

HIGH OLEIC ACID CONTENT IN SUNFLOWER GENOTYPES IN RELATION WITH RESISTANCE TO DISEASES

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ABSTRACT

The range of sunflower oil has been expanded and its quality largely diversified, mainly with respect to different fatty acids. Special achievements have been obtained with oleic acid which represents 82-85% of the total fatty acids content of some commercial hybrids. Our investigations to incorporate the genes for the high oleic acid in sunflower lines are correlated, at the same time, besides other valuable characteristics, with resistance to sunflower diseases. For a better constancy of high oleic acid content, both parental forms of F₁ hybrids should possess this characteristics. Twelve lines with different resistance to *Phomopsis helianthi* and to the parasite *Orobanche cumana* Wallr. have been studied using observations on natural attack besides artificial infections and infestation in relation with high oleic acid content. Susceptibility to *Phomopsis* was very significantly correlated with low oleic acid content and the frequency of F₂-seeds in classes with values of oleic acid content from 31.0 to 89.3%. The higher values of oleic acid content were in the case of the both F₁ hybrids parents tolerant to *Phomopsis*. Resistance to the parasite *Orobanche cumana* showed an influence on the high oleic acid content especially in F₂ generation. Differences were observed concerning the resistance of the restorer lines and of the hybrids produced with their participation, including their oleic acid content. There were differences between natural attack and artificial infections with *Phomopsis*, seven lines out of twelve showing a lower high oleic acid content under artificial infection conditions.

Key words: high oleic acid content, incorporation of genes, *Orobanche* attack, *Phomopsis* attack sunflower.

INTRODUCTION

The modern requirements of consumers for the nutritive value of food products need a special attention to the aspects of oil quality. Such quality may be improved by the composition of fatty acids, the main indicator of the quality of vegetal oil. There are two important acids in the sunflower oil: oleic and linoleic, which represent together 90% of the total acids, the remainder of 10% consisting in palmitic and stearic acid. In the commercial hybrids, the oleic acid values range between 10 and 50%, depending on the climatic conditions of the field, the temperature during the seeds growing in particular. A strong negative correlation between oleic and linoleic acids was re-

ported (Fernández-Martinez et al., 1986; Vrânceanu et al., 1995).

As with other vegetable oils, quality of sunflower oil is a feature which depends on its use. The high content of oleic acid increases the oil stability to oxidative degradation at high temperatures also, being used in particular in canned food industry and as additive lubricant for cars and textile industry equipment. In order to obtain margarine, oils with a higher unsaturation level are used, that is those oils with a high content of linoleic acid.

It has been acknowledged that sunflower oil with a high oleic acid content has positive nutritional qualities like the olive oil.

The first genotype with a high oleic content is Pervenetz variety obtained in the former Soviet Union after treating the seeds with dimethyl-sulphonate (Soldatov, 1976). The oleic acid content of this variety is of about 75% on an average, although with individual plants this content ranges between 50 and 80% (Miller and Zimmerman, 1983) and with individual seeds, the variation is often greater, between 19 and 94% (Urie, 1985). The offspring of this variety with a high oleic content were very stable even under various conditions of temperature, recording values of over 83% (Urie, 1985; Fernández-Martinez et al., 1989).

A few studies succeeded in elucidating the mechanisms of transmitting the oleic acid content of the germplasm derived from Pervenetz variety. Fick (1984) specified that this feature is controlled by only one partially dominant gene marked by *Ol*. Urie (1985) stated that the trait of high oleic acid was controlled by one modifying gene. Another hypothesis was proposed by Miller et al. (1987) who showed that high oleic phenotypes could be found in cool maturation environment when partially dominant gene *Ol* is combined with the recessive homozygote of a modifier gene *Ml*. Fernández-Martinez et al. (1989) deter-

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mined segregants that indicated the presence of one, two or even three major, complementary genes which control this trait, depending on the parental genotype with low oleic oil content. Baldini et al. (1992) achieved the transformation into genotype with high oleic acid content of six pollen fertility restorer lines and five cytoplasmic male sterile lines, with an oleic acid content of 62-86%.

Demurin and Škoric (1996) showed an unstable expression of *Ol* for high oleic acid content in sunflower seeds. Fernández-Martínez et al. (1993) published a comparative study which included four hybrids with a high and a low oleic acid content, reaching the conclusion that hybrids with a high oleic acid content yielded a bigger production of seeds and oil than those with a low oleic acid content. Salera and Baldini (1998) studied some sunflower genotypes with high and low oleic acid under different environmental conditions.

Sunflower diseases are very important for their effect on sunflower oil quantity and quality. A research programme for sunflower oil quality improvements was initiated at RICIC Fundulea by changing the chemical composition of fatty acids, with the purpose of studying the genetic control of this trait and its incorporation into the best restorer and male sterile lines, with a view to obtaining commercial hybrids with a high oleic acid content and useful agronomic characteristics.

This paper presents the breeding method used for obtaining genotypes with high oleic acid content and the influence of the pathogenic agent *Phomopsis helianthi* and of the parasite *Orobanche cumana* Wallr. on this trait.

MATERIALS AND METHODS

The sunflower material used as a source for the high oleic acid content was constituted of two lines: one line received from the University of Pisa, Italy and the other from the University of North Dakota, USA.

The low oleic acid content genotypes used as recurrent parents were represented by the fertile analogous lines (B) of the sterile commercial lines (A), as well as restorer lines created at RICIC - Fundulea and most of them

used in the breeding programme. The seeds of every generation were analysed by means of the gascromatograph.

Pursuant to the chemical analyses, such lines were selected for high oleic acid content. Some of the lines used as recurrent parents were lines with resistance to *Orobanche cumana* Wallr. or with a good tolerance to *Phomopsis helianthi* pathogenic agent attack.

The backcross method was used to transform the lines. The lines with a low oleic content were hand emasculated and pollinated with pollen from the donor line. The resulted seeds were analysed, obtaining F₁ plants with a high content of oleic acid. Further, such seeds were used as recurrent parent for the lines with a low oleic acid content. Six generations of backcross were achieved, followed by three generations of self-fertilization. After three generations of self-fertilization and selection, lines with a very high oleic acid content, over 85%, were identified (Vrânceanu et al., 1995; Figure 1).

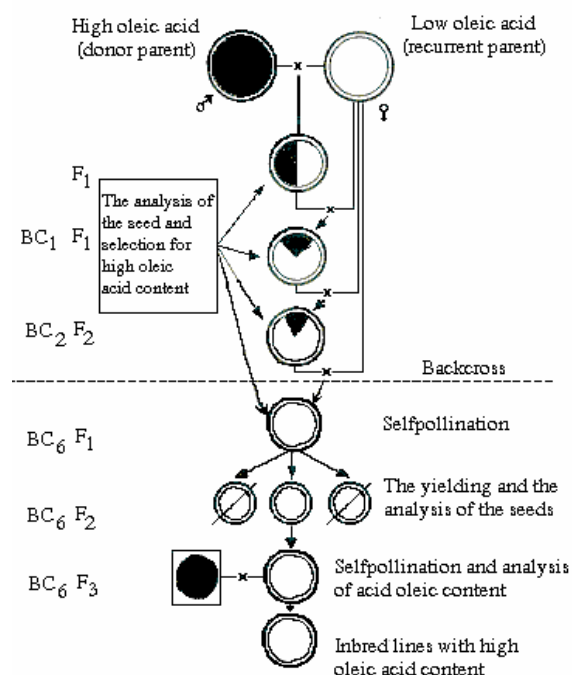


Figure 1. Selection scheme for introducing of high oleic acid content in fertility restores and maintainers of sterile lines (Vrânceanu et al., 1995)

The method of analysis used for the determination of the chemical composition of oil was the Downey and Harvel (1963) method, as modified by Conte et al. (1981).

In order to see the relation between the above-mentioned parasites attack and the oleic acid content, such genotypes were studied under conditions of natural infection / infestation respectively, in the field, but also under conditions of artificial infection / infestation. Artificial infection with *Phomopsis* was performed in a specially arranged field under irrigation by aspersion conditions and the study for the *Orobanche* attack under artificial infestation conditions was made in vegetation pots where a special mixture of earth, sand and broomrape seeds was introduced.

RESULTS AND DISCUSSIONS

The backcross breeding method followed by selection at the seed level may be applied because the dominant nature of the high oleic acid trait and the strong influence of the maternal genotype allow this procedure.

The lines obtained using the above-described method are phenotypically similar to the inbred lines with a low oleic acid content. The dominant nature given by this trait permits the incorporation of the gene in only one of the parental lines of a hybrid. According to other authors (Fernández Martínez et al., 1988), the strong embryogametophytic control and the involved genes show that the hybrids obtained may have a lower oleic acid content because the seeds of F_1 plants give F_2 seeds which segregate for the high, the intermediary and the low type. The average of the oleic acid content will be of 70% or even lower, if such hybrids are obtained without isolation. The combination of the lines with high oleic acid content, homozygote for the oleic acid, leads to obtaining combinations of over 90% oleic acid. This material will be stable and isolation is not necessary. Considering the foregoing considerations, throughout the breeding programme, the introduction of the genes for high oleic acid content was carried out both at the mother lines and at the restoring lines (Vrânceanu et al., 1995).

Table 1 presents the sunflower genotypes (inbred lines) after the introduction of the genes with high oleic acid content which have a different reaction to the attack of the two parasites under study.

Table 1. Sunflower inbred lines with high oleic acid content, after introducing *O1* gene

No.	The genotypes (inbred lines)	High oleic content (%)
1.	HO-842 -1 (very tolerant to <i>Phomopsis</i>)	89.8
2.	HO-97-842 -2 (tolerant to <i>Phomopsis</i>)	81.1
3.	HO-804 -1 (very tolerant to <i>Phomopsis</i>)	89.2
4.	HO-804 -2 (tolerant to <i>Phomopsis</i>)	80.0
5.	HO-850 (medium tolerant to <i>Phomopsis</i>)	88.2
6.	HO-822 (medium tolerant to <i>Phomopsis</i>)	83.5
7.	HO-837 (resistant to <i>Orobanche</i>)	89.0
8.	HO-884 -RF (medium tolerant to <i>Phomopsis</i>)	81.2
9.	HO-920 -RF (resistant to <i>Orobanche</i>)	89.1
10.	HO-875 -RF (medium tolerant to <i>Phomopsis</i>)	81.1
11.	HO-942 -RF (resistant to <i>Orobanche</i>)	85.0
12.	HO-918 -RF (tolerant to <i>Phomopsis</i>)	84.3

In the table, under numbers 1 and 2 the same genotype with good tolerance to *Phomopsis* is recorded, but which acquired the trait of high oleic acid content using the two different sources. In the first case, the source of high oleic acid content was also of a good tolerance to *Phomopsis* so that the distinction in percentage of oleic acid content of the genotype resulted following transformation is evident. The same thing happens in the case of the genotypes under number 3 and 4.

In table 2 the results concerning the oleic acid content with the same genotypes are presented, but under conditions of natural and artificial infection with the two parasites.

In the case of *Phomopsis*, under conditions of natural and artificial infection, the attack of the parasite displays higher values, pointing out at the same time a reduction of the oleic acid content in the case of a stronger attack.

A significant positive correlation between *Phomopsis* resistance and high oleic acid content was found. It may be noted that when HO-842-1, HO-804-1 and HO-918 RF lines were parents, the attack was very low, and oleic acid very high (Table 3).

Apparently, *O1* alleles could be associated with genes for resistance to *Phomopsis* attack.

The broomrape infestation does not influence so strongly the oleic acid content in the case of the inbred lines or of F_1 generation. A correlation between the strong attack of the parasite and the level of high oleic acid content was observed in F_2 generation (Table 4).

Table 2. The results concerning the tests for resistance to *Phomopsis* / *Diaphorte helianthi* Munt. Cvet et al. and *Orobanche cumana* Wall. of twelve sunflower inbred lines with high oleic acid content, Fundulea, Constanta, 1998-1999

No.	The genotypes	<i>Phomopsis</i>				<i>Orobanche</i>			
		natural attack		artificial infection		natural attack		artificial infestation	
		high oleic (%)	attack (%)	high oleic (%)	attack (%)	high oleic (%)	attack (%)	high oleic (%)	attack (%)
1.	HO-842 -1	82.0	1.7	80.0	7.8	89.0	68	88.7	77
2.	HO-842 -2	80.7	3.9	78.1	12.9	80.7	53	79.1	61
3.	HO-804 -1	84.2	0.9	81.1	1.7	88.3	57	87.4	66
4.	HO-804 -2	79.2	5.0	71.9	14.2	80.3	48	78.2	61
5.	HO-850	69.1	20.7	49.9	50.0	77.2	37	77.0	59
6.	HO-822	65.0	20.3	40.1	58.1	78.0	41	74.7	74
7.	HO-837	65.2	27.4	48.7	59.4	89.1	0	89.1	0
8.	HO-884 -RF	67.0	21.0	49.3	60.7	78.0	67	77.1	89
9.	HO-920 -RF	69.0	19.3	51.4	44.6	79.3	1	78.0	3
10.	HO-875 -RF	72.0	22.1	50.0	47.3	79.5	59	74.3	87
11.	HO-942 -RF	71.3	18.2	51.4	43.8	80.7	1	77.0	2
12.	HO-918 -RF	79.9	8.3	77.0	18.9	80.3	49	79.0	64
13.	Control 1 <i>Phomopsis</i>		78.4		94.0				
14.	Control 2 <i>Phomopsis</i>		1.1		3.1				
15.	Control 1 <i>Orobanche</i>						89		100
16.	Control 2 <i>Orobanche</i>						0		0
		LSD 5% = 6.7 0,1% = 13.3		LSD5 % = 7.6 0,1% = 15.3		LSD 5% = 6.2 0,1% = 12.4		LSD 5% = 6.9 0,1% = 13.1	

Table 3. Relations between *Phomopsis* resistance and high oleic acid content in F₂ generation

No	Cross	High oleic content (%)	<i>Phomopsis</i> attack %
1.	HO-842 -1 x HO-884 RF	81.1	7.3
2.	HO-842 -1 x HO-875 RF	80.0	11.0
3.	HO-842 -1 x HO-918 RF	89.3	0.4
4.	HO-842 -2 x HO-884 RF	66.6	27.3
5.	HO-842 -2 x HO-875 RF	52.4	31.5
6.	HO-842 -2 x HO-918 RF	80.1	10.7
7.	HO-804 -1 x HO-884 RF	80.0	5.2
8.	HO-804 -1 x HO-875 RF	77.2	13.7
9.	HO-804 -1 x HO-918 RF	89.1	1.7
10.	HO-804 -2 x HO-884 RF	67.2	33.5
11.	HO-804 -2 x HO-875 RF	50.0	29.7
12.	HO-804 -2 x HO-918 RF	79.8	12.8
13.	HO-850 x HO-884 RF	35.2	41.5
14.	HO-850 x HO-875 RF	34.5	39.1
15.	HO-850 x HO-918 RF	68.3	19.4
16.	HO-822 x HO-884 RF	37.7	47.1
17.	HO-822 x HO-875 RF	31.0	44.7
18.	HO-822 x HO-918 RF	61.3	32.4
LSD 5% = 6,3 0,1% = 11.8		r = -0.94***	

The relation between *Orobanche* attack and oleic acid content may be explained by the association of *Ol* alleles with some loci for adaptation to dry conditions.

CONCLUSIONS

There is a wide genetic variability concerning the oleic acid content (10-91%) of the converted inbred lines, which allows the successful development of the breeding pro-

Table 4. Relations between *Orobanche* resistance and high oleic acid content, in F₂ generation

No	Cross	High oleic content (%)	<i>Orobanche</i> attack %
1.	HO-837 x HO-884 RF	82.8	0
2.	HO-850 x HO-884 RF	60.3	72
3.	HO-822 x HO-884 RF	61.8	67
4.	HO-837 x HO-920 RF	81.7	0
5.	HO-842 -1 x HO-920 RF	84.7	9
6.	HO-804 -1 x HO-920 RF	81.0	11
7.	HO-850 x HO-920 RF	60.3	14
8.	HO-822 x HO-920 RF	68.5	17
9.	HO-837 x HO-875 RF	82.3	0
10.	HO-850 x HO-875 RF	66.6	87
11.	HO-837 x HO-942 RF	85.3	0
12.	HO-842 -1 x HO-942 RF	81.1	15
13.	HO-804 -1 x HO-942 RF	84.4	22
14.	HO-850 x HO-942 RF	71.2	19
15.	HO-822 x HO-942 RF	70.4	12
16.	HO-837 x HO-918 RF	85.3	0
17.	HO-850 x HO-918 RF	75.4	78
18.	HO-822 x HO-918 RF	71.9	83
19.	HO-842 -1 x HO-884	80.4	64
20.	HO-842 -1 x HO-875	82.8	77
LSD 5% = 5.5 0,1% = 10.1		r = -0.42*	

grammes for high oleic acid content. For a better constancy of the oleic acid content, in the cross-breeding, both improved parental forms need to be used.

Phomopsis attack is strongly correlated with the oleic acid, inclusively in F₂ generation so that *Ol* alleles could be associated with genes for resistance to *Phomopsis* attack.

Orobanche cumana Wallr. parasite influences the oleic acid content of sunflower genotypes, in a lower degree, but a stronger influence could be noticed in F₂ generation.

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Table 1

Sunflower inbred lines with high oleic acid content, after introducing OI gene

The genotypes (inbred lines)	High oleic content (%)
HO-842-1 (very tolerant to <i>Phomopsis</i>)	89.8
HO-97-842-2 (tolerant to <i>Phomopsis</i>)	81.1
HO-804-1 (very tolerant to <i>Phomopsis</i>)	89.2
HO-804-2 (tolerant to <i>Phomopsis</i>)	80.0
HO-850 (medium tolerant to <i>Phomopsis</i>)	88.2
HO-822 (medium tolerant to <i>Phomopsis</i>)	83.5
HO-837 (resistant to <i>Orobanche</i>)	89.0
HO-884-RF (medium tolerant to <i>Phomopsis</i>)	81.2
HO-920-RF (resistant to <i>Orobanche</i>)	89.1
HO-875-RF (medium tolerant to <i>Phomopsis</i>)	81.1
HO-942-RF (resistant to <i>Orobanche</i>)	85.0
HO-918-RF (tolerant to <i>Phomopsis</i>)	84.3

Figure 1 - Selection scheme for introducing of high oleic acid content in fertility restoring and supporting of sterile lines.

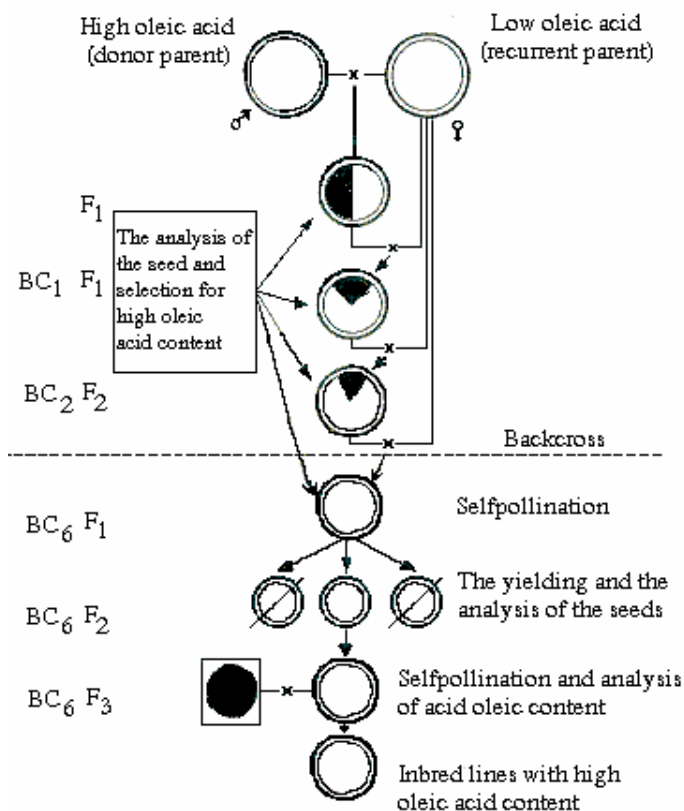


Table 2

The results concerning the tests for resistance to *Phomopsis* / *Diaphorte helianthi* Munt. Cvet et al. and *Orobanche cumana* Wall. of twelve sunflower inbred lines with high oleic acid content, Fundulea, Constanta, 1998-1999

No.	The genotypes	Phomopsis				Orobanche			
		natural attack		artificial infection		natural attack		artificial infestation	
		high oleic (%)	attack	high oleic (%)	attack	high oleic (%)	attack	high oleic (%)	attack
1.	HO-842-1	82.0	1.7	80.0	7.8	89.0	68	88.7	77
2.	HO-842-2	80.7	3.9	78.1	12.9	80.7	53	79.1	61
3.	HO-804-1	84.2	0.9	81.1	1.7	88.3	57	87.4	66
4.	HO-804-2	79.2	5.0	71.9	14.2	80.3	48	78.2	61
5.	HO-850	69.1	20.7	49.9	50.0	77.2	37	77.0	59
6.	HO-822	65.0	20.3	40.1	58.1	78.0	41	74.7	74
7.	HO-837	65.2	27.4	48.7	59.4	89.1	0	89.1	0
8.	HO-884-RF	67.0	21.0	49.3	60.7	78.0	67	77.1	89
9.	HO-920-RF	69.0	19.3	51.4	44.6	79.3	1	78.0	3
10.	HO-875-RF	72.0	22.1	50.0	47.3	79.5	59	74.3	87
11.	HO-942-RF	71.3	18.2	51.4	43.8	80.7	1	77.0	2
12.	HO-918-RF	79.9	8.3	77.0	18.9	80.3	49	79.0	64
13.	Control 1 <i>Phomopsis</i>		78.4		94.0				
14.	Control 2 <i>Phomopsis</i>		1.1		3.1				
15.	Control 1 <i>Orobanche</i>						89		100
16.	Control 2 <i>Orobanche</i>						0		0

LSD 5% = 6,7 LSD5 % = 7,6 LSD 5% = 6,2 LSD 5% = 6,9
0,1% = 13,3 0,1% = 15,3 0,1% = 12,4 0,1% = 13,1

Table 3
Relations between *Phomopsis* resistance and higholeic acid content in F2 generation-seeds

No	Cross	High oleic content (%)	<i>Phomopsis</i> attack
1.	HO-842-1 x HO-884 RF	81.1	7.3
2.	HO-842-1 x HO-875 RF	80.0	11.0
3.	HO-842-1 x HO-918 RF	89.3	0.4
4.	HO-842-2 x HO-884 RF	66.6	27.3
5.	HO-842-2 x HO-875 RF	52.4	31.5
6.	HO-842-2 x HO-918 RF	80.1	10.7
7.	HO-804-1 x HO-884 RF	80.0	5.2
8.	HO-804-1 x HO-875 RF	77.2	13.7
9.	HO-804-1 x HO-918 RF	89.1	1.7
10.	HO-804-2 x HO-884 RF	67.2	33.5
11.	HO-804-2 x HO-875 RF	50.0	29.7
12.	HO-804-2 x HO-918 RF	79.8	12.8
13.	HO-850 x HO-884 RF	35.2	41.5
14.	HO-850 x HO-875 RF	34.5	39.1
15.	HO-850 x HO-918 RF	68.3	19.4
16.	HO-822 x HO-884 RF	37.7	47.1
17.	HO-822 x HO-875 RF	31.0	44.7
18.	HO-822 x HO-918 RF	61.3	32.4

LSD 5% = 6,3
LSD 0,1% = 11,8 r = -0,94***

Table 4
Relations between *Orobanche* resistance and high oleic acid content, in F2 generation-seeds

No	Cross	High oleic content (%)	<i>Orobanche</i> attack
1.	HO-837 x HO-884 RF	82.8	0
2.	HO-850 x HO-884 RF	60.3	72
3.	HO-822 x HO-884 RF	61.8	67
4.	HO-837 x HO-920 RF	81.7	0
5.	HO-842-1 x HO-920 RF	84.7	9
6.	HO-804-1 x HO-920 RF	81.0	11
7.	HO-850 x HO-920 RF	60.3	14
8.	HO-822 x HO-920 RF	68.5	17
9.	HO-837 x HO-875 RF	82.3	0
10.	HO-850 x HO-875 RF	66.6	87
11.	HO-837 x HO-942 RF	85.3	0
12.	HO-842-1 x HO-942 RF	81.1	15
13.	HO-804-1 x HO-942 RF	84.4	22
14.	HO-850 x HO-942 RF	71.2	19
15.	HO-822 x HO-942 RF	70.4	12
16.	HO-837 x HO-918 RF	85.3	0
17.	HO-850 x HO-918 RF	75.4	78
18.	HO-822 x HO-918 RF	71.9	83
19.	HO-842-1 x HO-884	80.4	64
20.	HO-842-1 x HO-875	82.8	77

LSD 5% = 5,5 r = -0,42*
0,1% = 10,1