Original article

**Determination of the races of *Plasmopara halstedii* (Farl.) Berl. & de Toni, the causal agent of sunflower downy mildew in Turkey and reactions of some commercial sunflower varieties against these races**

Ajçiçeği mildiyösü etmeni *Plasmopara halstedii* (Farl.) Berl. & de Toni’nin Türkiye’deki ırklarının tespiti ve bazı ticari ajçiçeği çeşitlerinin bu ırklara karşı reaksiyonlarının belirlenmesi

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**ABSTRACT**

Downy mildew of sunflower caused by *Plasmopara halstedii*, is the most important disease of sunflower throughout the world including Turkey. Within the scope of the study, surveys were performed in Tekirdağ, Edirne, Kırklareli, Ankara, Bursa, Samsun, Tokat and Adana provinces where sunflower is widely grown in the period of 2009-2015. During the surveys, sixty-five *P. halstedii* isolates were obtained and purified. Using the race differentials set, nine races of the pathogen (100, 102, 110, 300, 500, 502, 510, 702 and 712) were determined. All races determined are first records for Turkey, whereas the races 102, 510 and 110 are the first record for the world. Almost 71% percent of the races were belonged to race 100. The reactions of 19 commercial sunflower cultivars against *P. halstedii* were determined by using isolates representing different races of downy mildew. None of the commercial sunflower cultivars showed resistance against all the races of *P. halstedii* at the same time. Among varieties tested LG5580 was found to be resistant to four races, whereas Sanay MR, Sanbro MR, LG540HO, and Sanbro varieties were found to be 3, 3, 2 and 1 races, respectively.

**INTRODUCTION**

Sunflower (*Helianthus annuus* L.) is an important source of vegetable oil and keeps gaining popularity because of its high oil percentage and quality, short growth duration and thermos-photo insensitiveness. It provides 46% of the total vegetable crude oil production in Turkey.
Most of the oilseed sunflower of Turkey is grown in Thrace and Marmara region (47.2%) and it is followed by Central Anatolia (29.2%), Black Sea (12%) and Mediterranean region (8.7%). About 2,000,000 tons of sunflower production is obtained from 795,215 ha cultivated area (Anonymous 2018). Sunflower oil consumption in Turkey is approximately 650,000 tons annually in which 400,000-450,000 tons provided by domestically.

More than 30 diseases of sunflowers have been documented worldwide (Gulya et al. 1991). Downy mildew is the most destructive disease affecting sunflower worldwide (Kolie 1985). The presence of sunflower downy mildew in Turkey was firstly reported in 1958 in Sakarya province (Karel 1958). In 2007 and 2008, a sunflower downy mildew outbreak occurred in Thrace and Marmara regions at four leaf period and crop losses up to 85% occurred (Göre 2009).

Plasmopara halstedii (Farl.) Berl. & de Toni is an obligate oomycete plant pathogen which remains viable in the soil as oospores for up to 10 years (Hall 1989, Nishimura 1926, Novotelnova 1966, Sackston 1981). Under favourable conditions, oospores germinate to give zoosporangia. Zoospores formed in the zoosporangia cause primary infections of sunflower radicles, leading to systemic infection that cause most of the losses. The systemic infection of the plants results stunting, leaf chlorosis, horizontal head, poor seed set and severe yield reduction (Komjati 2010). Disease severity may considerably varies according to region, year and growing conditions. The disease is especially severe when high moisture and cool temperatures (14–16 ºC) prevail at 2-4 leaf growth stages. The prevalence of sunflowers contaminated with P. halstedii in a field may be ranged from traces to near 50% or even up to 95% (Sackston 1981).

P. halstedii was originated from the North America and spread into Europe in the early 1940’s. It remained pathologically uniform until the introduction of resistant sunflower cultivars. The physiological races (pathotypes) of P. halstedii display variation in the interaction with sunflower genotypes. The nomenclature of downy mildew pathotypes is based on an internationally accepted methodology using a set of sunflower differential lines with distinct resistance-susceptibility reactions (Gulya et al. 1998). The most detailed and up-to-date list of global distribution of P. halstedii pathotypes has been compiled by Gulya (2007) and Viranyi et al. (2015). In this accurate overview, he comprised as many as thirty-five pathotypes, an unbelievably high number considering the fact that in most sunflower producing countries from just a few to 12 well-distinguished virulence phenotypes exist (Viranyi 2008). The quantity and composition of pathogen races vary in different countries and the determination of these is the main objective of the studies by leading phytopathologists (Kormany and Viranyi 1997, Masirevic 1992, Molinero-Ruiz et al. 2002, Penaud et al. 1997, Rozynek and Spring 2001, Shindrova 2005, 2010, Shirshikar 2005, Tourvieille de Labrouhe et al. 2000).

While the number of pathotypes increasing, new pathotype evaluation techniques are emerging. Sedlarova et al. (2016) determined two new races (race 705 and 715) from, from the single site in The Check Republic by evaluating the resistance/susceptibility by five-digit code.

In the study carried out by Shindrova (2010) in Bulgaria, the race composition of P. halstedii was found to be quite stable in the years 1988-2000 and with the introduction of tolerant or resistant cultivars, the number of the races increased up to five.

There has not been a study on race differentiation of sunflower downy mildew up to now in Turkey, although Viranyi et al. (2015) listed 9 races, most of them belonging over the seven hundreds, from 700 to 774. They did not quote any reference about those races, some of which maintained by regional Agricultural Research Institute of Thrace and May Seed company.

The composition of sunflower cultivars widely grown in Turkey has been changed a lot and newly bred hybrid varieties have been introduced and grown in various places of Turkey. Until 2019, no research related with races of P. halstedii had been carried out. The aim of this study is to determine the races of P. halstedii according to the three-digit nomenclature system and the reaction of some commercial sunflower cultivars against all the determined pathotypes of downy mildew in Turkey.

MATERIALS AND METHODS

Plasmopara halstedii populations

Sixty-five P. halstedii populations were collected from sunflower fields in eight provinces of Turkey; in eight different sunflower fields of Turkey; namely Edirne, Tekirdağ, Kırklareli, Bursa, Adana, Samsun, Tokat and Ankara provinces, during years 2009 and 2015. The zoosporangia of downy mildew were directly recovered from individual plants showing sporulation on their lower sporulation on lower surface of leaves.

Obtain monozoosporangial isolates

Inoculum was obtained from infected leaves (Figure 1). Each sporulated leaf was placed in a 50 ml Falcon conical tube containing 20 ml of NaCl solution (9 g NaCl + 1 liter distilled water) and shaken. A hundred microlitres of inoculum
The surface of sunflower seeds were disinfected in 15% sodium hypochloride solution for 10-15 min, washed with tap water, placed on moistened filter paper and put in a dark climate chamber at 24 °C. Seed inoculation method of *P. halstedii* described by Cohen and Sackston (1973) and modified by Molinero-Ruiz et al. (2002) was used for determination resistance/susceptibility. When the radicle was 2–5 mm long, they were immersed for 4 h at 18 °C in an inoculum suspension 3x10⁴ zoosporangia/ml containing 25 µg/ml riboflavin and 2 mg/ml glucose to increase the activity of zoosporangia. Then five seedlings were sown in each plastic pot (9 cm diameter) filled with perlite/sand mixture (2/3, v/v). In a climate chamber (24 ºC) in 16 h photoperiod, plants were grown for 10–12 days until first pair of true leaves (in size of mouse ears) developed. Then the plants and inside of the pots were moistened with distilled water to obtain relative humidity. Inoculated sunflower seedlings were placed in a dark climate chamber overnight to initiate sporulation on cotyledons and/or the first pair of true leaves of susceptible seedlings (Figure 2) (Molinero-Ruiz et al. 2002).

Race characterization

The method used in race characterization was the same as used at inoculum preparation stage (Cohen and Sackston 1973, Molinero-Ruiz et al. 2002). Sunflowers were scored as resistant (R) if no sporulation was seen on cotyledon and as susceptible (S) if sporulation was observed in cotyledons and the first pair of true leaves. The races of the *P. halstedii* populations were identified by using the triplet coding

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**Figure 1.** An infected sunflower plant by *Plasmopara halstedii* having profuse sporulation used to obtain mono-zoosporangial isolates

**Figure 2.** Initiate sporulation on cotyledons and/or the first pair of true leaves of susceptible seedlings
system (Table 1) that records the R/S reactions of each nine lines of sunflower differential set by Gulya et al. 1998 (Table 2). There were five replications per each differential line (5 seedlings in each replication), and the entire experiment was repeated twice.

**Table 1. Triplet coding system for defining sunflower downy mildew races (Gulya et al. 1998)**

| Differential Line | D-1 | D-2 | D-3 | D-4 | D-5 | D-6 | D-7 | D-8 | D-9 | Triplet Code |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------------|
| Value if S.       | 1   | 2   | 4   | 1   | 2   | 4   | 1   | 2   | 4   |             |
| European race     | S   | R   | R   | S   | R   | R   | S   | R   | R   | 100         |
|                   | 1 + 0 + 0 = 1 | 0 + 0 + 0 = 0 | 0 + 0 + 0 = 0 |             |
| Red River race    | S   | S   | R   | R   | R   | R   | R   | R   | R   | 300         |
|                   | 1 + 2 + 0 = 3 | 0 + 0 + 0 = 0 | 0 + 0 + 0 = 0 |             |
| Race xyz          | S   | S   | S   | S   | R   | S   | R   | R   | R   | 752         |
|                   | 1 + 2 + 4 = 7 | 1 + 0 + 4 = 5 | 0 + 2 + 0 = 2 |             |

S = susceptible; R = resistant; Race “xyz” = hypothetical race

**Table 2. Sunflower differential lines for downy mildew race identification**

| Designation | Original Name | Pedigree | Source of Resistance |
|-------------|---------------|----------|----------------------|
| D-1         | Ha-89 (USDA)* | -        | -                    |
| D-2         | Rha-265 (USDA) | Peredovik/953-102 | 953-102 (Canada) |
| D-3         | Rha-274 (USDA) | HA-119/HA-62 | 953-88 (Canada) |
| D-4         | DM-2 (INRA)** | selection of PMI-3 | Novinka (Russia) |
| D-5         | PMI-17 (USDA) | PI 406022 | ? (Iran) |
| D-6         | 803-1(IFVC)*** | H. tuberosus | H. tuberosus |
| D-7         | HAR-4 (USDA) | Saenz-Pena 74-1-2 | ? (Argentina) |
| D-8         | HAR-5 (INRA) | QHP-1 (INRA) | Guayacan INTA (Argentina) |
| D-9         | HA-335 (USDA) | HA-89 x wild H. annuus | wild H. annuus |

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system (Table 1) that records the R/S reactions of each nine lines of sunflower differential set by Gulya et al. 1998 (Table 2). There were five replications per each differential line (5 seedlings in each replication), and the entire experiment was repeated twice.

Reactions of some commercial sunflower cultivars against downy mildew races

The reaction of nineteen commercial sunflower cultivars (LG 540HO, LG 5580, LG 5550, LG 5650 CL, Sanbro MR, Sanay MR, Sanbro, Oleko, Alhaja, Transol, Dkf 2525, Bosfora, Dkf 3518, Biser Cl, Sirena, Armada Cl, Es Primus, Es Amira and Aitana) against *P. halstedii* was determined by using isolates representing each of nine races. The method used in race characterization was also used for inoculum preparation (Cohen and Sackston 1973, Molinero-Ruiz et al. 2002). Sunflowers were scored as mentioned above for race characterization. There were five replications per each cultivar (5 seedlings in each replication), and the entire experiment was repeated twice per each race.
RESULTS

Occurrence of sunflower downy mildew in Turkey

A total of 65 fields were found to be infested by downy mildew, out of the 296 sunflower fields inspected between the years of 2009 and 2015. The average incidence of affected fields was 25%, which we expected yield losses to occur (Figure 3). The survey area comprised four climatically different regions of Turkey; Thrace and Marmara, Black Sea, Central Anatolia and Mediterranean regions.

Races of Plasmopara halstedii in Turkey and their distribution

Analysis of 65 monozoosporangial isolates of *P. halstedii* obtained from the same number of fields, yielded 9 races determined by the reactions of three sets of differential varieties. Distribution of the races is given in Table 3.

The most widespread race was “race 100” which was obtained from all the provinces and 70.7% of the infested fields. The second most widespread race was “race 500” obtained 10 (15%) samples from four of the provinces. Race 300 was found in 4 of the samples while races 102, 110, 502, 510, 702 and 712 were obtained from only one sample each.

The highest number of races were obtained from Kırklareli, followed by Tekirdağ and Edirne provinces, which all are located in Eastern Thrace region and comprise the highest amount of sunflower cultivated area in Turkey.

Reactions of some commercial sunflower cultivars against *Plasmopara halstedii*

Fifteen of the sunflower cultivars did not show any resistance against the all of *P. halstedii* determined in Turkey (Table 4). Five cultivars; LG 5580, LG 5400 HO, Sanay MR, Sanbro and Sanbro MR, were resistant to race 100. There were not any resistance against the races 102, 110, 510, 702 and 710. LG 5580 showed resistant reaction against four races.

Figure 3. A sunflower field showing dwarfed sunflowers infected by *Plasmopara halstedii* near Edirne province

### Table 3. Distribution of the races of *Plasmopara halstedii* in Turkey

| Provinces      | 100 | 102 | 110 | 300 | 500 | 502 | 510 | 702 | 712 |
|----------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Adana          | 3   | -   | -   | -   | -   | -   | -   | -   | -   |
| Ankara         | 2   | -   | -   | -   | -   | -   | -   | -   | -   |
| Bursa          | 7   | 1   | -   | -   | 1   | -   | -   | -   | -   |
| Edirne         | 3   | -   | 1   | 2   | 3   | -   | -   | -   | -   |
| Kırklareli     | 14  | -   | -   | 1   | 5   | 1   | 1   | 1   | 1   |
| Samsun         | 2   | -   | -   | -   | -   | -   | -   | -   | -   |
| Tekirdağ       | 11  | -   | -   | 1   | 1   | -   | -   | -   | -   |
| Tokat          | 3   | -   | -   | -   | -   | -   | -   | -   | -   |
| Total          | 45  | 1   | 1   | 4   | 10  | 1   | 1   | 1   | 1   |
Table 4. Reactions of twenty sunflower cultivars against nine races of *Plasmopara halstedii*

| Cultivars tested  | Plasmopara halstedii Races |
|-------------------|-----------------------------|
|                   | 100 | 102 | 110 | 300 | 500 | 502 | 510 | 702 | 712 |
| LG 5580           | R   | S   | S   | R   | R   | R   | S   | S   | S   |
| SANAY MR          | R   | S   | S   | R   | R   | S   | S   | S   | S   |
| SANBRO MR         | R   | S   | S   | R   | R   | S   | S   | S   | S   |
| LG 5400 HO        | R   | S   | S   | R   | S   | S   | S   | S   | S   |
| SANBRO            | R   | S   | S   | S   | S   | S   | S   | S   | S   |
| LG550             | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| LG 5650 CL        | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| OLEKO             | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| ALHAJA            | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| TRANSOL           | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| DKF 2525          | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| BOSFORA           | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| DKF 3518          | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| BISER CL          | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| SIRENA            | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| ES PRIMUS         | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| ES AMIRA          | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| AITANA            | S   | S   | S   | S   | S   | S   | S   | S   | S   |
| ARMADA CL         | S   | S   | S   | S   | S   | S   | S   | S   | S   |

(S = susceptible; R = resistant)

**DISCUSSION**

A total of 296 sunflower fields were inspected for *Plasmopara halstedii* incidence between 2009 and 2015 (Figure 3) and about 25% of them were found be infested. The rate of the infestation is expected to increase since *P. halstedii* survive in the soil a long time and a lot of new cultivars have been introducing which could bring new races although the rate of seed transmission is very low.

Although there is a review by Viranyi et al. (2015) giving the number of the races of *P. halstedii* in Turkey, no citation, related with them, has been mentioned in this paper. Races of *P. halstedii* were firstly reported with this study. By the past 6 years occurrence or introduction of new races could be possible since many new sunflower cultivars have been either imported or bred. It is highly probable that the races mentioned by Viranyi et al. (2015) exist in Turkey.

Races of *P. halstedii* were determined by using nine differential lines. About 70% of the population of *P. halstedii* were belong to race 100 (45 out of 65), followed by race 500 and 300. Races 100, 102, 110, 300, 500, 502, 510, 702 and 712. Races 102, 110 and 510 were recorded for the first time in the world. Recently, race determination with nine differentials has been found insufficient for some races and a new set comprising 15 lines have been adopted. Mostly new races over 7 digits have increased especially after the introduction of new resistant cultivars (Molinero-Ruiz 2018, Sedlarova et al. 2016, Shindrova 2010).

There is only one study on the reactions of some sunflower...
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