

## UP-TO-DATE RESULTS ON BIOCHEMICAL GENETICS OF SUNFLOWER IN VNIIMK

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### SUMMARY

Forty-seven specimens from the world germplasm collection of VIR were crossed as male with a high oleic line HA89A OL as female. The mean of 10 seed samples of F<sub>1</sub> was 83% oleic acid content. High oleic trait was mainly dominant. Two crosses showed intermediate inheritance with the means of 62% and 68%. The range of single-seed variability of these two crosses was from 53% to 89% oleic acid. The genotypes showing abnormal segregation in F<sub>1</sub> have been selected to develop a set of normal lines with a suppressor for Ol mutation. The inheritance of mid- (increased) oleic content (about 62%) was studied in the F<sub>1</sub>, F<sub>2</sub> and BC generations of the inbred line LG27 crossed with the high oleic line HA89 OL. Mid-oleic acid content was shown to be controlled by a recessive allele of ol gene, designated ol<sup>l</sup>. An inbred line with 92% oleic content was obtained to investigate a super high oleic genotype. Another high oleic line was selected for low stearic content, about 1%. High palmitic (about 24%) line is being developed. High-oleic mutation is quite detectable both in immature and germinated seeds. Mutations for tocopherol composition, *tph*<sub>1</sub> and *tph*<sub>2</sub>, have a high level of expressivity in different parts of the plant. It was shown for *tph*<sub>2</sub> mutation that its heterozygotes could be clearly identified by the analysis of pollen from a single head. The open pollinated variety Favorite was developed in VNIIMK in 1993. It was shown that seed oil of the variety had about three times higher hydrolytic stability estimated as the lower level of free fatty acid value of crude oil.

**Key words:** fatty acid mutant, tocopherol, free fatty acids

### INTRODUCTION

Research on biochemical genetics of the characters that influence seed oil quality was carried out at VNIIMK since 1982. Presently, it includes three main areas—fatty acid profiles, tocopherol composition and free fatty acid content.

A high-oleic mutation from Pervenetz germplasm was shown to be controlled by both a dominant *Ol* gene and a genotypic factor of reversion (suppressor) with unstable action (Demurin and Škorić, 1996).

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The open pollinated variety Favorite with low level of free acid content in seed oil was developed in VNIIMK in 1993 (Surovikin and Borodin, 1995).

## MATERIALS AND METHODS

The pollen from individual plants of 47 specimens of the world germplasm collection of VIR (St. Petersburg) was collected in the field condition of Cuban Experiment Station of VIR (Krasnodar region) in summer 2001. Female plants of the high-oleic tester HA89 A OL were grown the same season in the experiment field of VNIIMK. All crosses were made using the head-by-head scheme.

Biochemical determination of fatty acids (GC), tocopherols (TLC) and free fatty acids was performed using standard techniques.

## RESULTS AND DISCUSSION

Forty-seven specimens of the world germplasm collection of VIR were crossed as male with a high oleic line HA89A OL as female. The mean of 10 seed samples of F<sub>1</sub> was 83% oleic acid content. High oleic trait was mainly dominant. Two crosses showed intermediate inheritance with the mean of 62% and 68%. The range of single-seed variability of these two crosses was from 53% to 89% oleic acid (Table 1). The genotypes showing abnormal segregation in F<sub>1</sub> were selected to develop a set of normal lines with a suppressor for *Ol* mutation.

Table 1: Oleic acid content of F<sub>1</sub> seeds in crosses of CMS HA89 OL as the female tester

Male genotype	Bulk F <sub>1</sub> seed mean	Min.	Max.
45 accessions	84	70	89
VIR 721-3	62	57	87
VIR 721-4	68	53	89

An inbred line with 92% oleic content was obtained to investigate a super high oleic genotype. Another high oleic line was selected for low stearic content, about 1%. High palmitic (about 24%) line is currently being developed (Table 2).

Table 2: Genetic variants for fatty acid composition (%)

Genotype	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic
Super high oleic	3.7	0.1	2.2	92.3	1.7
Low stearic	4.0	0.2	1.4	91.3	3.1
High palmitic	23.9	3.4	2.0	20.0	50.7

High-oleic mutation is quite detectable both in immature seeds and germinated seeds (Table 3). It is important to develop a method of rapid conversion of low-oleic lines to high-oleic ones. Methods include the half-seed technique. It should be emphasized that fatty acid composition must be determined first or second day after cotyledons emerge in the field, before they turn green. Green tissues are known to contain about 50% of linolenic acid. In that case, high-oleic mutation is not detectable.

Table 3: Expressivity of high-oleic mutation in seed cotyledons

Seed status	Genotype	Oleic acid, %
immature, 12-14 day after flowering	low oleic	45
	high oleic	80
germinating, first day after emergence in field	low oleic	43
	high oleic	79

The mutations for tocopherol composition  $tph_1$  and  $tph_2$  have a high level of expressivity in different parts of the plant. It was shown for  $tph_2$  mutation that heterozygotes for the gene could be clearly identified by the analysis of pollen from a single head. That is possible due to heterozygotes producing a mixture of heterogeneous pollen grains of two types in 1:1 ratio. It is suitable for backcrossing to produce analogs (Table 4).

Table 4: Expressivity of  $tph_2$  mutation in pollen from a single head

Genotype	Tocopherol composition, %			
	Seeds		Pollen grains	
	$\alpha$	$\gamma$	$\alpha$	$\gamma$
$Tph_2 Tph_2$	90	10	85	15
$Tph_2 tph_2$	90	10	60	40
$tph_2 tph_2$	5	95	5	95

The open pollinated variety Favorite was developed in VNIIMK in 1993. It was shown that seed oil of the variety has about three times higher hydrolytic stability and a low level of free fatty acid value of crude oil.

Table 5: Free fatty acids value in crude seed oil (%)

Genotype	Before lipase activation	After lipase activation
Favorite	0.32	0.53
Yubilejny 60	0.73	1.67

The main reason for that is believed to be the decrease in seed lipase enzyme activity for about 1.8 times (Table 5). Nevertheless, the conclusive explanation is still being sought for.

## CONCLUSIONS

Research activity on biochemical genetics of sunflower in the genetics laboratory of VNIIMK includes investigation of variability and inheritance of the seed traits that influence oil quality. Most interesting results have been obtained for fatty acid and tocopherol composition. Free fatty acid content is considered to be a new promising research object.

## REFERENCES

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## LOS RESULTADOS MÁS RECIENTES DE LA GENÉTICA BIOQUÍMICA DE GIRASOL, OBTENIDOS EN EL INSTITUTO VNIIMK

### RESUMEN

De la colección mundial de germoplasma del Instituto VIR se ha tomado 47 muestras y cruzado como la componente paterna con la línea altamente oleica HA89A, como la línea de madre. El promedio de contenido del ácido oleico para 10 semillas en la generación  $F_1$  era 83%. La propiedad de alto contenido del ácido oleico, fue dominante en la mayor parte. En dos cruces se ha notado el modo intermediario de herencia con el promedio de 62 y 68%. El rango de variación del contenido del ácido oleico de una semilla en estos dos cruces era 53-89%. Los genotipos con la segregación anormal en la generación  $F_1$  fueron elegidos con el motivo de formación de líneas normales con el gen supresor para la mutación *Ol*. La herencia del contenido medio (aumentado) del ácido oleico de unos 62% líneas inbred LG27 en los cruzamientos con la línea altamente oleica HA89 OL se ha investigado en las generaciones  $F_1$ ,  $F_2$  y BC. Los resultados han mostrado que el contenido medio del ácido oleico está controlado por parte del alelo recesivo del gen *ol*, marcado con *ol<sup>l</sup>*. Para investigar un genotipo altamente oleico super, se ha creado una línea inbred con 92% del ácido oleico. También se ha seleccionado una línea altamente oleica con bajo contenido del ácido esteárico (cca. 1%). Está en curso la formación de una línea con alto contenido del ácido palmítico (cca. 24%). La mutación altamente oleica puede ser detectada fácilmente tanto en la semilla inmadura, como en la semilla germinada. Las mutaciones del contenido tocoferólico *tph<sub>1</sub>* y *tph<sub>2</sub>* tienen alto nivel de expresión en diferentes partes de la planta. En la mutación *tph<sub>2</sub>*, se ha mostrado que los heterocigotos para este gen pueden identificarse claramente tras el análisis del contenido tocoferólico de polen de la cabeza particular. La variedad de polinización abierta Favorite, fue creada en VNIIMK-u 1993. g. Se ha mostrado que el aceite de semilla de esta variedad, tiene alrededor de tres veces mayor la estabilidad hidrolítica, evaluada como el nivel más bajo de valores de los ácidos grasos libres en el aceite crudo.

**TOUT DERNIERS RÉSULTATS DE GÉNÉTIQUE  
BIOCHIMIQUE DU TOURNESOL À L'INSTITUT VNIIMK**

## RÉSUMÉ

Quarante-sept échantillons ont été pris dans la collection de germoplasmes de l'institut VIR pour être croisés en tant que composantes parentales mâles avec la ligne très oléagineuse HA89A en tant que ligne mère. La moyenne du contenu d'acide oléique pour 10 graines dans la génération  $F_1$  était de 83%. Un important contenu d'acide oléique était en général la caractéristique dominante. Pour deux croisements, on a noté une méthode intermédiaire de transmission avec une moyenne de 62 et de 68%. La portée de variation du contenu d'acide oléique d'une graine était de 53-89% pour ces deux croisements. Les génotypes avec ségrégation anormale dans la génération  $F_1$  ont été choisis pour créer des lignes normales avec gène de suppression pour la mutation *Ol*. La transmission d'un contenu d'acide oléique moyen (augmenté) d'environ 62% de la ligne inbred LG27 dans les croisements avec la ligne à haute teneur oléagineuse HA89 OL a été examinée dans les générations  $F_1$ ,  $F_2$  et BC. Les résultats ont montré que le contenu moyen d'acide oléique est contrôlé par un allèle récessif du gène *ol*, noté *ol*<sup>1</sup>. Pour pouvoir examiner un génotype supra grandement oléagineux, on a créé une ligne inbred avec 92% d'acide oléique. On a aussi sélectionné une ligne hautement oléagineuse à faible contenu d'acide stéarique (environ 1%). Une ligne à haute teneur d'acide palmitique (environ 24%) est aussi en cours de création. On peut assez facilement détecter une mutation à haute teneur oléagineuse autant dans la graine immature que dans la graine germée. Les mutations de contenu de tocophérol *tph*<sub>1</sub> et *tph*<sub>2</sub> ont un haut niveau d'expression dans différentes parties de la plante. Dans la mutation *tph*<sub>2</sub> il a été démontré que les hétérozygotes de ce gène peuvent être clairement identifiés après l'analyse du contenu de tocophérol du pollen de certaines têtes. La variété Favorite fécondée par pollinisation libre a été créée à VNIIMK en 1993. Il a été démontré que l'huile de la graine de cette variété avait une stabilité d'hydrolyse environ trois fois plus grande évaluée comme un niveau plus faible de la valeur des acides gras libres dans l'huile brute.

