

Research Article

## Resistance evaluation of some commercial *Vitis vinifera* varieties to powdery mildew *Erysiphe necator* Schwein. in two regions of Iran

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**Abstract:** Powdery mildew, caused by *Erysiphe necator* is a common and severe fungal disease of grapevine all over the world including Iran. Although the application of chemical fungicides is the most common control method, the use of resistant varieties may be the most effective and environmentally sound strategy for managing the disease. Twenty two grape varieties were evaluated against powdery mildew under natural infection and artificial inoculation during 2011–2013 in Ardabil and Qazvin provinces of Iran. In this study, Perlett, Robee seedless, Flame seedless and Tompson seedless varieties were immune; Mish-Pestan, Khosnav and Torkamanestan 8 were highly resistant; Torkamanestan 4 and Superior seedless were resistant; Shahani-e-Qazvin and Shast-e-Arous were tolerant; Ghermez-e-Bidaneh, Sefid-e-Bidaneh, Fakhri, Sahebi-e-Ghermez, Peikami, Rishbaba-e-Sefid, Siyah-e-Samarghandi and Rotabi were susceptible and Rishbaba-e-Ghermez, Asgari, and Shahroodi were highly susceptible varieties to grape powdery mildew. In general, findings of this research may be used as resistance genetic sources for management of the disease in national and international programs.

**Keywords:** Grape varieties, powdery mildew, resistance, *Uncinula necator*

### Introduction

Fungal diseases are a major problem in grapevine cultivation, and one of the most threatening pathogens is the fungus *Erysiphe necator* (*Uncinula necator*), the causal agent of powdery mildew. These biotrophic ascomycetes invades host epidermal cells and colonizes leaves, rachis, and grapes, causing a decrease of vine growth, yield, and fruit

quality (Calonnec *et al.*, 2004; Karbalaee Khiavi *et al.*, 2014; Wan *et al.*, 2007). The incidence of powdery mildew has increased in recent years in Europe. Climatic conditions and reduced efficacy of fungicides have been suggested as possible reasons (Dean and Gray, 2008; Staudt, 1997). The disease was reported from Iran in 1946 for the first time and since then it has been observed in many grape growing provinces of the country (Karbalaee Khiavi *et al.*, 2012). It is believed that *U. necator*, the causal agent of the disease had been first identified in North America and then it spread to Europe in 1840's and was

Handling Editor: Naser Safaie

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Received: 14 March 2015, Accepted: 5 March 2016

Published online: 10 April 2016

officially reported from Europe in 1945 (Pearson and Goheen, 1990; Staudt, 1997).

Mechanisms of resistance to grape powdery mildew are as follows: penetration resistance that prevents haustorial formation; R- gene mediated resistance associated with programmed cell death or quantitative resistance that reduces pathogen fecundity (Eibach *et al.*, 2007; Mahanil *et al.*, 2007; Pauquet *et al.*, 2001; Peros *et al.*, 2006; Savocchia *et al.*, 2004). The most efficient way to minimize powdery mildew is to grow resistant cultivars; however, the elite cultivars originate from *Vitis vinifera* which is the most susceptible host species. Monogenic resistance to *E. necator* has been studied in *Muscadinia* spp. and its hybrids with *Vitis vinifera* (Korbuly, 1999; Kozma *et al.*, 2009; Molnar *et al.*, 2007; Pauquet *et al.*, 2001). In the *Vitis* genus, resistance appears to be due to several genes (Fisher *et al.*, 2004). Methods are needed to identify valuable sources of resistance and to assist breeders in production of resistant cultivars or hybrids against powdery mildew. Determination of host resistance was based on field observations (Staudt, 1997), tests on leaf disks (Korbuly, 1999; Wang *et al.*, 1995) or in vitro experiments (Kitao and Doazan, 1990).

Natural sources of disease resistance are normally found in geographic regions where populations of pathogens and host plants have co-evolved. The germplasm native to temperate zones of North America was investigated by grape breeders for sources of powdery mildew (PM) resistance (Barker *et al.*, 2005; Lenne and Wood, 1991). Powdery mildew resistance has also been explored in grapes of the genus *Muscadinia*. The first studied resistance locus against PM was *Run1* (Donald *et al.*, 2002; Korbuly, 1999). *Run1* was introduced from *M. rotundifolia* by conventional breeding and was localized on linkage group 12 (Barker *et al.*, 2005). Almost all *V. vinifera* cultivars and its hybrids are susceptible to powdery mildew disease (Li, 1993; Stummer and Scott, 2000; Wang, 1993; Wang *et al.*, 1995). In a study by Staudt (1997) conducted to evaluate different Asian and American genotypes for resistance to

*U. necator*, it was found that some of the tested genotypes were highly resistant to powdery mildew disease.

In Ardabil and Qazvin provinces of Iran where 22 different commercial grape varieties are grown, table grapes are a major fruit and play very important role in the provincial agriculture. Due to the importance of powdery mildew in the Ardabil and Qazvin vine-yards, this study was conducted during 2011–2013 to evaluate resistance of some native grape varieties to the disease order to identify the degree of resistance or susceptibility of varieties aimed for use in the national and international grape research centers.

## Materials and Methods

### Grape varieties

Twenty two grape varieties (9 of Ardabil and 13 of Qazvin provinces) were separately evaluated for disease resistance during 2011–2013 in Meshginshahr and Takestan regions of Ardabil and Qazvin provinces. These varieties were collected from different parts of the provinces and were preserved in nursery of Meshginshahr Horticultural Research and Takestan Research Stations. The experiments and practices were carried out according to common procedures and no fungicides were used in the experimental sites. Disease evaluation was carried out on leaves and clusters according to the following procedures:

### Evaluation of natural infection on leaves and clusters

The natural infection evaluation was carried out when powdery mildew symptoms widely appeared on the leaves and clusters during July, August and September. For disease evaluation, 150 leaves and 30 clusters from each variety (several plants) were randomly examined at different time intervals during July–September. The infection severity on leaves and clusters was determined based on percent of disease spots observed on the entire leaf and cluster area according to the procedure described by

Voytovich (1987) and Wang (1993) and six levels of reactions were identified as follows: 0–I, Immune = 0; 1–HR, Highly Resistant = 0.1–5%; 2–R, Resistant = 5.1–10%; 3–T, Tolerant = 10.1–25.0%; 4–S, Susceptible = 25.1–50%; 5–HS, Highly Susceptible = 50.1–100%. For comparison of varieties, average infection severity index from July– September was used for both regions.

#### **Evaluation of infection in using artificial inoculation of leaves and clusters**

In order to verify the results of the above experiment (natural infection), another experiment was conducted on the varieties with fungal inoculation of leaves and clusters. In this experiment, first, fungal conidia which were collected from infected leaves, were washed with 0/78% glucose solution and were then suspended in sterile water. Fifty leaves from each variety were inoculated with conidial suspension at the rate of  $2 \times 10^5$  conidia/ml by spraying the upper surface of the leaves. Inoculated leaves were immediately covered by thin paper bags (Fang, 1979). Disease severity was evaluated 3 weeks after inoculation according to the procedure described above.

#### **Disease progress in relation to plant phenology**

During 2011–2013, the experimental sites and grape plants were surveyed and monitored weekly and diseased samples were collected for evaluation of disease progress. As soon as the disease symptoms appeared, the development of the disease and its progress on the leaves and clusters were evaluated in the course of time (Wan *et al.*, 2007). In this evaluation Voytovich (1987) and Wang (1993) procedures were followed and the appearance of disease symptoms on different varieties in relation to plant phenology was investigated.

#### **Data analysis**

After collecting all data for reactions of leaves and fruits against powdery mildew, SPSS

software (Version 18) was used for cluster analysis and generating dendrogram for grouping of grape genotypes.

#### **Results and Discussion**

Resistance to grape powdery mildew *Erysiphe necator* Schwein. was studied in 22 *Vitis vinifera* varieties grown in Ardabil and Qazvin provinces. The infection was evaluated under natural infection conditions and artificial inoculation on leaves and clusters. For all of the years, the climatic conditions were favorable for *E. necator* development. Powdery mildew disease symptoms were not observed on twigs and leaflets of grape plants in early spring in the experimental sites. However, different disease symptoms appeared from mid June on different parts of grape plants including leaf, shoot and fruit (cluster). The reactions of different varieties to powdery mildew disease during 2011–2013 are shown in tables 1 and 2. According to the results, in both natural and artificial inoculation and based on the symptoms which appeared on the leaves, varieties Perlett, Robee seedless, Flame seedless and Tompson seedless were immune (I), Mish Pestan, Khoshnav and Torkamanestan 8 were highly resistant (HR), Torkamanestan 4 and Superior seedless were resistant (R), Shahani-e-Qazvin and Shast-e-Arous were tolerant (T), Ghermez-e-Bidaneh, Sefid-e-Bidaneh, Fakhri, Sahebi-e-Ghermez, Peikami, Rishbaba-e-Sefid, Siyah-e-Samarghandi and Rotabi were susceptible (S) and Rishbaba-e-Ghermez, Asgari and Shahroodi were highly susceptible (HS) to powdery mildew (Table 1). Disease evaluation results based on fruit symptoms were similar to those of leaves (Table 2). Results also showed that in both natural infection and artificial inoculation the reaction of different varieties to the pathogen was similar and this can be observed in tables 1 and 2.

**Table 1** Severity index and resistance rating of grape powdery mildew caused by *Erysiphe necator* on the leaves of different commercial grape varieties during 2011-2013.

No.	Variety	Natural Infection					Artificial Inoculation				
		2011	2012	2013	Mean	Rating	2011	2012	2013	Mean	Rating
1	Perlett	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
2	Robee seedless	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
3	Flame seedless	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
4	Tompson seedless	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
5	Superior seedless	6.12	8.20	7.18	7.17	R	4.31	7.50	5.59	5.77	R
6	Mish pestan	4.30	4.80	4.20	4.44	HR	4.00	3.90	4.10	4.00	HR
7	Khosnav	4.30	4.60	4.10	4.34	HR	2.00	2.10	1.90	2.00	HR
8	Torkamanestan 8	4.70	4.50	4.10	4.44	HR	3.90	4.20	4.10	4.10	HR
9	Torkamanestan 4	7.90	9.40	8.60	8.64	R	5.00	5.32	5.10	5.14	R
10	Ghermez-e-Bidaneh	30.20	33.30	29.90	31.14	S	29.00	30.25	28.90	29.39	S
11	Sefid-e-Bidaneh	45.00	48.20	46.20	46.47	S	41.10	45.65	40.65	42.47	S
12	Shahani-e- Qazvin	22.00	24.20	21.20	22.47	T	19.70	20.00	19.00	19.57	T
13	Shast-e- Arous	22.00	21.30	21.10	21.47	T	11.00	12.60	10.90	11.50	T
14	Fakhri	47.10	48.20	45.20	46.84	S	27.00	28.64	26.60	27.41	S
15	Sahebi-e-Ghermez	44.00	45.80	41.40	43.74	S	39.00	45.20	41.20	48.40	S
16	Peikami	33.00	35.00	32.00	33.34	S	30.00	30.80	30.10	30.30	S
17	Rishbaba-e- Sefid	47.00	48.25	46.15	47.14	S	27.10	28.40	28.10	27.87	S
18	Rishbaba-e-Ghermez	51.00	53.20	50.81	51.67	HS	40.00	43.20	40.20	41.14	S
19	Siyah-e-Samarghandi	31.00	31.00	29.00	30.34	S	31.00	31.00	29.00	30.34	S
20	Rotabi	31.00	31.00	44.00	35.34	S	31.00	31.00	44.00	35.34	S
21	Asgari	52.00	53.20	53.70	53.00	HS	58.00	60.20	58.20	58.80	HS
22	Shahroodi	69.00	70.32	68.10	68.69	HS	80.00	83.20	78.20	80.47	HS

I: Immune, HR: Highly resistant, R: Resistant, T: Tolerant, S: Susceptible, HS: Highly susceptible.

**Table 2** Severity index and resistance rating of grape powdery mildew caused by *Erysiphe necator* on fruits (cluster) of different commercial grape varieties during 2011-2013.

No.	Variety	Natural Infection					Artificial Inoculation				
		2011	2012	2013	Mean	Rating	2011	2012	2013	Mean	Rating
1	Perlett	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
2	Robee seedless	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
3	Flame seedless	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
4	Tompson seedless	0.00	0.00	0.00	0.00	I	0.00	0.00	0.00	0.00	I
5	Superior seedless	7.95	8.80	8.15	8.30	R	4.25	4.25	4.15	4.22	HR
6	Mish pestan	3.80	3.90	3.50	3.74	HR	3.00	3.90	2.90	3.27	HR
7	Khosnav	2.00	2.10	2.30	2.14	HR	2.00	2.10	2.20	2.10	HR
8	Torkamanestan 8	4.00	4.20	4.10	4.10	HR	4.00	4.20	3.70	3.97	HR
9	Torkamanestan 4	5.00	5.32	5.15	5.16	R	4.00	5.32	4.30	4.54	HR
10	Ghermez-e-Bidaneh	29.00	30.25	29.90	29.72	S	28.00	30.25	28.90	29.10	S
11	Sefid-e-Bidaneh	44.00	45.65	43.95	44.54	S	43.00	45.65	44.50	44.39	S
12	Shahani-e- Qazvin	20.00	20.00	20.00	20.00	T	20.00	20.00	20.00	20.00	T
13	Shast-e- Arous	12.00	12.60	12.20	12.27	T	11.00	12.60	11.90	11.84	T
14	Fakhri	26.00	28.64	27.71	27.45	S	27.00	28.64	27.64	27.76	S
15	Sahebi-e-Ghermez	43.00	45.20	44.20	44.14	S	43.00	45.20	43.20	43.80	S
16	Peikami	30.00	30.80	30.60	30.47	S	30.00	30.80	29.60	30.14	S
17	Rishbaba-e- Sefid	28.00	28.40	28.10	28.17	S	26.00	28.40	27.50	27.30	S
18	Rishbaba-e- Ghermez	40.00	43.20	42.20	41.80	S	42.00	43.20	41.80	42.34	S
19	Siyah-e-Samarghandi	31.00	31.00	29.00	30.34	S	31.00	31.00	29.00	30.34	S
20	Rotabi	31.00	31.00	44.00	35.34	S	31.00	31.00	44.00	35.34	S
21	Asgari	60.00	60.20	60.10	60.10	HS	60.00	60.20	60.30	60.17	HS
22	Shahroodi	80.00	83.20	81.10	81.44	HS	78.00	83.20	79.20	80.14	HS

I: Immune, HR: Highly resistant, R: Resistant, T: Tolerant, S: Susceptible, HS: Highly susceptible.

Evaluation of different varieties in reactions to powdery mildew disease under natural infection and artificial inoculation indicated that in natural field infection from among 22 test varieties four varieties were immune, three showed high resistance, two ranked as resistant, two were tolerant, eight showed susceptibility and finally three varieties were highly susceptible to powdery mildew causal pathogen. Results of artificial fungal inoculation on different grape varieties in vitro were in agreement with those of natural field infection. In this experiment varieties showed very different reactions to powdery mildew disease. In other words like natural infection experiments, varieties ranked in six categories as immune, highly resistant, resistant, tolerant, susceptible and highly susceptible.

In general, results of the experiments indicated that 18.18% of tested varieties were immune to the disease, 13.63% were highly resistant, 9.10% were resistant, 9.10% were tolerant, 36.36% were susceptible and 13.63% highly susceptible to powdery mildew disease (Table 1). Results of our study and the differences in the reaction of varieties to powdery mildew disease are in agreement with those of previous studies (Barker *et al.*, 2005; Eibach *et al.*, 2007; Korbuly, 1999; Li, 1993; Wan *et al.*, 2007). Voytovich (1987), Wang *et al.* (1995), Staudt (1997), Donald *et al.* (2002), Fisher *et al.* (2004), Kozma *et al.* (2009), Molnar *et al.* (2007) who studied the reaction of different grape varieties to powdery mildew disease in different countries have reported more or less the same results.

Approximately 2 weeks after bloom in 2011 to 2013, the incidence of powdery mildew was significantly and substantially higher on fruit clusters than on foliage. Foliar mildew was not detected or remained at trace levels for several weeks post bloom. Thereafter, the incidence of foliar mildew increased rapidly. In contrast, incidence of powdery mildew on fruit clusters, although initially was higher than on foliage, did not increase significantly after first detection. As in the case of disease incidence on fruit clusters, disease severity on fruit clusters did not increase significantly after 2 weeks post bloom.

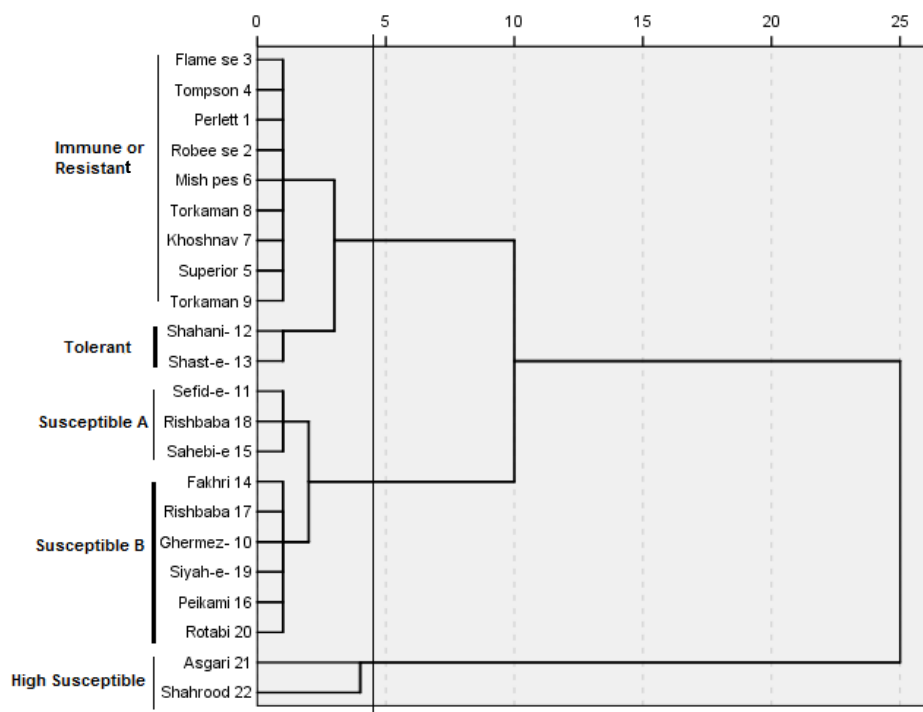
Disease indexes presented in the tables are the averages of disease indexes during July–September period. Study on the disease progress in relation with plant phenology indicated that during 2011–2013, growth activity of plants (budding and appearance of first true leaves) began in early May and early April respectively and first symptoms of disease appeared as pale small spots on the leaves around mid-June. In late June as the clusters formed, disease symptoms as fungal conidia were observed on fruits as small spots. In the course of the time and as the air temperature increased, the number of disease spots on fruit (clusters) expanded and in late June disease index was very high on the older leaves and the symptoms appeared on both sides of the leaves.

During the rapid ripening of the fruit (late June, early July) the sides of infected old leaves became crumpled and numerous disease spots appeared on the leaves, fruits and the twigs. During the period of July 27–August 4, severity of the disease was very high on the leaves and fruits of susceptible varieties (about 50%) and was then increased to about 100% in late August and early September. Pathogenic activity of the fungal causal agent continued until mid-September and was then gradually reduced and stopped in late September. The time period of disease development was similar for both regions. Despite the predominance of foliar tissue with respect to target area for infection during all phases of vine growth, we found powdery mildew colonies earlier in the growing season and at a substantially higher frequency on fruit clusters than on leaves. Colonies on the first formed leaves supposedly provide a pool of inoculum for the later-developing clusters (Pearson and Goheen, 1990). Like in our study, the results of their study also showed that different varieties indicated variable reactions to the disease and they ranked from immune to highly susceptible. The overall results of this study show that it may be possible to introduce some resistant and tolerant domestic grape varieties to powdery mildew disease. These varieties may be used as a genetic source in the development and production of resistant grape varieties both on national and international scales.

Cluster analysis of grape cultivars based on the reactions to powdery mildew infections on the leaf and fruit is shown in figure 1. Three cultivars Shahroodi, Rasmi, Asgari were separated with maximum distance from all the other cultivars, while the other cultivars were grouped into two main clusters. The first main cluster consisted of fifteen cultivars having immune, highly resistant, resistant, or tolerant reactions to powdery mildew. In the first cluster two sub-clusters were observed of which five cultivars (entries 13, 14, 15, 16 and 18) had resistant or tolerant reactions and ten cultivars had immune or highly resistant reactions which are placed in another sub-cluster. Ten cultivars were included in the second main cluster. These cultivars showed susceptible reactions. They were placed in two sub-clusters. Diversity among tested cultivars was considerable in the disease parameters and cluster analysis based on reactions to powdery mildew confirmed this diversity, which reflects their diversity at the genetic level. Kronstad (1996) proposed the use of wide genetic bases in cross breeding programs, in

order to avoid uniformity of the resulting progenies and genetic mono-culturing in any sense. The diversity recorded in this work may be exploited in further breeding for developing improved cultivars. This will help to avoid mono-culturing in terms of resistance genes.

Since powdery mildew disease caused by *E. necator* is a serious disease of grape around the world, results of such studies may be promising and could be used in the formulation of integrated control strategies for the management of this destructive disease around the world. The results of this study may have practical applications in formulation of disease management strategies to control powdery mildew in a safe environment. The use of resistant and tolerant varieties to manage different plant diseases including grape powdery mildew can potentially replace or minimize the application of harmful chemical fungicides and could be used as an important component of Integrated Pest Management (IPM) which is a promising approach to sustainable horticulture.



**Figure 1** Denderogram of cluster analysis for 22 grape genotypes based on reactions to powdery mildew infections on the leaf and fruit.

## Conclusion

Grape powdery mildew in Iran has caused significant crop loss and resulted in unprecedented costs in chemical control expenditure in epidemic seasons. It can be anticipated that control measures will be largely based on the development and release of resistant cultivars, although chemical control may have a place in high input/high yield situations. In general, Results of our study and the differences in the reaction of varieties to powdery mildew disease are in agreement with those of previous studies. Results of this study were promising and some immune, highly resistant and resistant cultivars to *E. necator* were identified and they may be used as a resistance genetic source for management of the disease in national and international programs.

## Acknowledgement

This work was supported scientifically by Dr. A. Heydari, Plant Disease Research Department, Iranian Research Institute of Plant Protection; hereby the authors express their gratitude.

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## ارزیابی مقاومت برخی از واریته‌های تجاری انگور *Vitis vinifera* نسبت به بیماری سفیدک پودری انگور *Erysiphe necator* Schwein.

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دریافت: ۲۳ اسفند ۱۳۹۳؛ پذیرش: ۱۵ اسفند ۱۳۹۴

**چکیده:** بیماری سفیدک پودری انگور *Erysiphe necator* Schwein. یکی از بیماری‌های قارچی مهم و رایج انگور در سراسر جهان از جمله ایران است. اگر چه کاربرد قارچ کش‌های شیمیایی، رایج‌ترین روش کنترل بیماری است اما استفاده از ارقام مقاوم ممکن است موثرترین و از لحاظ محیطی سالم‌ترین روش مدیریت بیماری باشد. در این پژوهش مقاومت ۲۲ رقم انگور نسبت به بیماری سفیدک پودری تحت شرایط آلودگی طبیعی و مایه‌زنی مصنوعی طی سال‌های ۱۳۹۰ تا ۱۳۹۲ در استان‌های اردبیل و قزوین ارزیابی شدند. نتایج بررسی‌ها نشان داد که ارقام Perlett، Robee seedless، Flame seedless و Torkamanestan 4 و Superior seedless مقاوم؛ ارقام Shahani-e-Qazvin و Shast-e-Arous متحمل؛ ارقام Rishbaba-، Peikami، Sahebi-e-Ghermez، Fakhri، Sefid-e-Bidaneh، Ghermez-e-Bidaneh و e-Sefid، Rotabi و Siyah-e-Samarghandi حساس و ارقام Rishbaba-e-Ghermez، Asgari و Shahroodi خیلی حساس به بیماری سفیدک پودری انگور بودند. به‌طور کلی نتایج امیدبخشی از این بررسی به‌دست آمد و برخی از ارقام مصون، مقاوم و خیلی مقاوم به بیماری سفیدک پودری انگور ممکن است در برنامه‌های ملی و بین‌المللی به‌عنوان منابع ژنتیکی مقاوم برای مدیریت بیماری مورد استفاده قرار گیرند.

**واژگان کلیدی:** واریته انگور، سفیدک پودری، مقاومت، *Uncinula necator*