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Evaluation of horizontal resistance of sunflower (*Helianthus annuus* L.) 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 was to evaluate the horizontal resistance of elite and prospective lines to downy mildew in field conditions. Experiments were conducted at All-Russia Research Institute of Oil Crops (VNIIMK) in 2016–2020. Released and prospective sunflower lines bred at VNIIMK were studied, divided into four groups on the base of their race-specific resistance in laboratory tests. Experimental design was randomized blocks with two replications. Evaluation was made by recording all the recognizable symptoms of downy mildew from emergence till flowering. The results obtained allowed us to identify the lines with the highest race non-specific resistance to downy mildew: VK 678 (5.3%), VK 653 (7.3%), VA 760 (3.3%), VA 93 (2.0%) and VK 732 (6.3%). The final aim is the development of sunflower hybrids with the most durable resistance to downy mildew, combining one parental line with the major gene (Pl_{15}) most effective for the specific location and the second parental line with a high level of horizontal resistance to downy mildew.

Keywords: sunflower / line / resistance / downy mildew / breeding

Résumé – **Évaluation de la résistance horizontale du tournesol (*Helianthus annuus* L.) au mildiou (*Plasmopara halstedii*).** Le mildiou est l'une des maladies les plus importantes de la culture du tournesol dans le monde, causée par *Plasmopara halstedii (Farl.) Berl.et de Toni*. L'objectif de notre étude était d'évaluer la résistance horizontale au mildiou des lignées élites et prospectives dans des conditions de terrain. Les expériences ont été menées à l'Institut de recherche panrusse des oléagineux (VNIIMK) en 2016–2020. Des lignées de tournesol issues du programme de sélection du VNIIMK ont été étudiées, divisées en quatre groupes sur la base de leurs résistances race-spécifique déterminées par tests de laboratoire. Le plan expérimental était en blocs randomisés avec deux répétitions. L'évaluation a été faite en enregistrant tous les symptômes reconnaissables du mildiou, de la levée à la floraison. Les résultats obtenus nous ont permis d'identifier les lignées présentant la plus forte résistance horizontale (non spécifique d'une race du pathogène) au mildiou : VK 678 (5,3 %), VK 653 (7,3 %), VA 760 (3,3 %), VA 93 (2,0 %) et VK 732 (6,3 %). L'objectif final est le développement d'hybrides de tournesol présentant une résistance durable au mildiou, en combinant une lignée parentale avec un gène majeur de résistance (Pl_{15}) le plus efficace localement et une seconde lignée parentale dotée d'une résistance horizontale élevée au mildiou.

Mots clés : tournesol / lignée / résistance / mildiou / sélection

1 Introduction

Downy mildew (DM) is one of the most important diseases of sunflower around the world caused by *Plasmopara halstedii* (Farl.) Berl. et de Toni (Novotelnova, 1962; Goossen and Sackston, 1968; Sackston, 1992; Gulya *et al.*, 1997; Jocić *et al.*, 2012). There are a few ways to control DM including agrotechnical, chemical, and breeding methods. Seed chemical treatment and cultivation of resistant sunflower hybrids to DM are the most effective control measures (Gulya *et al.*, 1997; Vear *et al.*, 1997). Breeding for resistance to DM usually

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[☆] Contribution to the Topical Issue "Sunflower / Tournesol".
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concentrates on the search for and incorporation of major genes designated *Pl* into elite sunflower lines. Simple inheritance of DM genes was discovered rather early (Vranceanu and Stoenescu, 1970; Zimmer, 1974), with different race-specific single dominant resistance genes used worldwide by sunflower breeders (Vear *et al.*, 2008a). This causes the DM pathogen to evolve new races (Ahmed *et al.*, 2012). After race 100 and 300 appeared, then 710, 730, 304, 307, 314, 334, 704 and 714 evolved (Carson, 1981; Gulya *et al.*, 1991b; Molinero-Ruiz *et al.*, 1998, 2002; Tourvieille de Labrouhe *et al.*, 2000; Gulya, 2007). Now 35 *Pl* genes have been discovered and incorporated into commercial sunflower hybrids (Tourvieille de Labrouhe *et al.*, 2008; Qi *et al.*, 2016, 2019; Trojanova *et al.*, 2017).

Identification of races is based upon the reaction of the host (an internationally accepted set of differential lines) to the pathogen (Gulya *et al.*, 1991a; Gulya, 1995; Tourvieille de Labrouhe *et al.*, 2000, 2012). Molecular markers have been used in *P. halstedii* race differentiation (Roeckel-Drevet *et al.*, 2003; Gascuel *et al.*, 2015) and in sunflower maker assisted selection for resistance (Mulpuri *et al.*, 2009).

Wild sunflower species are often used as a source of new DM resistance genes (Seiler, 1992; Vear, 2004, 2010; Vear *et al.*, 2008a; Kaya *et al.*, 2012).

Appearance of new races forces sunflower growers to use chemical control with seed-applied fungicides. Usually, seeds are treated by metalaxyl or mefenoxam (Melero-Vara *et al.*, 1982; Albourie *et al.*, 1998; Gulya, 2002; Molinero-Ruiz *et al.*, 2005). However, in 1995 metalaxyl tolerant isolates were discovered (Molinero-Ruiz *et al.*, 2005; Spring *et al.*, 2006) and a little later – mefenoxam-tolerant isolates (Molinero-Ruiz *et al.*, 2005).

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.*, 2008b). 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 DM.

The aim of this study was to evaluate the horizontal resistance of elite and prospective VNIIMK breeding lines to DM under field conditions.

2 Material and methods

The experiments were conducted at the Central Station of All-Russia Research Institute of Oil Crops (VNIIMK), Krasnodar, Russia in 2016–2020. The Krasnodar region is situated in the Southern part of Russia near the Black Sea. Climatic conditions are very favorable for sunflower production. Sunflower production usually covers about 0.5 million ha in this region. Downy mildew is one of the most dangerous pathogens for sunflower in the region. During the last decade, new races of DM (710, 730 and later 334) (Iwebor *et al.*, 2016) appeared to become common and all the commercial VNIIMK hybrids (resistant to only race 330) became susceptible to DM (Gontcharov, 2014).

Released and prospective sunflower lines of the VNIIMK breeding program were used in the study and divided into four Groups based their race-specific resistance in laboratory tests. All lines used in the study are the property of the V.S. Pustovoit All-Russian Research Institute of Oil Crops (VNIIMK), and could be available through a specific agreement. The first Group includes 18 lines without any major genes for resistance to DM. The lines in this Group were highly susceptible to race 330 laboratory tests. The second Group included 17 lines resistant to the race 330, but susceptible to the race 710 and 730 consisted of 17 lines resistant to the mixture of races – 330, 710 and 730 (mainly with Pl_8). The fourth Group included five lines with Pl_{15}, resistant to all the downymildew races identified in the region up to 2016. All the pathogen samples used in the experiment were collected in the field of the Krasnodar region and identified as to race.

The experimental design was a randomized block with two replications. Each replication had two rows for each line with an area of 12.2 m^2. Seeds were not treated with any chemicals. Evaluation was made by recording all the visible symptoms of DM from emergence until flowering. Low air temperatures and an abundance of precipitation at emergence and early growth stages produced favorable conditions for the pathogen (Cohen and Sackston, 1973) in both 2016 and 2017. However, in 2018, 2019 and in 2020 they were unfavorable for DM with higher temperatures and low precipitation in the early growth stages.

3 Results

Many sunflower plants had DM symptoms in the field in 2016 and 2017. Favorable weather conditions stimulated the pathogen development. This allows for the identification of all the sunflower material with resistance to DM in the field without any artificial inoculation. Downy mildew infection was present in all Groups, except one, Group 4 (lines with Pl_{15} gene) where there was no infection (Tab. 1).

Sunflower lines from Group 2 (resistant to the race 330) were damaged to a greater extent than those from Group 1. Therefore, this major gene for resistance to DM was no longer effective. Sunflower lines from the Groups 1 and 2 were analyzed together.

Sunflower lines from Group 3 (resistant to the most frequently appearing races) were damaged by DM to a lesser extent.

The situation in the following years (2018–2020) was quite different with DM rarely occurring, indicating a low infection pressure.

Sunflower lines included in Groups 3 and 4 have major gene(s) effectively protecting them from DM attacks. Lines from Groups 1 and 2 demonstrated rather high damage rate depending on year condition and their level of horizontal resistance (Tabs. 2 and 3).

The results allowed for the identification of lines with the highest nonspecific race resistance to DM: VK 678 (5.3%), K 653 (7.3%), VA 760 (3.3%), VA 93 (2.0%) and VK 732 (6.3%). Presence of horizontal resistance can vary greatly depend on the quantity of available spores for infection and proper environmental conditions. Some lines were damaged up to 100%. It demonstrates that weather conditions were favorable for the pathogen development and a sufficient quantity spores were available in the field for infection.

Similar results were obtained for the Group 2. In general estimation of sunflower resistance was close to the previous

Table 1. Resistance of sunflower lines Groups to downy mildew (VNIIMK, Krasnodar, Russia).

Group	Average percent downy mildew infection				
	2016	2017	2018	2019	2020
Group 1 (without major genes for resistance)	21	22	6.6	0.9	0.8
Group 2 (resistant to race 330)	30	24	6.4	1.6	0.9
Group 3 (resistant to a mixture of races (330, 710 and 730)	2	1	2	0	0
Group 4 (with Pl_{15} gene)	0	0	0	0	0

Table 2. Evaluation of sunflower lines horizontal resistance to downy mildew (VNIIMK, Krasnodar, Russia).

Line of the Group 1 (without major genes of resistance to downy mildew)	Infected plants (%)				
	2016	2017	2018	2019	Average
VK 276	70	77	15	0	40.5
VK 585	100	20	0	1	30.3
VK 700	46	34	5	2	21.7
VA 760	0	6	6	1	3.3
VA 93	4	1	1	2	2.0
VK 905	29	51	25	0	26.3
SL 24	8	16	22	0	11.5
SL 4	14	16	9	0	9.8
VK 934	15	26	0	1	10.5
VK 678	7	10	3	1	5.3
VK 935	17	19	0	1	9.3
VK 934	15	25	0	1	10.3
VK 101	16	23	2	0	10.3
SL 3844	4	16	4	1	6.3
VK 653	1	9	19	0	7.3
VK 732	5	16	0	4	6.3
VK 680	10	25	2	1	9.5
Average	21.2	22.9	6.6	0.9	

Group (Tab. 3). Race 330 is no longer the predominant race so the major gene for resistance to DM is no longer effective.

Field evaluation from the favorable years (2016 and 2017) allowed for the differentiation of susceptible lines for horizontal (race-nonspecific) resistance to DM. Lines with the less than 10% of infected plants were considered to be resistant, with 10 to 40% of infected plants, mildly resistant, and with more than 40% of infected plants susceptible (Fig. 1).

Field evaluation made in the unfavorable years (2018 and 2019) for pathogen development were not useful since all lines showed resistance (Fig. 1).

The final aim of the study was the development of sunflower hybrids with durable resistance to DM, combining one parental line with the most effective major gene (Pl_{15} for example) for the specific location and the second parental line with a high horizontal resistance to DM.

4 Discussion

Field evaluation during favorable years showed the presence of high genetic variability for horizontal resistance to DM in a set of sunflower inbred lines developed by VNIIMK. This has been shown in different materials reported by other researchers (Tourvieille de Labrouhe *et al.*, 2008; Vear *et al.*, 2008b; Ahmed *et al.*, 2012; Gontcharov, 2014).

To evaluate the horizontal resistance properly, it is necessary to test the number of susceptible to DM sunflower lines during at least three years in a field conditions. However,

Table 3. Evaluation of sunflower lines horizontal resistance to downy mildew (VNIIMK, Krasnodar, Russia).

Line of the Group 2 (without major genes of resistance to downy mildew)	Infected plants (%)				
	2016	2017	2018	2019	Average
VK 580	23	18	15	1	14.3
VK 794	17	31	0	0	12.0
VK 551	13	3	5	0	5.3
VK 591	13	13	6	1	8.3
VD 541	32	19	1	0	13.0
VK 989	39	18	25	2	21.0
VK 536	5	9	22	1	9.3
VK 941	36	41	9	2	22.0
VK 776	6	36	0	0	10.5
VK 780	34	30	3	0	16.8
VK 787	69	47	0	1	29.3
VK 788	6	14	0	1	5.3
VK 930	72	30	2	1	26.3
VK 914	30	35	4	1	17.5
VK 915	62	33	19	2	29.0
VK 917	11	8	0	2	5.3
VK 920	44	29	2	2	19.3
Average	30.1	24.4	6.6	1.0	

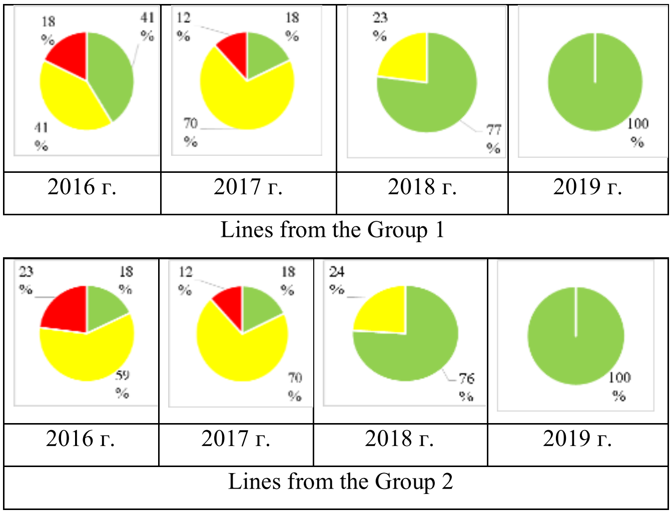

Fig. 1. Differentiation of susceptible sunflower lines for horizontal (race nonspecific) resistance to downy mildew depending on the year. Green: Resistant, less than 10% of damaged plants; Yellow: Medium resistant, 10 to 40% of damaged plants; Red: Susceptible, more than 40% of damaged plants.

we didn't meet any published material with such kind of information.

This study agrees with other researchers (Tourvieille de Labrouhe *et al.*, 2004; Vear, 2004; Vear *et al.*, 2008b) that durable resistance in sunflower could be achieved by combining race-specific (vertical) and non-race-specific (horizontal) resistance in one hybrid. To achieve this, it is possible to use one parental line with the most effective specific location gene and the second parental line with a high horizontal resistance to DM.

5 Conclusion

Results of this study allowed for the identification of lines with the highest nonspecific race resistance to DM: VK 678 (5.3%), VK 653 (7.3%), VA 760 (3.3%), VA 93 (2.0%) and VK 732 (6.3%). The major Pl_{15} gene was highly effective in preventing DM infection. The final aim was the development of sunflower hybrids with durable resistance to DM, combining one parental line with the specific effective major (Pl_{15}) gene and the second parental line with a high horizontal resistance to downy mildew.

Conflicts of interest. The authors declare that they have no conflicts of interest in relation to this article.

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