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Research Article

Screening eight tomato varieties for resistance to Fusarium wilt disease

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Abstract

Introduction: Fusarium wilt caused by soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici* (Fol) is one of the most important diseases of tomatoes in the world. Identification and cultivation of resistant varieties is an environmentally friendly method of disease management that leads to the production of a chemical-free and clean yield. This research was conducted to evaluate the reactions of eight tomato varieties to the disease and their correlation with peroxidase enzyme. **Materials and Methods:** Wilted tomato plants were sampled in the greenhouses of southwestern Iran and after isolation and purification and studying the morphological characteristics, two Fol isolates were identified. The reactions of eight varieties *vs.* Kingston, Dafnis, Super chef, Karnak, CHpooya, Earlypooya, Superpooya, and CH to these two isolates were investigated in a factorial experiment with a completely randomized statistical design with four replications for each treatment under greenhouse conditions. The disease severity index and the vegetative and reproductive indices of the plant were determined. The level of peroxidase enzyme in the leaves of plants of each treatment was measured by spectrophotometric method at 470 nm wavelength and its correlation coefficient was determined with the disease severity index. **Results:** Analysis of the variance of these experimental data revealed a significant difference between Fol isolates in pathogenicity and interaction between Fol isolates and varieties. Fol2 isolate was more aggressive than Fol1 and CH was moderately susceptible, and Earlypooya, Karnak, Superpooya, Dafnis, CHpooya, were moderately resistant, while Kingston and Super chef were completely resistant to this hyper-virulent isolate of the pathogen and disease. The peroxidase level had negatively correlated with the disease severity index. **Conclusion:** Among these varieties, Kingstone and Super chef are resistant to the disease. Peroxidase levels can be used as a resistance marker to assess how different tomato varieties react to the disease.

Keywords: *Fusarium oxysporum*, Peroxidase, Resistant, Tomato, Wilt

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مقاله پژوهشی

غربالگری هشت رقم گوجه‌فرنگی برای مقاومت به بیماری پژمردگی فوزاریومی

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چکیده

مقدمه: پژمردگی فوزاریومی ناشی ازقارچ خاک‌زی *Fusarium oxysporum* f. sp. *lycopersici* (Fol) یکی از بیماریهای مهم گوجه‌فرنگی در دنیا است. شناسایی و کشت رقم‌های مقاوم روشی دوستدار محیط زیست مدیریت بیماری است که به تولید محصولی عاری از مواد شیمیایی و تمیز می‌انجامد. این پژوهش برای تعیین واکنش هشت رقم گوجه‌فرنگی به بیماری و همبستگی واکنش آنها با آنزیم پراکسیداز انجام شد. **مواد و روش‌ها:** بوته‌های پژمرده گوجه‌فرنگی در گلخانه‌های جنوب غربی ایران نمونه‌برداری شدند و پس از جداسازی و خالص‌سازی و مطالعه خصوصیات ریختی دو جدایه Fol شناسایی شدند. واکنش‌های هشت رقم کینگ‌استون، سوپرچف، دافنیس، کارناک، سی‌اچ‌پویا، ارلی‌پویا، سوپرپویا، سی‌اچ نسبت به این دو جدایه در آزمایشی فاکتوریل با طرح آماری کاملا تصادفی با چهار تکرار برای هر تیمار در شرایط گلخانه بررسی شد. شدت بیماری و شاخصهای رویشی و زایشی گیاه اندازه‌گیری شدند. میزان آنزیم پراکسیدازدر برگهای بوته‌های هر تیمار به روش اسپکترو فتومتری در طول موج ۴۷۰ نانومتر اندازه‌گیری و ضریب همبستگی آن با شدت بیماری تعیین شد. **یافته‌ها:** تجزیه واریانس داده‌های این آزمایش نشان داد که بین جدایه‌های Fol از نظر قدرت بیماری‌زایی و تاثیر متقابل جدایه‌های بیمارگر و رقم‌ها تفاوت معنی‌داری وجود دارد. جدایه Fol2 پرآزارتر از Fol1 بود و رقم سی‌اچ نیمه‌حساس ، رقمهای دافنیس، کارناک، سی‌اچ‌پویا، ارلی‌پویا و سوپرپویا نیمه‌مقاوم، رقم‌های کینگ‌استون و سوپرچف کاملا مقاوم به این جدایه پرآزار بیمارگر و بیماری بودند. سطح آنزیم پراکسیداز در گیاه با شدت بیماری همبستگی منفی داشت. **نتیجه‌گیری:** کینگ‌استون و سوپرچف در بین این رقم‌ها مقاوم به بیماری هستند. اندازه‌گیری سطح آنزیم پراکسیداز می‌تواند به عنوان یک شاخص مقاومت برای تعیین واکنش رقم‌های گوجه‌فرنگی به بیماری استفاده شود.

واژگان کلیدی: پراکسیداز، پژمردگی، گوجه‌فرنگی، مقاومت، *Fusarium oxysporum*

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Introduction مقدمه

Fusarium wilt caused by soil-borne fungus *Fusarium oxysporum* f. sp. *lycopersici* W.C. Snyder & H.N. Hansen (Fol) is one of the most common tomato diseases worldwide. The disease was first reported in the United States of America by Smith in 1899, and developed enough to prevent tomato cultivation in the state of Florida. Tomato's yield decrease between 30 to 40 percent by the disease (Gordon, 2017). It was reported from Hormozgan Province in the south of Iran for the first time, and the only known fungal agent was *Fusarium oxysporum* f. sp. *lycopersici* (Fasihiani, 1985). Then it has been reported from central region of Iran with 27.3% losses, northeastern and southwestern Iran (Etebarian, 1992; Sadravi and Setayeshmehr, 2008; Rahimizadeh and Sadravi, 2020).

Since tomato is one of the most important crops in the world and Fusarium wilt is one of the most important diseases of this crop, to avoid yield losses. Due to possible contamination of the agricultural products as well as environmental pollution chemical control is considered a threat to food, and environmental health. Another consequence of chemical control is the pathogen resistance. One of the choices of the disease management to reduce the negative consequences of chemical control is use of resistant varieties (Bawa, 2016; Njiru, 2012). In independent studies a number of tomato varieties have been evaluated for their susceptibility to Fusarium wilt (Sadravi and Setayeshmehr, 2008; Akram et al., 2014; Arici et al., 2018). Also, peroxidase enzyme activity associated with plants resistance to Fusarium wilt has been investigated (Aguilar et al., 2000; Madadkhah et al., 2012). Due to importance of the disease in the world, the objective of this study was to assess the susceptibility of eight tomato varieties against Fusarium wilt, to find an environmentally friendly method of the disease management that leads to the production of a chemical-free and clean yield.

Materials and Methods مواد و روش‌ها

Sampling, isolation and detection of pathogen

Tomato's production greenhouses were visited in southwestern Iran and sampled from yellow and wilted bushes. The pathogen was isolated after surface disinfection of the disease's tissue on potato dextrose agar (PDA) medium. Carnation-leaf-agar (CLA) medium was used for producing macro-conidia. The isolates were identified by studying their morphological characteristics of colonies and spores (Leslie and Summerell, 2006).

Evaluation of the reaction of eight tomato varieties to two pathogen isolates

Eight varieties of tomatoes *vs.* Super chef, Kingston, CHpooya, Earlypooya, Karnak, CH, Superpooya, Dafnis, were selected. They were seeded after disinfection seeds with 0.5% sodium hypochlorite solution for one minute, and then washed with distilled water, in transplant tray. The roots of seedlings with 2-4 leaves were inoculated with a suspension of 10^6 conidia/ml of each pathogen isolate by root-dip method (Dhingra and Sinclair, 2017), and cultivated in pots filled with sterile perlite, sand and soil in a ratio of 1:1:1. Distilled water was used in control seedlings. The seedlings were kept in greenhouse, with a temperature of 20 to 30 degrees Celsius, for 72 days. Disease severity index (DSI) was evaluated, based on the wilting percentage of the bushes, and the reaction of the tested varieties to pathogen isolates was determined using the following criteria: if the value of DSI is equal to 0%=Immune, 1-10%=Resistant(R), 10-20%=Moderately resistant(MR), 21-40%=Moderately susceptible(MS), 41-70%=Susceptible(S), 71-100%=Very susceptible (Akram et al., 2014, Song et al. 2004). Also, growth indicators of plants including: bushes height, and number of flowers were measured. This experiment was carried out as a factorial experiment (Factor 1:

eight tomato varieties, Factor 2: two isolate of the pathogen) using a completely randomized design, with five replications for each treatment.

Peroxidase enzyme level assessment

Sixty-five days after the experiment's beginning, the level of peroxidase enzyme in young bush leaves, by Madadkhah et al. (2012) method. In the tested varieties, the correlation coefficient of this enzyme to the disease severity index was calculated.

Results and Discussion یافته‌ها و بحث

Morphological characteristics of the pathogen

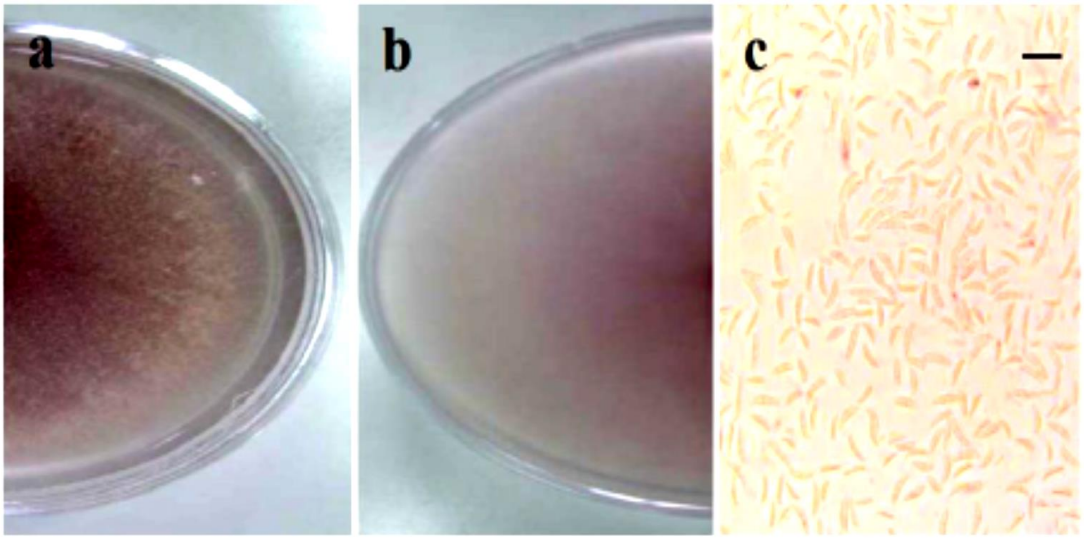
Colony with light purple to dark-colored, 90 macroconidia small-to-medium-sized, 3–5 cells straight, relatively thin wall, gradually narrowed and the ends bent, as shown in the Figure 1. The microconidia were ovate shape, with one to two cells.

Fusarium oxysporum f.sp. *lycopersici* has been recognized as a major cause of tomato wilt disease worldwide (Srinivas et al., 2019; Fasihiani, 1985; Etebarian, 1992; Sadravi and Setayeshmehr, 2008; Akram et al., 2014; Arici et al., 2018; Rahimizadeh and Sadravi, 2020).

The reactions of eight tomato varieties to Fusarium wilt disease

Analysis of the variance of the greenhouse experiment data revealed a significant difference at the level of 5%, between the virulence of Fol isolates and their interaction with the varieties. Fol2 isolate was more aggressive than Fol1, and CH is moderately –susceptible, and Earlypooya, Karnak, Superpooya, Dafnis, CHpooya, are moderately – resistant, while Kingston and Super chef are completely resistant to this hyper-virulent isolate of pathogen and disease (Table 1). Also, between these eight varieties, CH is moderately- susceptible to Fol2 and moderately-resistant to Fol1 (Figure 2).

Some researchers have also evaluated other tomato varieties react to the disease. Super strain b was resistant to the disease, but Mobil, Falat, and PS were susceptible (Sadravi and Setayeshmehr, 2008). In Pakistan, screening of 23 tomatoes revealed the semi-susceptibility of



شکل ۱. *Fusarium oxysporum* f. sp. *Lycopersici* ، a. سطح روی پرگنه، b. پشت پرگنه، c. ماکروکنیدیوم‌ها (خط مقیاس= ۲۰ میکرومتر)

Figure 1. *Fusarium oxysporum* f. sp. *lycopersici* , a. Above surface of colony, b. Under surface of colony, c. Macrconidia (bar=20µm).

جدول ۱. واکنش هشت رقم گوجه‌فرنگی به دو جدایه (Fol) *Fusarium oxysporum* f.sp. *lycopersici* از جنوب غربی ایران*.

Table 1. Reaction of eight varieties of tomato to two *Fusarium oxysporum* f.sp. *lycopersici* (Fol) isolates from southwestern Iran*.

Treatment	Disease Severity index**	Reaction**	Bush height (Cm)	Number of flowers
CH : Fol2	26.26 a	MS	40.56 fgh	8 b-f
Earlypooya : Fol2	14.65 b	MR	38.28 gh	5 def
CHpooya : Fol2	14.40 b	MR	43.18 d-h	10 a-e
Superpooya : Fol2	12.32 b	MR	36.30 h	5 c-f
Earlypooya : Fol1	12.08 b	MR	40.50 f-h	7 c-f
Dafnis : Fol2	12.02 b	MR	46.26 c-h	3 f
CH : Fol1	11.46 b	MR	41.62 d-h	11 a-d
Karnak : Fol2	10.76 b	MR	72.39 a	10 a-f
Super chef : Fol2	9.62 bc	R	45.90 c-g	4 ef
Superpooya : Fol1	9.24 bc	R	40.30 fgh	6 c-f
Dafnis : Fol1	5.38 c	R	48.27 b-f	5 def
Karnak : Fol1	4.98 c	R	72.65 a	10 a-f
CHpooya : Fol1	4.31 c	R	45.68 c-g	12 abc
Kingston : Fol1	3.60 c	R	50.50 b-e	8 c-f
Super chef : Fol1	2.42 c	R	47.53 c-g	5 def
Kingston : Fol2	0.71 c	R	48.22 b-f	5 def
Super chef check	0.00 c	-	50.90 bcd	10 a-f
Dafnis check	0.00 c	-	52.58 bc	5 def
Superpooya check	0.00 c	-	41.17 e-h	9 a-f
CH check	0.00 c	-	44.47 c-h	14 ab
Karnak check	0.00 c	-	80.06 a	11 a-d
Earlypooya check	0.00 c	-	41.17 e-h	7 c-f
Kingston check	0.00 c	-	56.88 b	10 a-e
CHpooya check	0.00 c	-	46.15 c-g	15 a

*اعدادی که با حروف مشابه نشان داده شده‌اند، اختلاف معنی‌داری در سطح ۵ درصد ندارند (DMRT).

** شاخص شدت بیماری و واکنش رقمها براساس معیار اکرم و همکاران (۲۰۱۴) تعیین شده است.

*The numbers shown with the same letters do not have a significant difference at the 5% level (DMRT).

** The disease severity index and the reaction of varieties is determined based on the criteria of Akram et al. (2014).

Pride-Burn, Red-Power, and Sun-Grape to the disease (Akram et al., 2014). Mercury, Polaris, Alsancak, Kuzeykoy, Kahraman, Tayfun, and Tory were resistant to the disease in Turkey, while Arzum, Dorit Alaturka, and Asil were semi-susceptible (Arici et al., 2018).

شـــکل ۲. دو نوع واکنش رقمهای گوجه‌فرنگی به بیماری پژمردگی فوزاریومی: a. نیمه حســـاس (MS)، b. نیمه مقاوم(MR).

Figure 2. Two types of reaction of tomato varieties to Fusarium wilt disease: a. Moderately susceptible (MS), b. Moderately resistant (MR).

Coefficient of correlation between the level of the peroxidase enzyme and the disease severity index.

Peroxidase enzyme level ranged from 0.015 to 0.250 (l/gr) between treatments, and a Pearson correlation coefficient of -0.523 was found between enzymatic data and the disease severity score. This suggests that the increase in enzyme activity is caused by the host's resistance to the disease and reduces the pathogen's function.

Study on the activity of peroxidase (POX), polyphenol oxidase (PPO) enzymes, and phenolic compounds (PCs), in 45 local genotypes of melon after inoculation with *Fusarium oxysporum* f. sp. *melonis*, showed that, although the POX and PPO activities and PCs content in both resistant and susceptible groups increased in response to infection by the pathogen, this significant increase was more prominent in resistant genotypes (Madadkhah et al., 2012). The peroxidase enzyme, plays an important role in the biosynthesis of lignin, which is involved in the cell wall, xylem and phloem of plants, and the production of some phytoalexins (Agrios, 2005).

Conclusion نتیجه‌گیری

Among eight tomato varieties screened for resistance to Fusarium wilt disease, CH is moderately susceptible, and Karnak, Dafnis, Earlypooya, Superpooya, CHpooya, are moderately resistant, and Kingston, and Super chef are resistant to the disease. Additionally, peroxidase levels may be used as a resistance marker to evaluate how different tomato varieties react to the disease.

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