Iran. J. Plant Path., Vol. 48, No. 4, 2013: 169-176

EVALUATION OF SUNFLOWER CULTIVARS AND HYBRIDS FOR THEIR RESISTANCE TO *Plasmopara halstedii* IN WEST-AZARBAIJAN, IRAN*

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(Received : 29.10.2012; Accepted : 2.1.2013)

Abstract

Sunflower downy mildew is one of the most important and destructive diseases of this crop in West-Azarbaijan. The disease causes epidemic damages and yield losses at favorite environmental conditions for the pathogen activity. Application of disease resistant cultivars is considered as one of the effective control methods. During the growing season of 2009 in West-Azarbaijan, infected sunflower plants were collected and used as a source of *Plasmopara halstedii* for inoculation of standard differential lines to identify the variation of pathogenicity. The resistance of seven open-pollinating cultivars and two hybrids of sunflower to the dominant race (race 100) of *P. halstedii*, was evaluated employing the whole seedling immersion (WSI) method in the greenhouse. In this investigation, nine macroscopic disease symptoms including damping-off, sporulation on cotyledons and leaves, sporulation on leaves, stunting, sporulation on cotyledons, mosaic on leaves, deformation of leaves, lesion on hypocotyls and root reduction on the inoculated plants were considered for disease rating and calculating the disease severity index (D.S.I). The experiment was conducted in three replications for each cultivar. The analysis of variance (ANOVA) was performed and the means were compared using Duncan multiple range test (P = 0.05) in SAS ver. 9.1 (SAS institute Inc., Cary, NC.). According to the analysis of variance (ANOVA) for disease severity index, cultivars and hybrids were classified into five significantly different groups (P< 0.05): resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible. Cultivars Lakumka and Master were in the same group as resistant cultivars, whereas Record was highly susceptible.

Keywords: Sunflower, Downy mildew, Resistance, Cultivars and hybrids.

*: Part of MSc. Thesis of the First Author, Submitted to College of Agriculture, University of Zanjan, Zanjan, Iran.

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Introduction

Downy mildew of sunflower caused by the pathogenic oomycete *Plasmopara halstedii* (Farl.) Berl. and de Toni, is one of the most important diseases on this crop in Iran (Rahmanpour *et al.* 2000[a]) and it has also been reported in all sunflower growing regions of the world except Australia(Roeckel- Drevet *et al*.,2003).

The pathogen is a biotrophic oomycete which reproduces asexually by means of motile zoospores released from sporangia, and sexually through soil-borne oospores (Shindrova 2005). It is believed that this disease originated from North America and was spread by seed to major sunflower growing areas of the world (Leppik 1966, Sackston 1981). Resistance in sunflower against downy mildew was first reported in Canada in 1964. This resistance is conferred by major genes, denoted *Pl.* (Vear & Leclercq 1971). The incorporation of genes of resistance to *P. halstedii* is a common way to control the disease. Until 1980s, two dominant genes, Pl_1 and Pl_2, responsible for resistance to races 100 and 300, respectively, provided effective control of downy mildew (Zimmer 1974). During the last twenty years new races of *P.halstedii* have evolved and have been identified worldwide. New resistance sources have been found, resistance genes have been incorporated into hybrids and breeding lines, and some of these lines have been used as differentials of new races (Fick *et al*., 1975). These genes are effective against one or more races of the pathogen, but they are overcome by more virulent races after continuous cropping of resistant hybrids (Molinero-Ruiz *et al*. 2003). In Europe and America most researchers have used an artificial inoculation method using oospore as the inoculum. This method is time consuming and the results are different from field observations (Sackston 1978). In 1969, another artificial inoculation technique based on zoospores released from zoosporangia was established, in which, suspension of zoospores was sprayed on four day old seedlings of sunflower (Rahmanpour *et al*., 2000[b]). In 1975, this method was used in Iran to evaluate resistance of sunflower cultivars against downy mildew (Rahmani & Majidiye-Gasemi 1975). Whole seedling immersion (WSI) has been introduced as another inoculation method (Viranyi 1977). Rahmanpour and coworkers (2000) used the same method for resistance evaluation of different sunflower cultivars and hybrids against *P. halstedii.* In investigation of the resistance of sunflower cultivars and hybrids, most researchers (Molinero-Ruiz *et al.* 2002, Rashid, 1993, Baldini *et al*. 2008) believe that sporulation of this pathogen on the first leaves and/or cotyledons is indicative of susceptibility, whereas resistance is indicated by the absence of sporangia or weak sporulation, often simultaneous with a hypersensitive reaction, only on cotyledons.

The objective of this research was to evaluate the resistance of a number of sunflower cultivars and hybrids against the dominant race of *P. halstedii* in West-Azarbaijan, Iran.

Materials and methods

Sunflower genotypes

Seven open – pollinated cultivars and two hybrids of sunflower were prepared for evaluation experiments. The cultivars were Record, Armaviresky, Pesteii, Ablagh, Master, Lakumka, Alstar and the hybrids included Euroflor and Azargol. Pesteii and Ablagh are confectionary cultivars whereas the others are used in oil industry for cooking purposes. All seeds were provided by the Seed and Plant Improvement Research Institute (SPII), Iran.

Inoculum preparation

Isolates of *P. halstedii* used in these experiments were those collected by Shapouran and coworkers (2010) from sunflower plants demonstrating systemic symptoms of downy mildew in different areas of Urmia during the growing season of 2009. The isolates were inoculated and kept on a sensitive cultivar of sunflower (Record) under greenhouse conditions, using WSI inoculation method. Zoosporangia of the pathogen were collected directly from individual plants showing pathogen sporulation on leaves, the inoculum was a zoosporangial suspension in deionized water filtered through sterile gauze. The concentration was adjusted to 2×10^4 sporangia per ml using a hemacytometer. The seedlings of Record, were immersed in the zoosporangial suspension for 4 to 5 h at 15 °C. Then inoculated seedlings were planted in pasteurized soil including sand: perlite mixture (3:2, vol/vol) in plastic trays under greenhouse conditions (temperature: 18 to 22 °C, photoperiod of 14 h with light irradiance of 100 u E. $m^{-2}.s^{-1}$) (Gulya *et al*. 1991). According to the

results obtained by Shapouran and coworkers (2010), all of the isolates had been recognized as race 100. Among them isolate PH22 from a field in Tala-Tapeh was employed for cultivar evaluation tests. This isolate had more growth rate and more sporulation which made it easier to use in evaluation experiments (Fig. 1). Zoosporangia of the pathogen were collected directly from individual plants showing pathogen sporulation on leaves.

The seeds of sunflower cultivars and hybrids were surface-sterilized by immersing them in 5% sodium hypochlorite for 10 to 15 min, then thoroughly rinsed in deionized water and incubated in darkness at 24- 28°C and saturated humidity, until radicles were 3 to 5 mm long.

Evaluation experiments

The evaluation tests were conducted under greenhouse conditions. The resistance of seven sunflower cultivars and two hybrids against *P. halstedii* was evaluated by applying whole seedling immersion method (WSI) following Rahmanpour and coworkers (2000).

For each cultivar and hybrid, 15 seedlings (with rootlet length of 3 to 5 mm) were immersed in the zoosporangial suspension. Then inoculated seedlings, were sown in pasteurized soil including sand: perlite mixture (1:1, vol/vol) in plastic pots (diameter of 12 cm and height of 10 cm). For each cultivar and hybrid three pots each containing five seeds were used and incubated in growth chamber at 18 to 22 °C, photoperiod of 14 h with light irradiance of 100 u E. m-2.s-1. Control seedlings were treated similarly, but we used deionized water instead of fungal suspension. Plants were irrigated daily and fertilized with complete nutrient solution. After emergence of the first pairs of true leaves, the greenhouse conditions were adjusted to 16°C temperature, 90-100 percent relative humidity and darkness for 24 h. Then the reaction of cultivars and hybrids to the pathogen was evaluated. In this investigation, nine macroscopic disease symptoms including damping-off, sporulation on cotyledons and leaves, sporulation on leaves, stunting, sporulation on cotyledons, mosaic on leaves, deformation of leaves, lesion on hypocotyls and root reduction on the inoculated plants were considered for disease rating as disease severity index (Table 1) and cultivars were classified in five groups of resistant, moderately resistant, moderately susceptible, susceptible, and highly susceptible (Rahmanpour *et al*. 2000[b]) (Table 2). The experiment was performed as a completely randomized design with three replications. For each cultivar and hybrid, three replications of inoculation by sterile distilled water were considered as the control treatments. The analysis of variance (ANOVA) was performed on data of disease severity index (D.S.I.) and the means were combined by using a Duncan's multiple range test in SAS ver. 9.1.

Results and discussion

For all cultivars and hybrids, inoculation with sporangial suspension resulted in disease symptoms. Damping-off was considered as highly severe infection followed by sporulation on leaves and cotyledons. The symptoms were different and disease severity index, by calculating the scores of each symptom, varied from zero (for a few replications) to 100. The existence of symptoms was converted to quantitative data based on their importance and frequency (Rahmanpour *et al.*, 2000[b]). The mean disease severity index varied from 11 (Lakumka) to 80.33 (Record). Control plants did not show infections. The analysis of variance showed significant difference ($P < 0.05$) among cultivars and hybrids in terms of reaction to race 100, the dominant race of *P. halstedii* on sunflower in Urmia (Shapouran *et al.*, 2010), (Table 3). According to the classification method described previously (Rahmanpour *et al*. 2000[b]), three groups were distinguished among cultivars (Table 4), whereas according to the results from Duncan's multiple range test, the plants were classified in five significantly different groups. Record was the most sensitive cultivar, whereas Master and Lakumka were the most resistant ones (Fig. 2). In this investigation, Record, as a susceptible cultivar, was used for reproduction of inocula.

Wild sunflower species are important source of resistance to many sunflower pathogens, including *P. halstedii* (Georgieva- Todorova 1993). They are used as sources of resistance genes for sunflower breeding programs (Vranceanu *et al.* 1981). Different modes of action have been described in sunflower for resistance to *P. halstedii* such as complementarity, allelism and epistasis (Garcia & Gulya 1991, Miller & Gulya 1991), but the most

Fig.1. Sporulation of *Plasmopara halstedii* on Record (a sensitive sunflower cultivar).

Table 1. Disease symptoms of downy mildew on sunflower and their rating according to the importance of symptoms (Rahmanpour *et al.* 2000[b])

Symptoms	score
Damping-off	100
Sporulation on cotyledons and leaves	60
Sporulation on leaves	50
Stunting	15
Sporulation on cotyledons	10
Mosaic on leaves	10
Deformation of leaves	5
Lesion on hypocotyls	5
Root reduction	5

Table 2. Classification of sunflower cultivars and hybrids in five groups according to disease severity index (D.S.I) caused by *Plasmopara halstedii*. (Rahmanpour *et al.* 2000[b])

Reaction	Disease severity index (D. S. I.)
resistant	0-20
moderately resistant	20-30
moderately susceptible	30-50
susceptible	50-95
highly susceptible	95-100

Table 3. Analysis of variance for disease severity data of nine sunflower cultivars inoculated with *Plasmopara halstedii* race100.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Genotypes	8	17097.41	2137.17	115.87	<.0001*
Error	18	332.00	18.44		
Corrected Total	26	17429.411			

*: Genotypes are significantly different

Table 4. Reaction of sunflower cultivars and hybrids to downy mildew (race 100) based on the disease severity index (D. S. I.).

cultivars and hybrids	Reaction	Mean Disease severity index (D. S. I.)	Duncan grouping	SD (Standard Deviation)
Record	susceptible	80.33	A	1.1
Armavirесky	susceptible	70.33	B	4.7
Alstar	susceptible	68	B	7
Ablag	susceptible	57.33	C	5.5
Pesteii	susceptible	54.33	C	5.1
Euroflor	moderately resistant	25.66	D	1.5
Azargol	moderately resistant	25	D	2.6
Master	resistance	11.66	E	3.7
Lakumka	resistance	11	E	3.6

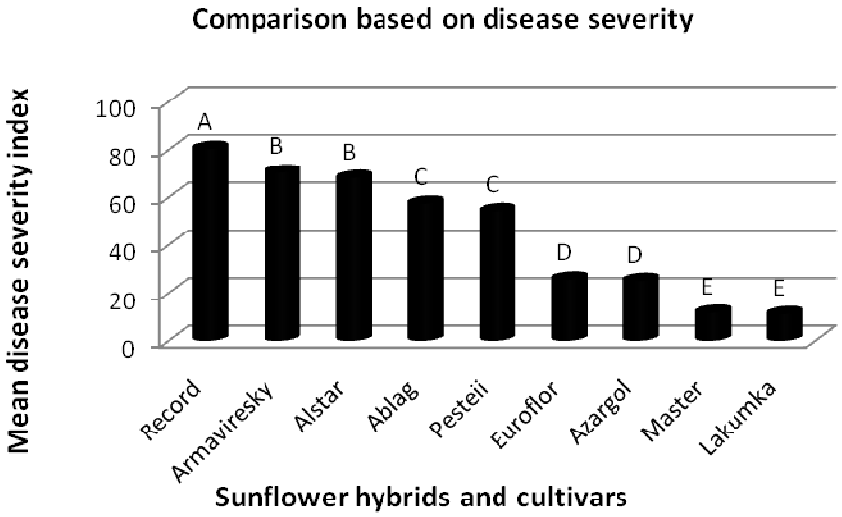

Fig. 2. Comparison of sunflower cultivars and hybrids based on the average downy mildew severity index

effective method to control disease is the use of a single dominant gene (*Pl*) (Vear & Leclercq 1971). Several decades after the introduction of the *Pl* genes into the cultivated sunflower, typical symptoms of *P. halstedii* were again found on cultivated plants. Extensive search for new sources of resistance started after investigations showed the development of a new physiological race of the pathogen (Gulya *et al.*, 1997). So far, eleven *Pl* genes have been reported that confer resistance to one or all identified downy mildew races (Miller & Gulya 1991). Genetic analysis revealed that some of the genes are tightly linked (Roeckel- Drevet *et al*., 2003). It is not clear whether these loci are single resistance genes conferring non-race-specific resistance or clustered genes giving resistance to individual races following the gene-for- gene hypothesis, but the sunflower resistance to most races is controlled by single dominant genes (Molinero-Ruiz *et al.* 2003). However, the accurate phenotype assessment can be very difficult because the incidence of the disease in susceptible control plants in an experiment does not usually reach 100%. Thus, a formula to calculate a correction factor was used to correct the observed susceptible seedling numbers in the resistance study (Molinero-Ruiz *et al*. 2003).

Despite worldwide distribution of downy mildew of sunflower the risk of economic loss is low because the resistance to the disease is mainly the product of the dominant gene, *Pl* (Vear & Leclercq 1971). West Azarbaijan Province is considered as the oldest and largest region in which sunflower cultivars, suitable for confectionary, is cultivated. Although the farmers did not have enough knowledge about the disease and its causal agent, they eliminated the diseased plants (with systemic symptoms) through pulling them out to have better quality of seeds and crop. Therefore by doing this during decades, it might be likely that seeds used for planting have been selected and resistance of local confectionary varieties has been increased by choosing the healthy plants in infected areas. But proving this hypothesis needs more investigation. The old cultivars of sunflower used for oil production, demonstrate considerable sensitivity to the disease explaining the lack of resistance genes. Currently used cultivars such as Lakumka and Euroflor confer the genes expressing resistance.

Gene-for-gene interactions have been demonstrated between this pathogen and its host plant (Tourvieille de Labrouhe *et al*. 2000), and many physiological races (or pathotypes) have been characterized worldwide (Gulya *et al*., 1991). The current identified race (100) in North-West of Iran has some physiological similarities with the race identified by Alizadeh and Rahmanpour (2005) in terms of reaction of several differential lines against it. Therefore race 100 is likely to be close to that identified by them. Furthermore, most differential lines used by them have been discarded in the last nomenclature system. During the last twenty years new races of *P. halstedii* have been evolved and identified (Molinero-Ruiz *et al*. 2003).

Since genes against the race 100 are not available, other genes in the genome of resistant cultivars can be exploited. Although in our evaluation of resistance in sunflower cultivars and hybrids against race 100, the dominant race of the pathogen in Urmia, we found no completely resistant cultivar or hybrid, but five significantly different groups were recognized. Dueto the highest rank of resistance against the disease in Lakumka and Master, these two cultivars are recommended to be used in cultivar breeding programs in the studied region to reach a reasonable level of resistance. Two hybrids, Azargol and Euroflor included in this research were ranked as partially resistant hybrids. These hybrids are also recommended for development of growing areas. In comparison with open- pollinate cultivars, application of sunflower hybrids are preferable, because sunflower hybrids have usually more seed yield and oil and earlier maturity in comparison to open-pollinated ones. Most hybrids are resistant against draught, many pests and diseases. It is possible to use chemicals against pests on the hybrids without considering the effects of chemical control on pollination rate. It is also important to know that in some occasions a few plants from resistant cultivars and hybrids of sunflower may be infected with *P. halsedii* (Viranyi & Mohamed 1985). According to the potential of emergence of more new races of the pathogen in sunflower cultivation areas, it is essential to perform a continuous program of race identification of this pathogen to employ appropriate resistance gene against the dominant race or races in each region.

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