

Screening of Potato Lines Including Department Genetic & National Plant Gene Bank of Iran for Resistance to Early Blight (*Alternaria. solani*) Using Culture Filtrate Produced by the Fungus

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ABSTRACT

Potato is a plant that can be influenced by early blight disease. Early blight is one of the most destructive plant diseases, especially in the members of *solanaceae* family. This disease can reduce yield. Experiment design was a factorial randomized complete design to evaluate the isolate pathogenic *Alternaria solani* in National Plant Gene Bank of Iran, during 2008-2009. This experiment was conducted with three replications, and the factors include test methods and three lines 9506, 10908-05 and 10908-18 with genotypes of Agria, Casmos and Desiree (susceptible) and Delta (resistant control). Analysis of variance table was based on the area under the disease progress curve, indicating the effects of the experimental methods; genotype and their interactions are significant at the 1% level. In greenhouse evaluation, the symptoms appeared on, the first day. In this method the area under the curve progression was noted in 10 times, but *in vitro* method, the symptoms of this evaluation appeared on the first or second day. The Evaluation was carried out after inoculation, and was noted 6 times. The results indicate that genotypes and lines are located in four different groups. In this case, line 9506 and Cosmos were quite sensitive to the surface, and Desiree, is located in a sensitive area. Line 10908-18 and Agria, located approximately at the level of resistance, and line 10908-05 and Delta, have been resistant surface.

Keywords: Screen; Culture Filtrate; Early Blight; Severity

1. Introduction

The Potato crop is considered one of the strategic products. According to the Food and Agriculture Organization (2007) we have been producing potatoes up to 321,736 tons in the world, and 5240 tons in Iran [1].

Early blight of potato is the most important foliar diseases [2]. It reduces the level of product yield, and there's a wide world influence [3]. This disease, caused by the *A. solani* fungus, is one of the main diseases of potatoes in tropical climates, especially where potatoes are grown under irrigation [4]. The first reports are relevant to the discussion of *A. alternata* under its synonym (*A. tenuis* from wheat, *A. brassicae* and *A. solani* of tomato, cabbage) in Iran [5]. The first written report of the disease in Iran goes back to 1977 [6].

Ellis and Martin described the first symptoms on potato leaves. Initially, the symptoms were seen in older and lower leaves [7,8]. Based on morphological studies two *Alternaria species* i.e., *A. tenuissima* and *A. interrupta* have been recovered from potato in Iran [9]. The inter-relationship between *Alternaria species* and some substrates had also been recorded and the dangers this can pose to man, as a consumer were highlighted [10-12].

Agria genotype has been reported as susceptible genotypes, isolates of *A. solani* [13]. The cultivar Desiree is used as a susceptible cultivar and Delta genotypes were reported as resistant genotypes [14]. The specific procedures include the use of low nutrient media, such as PCA, temperature 20°C - 25°C under a light/dark 8/16 h using a white fluorescent lamp [15-18].

Severity values were plotted against time and the area under the disease progress curve (AUDPC) was calculated.

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ed [19]. Evaluation of susceptibility or resistance exists in potato cultivars with some of these methods [20]. Evaluation of *in vitro* methods, e.g. spraying the culture filtrate on the plant was introduced first by Locke [21].

2. Material and Method

2.1. Plant Material

The experiment was conducted *in vitro* and greenhouse conditions from Genetics and National Plant Gene Bank of Iran during 2008-2009. In this evaluation, the three lines 9506, 10908-05 and 10908-18 with genotypes of Agria, Casmos and Desiree (susceptible) and Delta (resistant control) were evaluated in terms of virulence of pathogenic *A. solani*. The genotypes were maintained on MS medium in the growth chamber conditions ($24^{\circ}\text{C} \pm 1^{\circ}\text{C}$, a period of 16 h light and 8 h dark) for 4 weeks.

2.2. Spore Production and Inoculation

All experiments were performed with a single-spore *A. Solani* isolate obtained from the Genetic and National Plant Gene Bank of Iran. For inoculum production, sporulation was induced in 10-day-old *A. solani* were grown in plastic Petri plates on potato dextrose agar (PDA) in continuous (8/16) light/darkness to maximize spore production. Superficial mycelium was removed with 10 ml of sterile distilled water (SDW) and a clean paintbrush and the suspension was discarded. To collect conidia, colonies were scraped with a sterile scalpel and conidia were transferred to a plate containing SDW.

Then suspension with 10^5 conidia/ml were placed in 500 ml glass flasks containing 100 ml of potato dextrose broth (PDB) medium and maintained in the dark at $28^{\circ}\text{C} \pm 2^{\circ}\text{C}$. After 12 days the contents of glass flasks were filtered through the filter $0.2 \mu\text{m}$ and concentrated to centrifuge at 2000 - 2500 g for 10 - 15 min and the samples are centrifuged at a time.

2.3. Greenhouse Evaluation

In this evaluation, the explants were transferred to a greenhouse environment. These plantlets obtained from each single nodal cutting were transplanted into a sterile mixture of peat moss and perlite (2:1 by volume) in a greenhouse. To maintain higher humidity around the cuttings for longer period of time, the trays were covered with plastic covers. After 25 to 30 days, the fungal spore suspension was sprayed using content geysers, and suspension of inoculum was spread uniformly on the leaves.

2.4. In Vitro Selection by Droplet Using of Culture Filtrate

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 $10 \mu\text{l}$ droplet of the *A. solani* culture filtrate. The test tubes were placed in a growth chamber at a temperature of $20^{\circ}\text{C} - 25^{\circ}\text{C}$. The leaflets were rated according to **Table 1** for reaction to the treatments 1 - 2 days after inoculation until 6 days.

2.5. In Vitro Selection by Direct Using of Culture Filtrate

Three replications per cultivar were inoculated by placing whole *in vitro* plantlets in an 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 replication. The test tubes were placed for 6 - 7 days in a growth chamber at $20^{\circ}\text{C} - 25^{\circ}\text{C}$, with a photosynthetic photon flow density of $100 \mu\text{E/m/s}$ and a day length of 16 h [20]. During *in vitro* assay the *A. solani* symptoms appear 1 - 3 days until 6 day. For evaluation of the damage produced by *A. solani* using the scale described in **Table 1**.

2.6. 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 [6].

3. Result

In these experiments, the symptoms began, the first and second day. Analysis of variance table based on area under the disease progress curve, indicating that the effects of the experimental methods; genotype and their interactions are significant at the 1% level (**Table 2**).

3.1. Greenhouse Evaluation

In this evaluation, the symptoms appeared, the first day

Table 1. Scale for evaluation of the damage produced by *Alternaria species* in potato *in vitro* and greenhouse Plants [22].

Rating of affection	Description of symptoms
1	No lesion development
2	Lesions < 1-mm diameter
3	Lesions 1- to 5-mm diameter
4	Lesions > 5-mm diameter

(Figure 1). Area under the curve progression in 10 in-nings notes, show that 9506 line is located, the highest level (sensitivity), and 10908-05 line is located, at the lowest level (resistance). Cosmos and Desiree genotype are located at the next level, and they are as susceptible genotypes, however, the genotype of Agria is equal to the genotype of Delta, and it is relatively stable. Line of 10908-18, is located in the lower level of the Delta, and it is named as resistant (Table 3).

3.2. Evaluation of *in Vitro*—A Drop

The symptoms of this evaluation appeared the first day (Figure 2). The Evaluation was carried out after inoculation, in 6 times notes. In this evaluation, the 9506 line is equal to the Desiree and the Cosmos, and a susceptible genotype, and lines 10908-05 and 10908-18 are almost at equality with Delta, and it can be considered as resistant. Agria has moderate sensitivity, and it is not quite as susceptible genotypes (Table 4).

3.3. *In Vitro* Evaluation of Direct

In this evaluation, the symptoms appeared the second day (Figure 3). The evaluation was carried out after inoculation, notes of in 6 times. Cosmos genotype was equal in 9506 line and Desiree, and it is a sensitive genotype. 10908-05 and 10908-18 lines, are at lower levels of Agria, and almost equal to the data, which can be named as resistant. Also, Agria had moderate sensitivity, and it is not quite as susceptible genotypes (Table 5).

Table 2. Analysis of variance assessed *in vitro* (drop-Direct) and greenhouse conditions for pathogenic *A. solani*.

Source	df	Mean Square	F
Method	2	15315.965	261669.373**
Genotype	6	146.039	2495.045**
Method × genotype	12	54.581	932.503**
Error	42	0.059	
Total	62		

Table 3. Comparison mean of potato genotypes with AUDPC by Tukey at 5% level (greenhouse method).

Genotype	AUDPC Mean	
10908-05 line	49.00	f
10908-18 line	51.66	e
Delta	56.16	d
Agria	56.50	d
Desiree	65.16	c
Casmos	70.00	b
9506 line	71.00	a

Table 4. Comparison mean of potato genotypes with AUDPC by Tukey at 5% level (*in vitro* method-drop).

Genotype	AUDPC Mean	
Delta	12.66	d
10908-05 line	15.08	c
10908-18 line	15.25	bc
Agria	15.58	b
9506 line	17.50	a
Desiree	17.75	a
Casmos	17.91	a

Table 5. Comparison mean of potato genotypes with AUDPC by Tukey at 5% level (*in vitro* method-direct).

Genotype	AUDPC Mean	
Delta	7.83	e
10908-05 line	8.58	d
10908-18 line	10.08	c
Agria	11.25	b
Desiree	12.50	a
9506 line	12.50	a
Casmos	12.66	a



Figure 1. Greenhouse evaluation—View chlorotic and necrotic spots caused by pathogenic *A. solani*.



Figure 2. *In vitro* evaluation of drop method—View necrosis and leaf chlorosis.

3.4. Correlations between Traits

The correlation table shows that there is a high correla-

tion between test methods. The highest correlation is between the two *in vitro* methods direct and drop. The correlation between these methods is positive. Correlation indicates that evaluation of each of these methods, have similar results together, and can be evaluated; use either of these methods separately (Table 6).

3.5. Cluster Analysis

The results of cluster analysis indicate that genotypes and lines are located in four different groups. In this case, line 9506 and Cosmos were quite sensitive to the surface, and Desiree, is located in a sensitive area. Line 10908-18 and Agria, located approximately at the level of resistance, and line 10908-05 and Delta, have been resistant surface (Figure 4).

4. Discussion

Mirkarimi *et al.*, showed that the Cosmos are more sensitive genotypes than isolates *A. tenuissima*, and our results also indicate that this is a sensitive genotype [4].

Dita Rodriguez *et al.* reported that the susceptible genotype Desiree, genotype Delta is resistant. These findings are consistent with our results [20].



Figure 3. *In vitro* evaluation of direct method—View necrosis and leaf chlorosis.

Table 6. Correlation between *in vitro* and greenhouse methods based on area under the disease progress curve.

		greenhouse	drop	direct
Greenhouse	Pearson Correlation	1	0.747**	0.817**
	Sig. (2-tailed)		0.000	0.000
	N	21	21	21
Drop	Pearson Correlation	0.747**	1	0.941**
	Sig. (2-tailed)	0.000		0.000
	N	21	21	21
Direct	Pearson Correlation	0.817**	0.941**	1
	Sig. (2-tailed)	0.000	0.000	
	N	21	21	21

*Correlation is significant at the 0.05 level. **Correlation is significant at the 0.01 level.

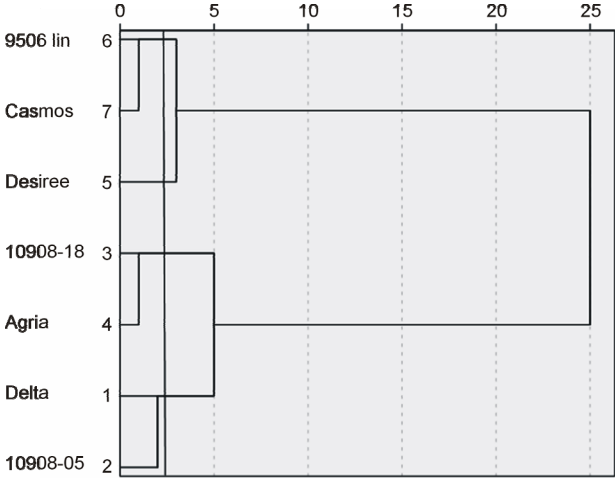


Figure 4. Cluster analysis based on two *in vitro* conditions and greenhouse methods.

In other studies, Nasr Esfahani, stated that shortening is a sensitive genotype, and the evaluation shows that this genotype, has been resistant surface. These results could be due to the effects of different fungal isolates, and better yet, researchers have not used it as a susceptible genotype in our experiments [13].

Finding resistant or immune genotypes to the disease, is highly effective in controlling it. Therefore, finding the resistant genotypes could be considered an advantage in the cases of crop cultivars [17]. Based on this test, line 05-10908, can be considered as a resistant line, and it can be introduced to farmers, after physiological and agronomic tests.

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