HYBRID SUNFLOWER BREEDING FOR RESISTANCE TO *FUSARIUM*

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Abstract

Earlier *Fusarium* fungi were usually only minor pathogens on the sunflower crop, but now they have become a serious problem in Russia. The aim of our work was to start breeding for resistance to this new pathogen, and to evaluate the possibility of using a laboratory testing method in the VNIIMK hybrid sunflower breeding program. Four segregated hybrid combinations selected on the basis of their field resistance to different pathogens were used as breeding material. Three of them were three-line combinations: F3 R-14 x (VK-591 x VK-539), F4 VK-623 x (HA-385 x VK-653) and F4 (VK-678 A x VK-464) x VK-541 and the last, F3 VK-680 x O.P. variety 'Leader.' Two methods of artificial infection were used: laboratory (seedling infection) and field (soil infection). It was shown that a combination of laboratory tests with individual selection can rapidly improve a segregated population for *Fusarium* resistance. The share of families without any plants with *Fusarium* damage symptoms was 62.5% among the selected plants' offspring while only 18.2% of such families were found in the breeding nursery. The average ratio of plants with different symptoms of *Fusarium* damage was 21% in the nursery of breeding material, but only 11% of plants were damaged among the offspring of plants selected by the laboratory test. It was also shown that breeding with application of two methods of artificial infection (laboratory and soil infection) was highly effective for *Fusarium* resistance breeding. New elite breeding line development is under way now.

Introduction

Sunflower (*Helianthus annuus* L.) is the main oil crop in Russia. Sunflower acreage was about 3.5-4.5 million ha during the last decade. Breeding for resistance (or at least tolerance) to pathogens is always considered to be the most important aim in sunflower breeding. *Fusarium* is one of the most important pathogens for many crops, but for sunflower it was earlier considered to be of less importance or a minor pathogen (Tikhonov, 1992; Gulya et al., 1997). During the last few years *Fusarium* has become a serious problem for the sunflower crop in Russia. The first report was made in the 1990s, when five species of *Fusarium* were found in the sunflower fields of he Voronezh and Belgorod regions of Russia (Yakutkin, 1995). In the Krasnodar region of Russia 12 different species and varieties of *Fusarium* (*F.*

oxysporum, F. oxysporum var. *orthoceras, F. sporotrichiella* var. *poae, F. sporotrichiella* var. *tricinctum, F. sporotrcihiella* var. *sporotrichioides, F. semitectum, F. gibbosum, F. moniliforme, F. solani, F. solani* var. *argillaceum, F. javanicum* and *F. heterosporum*) were found during the phytopathology surveys in 1999-2001 (Antonova et al., 2002). Its pathogenity for the sunflower plant was also demonstrated. Among the species, *F. oxysporum* var. *orthoceras* was the most widely spread, and *F. sporotrcihiella* var. *sporotrichioides* was the most aggressive. During the last decade *Fusarium* is regularly registered in the breeding nurseries of All-Russia Research Institute of Oil Crops (Krasnodar). As special breeding program for resistance to *Fusarium* started here in 2001 using the laboratory test developed in VNIIMK. Field experiments demonstrated high levels of resistance in newly bred lines to this pathogen.

Materials and Methods

Plant and Fungi Materials. For the testing we used two different samples of *Fusarium*: *F. oxysporum* var. *orthoceras* as the most widely spread *Fusarium* pathogen on sunflower plants in the Krasnodar region, and *F. sporotrcihiella* var. *sporotrichioides* as the most aggressive one.

Plant material was presented by four segregated hybrid combinations between promising and released inbred lines, selected on the base of the field evaluations of their resistance to the different pathogens. Three of them were three-line combinations: F3 R-14 x (VK-591 x VK-539), F4 VK-623 x (HA-385 x VK-653) and F4 (VK-678 A x VK-464) x VK-541, and the last one F3 VK-680 x O.P. variety Leader.

Methods. For the testing we used a method developed at the Immunology Laboratory of VNIIMK (Saukova, 2001). Two-day-old sunflower plantlets were placed for 6 hours on the perforated covers of Petri dishes with 15-day-old *Fusarium* cultures. Two days later they were incubated in the moisture chamber. After the plantlets were visually evaluated, their roots were measured, and the best plants placed in the soil and cultivated in the greenhouse till maturity. An analysis of variance was used to statistically compare the mean root length values of the control and pathogen-influenced plantlets. After self-pollination, seeds were collected and used for field resistance tests and breeding purposes. The second method was used in the next stage of breeding process, testing sunflower resistance in artificially infected soil. A specimen of *Fusarium sporotrichiella* var. *sporotrichioides* was cultivated on sterilized sunflower seeds, then dried at the room temperature and ground. The material obtained was mixed with the soil and used for infection in two different quantities, 0.5 and 1.0 g per seed bed. Seeds of tested sunflower inbred lines were planted by the usual VNIIMK method with two seeds per seed bed simultaneously with the soil-infection mixture. Hybrid Kubanskiy 930 was used as a check variety.

Results and Discussion

Breeding nurseries of the All-Russia Research Institute of Oil Crops are regularly inspected by the phytopathologists. During the last decade different species of *Fusarium* fungi became usual pathogens for sunflower plants. For example, in 2001 the average number of plants with different symptoms of *Fusarium* damage was 21% in the breeding nursery material, varying from 0% up to 100% in different lines.

Four segregating populations of sunflower hybrids were selected for our work in the breeding nursery. They showed maximal field resistance to all registered pathogens among prospective breeding material. Their seeds were used for selection by the laboratory method. After treatment by *F. oxysporum* var. *orthoceras*, seedlings had different levels of damage from hardly visible necrosis to full destruction. ANOVA showed significant reduction of root length of treated plants compared with check plants treated by water without the pathogen (Table 1). Individual plantlets with minimal visible symptoms of *Fusarium* damage and maximal root growth rate were selected and transplanted in greenhouse.

Table 1. Sunflower seedling root length (mm) in the laboratory test for *Fusarium* resistance.

Origin	Experiment (*Fusarium oxysporum* culture)	Check (water)
F4 (VK-678 A x VK-464) x VK-41	15.9	22.8
F3 R-14 x (VK-591 x VK-539)	16.3	18.3
F4 VK-623 x (HA-385 x VK-653)	22.3	22.8
F3 VK-680 x Leader	10.3	28.2

Treatment by *F. sporotrcihiella* var. *sporotrichioides* gave more severe results; all seedlings had greatly reduced root length (less than 5 mm) as a result of damage. Plantlets with maximal shoot growth rate with green cotyledons (the majority were of a brownish color) were selected for transplanting.

An equal number of plants was selected from all four populations after treatment by both pathogens. Sunflower plantlets treated by *F. sporotrichiella* var. *sporotrichioides* had to form additional roots because their main roots were heavily damaged. Transplanted plants were self-pollinated and seeds after harvesting were used to detect their field resistance.

The field experiment was conducted in 2001. Spring was unusually cold with high soil humidity followed by a hot dry summer. Such weather conditions influenced the phytopathologic situation greatly; *Phomopsis helianthi* (the most frequent pathogen previous years) was registered rarely and on the most susceptible specimens only, but *Fusarium* fungi coupled with *Verticillium* were the most frequent pathogens on sunflower plants this year. The average ratio of plants with different symptoms of Fusarium damage was 21% in the nursery of breeding material, but only 11% of plants were damaged among the offspring of plants selected by the laboratory test. The ratio of families without any visible *Fusarium* symptoms was 62.5% among those offspring, while only 18.2% of such families were found in the breeding nursery (Table 2).

Table 2. Frequency of sunflower families with different levels of field *Fusarium* resistance after individual selection.

	Damaged plants (%)			
Nursery	0 %	1-39 %	40-60 %	61-100%
Breeding material (check)	18.2	67.0	14.8	0.0
Offspring of selected plants	62.5	29.2	8.3	0.0

From the first set of experiments we made a conclusion that a combination of a laboratory test developed by VNIIMK Immunology Laboratory with individual selection can rapidly improve a segregated population for *Fusarium* resistance.

The next step of breeding was made using another method of artificial infection via the soil. Sunflower material obtained earlier was planted in the soil simultaneously with the infection of *Fusarium sporotrcihiella* var. *sporotrichioides*. Individual plants without visible damage were selected and self-pollinated. After two cycles of individual selection with artificial infection the best lines were tested by the laboratory method described earlier. Lines derived from the same source populations, but selected for *Fusarium* resistance on the basis of field observations only, were used as a check. It was shown that breeding with application of two methods of artificial infection was highly effective for *Fusarium* resistance (Table 3).

Table 3. Increase of sunflower seedling resistance to *Fusarium sporotrichiella* var. *sporotrichioides* after two individual selections.

Line	Seedling death in a source population (%)	Share of resistant seedlings after selection (%)
L-1661-1	60	70
L-1731-1	70	40
L-1731-2	55	50
L-1746-2	90	60
L-1748-1	95	60

Conclusions

Evaluation of the laboratory method of *Fusarium* resistance testing developed by VNIIMK Immunology Laboratory (seedling infection) has shown its effectiveness. Combination of this method with individual selection can rapidly improve a segregated sunflower population for *Fusarium* resistance. Breeding with application of two different methods of artificial infection was highly effective for *Fusarium* resistance breeding. New elite breeding line development is under way now.

References

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