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Full Length Research Paper

Evaluation of different grape varieties for resistance to powdery mildew caused by *Uncinula necator*

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Powdery mildew is one of the most serious and destructive diseases of grapes around the world, including Iran. Although the application of chemical pesticides is the most common control method, the use of resistant varieties may be the most effective and environmentally sound strategy for managing the disease. Fourteen grape varieties were evaluated for testing powdery mildew (*Uncinula necator*) resistance under natural infection conditions and artificial inoculation methods were used between 2007 and 2009 in Ardabil province of Iran. Disease severity on leaves and fruit (cluster) were evaluated by the following procedures: 0: immune, 1: highly resistant, 2: resistant, 3: tolerant, 4: susceptible, and 5: highly susceptible. Results of the experiments showed that Shahani, Yagothi and Tabrizkishmishi cultivars were immune, Sahibi cultivar was highly resistant, Agshilig and Tokoulgan were resistant, Kishmishi, Khalili and Tabarzeh cultivars were tolerant, Aldarag and Koupakbogan were susceptible and Garashilig, Seyrakpousteh and Rasmi cultivars were highly susceptible to the disease. In general, results of this study were promising and some immune, highly resistant and resistant cultivars to *U. necator* were identified and they may be used as a resistance genetic source for management of the disease in national and international programs.

Key words: Grape, *Uncinula necator*, powdery mildew, resistance.

INTRODUCTION

Grape is one of the most important fruits around the world and it is believed that it has been cultivated in many regions of the world for thousands of years. Over the time, different pests and diseases spread over grape vineyards and became one of the most important yield reducing factors in the world's vineyards (Carisse et al., 2006). Powdery mildew disease caused by plant pathogenic fungus *Uncinula necator* is one of the most important and destructive diseases of grape in many countries of the world, including Iran. It can cause serious damage to grape production in conducive environmental conditions, affect the production and the yield quantitatively and qualitatively, and increase the production cost significantly (Behdad, 1988; Wayne and Wilcox 2003; Carisse et al., 2006; Wan et al., 2007). The disease was reported from Iran grape vineyards in 1946 for the first time and since then, it has been observed in many grape growing provinces of the country (Behdad, 1988). 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 before the 1840's and was officially reported from Europe in 1945 (Staudt, 1997). In general, almost all *Vitis vinifera* cultivars and its hybrids are susceptible to powdery mildew disease (Di Vecchi-Staraz et al., 2009). The need for providing food for world increasing population has made scientists to use different methods, particularly breeding strategies for management of plant diseases which are among the

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major limiting factors for the crop production and the yield in the agriculture. To achieve these goals, identification of tolerant and resistant plant genotypes to certain diseases and development and introduction of tolerant or resistant varieties are very important and critical (This et al., 2006). Some of the wild and native grape genotypes showed natural resistance to powdery mildew disease in different countries which can be used as a genetic source for development of resistant and tolerant varieties (Nelson et al., 1995; Adam-Blondon et al., 2004; Hoffmann et al., 2008). The repertoire of grapevine resistance genes against fungal diseases has recently embraced a novel member, the first naturally present in a genome of the cultivated species *V. vinifera* (Dalbó et al., 2001; Welter et al., 2007). 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 test genotypes were highly resistant to powdery mildew disease (Staudt, 1997). The results of other studies in this regard in China showed that this country is one of the most important genetic resources for the grape and China is the source of 27 out of 70 known grape varieties in the world. Thus, Chinese wild grape cultivars can be used as genetic sources for resistance to powdery mildew (Eibach, 1994; Nelson et al., 1995). Russian breeders sought after sources of resistance in Central Asian varieties, which kindled hopes of finding resistance genes in cultivated vines. Kishmish vatkana is able to mount a post-penetration reaction against *Erysiphe necator*, a trait controlled by the dominant gene REN1. (Voytovich, 1987; Dalbó et al., 2001). REN1 is a novel resistance gene located on chromosome 13, different from those that had been previously found in wild North American grapes and their interspecific hybrids (Donald et al., 2002; Welter et al., 2007; Fung et al., 2007; Heuertz et al., 2008; Obón de Castro et al., 2008; Cipriani et al., 2008). Researchers believe that Chinese wild grape cultivars can be used as genetic sources for resistance to powdery mildew. Iran is one of the major grape growing countries in the world. In Ardebil province of Iran where 14 different grape varieties are grown, table grapes are a major fruit tree and play very important role in the provincial agriculture. Due to the importance of powdery mildew in the Ardebil vine yards, this study was conducted and completed between 2007 and 2009 to evaluate resistance of some native grape varieties to the disease in order to identify the resistant and tolerant varieties for recommendations to the national and international grape research centers.

MATERIALS AND METHODS

Grape varieties

Fourteen grape varieties were evaluated for disease resistance between 2007 and 2009 in Meshkin Shahr region of Ardebil province. These varieties were collected from different parts of the province and were preserved in the nursery of Meshkin Shahr agricultural research station. The experiments and practices were carried out according to common procedures and no pesticides 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 between July, August and September. For disease evaluation, 150 leaves and 30 clusters from each variety (several plants) were randomly examined at different time intervals between July and 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 to 5% ; 2 – R , resistant = 5.1 to 10%; 3 – T, tolerant = 10.1 to 25.0%; 4 – S, susceptible = 25.1 to 50%; 5 – HS, highly susceptible = 50.1 to 100%. For comparison of varieties, average infection severity index from July to September was used.

Evaluation of infection in using artificial inoculation of leaves and clusters

In order to verify the results of the aforementioned experiment (natural infection), another experiment was conducted on the varieties with fungal inoculation of leaves and clusters. In this experiment, first, fungal conidia were collected from infected leaves, were washed with 78% glucose solution and were then suspended in sterile water. Fifty leaves from each variety were then inoculated with conidial suspension at the rate of 2×10^5 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 afore described.

Study on disease progress in relation to plant phenology

Between 2007 and 2009, 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 over time (Wan, 2007). In this evaluation, Voytovich (1987) and Wang's (1993) procedures were followed and the appearance of disease symptoms on different varieties in relation with plant phenology was investigated.

RESULTS AND DISCUSSION

Powdery mildew disease symptoms were not observed on twigs and leaflets of grape plants at early spring in the experimental sites. However, different disease symptoms appeared from mid June on different parts of the plants. As the figures show, powdery mildew various symptoms were observed on different parts of grape plants including leaf, shoot and fruit (cluster). Results of the experiments in the reactions of different varieties to powdery mildew disease between 2007 and 2009 are shown in Tables 1 and 2. According to the results, in both natural and

Table 1. Average disease index (based on 0-5 scale) of grape powdery mildew caused by *U. necator* on the leaves of different varieties between 2007 and 2009.

Variety	Natural infection					Artificial inoculation				
	2007	2008	2009	Mean	Rating	2007	2008	2009	Mean	Rating
Ishahan	0	0	0	0	I	0	0	0	0	I
Kishmishi	3	3	3	3	T	2	2	2	2	R
Rasmi	5	5	5	5	HS	5	4	4	4.3	HS
Tokoulgan	2	2	2	2	R	2	1	2	1.7	R
Garashilig	5	4	5	4.7	HS	4	4	4	4	S
Seyrakpousteh	5	4	5	4.5	HS	4	3	4	3.7	S
Khalili	3	3	3	3	T	2	2	2	2	R
Tabarzeh	3	3	3	3	T	3	2	3	2.7	T
Koupakbogan	4	4	4	4	S	4	3	4	3.7	S
Aldarag	4	4	4	4	S	3	3	3	3	T
Tabrizkishmishi	0	0	0	0	I	0	0	0	0	I
Agshilig	2	2	2	2	R	2	1	2	1.7	R
Yagothi	0	0	0	0	I	0	0	0	0	I
Sahibi	1	1	1	1	HR	1	1	1	1	HR

I: Immune, HR: highly resistant, R: resistant, T: tolerant, S: susceptible, HS: highly susceptible.

Table 2. Average disease index (based on 0–5 scale) of grape powdery mildew caused by *U. necator* on fruits (cluster) of different varieties between 2007 and 2009.

Variety	Natural infection					Artificial inoculation				
	2007	2008	2009	Mean	Rating	2007	2008	2009	Mean	Rating
Shahani	0	0	0	0	I	0	0	0	0	I
Kishmishi	3	3	3	3	T	2	2	2	2	R
Rasmi	5	5	5	4.5	HS	5	4	4	4.3	HS
Tokoulgan	2	2	2	2	R	2	1	2	1.7	R
Garashilig	5	4	5	4.7	HS	4	4	4	4	S
Seyrakpousteh	5	4	5	4.7	HS	4	3	4	3.7	S
Khalili	3	3	3	3	T	2	2	2	2	R
Tabarzeh	3	3	3	3	T	3	2	3	2.7	T
Koupakbogan	4	4	4	4	S	3	3	3	3	T
Aldarag	4	4	4	4	S	3	2	3	2.7	T
Tabrizkishmishi	0	0	0	0	I	0	0	0	0	I
Agshilig	2	2	2	2	R	2	1	2	1.7	R
Yagothi	0	0	0	0	I	0	0	0	0	I
sahibi	1	1	1	1	HR	1	1	1	1	HR

I: Immune, HR: highly resistant, R: resistant, T: tolerant, S: susceptible, HS: highly susceptible.

artificial inoculation and based on the symptoms which appeared on the leaves, Shahani, Yaghuti and Tabriz Keshmeshi varieties were immune (I), Sahebi variety was highly resistant (HR), Aghshaligh and Tukilgen were resistant (R), Kesmeshi, Khalili and Tabarzeh were tolerant (T), Alderegh and Kopak Boghan were susceptible (S) and Ghara Shaligh, Sirk Poosteh and Rasmi 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. Disease indexes presented in the tables are the averages of disease indexes between July and September. Study on the disease progress in relation with plant phenology indicated that between 2007 and 2009, 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

Figure 1. *U. necator*-induced symptoms on a susceptible (top) and a resistant (bottom) *V. vinifera* variety. Left and center panels: appearance of PM-infected leaves and clusters, Right panel: Aniline blue-stained fungal structures as observed under a compound microscope. Note the profuse and sparse fungal growth on the susceptible and resistant genotype, respectively. Scale bar: 100 µm.

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. Over time and as the weather became warmer and 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. Between the period of July 27 to 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 (Figure 1). Evaluation of different varieties in reactions to powdery mildew disease under natural infection and artificial inoculation indicated that in natural field infection from among 14 test varieties, three varieties were immune, one showed high resistance, two ranked resistant, three were tolerant, two showed susceptibility and finally, three varieties were high susceptible to powdery mildew causal pathogen. Results of artificial fungal inoculation on different grape varieties 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 experiment indicated that 21.4% of test varieties were immune to the disease, 7.2% showed high degree of resistance, 14.3% were resistant, 21.4% showed tolerance, 14.3% were susceptible and 21.4% highly susceptible to powdery mildew disease. Results of our study and the differences in the reaction of varieties to powdery mildew disease are in agreement with those of previous studies (Eibach, 1994; Nelson et al., 1995; Dalbó et al., 2001; Jarvis et al., 2002; Wan et al., 2007). Voytovich (1987), Wang et al. (1995), Staudt (1997), Donald et al. (2002) and Fung et al. (2007) studied the reaction of different grape varieties to powdery mildew disease in different countries (Wang et al., 1993; Eibach, 1994; Nelson et al., 1995; Wang et al., 1995; Jarvis et al., 2002; Hoffmann et al., 2008; Obon et al., 2008).

Like in our study, the results of their study also showed that different varieties showed 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. Since powdery mildew disease caused by *U. 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 for controlling 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 a sustainable agriculture.

Conclusion

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 *U. necator* were identified and they may be used as a resistance genetic source for management of the disease in national and international programs.

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