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Sunflower (*Helianthus annuus* L.) breeding for durable resistance to Downy mildew (*Plasmopara halstedii*)

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Abstract: Downy mildew is one of the most important diseases of sunflower crop around the world caused by *Plasmopara halstedii* (Farl.) Berl.et de Toni. The aim of our study is the sunflower hybrid development with the durable resistance to downy mildew, combining one parental line with the most effective for the specific location major gene (Pl_{15} for example) and the second parental line – with a high horizontal resistance to downy mildew. Experiments were conducted at All-Russia Research Institute of Oil Crops in 2016–2021. Experimental design was randomized blocks with three replications. CMS-lines with the highest level of horizontal resistance (VK 678, VA 732, VK 680, VK 934, VK 900 and new line SL 0516) were used as mother parents. Lines with Pl_{15}, resistant to all the downy mildew races were used as pollinators to create sunflower hybrids with durable resistance to downy mildew. A number of high-productive sunflower hybrids with durable resistance to DM (VK 732A × L 642-15, VK 732A × L 634-15, VK 680A × L 642-15, VK 680A × L 634-15, VK 934A × L 642-15 and VK 678A × L 634-15) were developed and tested.

Keywords: breeding; downy mildew; durable resistance; sunflower.

1 Introduction

Plant breeding for the disease resistance is a traditional and one of the most important aims. Plant diseases still cause significant damage to agriculture around the world. The well-known Serbian breeder S. Borojevic distinguished four main methods to control pathogenic organisms: agronomic, chemical, biological and

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breeding, considering the development of resistant varieties and hybrids to be the most effective and safe method (Borojević 1990).

For a long time, breeding for disease resistance was based exclusively on horizontal (Plank Van der 1984) or race-nonspecific resistance, since breeders earlier had no opportunity to distinguish individual races or carry out artificial infection. The process was based on the discarding of plants with symptoms of disease in the field. The effectiveness of such kind of breeding was quite low, especially in years unfavorable for the development and spread of the pathogen, as well as under a low infectious load. Nevertheless, decades of breeders' work in this direction have led to the creation of numerous cultivars with a sufficiently high level of field resistance to pathogens.

Development of methods of artificial infection and using of infectious and provocative backgrounds made a great progress in breeding for disease resistance. The next step has been achieved by creating collections of differentiator lines that allow determining the racial composition of the pathogen and identifying the most important races for the breeder (the most common or the most aggressive). Currently, the breeding programs of most crops aimed on the resistance to pathogens are based almost exclusively on the use of the major genes (vertical or race-specific resistance) (Gulya et al. 1997; Vear et al. 1997). The widespread introduction of new resistant varieties and hybrids into the fields leads to the loss of the established equilibrium in the parasite-host system and stimulates the race-developing process (Ahmed et al. 2012).

The emergence and spread of new pathogen races, results in epiphytoties, wide fungicides application and forces breeders to look for new resistance genes. As a result, the cycle repeats, and with the development of international trade, including seeds, the spread of new races of pathogens around the world is significantly accelerated. The constant replacement of existing varieties and hybrids by the cultivars resistant to new races of pathogens is becoming an urgent task for originators.

There is only one way out from this situation – breeding for durable resistance. To implement this, several basic strategies have been proposed: pyramiding resistance genes, creating multilinear varieties (Tourvieille de Labrouhe et al. 2004), and combining horizontal and vertical resistance in one genotype (Vear 2004). For crops implemented as first-generation hybrids, the third approach is the most attractive and realistic. Durable resistance could be achieved by combining race-specific (vertical) and non-race-specific (horizontal) resistance in a single hybrid (Tourvieille de Labrouhe et al. 2004; Vear 2004; Vear et al. 2008). To accomplish this, it is possible to use one parental line with the most effective major gene for the specific location and the second parental line – with high horizontal resistance to the pathogen.

Downy mildew (DM) is one of the most important diseases of sunflower crop around the world caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni (Goossen and

Sackston 1968; Gulya et al. 1997; Jocić et al. 2012; Novotelnova 1962; Sackston 1992). New races development are going fast – after race 100 and 300 appeared 710 and 730 and then 304, 307, 314, 334, 704 and 714, evolved (Carson 1981; Gulya 2007; Gulya et al. 1991; Molinero-Ruiz et al. 2002). Now more than 40 *Pl* genes have been discovered and incorporated into commercial sunflower hybrids (Molinero-Ruiz 2022; Qi et al. 2016, 2019; Tourvieille de Labrouhe et al. 2008; Trojanova et al. 2017).

Sunflower downy mildew must be tackled under the scope of Integrated Pest Management, including genetic resistance, some special agronomic practices, biocontrol alternatives and chemical treatments (Molinero-Ruiz 2022).

Our previous study showed high differentiation of elite and prospective lines of VNIIMK breeding for horizontal resistance to DM (Gontcharov & Goloschapova 2021).

The aim of our present study is the sunflower hybrid development with the durable resistance to DM, combining one parental line with the most effective for the specific location major gene (Pl_{15} in our case) and the second parental line – with a high horizontal resistance to DM.

2 Material and methods

Experiments were conducted at the Central Station (Krasnodar) of All-Russia Research Institute of Oil Crops (VNIIMK) in 2016–2021. During the last decade the new races of DM (710, 730 and later 334) (Iwebor et al. 2016) appeared to be common in this region and so all the commercial VNIIMK hybrids become susceptible to DM (Gontcharov 2014).

Among the released and prospective sunflower lines of VNIIMK breeding we identified CMS-lines with the highest level of horizontal resistance (VK 678, VA 732, VK 680, VK 934, VK 900 and new line SL 0516). They were used as mother parents. Lines resistant to a mixture of races – 330, 710 and 730 (mainly with Pl_8) and lines with Pl_{15}, resistant to all the downy mildew races registered in Krasnodar region of Russia by 2016 were used as pollinators to create sunflower hybrids with durable resistance to DM.

Experimental design was randomized blocks with three replications. Each replication had two rows for each line with the acreage 12.2 m^2. Seeds were not treated by any chemicals. Evaluation was made by recording all the recognizable symptoms of DM from the emergence till flowering.

3 Results

Our previous study allowed us to identify CMS-lines with the highest level of horizontal resistance (Gontcharov and Goloschapova 2021), so the next step to achieve durable resistance was to develop restorer lines with effective race-specific major gene. For this purpose we used Pl_{15}, identified by Nidera Company (Molinero-Ruiz 2022).

The main method of breeding was pedigree (Kaya et al. 2012; Vear 2010). Breeding material was crossed with the donor of Pl_{15} gene and self-pollinated during six generations with individual selection for resistance to DM and all other desirable traits (including branching). Used breeding material was obtained as a part of shuttle-breeding program (Gontcharov et al. 2021). All obtained lines were resistant to DM, both in field conditions and under the artificial infection. Main characteristics of new lines are shown in Table 1.

The final stage included the selection of pairs for crossing based on the combination ability of the lines and the evaluation of the resulting hybrids for economically valuable traits and resistance to DM (Tables 2 and 3).

Table 1: New sunflower lines resistant to downy mildew (2016–2018).

Line	Period emergency-flowering, day	Plant height, cm	Head diameter, cm	1000 seed mass, g	Oil content, %
L 642-15	62	102	12	27.3	42.0
L 645-15	59	130	14	36.3	40.3
L 622-15	65	130	11	22.3	49.5
L 634-15	61	105	13	29.0	40.1

Table 2: Evaluation of sunflower hybrids with durable resistance to downy mildew (VNIIMK, Krasnodar, Russia, 2020).

Hybrid	Yield		Oil content, %	Oil yield	
	t/ha	± to check		t/ha	± to check
Tayfoon (check)	2.69	–	51.8	1.28	–
SL 0516A × L 642-15	3.11	0.42	52.3	1.47	0.18
VK 732A × L 634-15	3.07	0.38	52.7	1.38	0.09
VK 101A × L 622-15	2.94	0.25	51.8	1.37	0.09
VK 900A × L 634-15	2.93	−0.35	51.8	1.36	0.08
VK 678A × L 634-15	3.03	0.35	49.5	1.35	0.07
SL 0516A × L 622-15	2.81	0.12	52.8	1.34	0.05
SL 0516A × L 634-15	2.9	0.22	50.9	1.33	0.04
VK 934A × L 642-15	2.88	0.19	49.5	1.3	0.02
VK 680A × L 642-15	2.74	0.06	52.4	1.29	0.01
VK 732A × L 642-15	2.78	0.1	50.7	1.27	−0.02
VK 680A × L 634-15	2.62	−0.07	51.9	1.22	−0.07
HCP $_{05}$	0.23			0.11	

Table 3: Evaluation of sunflower hybrids resistance to downy mildew (VNIIMK, Krasnodar, Russia, 2020–2021).

Hybrid	Infected plants in the field, %	Resistance to DM (artificial infection)[a], race					
		330	330, 710, 730	334	734	733	713
VK 732A × L 642-15	0	R	R	R	R	R	R
VK 732A × L 634-15	0	R	R	R	R	R	R
VK 680A × L 642-15	0	R	R	R	R	R	R
VK 680A × L 634-15	0	R	R	R	R	R	R
VK 934A × L 642-15	0	R	R	R	R	R	R
VK 678A × L 634-15	0	R	R	R	R	R	R
SL 0516A × L 642-15	0	R	R	R	R	R	R
SL 0516A × L 622-15	0	R	R	R	R	R	R
SL 0516A × L 634-15	0	R	R	R	R	R	R
VK 101A × L 622-15	0	R	R	R	R	R	R
VK 900A × L 634-15	0	R	R	R	R	R	R

[a]R, resistant.

High seed and oil yields were obtained in hybrid combinations SL0516A × L 642-15 (3.11 t/ha; 1.47 t/ha) and VK 732A × L 634-15 (3.07 t/ha; 1.38 t/ha). The results of the evaluation of obtained hybrids for resistance to DM are shown in Table 3.

As a result, a number of high-productive sunflower hybrids with durable resistance to DM (VK 732A × L 642-15, VK 732A × L 634-15, VK 680A × L 642-15, VK 680A × L 634-15, VK 934A × L 642-15 and VK 678A × L 634-15) were developed and tested.

Prevalence of such hybrids in fields will restrain DM race formation process in, successfully competing with other hybrids for seed and oil yield. This approach can be implemented on other pathogens and any crops using first generation hybrids.

4 Discussion

The discovery of new sources of resistance and the use of broad-spectrum resistance by combining effective and diverse *Pl* genes (gene pyramiding) consider being the best strategies for preventing downy mildew in sunflowers (Molinero-Ruiz 2022). So far, more than 40 of different downy mildew major resistance genes (Pl_v–Pl_z, Pl_1–Pl_{35} and Pl_{Arg}) have been found and reported (Molinero-Ruiz 2022; Qi et al. 2016, 2019; Trojanova et al. 2017). However, the co-evolution of sunflower plant and pathogen leads to the emergence of new races of *P. halstedii* able to infect previously resistant hybrids. Finally we consider with other researchers (Tourvieille de Labrouhe et al. 2004; Vear 2004; Vear et al. 2008) that durable resistance in sunflower could be achieved by combining race-specific (vertical) and non-race-specific (horizontal) resistance in one hybrid. Our data demonstrated the realization of such approach in practical breeding.

5 Conclusions

A number of high-productive sunflower hybrids with durable resistance to DM (VK 732A × L 642-15, VK 732A × L 634-15, VK 680A × L 642-15, VK 680A × L 634-15, VK 934A × L 642-15 and VK 678A × L 634-15) were developed and tested. The major gene Pl_{15} showed high effectiveness to prevent sunflower lines from downy mildew. Durable resistance in sunflower to DM was achieved by combining race-specific (vertical) and non-race-specific (horizontal) resistance in one hybrid.

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