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RESULTS OF IMMUNOLOGICAL ESTIMATION OF THE ORIGINAL AND BREEDING MATERIAL OF SUNFLOWER FROM VIS

N.P. Tavoljanskiy, V.T. Tikhomirov*, P.V. Chiryaev and V.I. Yakutkin

Veidelevka Institute of Sunflower (VIS), Ltd, Centralnaya 43a, Veidelevka, Belgorod region, 309720, Russia

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SUMMARY

The sunflower is susceptible to a large number of pathogens. Facultative fungal parasites are most harmful to sunflower yield. In this paper we have summarized the results of immunological research on the original and breeding material of sunflower carried out at Veidelevka Institute of Sunflower in recent years. Data on the efficiency of different disinfectants are also presented here.

Key words: sunflower, wild species, selection lines, facultative and obligate parasites, resistance, tolerance, disease, fungicide

INTRODUCTION

In a greater part of Russia, fungal phytopathogens and other pathogens stand in the following sequence of economic importance for sunflower yield (in descending order): *Fusarium* wilt - *Sclerotinia* (white rot) - *Botryotinia fuckeliana* (gray rot) - *Phomopsis* (stem cancer) - *Phoma* black stem - verticilliosis - downy mildew -rust - bacteriosis - *Alternaria* blight - broomrape. This sequence may vary in dependence of the region, weather conditions in production year and resistance or susceptibility to the enumerated diseases of most widely grown varieties and hybrids.

The majority of sunflower phytopathogens belong to the division of Fungi imperfecti. Those are facultative parasites or semi-saprophytes -white and gray rots (caused by *Sclerotinia sclerotiorum* and *Botrytis cinerea*, respectively), *Phomopsis* (*Diaporthe helianthi*), Phoma black stem (*Phoma macdonaldii*), *Fusarium* wilt (*Fusarium spp.*) and others. They infect plants on contact, typically without a clear varietal differentiation with regard to resistance or susceptibility, as is usually observed in obligate parasites -rust (*Puccinia helianthi*), downy mildew (*Plasmopara halstedii*), broomrape (*Orobanche cumana*).

* Corresponding author, e-mail: msch@mail.ru

In the former case, the resistance is horizontal, polygenic or quantitative and it is better to call it tolerance. In the latter case, the resistance is vertical. It is defined by a small number of major genes and it is a quantitative trait.

The immunological work on sunflower conducted at VIS under the conditions of natural and artificial infection was based on the postulates exposed in the previous paragraphs.

MATERIALS AND METHODS

Original and breeding material of sunflower used for immunological estimation included varieties, female and male lines, different sources of CMS and RF-genes, annual and perennial wild species, intra- and interspecific hybrids developed at VIS, collection material from INRA nursery (France), Ukrainian hybrids from Kharkov Breeding Institute and Zaporozhie Institute of Oil Crops, and various forms from Kazakhstan, Bulgaria, Romania, USA and other countries (Tavoljanskiy *et al.*, 2001; Tikhomirov *et al.*, 2002). More than 3 thousand samples were tested in the period 1999-2002.

More than 1600 samples were tested for resistance to ALMR under laboratory conditions. 2-4-day-old sunflower seedlings were infected by the pathogen by submersion of rootlets into *P. halstedii* suspension (in the concentration of 2 × 104 zoospores per 1 ml.) for 12 hours at the temperature of 13-14°C. The seedlings were then grown in controlled conditions for 6 days at the temperature of 22-24°C and 16-hour photoperiod. After that the seedlings were placed into a moisture chamber at the temperature of 14-15°C and 100% humidity for fungal sporogenesis stimulation. The reaction of plants to the infection was determined by the degree of conidial coating development on the cotyledon leaves and rootlets.

More than 1400 samples were tested for resistance to *Sclerotinia*, *Phomopsis* and broomrape under conditions of field infection in a quarantine plot. The technique of infection background creation for these diseases was described before (Tavoljanskiy *et al.*, 2001). The reaction of plants to gray rot, verticilliosis, rust and *Alternaria* blight inoculations was estimated in field nurseries of the original and breeding material under conditions of natural infection.

Finally, in 2002, in the laboratory of sunflower immunity a number of fungicides was tested as seed disinfectants on the variety Konditersky: TMTD (3.5 kg/t), TMTD -standard (4.0 kg/t), Maxim (5 l/t), Maxim + Apron gold (5.0 l/t + 3.0 l/t), Vincit (2.0 l/t). These preparations were compared against the untreated control. The size of experimental unit was 25 m^2. The experiment was conducted in 4 replicates. The influence of the fungicides on germination energy and seed viability was determined in laboratory and field conditions.

RESULTS AND DISCUSSION

The obtained results show that 15 samples were immune to downy mildew (0.9% of the total quantity studied). These were the lines RHA 373, RHA 397, RHA 398, RHA 854, RHA 855, RHA 856, RHA 858, RHA 859, VIR 655, VIS 2002, VIS 2003, VIS 2003-4. Approximately 20% of the samples showed various degrees of resistance to *Plasmopara halstedii* –that was mainly the breeding material of VIS. The remaining 80% were highly susceptible to the pathogen. The susceptible materials came from different collections.

In 2002, the severe drought in the initial period of plant development did not encourage the occurrence of infection symptoms of fungal diseases on plants grown in natural conditions. However, the intensity of the major diseases was increased in August: head form of grey rot, phomopsis, root form of white rot, verticilliosis, *Alternaria* blight and others. In the other years and on the artificial infection backgrounds in all years, the development of infection was sufficient to permit objective immunological estimation.

The estimation of breeding material response to broomrape was performed on the infection background consisting of a mixture of races C and D. The standard susceptible variety Giant 549 was infected 100%. Twelve samples of female lines remained uninfected by broomrape: VB 2, VB 110, VB 132, VB 171, VB 471, VB 4703, VKU 61, VKU 102, VKU 127, VKU 310, VKU 478 and VKU 701. The male line VB 273 was also immune. Almost all lines among the CMS sources of the no-RET type remained free of floriferous shoots of *Orobanche cumana*. The only exception was the combination VC_8 (VB 1002 / CMG-2 / 471) which was one infected at the rate of 11%. The infection among the intervarietal hybrids ranged from 9% to 87%. Generally, the breeding materials of VIS were resistant to this plant parasite.

The majority of the samples tested against the artificial infection background for white rot (the original material of the collections and the breeding varieties, hybrids and lines) were susceptible to a considerable extent. Still, among the interspecific hybrids *Helianthus sp.* from VIS we managed to select hybrids that were resistant to various forms of *S. sclerotiorum* (Table 1).

Another way to search for resistant forms relies on the anatomical and morphological particulars of the sunflower plant. It was found that white rot resistance exists in plants with a thin, tight and slightly bent head. Two types of such heads were observed.

Type 1. The head is thin, flat and regularly shaped, with a weakly developed parenchyma layer and a thick layer of cover tissues on the reverse side. The neck is thin, not exceeding 3 cm in diameter.

Type 2. The head is also correct regularly shaped, the layer of cover tissues is rather thin, but the parenchyma layer is strongly developed. The neck has a boot type and it is 7-8 cm long.

The lines of sunflower having these types of head architecture were selected on the basis of susceptibility to white rot (Table 2). Such heads do not hold moisture, which lowers the probability of disease development (Table 1). The hybrid VIS 200, which was less infected by *Sclerotinia*, was obtained from these lines.

Table 1: Results of white rot immunological test of interspecific sunflower hybrids (VIS, 2000-2002)

Wild species		CMS -forms, form of disease					
Species	Number of sample	RIG 1	MAX 1	ANN 1	ARG 1	GIG 1	PEF 1
H. praecox	18-28	1, 2, 3					
H. praecox	12-95	1, 3					
H. praecox	380	2, 3					
H. praecox	416	1, 2, 3					
H. petiolaris	815	1, 2, 3					
H. petiolaris	2011	1, 3					
H. petiolaris	8125					1, 2	
H. patiolaris	2203				1, 2, 3		
H. annuus	529	1, 2	2, 3			1, 2, 3	1, 2
H. annuus	H-216						
H. annuus	H-29				2, 3		
H. annuus	H-99			2, 3			
H. annuus	1173			2, 3			
H. annuus	H-98			2, 3			
H. annuus	376	1, 2, 3		1, 3			
H. annuus	H-41	1, 2, 3					
H. annuus	H-36						1, 2, 3
H. neglectus	1182	1, 2, 3		1, 2, 3			1, 2, 3
H. neglectus	460	1, 2, 3					
H. argophyllus	1812				1, 2, 3		1, 2, 3

Note: 1 -tolerance to root form;
2 -tolerance to stem form;
3 -tolerance to head form.

The erect position of leaves also provides resistance to the stem form of S. sclerotiorum because it enables better aeration of the crop. Improved resistance is also brought by a pear-shaped root collar of the stem.

Thus, the use of interspecific hybrids, specific characteristics of head structure, effective leaf position and pear-shaped root collar of the stem may be useful in the development of sunflower varieties and hybrids tolerant to white rot (Table 2).

Tolerance to *Phomopsis* in the sunflower breeding material from VIS had been described earlier (Tikhomirov *et al.*, 2002). At the same time, all INRA lines (France) received by VIS through GRESO-program were infected by this disease from 10% to 40%. In particular, 89 lines from the combinations NA 98 × LR 4-17 were infected more than 18%. Lines from the combinations NA 89 × LR 2-9 were infected up to 50%, although lines of the combination NA 89 × LR1F7 were infected

up to 20%. The reaction of the INRA lines to *Phomopsis* infection was similar. A sufficient number of immune and tolerant *Diaporthe helianthi* forms was selected from this material.

Table 2: Results of white rot immunological test of sunflowers with different types of head architecture (VIS, 1998-2001)

Forms of sunflower	Type of head architecture	Infected plants (%)			
		1998	1999	2000	2001
VIR 130 VB 181	1	9.1	22.5	18.4	11.2
VIR 160 VB 140	2	35.7	42.7	30.2	28.9
VIS 200		-	-	-	30.2
Voskhod, st.		79.7	84.2	75.4	70.2

The female lines VB 2, VB 171, VIR 130 and NA 371 and the male lines VB 166, VB 1617, VB 1618 (the last two lines were obtained from *H. argophyllus*), CM 620, RHA 408, RHA 856, VIS 2002, VIS 2003-1, VIS 2003-2, VIS 2003-3, GI 1, GI 3, GI 4 and GI 6 showed complex resistance and/or tolerance to all four infection backgrounds, except for a low level of *Verticillium* wilt.

CMS-forms of the no PET-1 type were tested against all infection backgrounds. They showed no symptoms of downy mildew, broomrape and *Sclerotinia*. However, almost all of these lines were infected by *Phomopsis* and *Phoma* at the end of the vegetative period. Only three lines turned out to be tolerant to these diseases: VS_3 (VB 1002 × DCS-1), VS_1 (VB 1002 × RIG 1) and VS (VB 1002 × ARG 3). Three hybrids having complex resistance or tolerance were selected from the group of of interspecific hybrids: MOZ × NA 89 (green stem), MOZ × VIS 130 and *H. argophyllus* × Na 89. The reaction to the diseases of the annual and perennial sunflower species had been described before (1, 2). Forms were selected from this group which were not infected by gray rot, verticilliosis, rust, *Alternaria* blight and *Phomosis*.

So, in general, VIS has a sufficient number of forms of original and breeding material which are not susceptible to diseases. They are widely used for breeding of sunflower varieties and hybrids which are immune, resistant and tolerant to fungal diseases.

In the field conditions, all tested seed disinfectants affected positively plant stand till harvest, yield and oil percentage of sunflower. Despite the dry conditions in the variants with disinfectants in conditions of natural infection, reductions in *Phomopsis* development and especially white rot were noted (Table 3). All preparations except Vincit failed to influence *Verticillium* wilt development.

In conclusion, it is necessary to note that the original (various collections, wild species, interspecific hybrids) and breeding material of sunflower (male and female lines, varieties, intraspecific hybrids, sources of CMS -*Rf* genes and so on) are infected by various fungal pathogens and by broomrape to a considerable extent.

It was particularly evident in the tests with artificial infection backgrounds. In these tests, the fungus *S. sclerotiorum* was distinguished for specific aggressiveness and high virulence.

Table 3: Results of economic and immunological tests of the sunflower variety Konditersky depending on disinfectants (VIS, 2002)

Var.	Preparation	Consumption norm (kg/t), (l/t)	Plant stand in the plot (pieces)	Yield per plot (kg)	Oil percentage (%)	Plants infected (%)		
						White rot	*Phomopsis*	*Verticilliose*
1	TMTD	3.5	106±3	6.26±1.35	48.4±0.8	0.0	0.9±0.2	17.8±1.2
2	TMTD	4.0	107±2	5.39±0.20	50.3±0.3	0.9±0.9	3.0±1.2	18.8±2.0
3	Maxim	5.0	114±2	5.29±0.09	49.3±0.7	0.0	2.0±1.3	18.0±2.2
4	Maxim + Apron gold	5.0+3.0	113±1	6.46±0.2	47.2±0.4	0.0	3.7±0.4	14.7±2.3
5	Vincit	2.0	114±2	6.83±0.39	48.3±0.8	0.0	1.0±0.5	10.6±3.5
6	Control	water	97±4	4.82±0.42	47.4±0.6	1.8±1.0	4.5±0.6	16.9±0.5

Nevertheless, it is possible to select forms and lines resistant or tolerant to individual diseases or to disease complexes by using infection backgrounds as a specific "sieve". The positive effect of the immunological sieve can be seen when comparing breeding and infection nurseries. For example, the former considerably exceed the original material in resistance to *P. halstedii.* It is also true in relation to the other pathogens and to their complexes, because the immunological estimation in VIS has been carrying out for many years. The breeding material has been formed in nurseries in these very years.

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RESULTADOS DE EVALUACIÓN INMUNOLÓGICA DEL MATERIAL ORIGINAL Y DE MEJORAMIENTO DE GIRASOL ENGENDRADO EN VIS

RESUMEN

Girasol es uno de los cultivos vegetales, al cual con más frecuencia atacan los patógenos. Los parásitos hongosos facultativos provocan los danos más significantes a los cultivos de girasol. En este trabajo se hizo el resumen de los resultados de las investigaciones inmunológicas del material original y mejorador de girasol, que a lo largo de varios anos anteriores se realizaron en VIS. También se exponen los datos sobre la eficacia de diferentes desinfectantes.

RÉSULTATS DE L'ÉVALUATION IMMUNOLOGIQUE DE MATÉRIEL ORIGINAL ET DE MATÉRIEL DE TOURNESOL CULTIVÉ, OBTENUS À VEIDELEVKA INSTITUTE OF SUNFLOWER(VIS)

RÉSUMÉ

Le tournesol est la plante le plus souvent attaquée par les différents pathogènes. Les parasites fongiques facultatifs sont les plus nuisibles au rendement de tournesol. Cet article présente les résultats des recherches immunologiques de matériel original et de matériel de tournesol cultivé sont présentés, qui s'étaient effectuées dans la période de quelques années précédentes à Veidelevka Institute of Sunflower(VIS). Les données sur l'efficacité de différents désinfectants y sont apportées.