

IDENTIFICATION OF RUST (*Puccinia helianthi* Schw.) RACES IN SUNFLOWER (*Helianthus annuus* L.) IN TURKEY

Tan, A.S.*

Ministry of Agriculture and Rural Affairs, General Directorate of Agricultural Research, Aegean Agricultural Research Institute, Plant Genetic Resources Department, PO Box: 9, Menemen, 35661 Izmir, Turkey

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SUMMARY

Sunflower (*Helianthus annuus* L.), with its high oil quality, is one of the major and most valuable oilseed crops in the world. Sunflower rust, caused by *Puccinia helianthi* Schw., is one of the major foliar diseases of sunflower. The pathogen is present wherever sunflower is grown in the world and causes important yield losses when severe epidemics occur. The objective of this study was to identify the races of sunflower rust under field conditions in the first and second crop production seasons. Experiments were conducted from 1991 to 2009 at Menemen, Izmir - Turkey. Race identification of *P. helianthi* was accomplished in field conditions in which seedlings of differential lines were naturally infected. Twenty-three differential genotypes were used to identify races of *P. helianthi*. Sunflower rust reactions of the differential genotypes were scored on a scale of 0 to 4, where 0 to 2 = resistant, 3 and 4 = susceptible. Races 1 and 3 of *P. helianthi*, the causal agent of sunflower rust, were identified at Menemen - Izmir.

Key words: *Puccinia helianthi*, *Helianthus* spp., sunflower, rust races

INTRODUCTION

Among the oilseed field crops, sunflower (*Helianthus annuus* L.) ranks second in the world for edible oil production and is produced mainly in Argentina, Russia, France, Ukraine, Spain, India, USA, China, Turkey, Romania, and Hungary (Anonymous, 2010).

Many diseases attack sunflowers, but only a few of them, such as sunflower downy mildew and sunflower rust (*Puccinia helianthi* Schw.), are considered to be important on the global scale and occur wherever sunflower is cultivated (Fick, 1978; Yücer and Karaca, 1978; Zimmer and Hoes, 1978; Demir and Tan, 1986; Miller, 1987; Gulya, 1990; Rashid, 1990; Tan, 1991; Tan, 1993; Tan, 1994).

* Corresponding author: Phone: +902328461331/470; e-mail: a_s_tan@hotmail.com

Sunflower rust is considered to be a major and destructive disease of sunflower globally and can cause serious yield losses on susceptible genotypes under conditions favorable for rust development (Putt and Rojas, 1955; Sackston, 1956; Putt and Sackston, 1957; Zimmer, 1971; Fick, 1978; Yücer and Karaca, 1978; Zimmer and Hoes, 1978; Demir and Tan, 1986; Gulya, 1990; Gulya *et al.*, 1990; Rashid, 1990; Tan, 1993; Gulya *et al.*, 1997; Gulya, 2006; Rashid, 2004).

There was a severe epidemic of rust in sunflower cultivars in Australia and yield losses were estimated to have been as high as 70% (Brown *et al.*, 1974). Singh (1974) reported that sunflower rust caused 60% yield losses in severe cases in Kenya. Sunflower acreage dropped dramatically from 24,280 ha in 1949 to 1,420 ha in 1952 in Manitoba (Putt and Rojas, 1955). Rashid (1993) reported that the incidence and severity of sunflower rust have steadily increased in Manitoba since 1988. Rust prevalence was 81% and severity was 52% in North Dakota, South Dakota, and Minnesota in 1992 (Gulya, 1993).

In the United States sunflower rust has not been of serious consequence since the mid-1990s (Gulya and Viranyi, 1994; Gulya *et al.*, 1997), but within the last six years its incidence and severity have been steadily increasing. Rust incidence was 17%, 44%, 60%, 68%, 77%, and 62% in 2002, 2003, 2005, 2006, 2007, and 2008, respectively (Rashid, 2004; Gulya, 2006; Gulya and Markell, 2009).

Zimmer and Zimmerman (1972) found that plants infected with *P. helianthi* produced smaller seeds with higher hull percentage and lower oil content. The potential yield loss occurs when rust pustules cover 5% of the lower leaves at or before anthesis (Gulya *et al.*, 1990). Fick and Zimmer (1975) reported that when the infection was moderately severe, yield losses were as high as 33%, and the susceptible hybrids produced seed significantly lower in 100 seed weight and oil content. Sunflower head area and the number of achenes per head were decreased during severe rust epidemics in Israel (Shtienberg and Zohar, 1992). Rust is the most common sunflower disease in the main production area in Turkey, and the average infection rate of *P. helianthi* was found to have been as high as 26.6% in one of the surveyed areas in Turkey (Yücer and Karaca, 1978).

The rust resistant sunflowers were first observed in 1949 (Putt and Sackston, 1957). Resistance of sunflower seedlings to *P. helianthi* was first demonstrated at Winnipeg in 1951 and 1952 (Sackston, 1962). *P. helianthi* is composed of several races (Miah and Sackston, 1970; Zimmer and Hoes, 1978; Gulya, 1990; Quresh *et al.*, 1991; Lambrides, 1992; Rashid, 1993). Tan (1993 and 1994) reported that race 1 of *P. helianthi* was found in the first and second crop production seasons in Menemen-Izmir, Balikesir, Aydin, and Denizli, and races 1 and 3 were found in the Edirne and Bursa provinces of Turkey. There are four known races of sunflower rust existing in North America. However, a race shift has occurred and races 3 and 4 have become the predominant races of *P. helianthi* in the last decade (Gulya, *et al.*, 1988; Gulya, 1990; Gulya, 1991; Rashid, 1990; Jan *et al.*, 1991). The change in virulence of *P. helianthi* occurred in Canada. As a result, in 1988, race 4 was identified in 67% of the isolates, followed by race 3 (33%). However, in 1990, race 3

was identified in 71% of the isolates, followed by race 4 (17%) and race 1 (12%) (Rashid, 1993). Resistance to rust was studied by Putt and Sackston (1957 and 1963) and their study indicated that resistance to *P. helianthi* was dominant. Miah and Sackston (1970) investigated rust resistance in sunflower for four races of *P. helianthi* and reported that resistance to rust was dominant and controlled by one or two genes. Rodriguez (1987) studied race 4 of *P. helianthi* in cultivated sunflowers and found that resistance to *P. helianthi* was controlled by a dominant gene. Genotypes with resistance to races of *P. helianthi* were registered (Gulya, 1985). Quresh *et al.* (1991) studied the genetic relationship between wild and cultivated species for sunflower rust resistance and reported that a single dominant gene was controlling resistance to races 1, 2, 3, and 4 of *P. helianthi*. Quresh and Jan (1993) studied the allelic relationship among genes for resistance to sunflower rust in wild accessions of *H. annuus*, *H. argophyllus*, and *H. petiolaris*. They reported that each of the wild accessions had rust-resistance genes unique to races 1, 2, 3, and 4 of *P. helianthi*.

Based on 300 samples evaluated so far from 2007 and 2008, 39 races have been identified, and races 334 and 336 were found to be dominant in both years. The most virulent race that can be identified with the current set of differentials, designated as race 777, was found in 1% of the 2007 samples and has not been identified yet in the 2008 samples. Ninety-three commercial and experimental hybrids were evaluated in greenhouse trials for resistance to race 336. Seven entries were rated as highly resistant and four as resistant, but none of the 93 entries were immune. Thus, 88% of the entries were susceptible to race 336, the most common race in 2007 and 2008 (Gulya and Markell, 2009).

P. helianthi is a major foliar pathogen that causes small, circular, orange to black pustules on the leaves and completes a sexual cycle every season (Zimmer and Hoes, 1978). The uredospores germinate and penetrate through the stomata. The pustules first appear on the lower leaves and the disease spreads as the season progresses and pustules can be seen on the younger leaves and the entire plant as well. Normally, chlorotic areas can be seen around the pustules (Zimmer and Hoes, 1978). Prud'Homme and Sackston (1989) reported that the germination percentage of spores on the leaves, growth rate of germ tubes, formation of appressoria, size of mycelial colonies within leaves, the size and density of pustules on the leaves, and spore production can be varied among the races of *P. helianthi*. Their study of the relative fitness of races 1 and 3 of rust in mixtures on susceptible sunflower resulted in the weight of spores produced per pustule of race 3 being lower than that of race 1, and the total number of spores of race 3 produced per unit of infected leaf area was greater. Crop rotation is an important way of reducing the rust inoculum and controlling this disease pathogen. Identification of the *P. helianthi* races and breeding for resistance are major objectives of most sunflower breeding programs, and this is also the most economical and effective way to control this pathogen. However, it is possible that new virulent races may arise by mutation and recombination; therefore, many of the hybrids may still be attacked by some of the newer rust races.

The main objective of this study was to determine races of *P. helianthi* in field conditions in Menemen, Izmir in Turkey.

MATERIAL AND METHOD

To obtain information about the occurrence, distribution and identification of rust races, 23 differential lines were used in Menemen, Izmir from 1993 to 2009 (Table 1). At the beginning of the study, 18 differential lines (Table 3) were used (Tan, 1994) in field trials in six provinces (Aydin, Balikesir, Bursa, Denizli, Edirne, and Izmir) of Turkey in 1992 (Table 3). The seed of the differential lines used in this study (Table 1) was obtained from the Oilseed Crops Research Section of USDA-ARS at Fargo, ND, USA. Race identification of *P. helianthi* was done under natural infection in field conditions. Each differential line was planted in individual rows. Each row consisted of 22 plants spaced 35 cm apart within rows 7.7 m in length and spaced 0.7 m apart. Germplasm accessions were sown in April-May (first crop) and June-July (second crop).

The resistance/susceptibility criterion of the differential lines to *P. helianthi* was used for the identification and determination of rust races. During the growing season, the occurrence and infection types of *P. helianthi* on the differential lines were observed. This was clear and specific to the differential genotypes, suggesting that the differential genotypes were homogeneous.

Table 1: Reactions of 23 sunflower differential lines to *P. helianthi*, races 1, 2, 3, and 4.

No.	Rust races differential genotypes	Reaction of rust races			
		1	2	3	4
1	ETAE - 307 - 2	S*	S	S	S
2	P - 386	R*	R	R	R
3	cms HA 89	S	S	S	S
4	HA 89	S	S	S	S
5	RHA 364	S	S	S	S
6	RHA 366	S	S	S	S
7	S 37	S	S	S	S
8	cms HA 371	S	R	S	S
9	HA 371	S	R	S	S
10	cms HA 372	S	R	S	S
11	HA 372	S	R	S	S
12	cms HA 370	R	R	S	S
13	HA 370	R	R	S	S
14	CM 90	R	R	S	S
15	CM 29	R	S	R	S
16	RHA 365	R	S	R	S
17	RHA 363	R	R	R	S
18	RHA 367	R	R	R	S
19	HAR 1	R	R	R	R
20	HAR 2	R	R	R	R
21	HAR 3	R	R	R	R
22	HAR 4	R	R	R	R
23	HAR 5	R	R	R	R

* R= Resistant, S=Susceptible

The reactions of each of these differentials to the four races of rust are presented in Table 1. The experiments were conducted during both the first and second crop production seasons at Menemen-Izmir but only in the first (main) production season in Aydin, Balikesir, Bursa, Denizli, and Edirne in 1992.

Since rust infection was sufficiently intense in the areas under study, the method produced satisfactory results for the race identification of *P. helianthi*.

The expected reaction of each differential line to the North American races of *P. helianthi* was determined according to Sackston (1962) and Gulya (1985).

The genotypes were analyzed and classified as R or S according to their reactions to sunflower rust. Rust severity in the differential genotypes was scored using a scale of 0 to 4, where 0 = immune, 1 and 2 = resistant, 3 and 4 = susceptible (Yang, 1986). The classification system shown in Table 2 was used to determine the reactions of differential genotypes to *P. helianthi* in this study. Scoring was done at 15-day intervals from stage R-1 through R-9 during the growing season (Schneider and Miller, 1981).

Table 2: Classification system for determining rust (*P. helianthi*) reaction of the genotypes (Yang, 1986).

Classification*	Description	Reaction type
0	No visible reaction	Immune
1	Small uredia (Pustule < 0.2 mm in diameter) in association with flecking	Resistant
2	Small uredia (0.2 - 0.4 mm in diameter) in association with necrosis and chlorosis	Resistant
3	Uredia (0.4 - 0.6 mm in diameter) which sporulate freely in association with chlorosis	Susceptible
4	Uredia (> 0.6 mm in diameter) which sporulate freely with little or no chlorosis	Susceptible

*Infection types 0, 1, and 2 were classed as resistant, while 3 and 4 were classed as susceptible

RESULTS AND DISCUSSION

Experiment results at Izmir, Aydin, Balikesir, and Denizli (Tan, 1994)

The reactions of the differentials to the races of *P. helianthi* in the field conditions at Izmir, Aydin, Balikesir, and Denizli are given in Table 3. The same reactions with the actual reaction types of the differential genotypes to the races of *P. helianthi* were obtained, and the genotypes RHA 365 and CM 29 were found to be resistant to *P. helianthi*; therefore, race 2 was not present. In this study race 3 was also not found in Izmir, Aydin, Balikesir, and Denizli, since CM 90, cms HA 370, HA 370 were found to be resistant to *P. helianthi*. A similar result obtained from the inbred lines RHA 363 and RHA 367, which were resistant to *P. helianthi*, implied that race 4 was absent. The genotypes HAR 3 and HAR 4 showed no visible reactions and were scored as resistant to *P. helianthi*.

However, the genotypes S 37, *cms* HA 89, HA 89, RHA 364, RHA 366, *cms* HA 371, HA 371, *cms* HA 372, and HA 372 were susceptible to *P. helianthi*, suggesting that only race 1 of *P. helianthi* exists in Menemen-Izmir, Çivril-Denizli, Koçarlı-Aydın, and Susurluk-Balikesir (Table 3).

Table 3: Race classification for sunflower rust (*P. helianthi*) using the differential genotypes in field conditions in Turkey in 1992 (Tan, 1994).

Rust races differential genotypes	Reaction of differentials in the provinces						
	Izmir		Aydın	Denizli	Balikesir	Bursa	Edirne
	Menemen	SC	Koçarlı	Çivril	Susurluk	Merkez	Merkez
	FC*	SC	FC*	FC*	FC	FC*	FC*
<i>cms</i> HA 89	S**	S	S	S	S	S	S
HA 89	S	S	S	S	S	S	S
RHA 364	S	S	S	S	S	S	S
RHA 366	S	S	S	S	S	S	S
S 37	S	S	S	S	S	S	S
<i>cms</i> HA 371	S	S	S	S	S	S	S
HA 371	S	S	S	S	S	S	S
<i>cms</i> HA 372	S	S	S	S	S	S	S
HA 372	S	S	S	S	S	S	S
<i>cms</i> HA 370	R	R	R	R	R	S	S
HA 370	R	R	R	R	R	S	S
CM 90	R	R	R	R	R	S	S
CM 29	R	R	R	R	R	R	R
RHA 365	R	R	R	R	R	R	R
RHA 363	R	R	R	R	R	R	R
RHA 367	R	R	R	R	R	R	R
HAR 3	R	R	R	R	R	R	R
HAR 4	R	R	R	R	R	R	R

* FC = First crop, SC = Second crop.

** R=Resistant, S = Susceptible.

Experiment results of Bursa and Edirne (Tan, 1994)

The reactions of the differentials to the races of *P. helianthi* in the field conditions in Bursa and Edirne in 1992 are given in Table 3.

The genotypes RHA 365 and CM 29 were resistant to *P. helianthi*, indicating that race 2 was not present. A similar result obtained from the lines RHA 363 and RHA 367, which were resistant to *P. helianthi*, implied that race 4 was absent. The genotypes HAR 3 and HAR 4 were rust-free and found to be completely resistant to *P. helianthi*. The genotypes S 37, *cms* HA 89, HA 89, RHA 364, RHA 366, *cms* HA 371, HA 371, *cms* HA 372, and HA 372 were susceptible to *P. helianthi*, suggesting that race 1 of *P. helianthi* is present in Bursa and Edirne.

P. helianthi attacked S 37, HA 89, *cms* HA 89, HA 371, *cms* HA 371, *cms* HA 372, HA 372, RHA 364, RHA 366 and the genotypes, HA 370, *cms* HA 370, CM 90.

However, the genotypes, CM 29, RHA 363, RHA 365, and RHA 367 were found to be resistant to *P. helianthi*. The results of natural inoculations with sunflower rust in Bursa and Edirne confirmed the existence of race 1 and a new race, 3 (Table 3).

The genotypes HA 89, *cms* HA 89, HA 371, *cms* HA 371, *cms* HA 372, HA 372, RHA 364, RHA 366 showed moderate levels of susceptibility to *P. helianthi*. However, the universally susceptible germplasm line S 37 showed the highest level of infection among the other susceptible lines in Bursa, Edirne and Izmir, Aydin, Balikesir, Denizli as well.

Based on the differential response to sunflower rust, *cms* HA 89, HA 89, *cms* HA 370 and HA 370, *cms* HA 371 and HA 371, *cms* HA 372, and HA 372 showed almost the same results of infection types of *P. helianthi* in the trials.

Experiment results of Menemen, Izmir (1993-2009)

The results compared with the data presented in Table 1 suggest the presence or absence of the races of *P. helianthi* in Menemen, Izmir. The reaction of the differentials to the races of *P. helianthi* in the field conditions in Menemen, Izmir is given in Table 4.

Table 4: Reaction of rust races in first and second crop production seasons at Menemen, Izmir (1991-2009).

No.	Rust races differential genotypes	Reaction of rust races in first and second crop production seasons.
1	ETAE - 307 - 2	S
2	P - 386	R
3	<i>cms</i> HA 89	S
4	HA 89	S
5	RHA 364	S
6	RHA 366	S
7	S 37	S
8	<i>cms</i> HA 371	S
9	HA 371	S
10	<i>cms</i> HA 372	S
11	HA 372	S
12	<i>cms</i> HA 370	S
13	HA 370	S
14	CM 90	S
15	CM 29	R
16	RHA 365	R
17	RHA 363	R
18	RHA 367	R
19	HAR 1	R
20	HAR 2	R
21	HAR 3	R
22	HAR 4	R
23	HAR 5	R

The same reactions with the actual reaction types of the differential genotypes to the races of *P. helianthi* were obtained, and the genotypes RHA 365 and CM 29 were found to be resistant to *P. helianthi*; therefore, race 2 was not present. RHA 363 and RHA 367, which were resistant to *P. helianthi*, implied that race 4 was absent. The genotypes HAR 1, HAR 2, HAR 3, HAR 4, and HAR 5 showed no visible reactions and were also found to be resistant to *P. helianthi*.

However, the genotypes S 37, *cms* HA 89, HA 89, RHA 364, RHA 366, *cms* HA 371, HA 371, *cms* HA 372, and HA 372 were susceptible to *P. helianthi*, suggesting that race 1 of *P. helianthi* exists in Menemen-Izmir (Table 4).

The genotypes RHA 365 and CM 29 were resistant to *P. helianthi*, indicating that race 2 was not present. A similar result obtained from the lines RHA 363 and RHA 367, which were resistant to *P. helianthi*, implied that race 4 was absent. The genotypes HAR 3 and HAR 4 were rust-free and found to be completely resistant to *P. helianthi*. The genotypes S 37, *cms* HA 89, HA 89, RHA 364, RHA 366, *cms* HA 371, HA 371, *cms* HA 372, and HA 372 were susceptible to *P. helianthi*, suggesting that race 1 of *P. helianthi* exists in Menemen, Izmir. Since *P. helianthi* attacked HA 370, *cms* HA 370, and CM 90, race 3 of *P. helianthi* also exists in Menemen, Izmir.

The results of natural inoculations with sunflower rust in Menemen - Izmir confirmed the existence of race 1 and race 3 (Table 4).

The susceptible germplasm line S 37 showed the highest level of infection among the susceptible lines.

The genotypes HA 89, *cms* HA 89, HA 371, *cms* HA 371, *cms* HA 372, HA 372, RHA 364, RHA 366 showed moderate levels of susceptibility to *P. helianthi*. However, the universally susceptible germplasm line S 37 showed the highest level of infection among the susceptible lines in all years.

The higher susceptibility of the genotypes (S 37, *cms* HA 89, HA 89, RHA 364, RHA 366, *cms* HA 371, HA 371, *cms* HA 372, and HA 372) in the second crop production season compared with the first could be due to more favorable conditions (especially temperature) for rust development.

This is the first study in which *P. helianthi* differential lines have been used to determine the races of sunflower rust in the main sunflower production areas (Edirne, Bursa, Balıkesir, Aydın, Denizli and Izmir) in Turkey.

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