

## GENE LINKAGE TEST FOR *imr* WITH *ol*, *tph1* AND *tph2* MUTATIONS IN SUNFLOWER

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### SUMMARY

The objective of this research was to check possibilities of combining imidazolinone resistance with mutations for seed oil quality characters in sunflower. An imidazolinone resistant line HA425 (*ImrImr*) with normal fatty acid and tocopherol compositions was used as female after hand emasculatation. An imidazolinone susceptible inbred line VK876 with high oleic content and changed tocopherol composition (*OiOl tph1tph1 tph2tph2*) served as male. Linkage test in the F<sub>2</sub> and F<sub>3</sub> generations has shown the *Imr* gene for imidazolinone resistance to be independently inherited from the high oleic mutation *Ol*, high β-tocopherol mutation *tph1* and high γ-tocopherol mutation *tph2*. Recombinant genotypes have been selected for development of inbred lines.

**Key words:** imidazolinone resistance, inheritance, oleic mutation, tocopherols

### INTRODUCTION

Resistance to imidazolinone herbicides was found in a population of wild sunflower (Al-Khatib *et al.*, 1998). This character was shown to be controlled by one semi-dominant gene *Imr1* and a modifier gene *Imr2* (Bruniard and Miller, 2001).

A high oleic genotype Pervenets was developed as a result of DMS chemical mutagenesis on the seeds of the variety VNIIMK 8931 (Soldatov, 1976). The first research on genetic control of the high oleic mutation led to the hypothesis of single dominant gene, *Ol* (Fick, 1984).

Two non-allelic unlinked genes, *Tph1* and *Tph2*, which control tocopherol composition in sunflower seeds were identified (Demurin, 1993). Recessive alleles of the genes were found by wide-scale screening and selfing as spontaneous mutations. *Tph1* gene controls the ratio of α- and β-tocopherols, whereas *Tph2* gene affects that of α- and γ-homologues. The *tph2* mutation has an epistatic action over *tph1* with δ-tocopherol appearance.

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The objective of this research was to test the linkage of the above genes. The answer will provide breeders with information of possibility to combine desired characters in one phenotype.

## MATERIALS AND METHODS

HA425, an imidazolinone resistant inbred line with normal fatty acid and tocopherol composition was used as female after hand emasculation. VK876, an imidazolinone susceptible inbred line with high oleic content and changed tocopherol composition (*OiOl tph1tph1 tph2tph2*) served as male.

Crosses were made at the experiment field of VNIIMK, Krasnodar, in the summer of 2003. F<sub>1</sub> plants were grown and self-pollinated in the greenhouse in the winter of 2003-2004. F<sub>2</sub> plants were grown and self-pollinated in the field in the summer of 2004. F<sub>3</sub> populations from F<sub>2</sub>-derived plants were tested in the field in the summer of 2005.

P, F<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> plants were treated at the V6 stage with imazethapyr (Pivot) at a 7 × rate (6 ml l<sup>-1</sup> or 245 g AI ha<sup>-1</sup> or 4 mg AI per a plant).

Fatty acid composition in seeds bulk was determined by gas chromatography of methyl esters.

Tocopherol composition was estimated with thin-layer chromatography (TLC) followed by Emmerie-Engel reaction without corrections of the different rates of homologue staining (Popov *et al.*, 1991).

## RESULTS AND DISCUSSION

The parent lines HA425 and VK876 confirmed to be resistant and susceptible (died after seven days) to imidazolinone herbicide, respectively. F<sub>1</sub> intermediate plants showed a definite degree of chlorosis symptoms. After combining the number of resistant and intermediate plants as survived in the F<sub>2</sub> and F<sub>3</sub> segregating populations, one semi-dominant *Imr* gene model was accepted (Table 1). Moreover, the five homogeneous and seven F<sub>3</sub> populations segregating for resistance fit an expected ratio of 1:2 ( $\chi^2_{1:1}=0.34 < \chi^2_{05}=3.84$ ). That was due to the lack of susceptible F<sub>3</sub> populations because susceptible plants had died after herbicide treatment in the F<sub>2</sub> generation. Thus the recessive homozygote *imr imr* was eliminated in F<sub>2</sub>.

Two out of twelve F<sub>3</sub> populations, numbered 1 and 4, were high oleic phenotypes with more than 82% of oleic acid. Two other populations, numbered 6 and 9, were clearly low oleic (Table 2). The ratio of 2 homogeneous high oleic: 10 segregated: 2 homogeneous low oleic fit the expected ratio 1:2:1 with  $\chi^2_{1:2:1}=3.33 < \chi^2_{05}=5.99$ .

Table 1: Inheritance of imidazolinone resistance in sunflower

Generation, population	Plant number		$\chi^2_{3:1}$ $\chi^2_{05}=3.84$
	Resistant	Susceptible	
HA425 ♀	20	0	-
HA876 ♂	0	14	-
F <sub>1</sub>	19	0	-
F <sub>2</sub>	98	32	0.01
F <sub>3</sub> 1	24	0	-
2	16	6	0.06
3	23	0	-
4	27	0	-
5	19	4	0.40
6	22	0	-
7	20	3	1.75
8	12	3	0.20
9	19	6	0.01
10	20	5	0.33
11	21	5	0.46
12	20	0	-

Table 2: Fatty acid composition in seed bulk of imidazolinone resistant F<sub>2</sub> -derived F<sub>3</sub> populations

Population index	Fatty acid composition, %			
	Palmitic	Stearic	Oleic	Linoleic
1	7	2	84	7
2	8	5	31	56
3	7	3	46	44
4	5	3	82	10
5	7	3	34	56
6	8	3	25	64
7	7	5	35	53
8	8	6	48	38
9	8	6	18	68
10	8	7	34	51
11	8	6	37	49
12	9	7	35	49

Theoretically, nine types of inheritance for two tocopherol genes can be observed in F<sub>3</sub> seeds at the ratio 4 (9 $\alpha$ :3 $\beta$ :3 $\gamma$ :1 $\delta$ ) : 2 (3 $\alpha$ :1 $\beta$ ) : 2 (3 $\alpha$ :1 $\gamma$ ) : 2 (3 $\beta$ :1 $\delta$ ) : 2 (3 $\gamma$ :1 $\delta$ ) : 1 (all  $\alpha$ ) : 1 (all  $\beta$ ) : 1 (all  $\gamma$ ) : 1 (all  $\delta$ ) when  $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$  correspond to normal, *tph1*, *tph2* and double mutation phenotypes. Six types were found with exception of 3a: 1 $\beta$ , all  $\alpha$  and all  $\delta$ . Three populations were homogeneous in tocopherol composition, 3 and 7 with  $\beta$ -form and 10 with  $\gamma$ -form (Table 3).

Table 3: Segregation for tocopherol genes in individual seeds of the F<sub>3</sub> populations

Population index	Number of seeds			
	<i>Tph1-Tph2-</i>	<i>tph1tph1 Tph2-</i>	<i>Tph1-tph2tph2</i>	<i>tph1tph1 tph2tph2</i>
1	0	0	15	4
2	5	2	8	1
3	0	19	0	0
4	0	15	0	4
5	9	2	5	2
6	15	0	4	0
7	0	19	0	0
8	0	0	14	5
9	1	11	4	3
10	0	0	38	0
11	0	12	0	6
12	2	1	14	2

Recombinant homozygous genotypes in F<sub>2</sub> *ImrImr* and *OIOI* (plant 1, 4), *ImrImr* and *tph1tph1* (3, 4), *ImrImr* and *tph2tph2* (1) were obtained (Table 4). It means that imidazolinone resistance was combined in one phenotype with high oleic acid content, high  $\beta$ -tocopherol content or high  $\gamma$ -tocopherol content.

Table 4: Deduced F<sub>2</sub> genotypes after estimation of F<sub>3</sub> populations

Plant index	Character			
	Imi-resistance	Oleic content	$\beta$ -tocopherol	$\gamma$ -tocopherol
1	<i>ImrImr</i>	<i>OIOI</i>	<i>Tph1tph1</i>	<i>tph2tph2</i>
2	<i>Imrimr</i>	<i>OIOI</i>	<i>Tph1tph1</i>	<i>Tph2tph2</i>
3	<i>ImrImr</i>	<i>OIOI</i>	<i>tph1tph1</i>	<i>Tph2Tph2</i>
4	<i>ImrImr</i>	<i>OIOI</i>	<i>tph1tph1</i>	<i>Tph2tph2</i>
5	<i>Imrimr</i>	<i>OIOI</i>	<i>Tph1tph1</i>	<i>Tph2tph2</i>
6	<i>ImrImr</i>	<i>olol</i>	<i>Tph1Tph1</i>	<i>Tph2tph2</i>
7	<i>Imrimr</i>	<i>OIOI</i>	<i>tph1tph1</i>	<i>Tph2Tph2</i>
8	<i>Imrimr</i>	<i>OIOI</i>	<i>Tph1tph1</i>	<i>tph2tph2</i>
9	<i>Imrimr</i>	<i>olol</i>	<i>Tph1tph1</i>	<i>Tph2tph2</i>
10	<i>Imrimr</i>	<i>OIOI</i>	<i>Tph1Tph1</i>	<i>tph2tph2</i>
11	<i>Imrimr</i>	<i>OIOI</i>	<i>tph1tph1</i>	<i>Tph2tph2</i>
12	<i>ImrImr</i>	<i>OIOI</i>	<i>Tph1tph1</i>	<i>Tph2tph2</i>

## CONCLUSIONS

The linkage test in the F<sub>2</sub> and F<sub>3</sub> generations showed the *Imr* gene for imidazolinone resistance to be independently inherited from the high oleic mutation *OI*, high  $\beta$ -tocopherol mutation *tph1* and high  $\gamma$ -tocopherol mutation *tph2*. Recombinant genotypes were selected for development of inbred lines.

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**PRUEBA DE VINCULACIÓN DEL GEN *IMR* CON MUTACIONES *ol*, *tph1* Y *tph2* EN GIRASOL**

## RESUMEN

El objetivo de esta investigación ha sido investigar la posibilidad de que exista un vínculo entre la resistencia hacia imidazolinona y las mutaciones en calidad del aceite en girasol. HA425 (*ImrImr*), la línea resistente hacia imidazolinona, pero con el normal contenido de ácidos grasos y tocoferol, fue utilizada como línea materna, tras la esterilización manual. La VK876, línea sensible hacia imidazolinona, y con el alto contenido del ácido oleico y cambiado contenido de tocoferol (*OiOl tph1tph1 tph2tph2*) fue utilizada como línea paterna. La prueba de vinculación, realizada en las generaciones F<sub>2</sub> y F<sub>3</sub>, demostró que el gen *Imr*, que condiciona la resistencia hacia imidazolinona, hereda, independientemente de la mutación con el alto contenido del ácido oleico *Ol*, la mutación con el alto contenido de β-tocoferol *tph1* y la mutación con alto contenido de γ-tocoferol *tph2*. Fueron elegidos los genotipos recombinantes para la formación de las líneas consanguíneas.

**TEST DE LIAISON DU GÈNE *IMR* AVEC LES MUTATIONS *ol*, *tph1* ET *tph2* CHEZ LE TOURNESOL**

## RÉSUMÉ

Le but de cette recherche était d'observer la possibilité de l'existence d'un lien entre la tolérance envers l'imidazolinone et les mutations dans la qualité de l'huile chez le tournesol. Une lignée résistante d'imidazolinone HA425 (*ImrImr*) avec un contenu normal d'acides gras et de tocophérol a été utilisée comme lignée mère après stérilisation manuelle. Une lignée autogame VK876 avec contenu élevé d'acide oléique et composition de tocophérol altérée (*OIOI tph1tph1 tph2tph2*) et sensible à l'imidazolinone a servi de lignée mâle. Le test de liaison dans les générations F<sub>2</sub> et F<sub>3</sub> a montré que le gène *Imr* qui conditionne la résistance envers l'imidazolinone est hérité indépendamment de la mutation avec contenu élevé d'acide oléique *Ol*, de la mutation avec contenu élevé de  $\alpha$ -tocophérol *tph1* et de la mutation avec contenu élevé de  $\beta$ -tocophérol *tph2*. Des génotypes recombinants ont été choisis pour le développement de lignées autogames.