oleic acid than that obtained from gemmule. The difference was about 12.7%. Other lines did not show any significant discrepancy. The phenomenon could be called environmental homozygous increase of oleic acid content. A wide range of distribution of heterozygous *Olol* F_1 seeds on oleic acid classes in the crosses of VK876×LG28 and VK876×K824 was observed. One half of the seeds belonged to the high oleic class (76 to 91%) and the other half varied in an intermediate class (46 to 76%). This abnormal variation of the F_1 seeds is believed to be due to the genetic suppressor of *Ol* mutation from normal lines. The oleic acid content of gemmule and cotyledon was the same for the F_1 seeds of the mutant class. On the contrary, a significant difference in oleic acid content was observed for the F_1 seeds of the intermediate class. In this case, the cotyledon had a lower content of oleic acid than that of gemmule with a difference of about 11.6%. This longitudinal seed gradient could be called an epigenetic heterozygous decrease in oleic acid content. The environmental homozygous change in oleic acid content is in the opposite direction to the epigenetic heterozygous variation. The portion of mosaic F_1 seeds was 23%. Only one type of mosaic heterozygous embryo was found, i.e. mutant gemmule and intermediate cotyledon.

Key words: heterozygote – mutation – oleic acid – seed gradient

INTRODUCTION

The sunflower achene (fruit) consists of a seed (kernel) and pericarp (hull). The seed includes a seed coat, endosperm and embryo (Seiler, 1997). The embryo is mostly made up of two cotyledons and a gemmule (seed tip), which consists of rootlet, hypocotyl and budlet. All parts of the embryo contain oil-rich cells with a maximum content of reserve lipids in cotyledons of about 65% (Dyakov and Perestova, 1975; Popov and Dyakov, 1975).

The spatial longitudinal difference between the embryo tip and the cotyledons of a sunflower seed in the oleic acid content was described for the first time for both the high oleic and normal homozygous genotypes. The oleic acid content changed from 87.5 for the seed tip to 91.3 mol% for cotyledons of a high oleic genotype and from 44.4 to 56.9 % for the seeds of normal inbred line. The increase in oleic acid percentage was 3.8 and 12.5, respectively (Garcés et al., 1989). Another type of spatial difference was associated with the decrease in oleic acid content from 82.1 to 77.5 % for inner seed cotyledon and cotyledon emerged out of the hull for a high oleic genotype (Garcés and Mancha, 1989).

The content of oleic acid was found to be higher, about 5% (that of linoleic acid being lower), at the distal end of the cotyledon comparatively to the seed tip for the seeds of normal line CAS-6. This phenomenon could be explained with a gradient of oxygen throughout the seed due to the oxygen impermeability of the seed coat resulting in its diffusion mainly through the contact between the seed and the capitulum. Obviously, the oxygen could be a limiting environmental factor of oleate desaturation. A contrarily longitudinal difference was observed for the high stearic mutant lines CAS-14. The percentage of oleic acid was about twice times lower at the distal end of the cotyledon (reduction from 39.3 to 16.8 %) due to the increase of stearic acid content (Fernández-Moya et al., 2003).

All of the above-mentioned cases of the seed longitudinal gradient of oleic acid content were observed for the homozygous genotypes of sunflower. The spatial heterogeneity of a heterozygous F_1 seed of the oleic acid content was described for the first time in the crosses of a high oleic mutant with normal inbred lines HA89 and VK678. About 35% of individual F_1 seeds were mosaic with only one type of “high oleic gemmule – normal cotyledon”. All of them belonged to the intermediate phenotypic class of whole seeds from 45 to 65% of oleic acid content (Demurin and Škorić, 1996).

This paper shows the results of the research on the seed oleic acid gradient in the crosses of a high oleic genotype with suppressor-carrying normal lines of sunflower. This was done in the development of hypothesis of incomplete penetrance of the *Ol* mutation.

MATERIALS AND METHODS

Seven inbred lines of sunflower were used: high oleic VK508 (*Ol*), VK876 (*Ol*); increased oleic LG27; low oleic RIL100, LG28, RHA416 and K824.

The plants were grown and self-pollinated in a field plot of VNIIMK, Krasnodar in summer 2004. The crosses were made with hand-emasculation. Each individual seed (embryo) of the inbred line or F_1 was cut with the razor into two parts, gemmule and cotyledons, which were analyzed separately. The oleic acid content of a whole seed was calculated with the weight ratio of 0.1 gemmule and 0.9 cotyledons.

Fatty acid composition of the oil from the seed parts was determined by gas chromatography of methyl esters.

RESULTS AND DISCUSSION

The oil obtained from cotyledons of the line LG27 had a higher content of oleic acid than that obtained from gemmule. This difference was about 12.7% (Table 1). Neither high oleic lines VK508, VK876 nor low oleic lines RIL100, LG28, RHA416, K824 showed any significant discrepancy. This type of spatial gradient along an embryo in the oleic acid content seems to be caused by an environmental lowering of oxygen concentration. The phenomenon could be called environmental homozygous increase in oleic acid content. These results agree with earlier research (Garcés et al., 1989; Fernández-Moya et al., 2003).

Table 1. Average oleic acid content in the oil of seeds and seed parts of inbred lines

Line	Oleic acid content, %				LSD_{05}
	embryo	gemmule	cotyledon	Δ^1	
VK508	92.3	92.2	92.3	0.1	0.4
VK876	89.2	88.8	89.2	0.4	1.9
LG27	72.0	60.6	73.3	12.7	2.3^2
RIL100	36.3	35.8	36.3	0.5	9.1
LG28	28.4	27.9	28.5	0.6	5.1
RHA416	27.3	24.8	27.6	2.8	3.7

[1] Difference between cotyledon and gemmule (five seeds per line)

[2] $p< 0.05$

A wide range of distribution of heterozygous *Olol* F_1 seeds on oleic acid classes in the crosses of VK876×LG28 and VK876×K824 was observed (Fig. 1). One half of the seeds belonged to the high oleic class from 76 to 91% and the other half varied within the intermediate class from 46 to 76%. This abnormal variation of the F_1 seeds is believed to be due to the genetic suppressor of *Ol* mutation of normal lines LG28 and K824. As a result of the incomplete penetrance of a dominant mutation *Ol* in a heterozygote can be detected.

The oleic acid content of gemmule and cotyledon was the same for the F_1 seeds of the mutant class with the embryo mean of 87.6% (Table 2). Differences between the cotyledon and the gemmule of the individual seeds of the mutant class varied accidentally. On the contrary, a significant directional difference in oleic acid content was observed for the F_1 seeds of the intermediate class with the embryo mean of 60.1%. In this case, the cotyledon had a lower content of oleic acid than that of the gemmule with a difference of about 11.6%. This spatial seed gradient could be called an epigenetic heterozygous decrease in oleic acid content. A heterozygous *Olol* embryo under the action of the suppressor seems to possess a reversion to the normal phenotype during mitotic division of the cotyledon cells and seed development. It should be noted that the environmental homozygous change in oleic acid content is in the opposite direction to the epigenetic heterozygous variation.

Table 2. Average (range) oleic acid content in the oil of F_1 seeds and seed parts.

Phenotypic class of whole seed	Number of seeds	Oleic acid content, %				LSD_{05}
		embryo	gemmule	cotyledon	Δ^1	
Mutant, high oleic	20	87.6 (77.3-91.4)	87.5 (75.6-90.7)	87.6 (77.3-91.5)	0.1	2.4
Intermediate	20	60.1 (45.8-75.6)	70.5 (51.4-87.3)	58.9 (44.7-75.3)	-11.6	6.6^2

[1] Difference between cotyledon and gemmule
[2] $p < 0.05$

The data on the phenotypic coincidence in oleic acid content between gemmule and cotyledon of the F_1 seeds are evidence of the above assumption (Table 3). The portion of mosaic F_1 seeds was 23% (9/40). It is very important to stress that only one type of mosaic heterozygous embryo was found, i.e. mutant gemmule and intermediate cotyledon. These results agree with our previous observation (Demurin and Škorić, 1996).

Table 3. Phenotypic coincidence in oleic acid content between gemmule and cotyledon of F_1 seeds.

Cross	Number of F_1 seeds				Portion of mosaic seeds
	G_m/C_m	G_m/C_{int}	G_{int}/C_m	G_{int}/C_{int}	
VK876×LG28	13	4	0	3	0.20
VK876×K824	7	5	0	8	0.25
total	20	9	0	11	0.23

G – gemmule, C – cotyledon, m – mutant (high oleic), int – intermediate oleic class

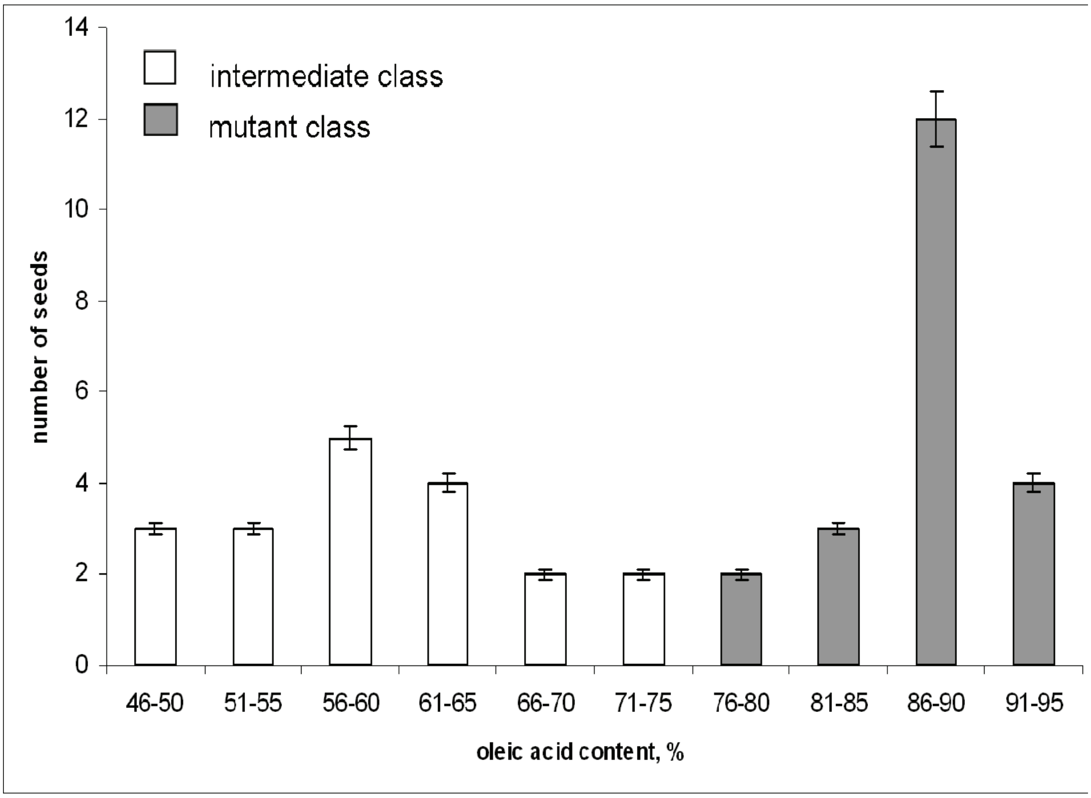

Fig. 1. Distribution of heterozygous *Olol* F_1 seeds on oleic acid classes in the crosses of VK876×LG28 and VK876×K824, n=40

In conclusion, the phenomenon of the longitudinal gradient of oleic acid content in the sunflower seeds has to be taken into account when the half-seed technique is used.

ACKNOWLEDGEMENTS

This work was supported by a grant № 06-04-97603 from the Russian Foundation of Basic Research (RFFI) and Administration of Krasnodar region, Russia.

REFERENCES

Demurin, Y., and D. Škorić. 1996. Unstable expression of *Ol* gene for high oleic acid content in sunflower seeds. p. 145-150 (Breeding). In: Proc. 14th Int. Sunflower Conf., Beijing/Shenyang, China.

Dyakov, A.B., and T.A. Perestova. 1975. Morphology and Anatomy of Sunflower Plant. p. 21-29. In: V.S. Pustovoit (ed.), Sunflower. Monograph. Kolos, Moscow.

Fernández-Moya, V., E. Martínez-Force, and R. Garcés. 2003. Temperature-related non-homogeneous fatty acid desaturation in sunflower (*Helianthus annuus* L.) seeds. Planta 216:834-840.

Garcés, R., J.M. García, and M. Mancha. 1989. Lipid characterization in seeds of a high oleic acid sunflower mutant. Phytochem. 28:2597-2600.

Garcés, R., and M. Mancha. 1989. Oleate desaturation in seeds of two genotypes of sunflower. Phytochem. 28:2593-2595.

Popov, P.S., and A.B. Dyakov. 1975. Ripening of Sunflower Seeds. p. 87-103. In: V.S. Pustovoit (ed.), Sunflower. Monograph. Kolos, Moscow.

Seiler, G.J. 1997. Anatomy and Morphology of Sunflower. p. 67-111. In: A.A. Schneiter (ed.), Sunflower Production and Technology. Agronomy Monograph 35. ASA, CSSA and SSSA, Madison, WI, USA.