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Assessment on early blight of potato in order to compare the two methods *in vitro* using pathogenic fungi *Alternaria solani*

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ABSTRACT

Potato (*Solanum tuberosum*) early blight, caused by *Alternaria solani* is one of the most destructive fungal foliar diseases. This research was done in order to study methods comparison of evaluation by culture filtrate of *A. solani* in *in vitro* condition for selecting resistance cultivars to early blight. Plantlets of potato viruse free were obtained from the National plant gene bank of Iran, and were inoculated *in vitro* methods with a culture filtrate of *A. solani*. In *in vitro* selection by droplet of culture filtrate method, leaflet received a 10 µl droplet of the *A. solani* culture filtrate and in *in vitro* selection by direct using of culture filtrate method, plantlets were placed in test tubes that include 5 µl *A. solani* culture filtrate. The experimental design was factorial on basis of completely randomized design (CRD) with two factors, three replications and six genotypes. During droplet method assay, the *A. solani* symptoms appeared 1 - 2 days until 6 days and during direct method they appeared 2 - 3 days until 6 days. The AUDPC values were submitted to the analysis of varience (ANOVA) and AUDPC means were compared by using Duncan test (α = 0.01%). In each method, significant difference among potato cultivars was observed for disease to early blight (p < 0.01). Results show that casmos cultivar is susceptible for resistance to early blight and *in vitro* methods experiment had the same result.

Keywords: Early Blight; AUDPC; Resistance; *Alternaria solani*; Potato

1. INTRODUCTION

Early blight is a very common disease of both potato and tomato. It causes leaf spots and tuber blight on potato, and leaf spots, fruit rot and stem lesions on tomato [1]. The disease can occur over a wide range of climatic conditions and can be very destructive if it is left uncontrolled. Infection can cause serious yield losses in susceptible cultivars [2,3]. Potato plants are susceptible to a wide variety of diseases that can severely reduce yield, quality and storability of tubers. Diseases can occur in the field or in storage and are caused by infectious bacteria, fungi, viruses and other related organisms. Early blight, caused by the *A. solani* fungus, is one of the main diseases of potatoes in tropical climates, especially where potatoes are grown under irrigation, causing yield-losses through defoliation of the plants. The fungicides used to control the disease are expensive and frequently inefficient [4]. Potato resistance to early blight is a quantitative trait, and obtaining successful resistant cultivars is not simple [5-7]. It has been observed that resistance to early blight is age-related: early-maturing cultivars are more susceptible than late-maturing cultivars. A droplet inoculation method was used for evaluation of tomato resistance to early blight, caused by *Alternaria solani* (Ellis & Martin) *Sorauer*. In this experiment method, leaflets are inoculated with small droplets of a conidial suspension in water or culture filtrate [8,9]. This method was first introduced by Locke (1948) to find sources of resistance to early blight (Locke, 1949). The droplet inoculation method has been used to evaluate early blight resistance components (O'Leary and Shoemaker, 1983). The direct method was described by [9,10]. Plantlets were inoculated in a 18 × 2 cm test tube, containing 5 ml of *A. solani* culture filtrate. Severity values were plotted against time and the area under the disease progress

curve (AUDPC) was calculated [11].

2. MATERIAL AND METHOD

2.1. Plant Material

The experiment was conducted during 2008-2009 under *in vitro* conditions. Virus free clones of potato cultivars were obtained from the National plant gene bank of Iran. Six cultivars were conducted Ells, Picasso, Maradona, Marfona, Casmos and Desiree that the cultivar Desiree is used as susceptible reference cultivar when screening potato genotypes in Brazil [6,8]. Plantlets were propagated through nodal cutting and kept in growth chamber at 23˚C ± 2˚C, light with a period of 16 h light and 8 h dark. After 4weeks-old the plants were transferred to *in vivo* conditions that plantlets were planted in pots (one seedling per pot). The planting bed is include pit/perlit/turb (2:1:1), temperature and humid about 27˚C - 33˚C, 75% - 80% respectively.

2.2. Sporulation and Culture Filtrate

The mycelia of an *A. solani* isolate were grown in plastic Petri plates on potato carrot agar (PCA) in the condition (8/16) light/darkness. After 10 days surface mycelium was removed with 10 ml of sterile distilled water (SDW) and a clean paintbrush and the suspension was discarded. Then suspension with10^5 conidia/ml were placed in 500 ml glass flasks containing 100 ml of potato dextrose broth (PDB) medium and maintained in the dark at 28˚C ± 2˚C. After 12 days the contents of glass flasks were filtered through the Whatman filter 0.2 µm and concentrated to centrifuge at 2000 - 2500 g for 10 - 15 min and the samples are centrifuged at a time.

2.3. *In Vitro* Selection by Droplet of Culture Filtrate Method

Three replications per cultivar were placed whole *in vitro* plantlets in an 18 × 2 cm test tube. The plantlets of potato into test tube were inoculated by droplet of culture filtrate method that the leaflet of potato received a 10 µl droplet of the *A. solani* culture filtrate. The test tubes were placed in a growth chamber at a temperature of 20˚C - 25˚C. The leaflets were rated according to **Table 1** for reaction to the treatments 1 - 2 days after inoculation until 6 days.

2.4. *In Vitro* Selection by Direct Using of Culture Filtrate Method

Three replication per cultivar were inoculated by placing whole *in vitro* plantlets in a 18 × 2 cm test tube each, containing 5 ml of *A. solani* culture filtrate. This study was conducted using factorial based on completely randomized design (CRD) with 3 replications. The test tubes were placed for 6 - 7 days in a growth chamber at 20˚C - 25˚C, with a photosynthetic photon flow density of 100 µE/m/s and a day length of 16 h [10]. During *in vitro* assay the *A. solani* symptoms appear 1 - 3 days until 6 days. For evaluation of the damage produced by *A. solani* using the scale described in **Table 1**.

Table 1. Scale for evaluation of the damage produced by *Alternaria* species in potato *in vitro* and greenhouse plants [12].

Rating of affectation	Description of symptoms
1	no lesion development
2	lesions < 1-mm diameter
3	lesions 1- to 5-mm diameter
4	lesions > 5-mm diameter

2.5. Pathogenicity Test

At the end of each of the above tests to ensure the absence of pathogens and other foreign pathogenicity tests, infected leaves after washing with tap water, placed in sterile distilled water for one minute. Then by sodium hypochlorite solution (% 0.5) for 35 seconds and re-sterilization were washed with sterile distilled water. Finally, the pieces are placed on sterile filter paper (for drying) and then transferred to the culture medium.

2.6. Statistical Analysis

The statistical analyses were accomplished using MSTATC. AUDPC values were submitted to analysis of variance (ANOVA) and treatment means were compared using Duncan test (% 0.01).

$$\text{AUDPC} = \sum\left[0.5\left(\text{Yi}+1+\text{Yi}\right)\left(\text{Ti}+1-\text{Ti}\right)\right]$$

Y = the response of plants based on Pryor & Michalides.

i = shift notes – T = date of the Inoculation.

3. RESULT

Variance analysis square shows that significant difference between methods, cultivars and interaction methods × cultivars (**Table 2**).

3.1. *In Vitro* Selection by Droplet of Culture Filtrate Method

Significant different had between cultivars (**Table 3**). Mean comparison showed that potato cultivars were grouped to two classes. Ells, Marfona, Casmos and Desiree cultivars were grouped at same class, and these cultivars had a low resistance (**Table 4**). Picasso and Maradona cultivars had a high level of resistance in comparison with other cultivars ($p < 0.01$).

Table 2. Variance analysis square for AUDPC mean in *in vitro* selection.

Source	Degree of Freedom	Mean square	F-value
Methods (A)	1	1018.674	4731.9032**
Cultivars (B)	5	113.774	528.4968**
A × B	5	1.207	5.6065**
Error	24	0.215	
total	35		
CV%	1.69%		

Table 3. Variance analysis square for AUDPC mean in methods pf evaluation.

Source	Degree of Freedom	F-value Droplet method	F-value Direct method
cultivars	5	317.827**	219.450**
Error	12		
Total	17		
CV %		1.39%	2.13%

Table 4. Mean comparison in *in vitro* method.

cultivars	AUDPC droplet		AUDPC direct	
Ells	35.66	A	24	B
Picasso	27	B	16.66	C
Maradona	26.50	B	17.33	C
Marfona	35.83	A	24.66	AB
Casmos	36.16	A	25.33	A
Desiree	35.50	A	25	AB

3.2. *In Vitro* Selection by Direct Using of Culture Filtrate Method

In direct method was observed significant difference among potato cultivars (**Table 3**). Mean comparison indicated that potato cultivars were grouped to four class (**Table 4**). Result showed that Casmos cultivar was the most sensitive in other cultivars. The other cultivars had a high level of resistance in comparison with other cultivars.

4. DISSCUSION

Disease severity is a valuable component for studying resistance to early blight. Disease severity assessments could was done on lower, middle and upper leaves but middle leaf assay is a useful factor for potato cultivars evaluation [1,6]. Therefore in this research was used from middle leaf for resistance level selection. Mean comparison *in vitro* method (**Table 4**) showed that in droplet method Desiree cultivar had low resistance level, however in direct method Desiree cultivar had high level the symptoms of early blight *in vitro* with were taken 1 - 3 days after inoculation that results is a similar too described by [6]. Culture filtrate was used in *in vitro* condition and caused leaf necrotic, were similar to those caused by infection through spores as was described by [9,10,13].

Rodriguez *et al*. showed that *in vitro* direct method is an effective in the evaluation of disease resistance of potatoes wave spots [10]. While Locke showed that drip into the glass, is a useful technique [14]. Our experiments show that the efficiency of both methods is almost identical. Thus, the results of both cover together.

5. CONCLUSION

Given the diversity of *Alternaria species* in the world, sources of resistance to the early blight disease are very small, and sometimes can be found in the wild plant. The transfer of genes from wild species is associated with many problems. Accordingly, resistance to diseases is considered as an advantage.

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