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In vitro and greenhouse evaluation for resistance to early blight of potato isolated from *Alternaria alternata*

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ABSTRACT

Early blight of potato is caused by the fungus *Alternaria alternata*, one of the most destructive foliar diseases, especially in hot climates under irrigation. In this study, the virus free potato seedlings were obtained from the National Plant Gene Bank of Iran and were inoculated *in vitro* with a culture filtrate of *A. alternate*. The leaflets received a 1000-µl droplet of the *A. alternata* culture filtrate and were inoculated by spraying with a suspension of 10^5 conidia/ml of isolate *A. alternata* in the greenhouse method. The experimental design was a completely randomized design (CRD) with three replications and seven genotypes, which have been infected with the two leaves of each iteration. *In vitro* selection of fungal isolates of *A. alternata*, chlorotic and necrotic symptoms began 1 to 2 days after inoculation, but the assessment of greenhouse symptoms appeared 6 - 10 days after inoculation. The area under the disease progress curve values were presented by analysis of variance (ANOVA), and they were compared using Duncan's test (a = 0.01%). In both methods, there was a significant difference between the potato genotypes (P < 0.01). For *In vitro* selection and evaluation greenhouse, Casmos were resistant to at least figure and Marfona genotype had the highest resistance.

Keywords: Potato; Early Blight; *Alternaria*; Inoculation

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. The disease can occur over a wide variety of climatic conditions and can be very critical if left uncontrolled. Potato plants are susceptible to a wide diversity 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. Early blight, caused by the *A. alternata* fungus, is one of the main diseases of potatoes in tropical climates, especially where potatoes are grown under irrigation. He described the new report of *A. alternata* that was caused by leaf blight of tomato in Pakistan [1]. The fungicides used to control the disease are expensive and frequently inefficient [2]. Potato resistance to early blight is a quantitative trait, and obtaining successful resistant cultivars is not simple [3-5]. It has been observed that resistance to early blight is age-related: early-maturing cultivars are more susceptible than late-maturing cultivars. *A. alternata* is a well-known pathogen on many crops but a few records report this fungus as a causal agent of leaf spot on deciduous trees. Glasshouse tests using spray inoculation of a conidial suspension on leaves are widely used for conidial inoculum production techniques [6]. *In vitro* selection is caused by the direct method that plantlets were inoculated in an 18 × 2 cm test tube each, containing 5 ml of *A. solani* culture filtrate [7]. Severity values were plotted against time and the area under the disease progress curve (AUDPC) was calculated [8].

2. MATERIAL AND METHOD

2.1. Plant Material

The experiment was conducted during 2008-2009 under *in vitro* and *ex vitro* conditions. Virus free clones of potato cultivars were obtained from the National plant

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gene Bank of Iran. Seven cultivars were conducted Ells, Picasso, Maradona, Marfona, Delta, Casmos and Desiree that were propagated through nodal cutting every three month and kept in growth chamber at 25˚C ± 1˚C light with a period of 16 h light and 8 h dark.

2.2. Tissue Culture

In vitro plantlets of potato were multiplied routinely by subculturing single node cuttings. Single node cuttings were propagated MS basal medium with 3% sucrose and 0.7% agar in petri dishes (25 × 100 mm). Cultures were placed in tissue culture growth room at 16 hour photoperiod and 25˚C ± 1˚C temperature system for 4 weeks. Fore week-old plantlets (4 - 6 cm long) were transplanted in a plastic cover into a sterile mixture of peat moss, perlite and turb (2:1:1) in pots (one seed- ling per pot), temperature and humid about 27˚C - 33˚C, 75% - 80% respectively. The plantlets were irrigated three times at every day that was increased moist and temperature.

2.3. Sporulation and Culture Filtrate

The mycelial (1 cm^2) of an *A. alternata* 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 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˚C ± 2˚C. After 12 days the contents of glass flasks were filtered through the what man filter 0.2 µm and concentrated to centrifuge at 2000 - 2500 g for 10 - 15 min and the samples are centrifuged at a time.

2.4. *In Vitro* Selection

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. alternata* culture filtrate. This study was conducted using factorial based on completely randomized design (CRD) with 3 replications. The test tubes were placed for 72 h in a growth chamber at 22˚C ± 2˚C. with a photosynthetic photon flow density of 100 µE/m/s and a day length of 16 h [7]. During *in vitro* assay the *A. alternata* symptoms appear 2 - 3 days until 6 day. For evaluation of the damage produced by *A. alternata* using the scale described in **Table 1**.

2.5. Greenhouse Evaluation

Three plant of each cultivar were inoculated by sporulation of conidial suspension with 10^5 conidial/ml of *A. alternata* isolate on leaflet in plant. After inoculation, plants were kept for 24 h in a plastic at 25˚C, 12 h photoperiod. After this time, plants were transferred to greenhouse conditions. Early blight was allowed to develop in the greenhouse through inoculation, recording the intensity of affectation 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 [9].

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

2.6. Statistical Analysis

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

3. RESULT

3.1. *In Vitro* Selection

During *in vitro* assay, the *A. alternata* symptoms appear 1 - 2 days after inoculation. Disease severity assessments were taken every day beginning at 1 day until 6 day (**Figure 1**). Severity value observed in the disease development curve. The severity value to area under the disease progress curve (AUDPC) was calculated. Significant different was observed amongst potato cultivars (**Table 2**). Mean comparison among the potato cultivars indicated the cultivars were grouped into five class (**Table 3**). Casmos had a high level of pathogenecity in comparison with other cultivars and Marafona cultivar was the highest resistant.

3.2. Greenhouse Evaluation

A. alternata chlorotic and necrotic symptoms appear 6 - 10 days after inoculation in the greenhouse plantlets. Disease severity assessments were taken every 2 days beginning at 3 day until 21 day. Severity value in observed the disease development curve (**Figure 2**). Early blight severity was calculated as AUDPC varied according to cultivar. Statistical analysis determined significant differences between cultivars (**Table 2**). Cultivar resistance levels was grouped into foure class. Results show that Casmos was the most sensitive cultivar and Marfona was the most tolerance cultivar.

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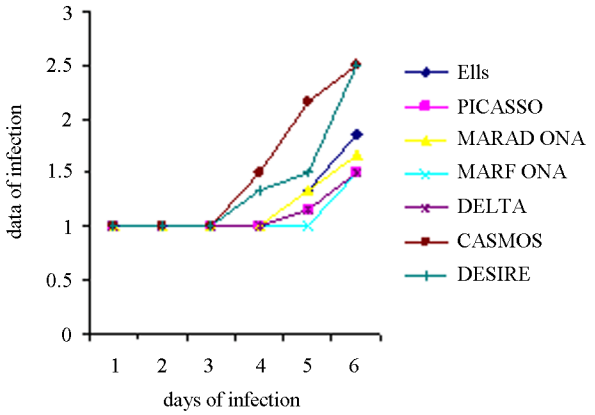

Figure 1. Disease development curve in *in vitro* condition.

Table 2. Variance analysis square for AUDPC maen in *in vitro* selecton and greenhouse evluation Obtained, on 10 notes.

Source	Degrees of Freedom	F-value (*in vitro*)	F-value (Greenhouse)
Genotype	**6**	**34.157****	**106.043****
error	**14**		
Total	**20**		
Cv%		**3.82%**	**4.41%**

** = Significant at level 1%.

Table 3. Mean comparison in *in vitro* and greenhouse condition for assessment of resistance level to early blight of potato isolated from *Alternaria alternate*.

Cultivars of potato	Greenhouse eveluation		*In vitro* selection	
ELLS	**40**	**c**	**5.58**	**cd**
PICASSO	**32.83**	**d**	**5.41**	**cd**
MARADONA	**44.16**	**bc**	**5.91**	**c**
MARFONA	**25.83**	**e**	**5.25**	**d**
DELTA	**29**	**de**	**5.41**	**cd**
CASMOS	**54**	**a**	**7.41**	**a**
DESIREE	**45.66**	**b**	**6.5**	**b**

The letter "a" is most sensitive, and the move to the next character, resistance is greater.

4. DISCUSSION

This study was done on middle leaves with a result similar to the observation [4] and middle leaf assay is a useful factor for potato cultivars evaluation. Results and disease symptom of *A. alternata* by infection culture filterate in *in vitro* plantlets of potato cultivars were similar to the symptom by infection sporulation in greenhouse method as was described by [7,10,11]. Lesion expansion rate has been used for assay observation and disease severity and lesion expansion rate can be evaluated with due scale. Disease severity assessments in *in vitro* were taken every 2 days beginning at 6 until 21

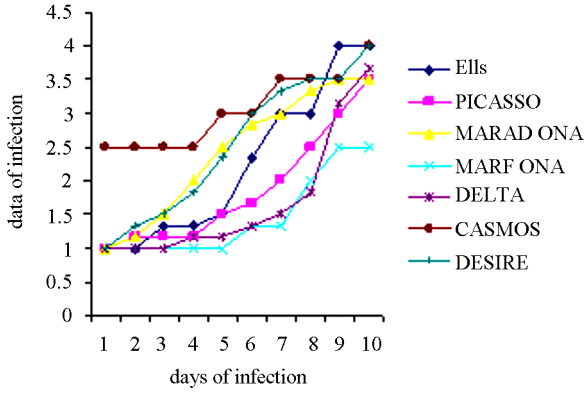

Figure 2. Disease development curve in greenhouse condition.

days post inoculation that were described [4]. Lack of resources or lack of resistance to *Alternaria* was declared among potato lines, as well as among commercial potato [12]. However, different levels of resistance have been observed in the wild diploid potato [13]. Thus, the possible genotypes are safe, with a high level of resistance to this disease, which can greatly affect wave spot disease resistance and production of resistant crop cultivars used.

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