

Full Length Research Paper

In vitro* evaluation of Atlantic genotype to early blight of potato caused by the fungus isolates of *Alternaria solani

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Early blight of potato has the most diverse in the world and Iran, and it plays an important role in reducing crop yields. The disease is found in plants such as potatoes and tomatoes. Therefore, this experiment was to test the factorial based on Completely Randomized Design (CRD) with two factors and three genotypes to be performed thrice. Genetic samples of potatoes were produced from genetically National Plant Gene Bank of Iran, and they were maintained as explants *in vitro*. Assessment resistance genotype of Atlantic was evaluated using both susceptible and resistant (Agria to Delta). In this assessment, symptoms began in the first and second day. Variance analysis table shows that the experimental methods, genetic samples, and their interactions are significant at the 1% level. According to the mean comparison, *in vitro* assessment of the drop, Atlantic genotypes were similar at Agria genotype. Since the genotype Agria is seen as crucial, therefore, the genotypes Atlantic is a genotype susceptible to this disease. These results are consistent with the results obtained *in vitro* evaluation of the direct method, which can prove the validity of the results.

Key words: Early blight of potato, *Alternaria*, evaluation *in vitro*.

INTRODUCTION

Potato crop is considered one of the strategic products. According to the Food and Agriculture Organization (FAO, 2007) report, it has been producing potatoes up to 321,736 tons of in the world, and 5,240 tons in Iran (Anonymous, 2009).

Early blight of potato is important in reducing crop yields. This disease causes chlorotic and necrotic spots (Mirkarimi et al., 2013). Important hosts for *Alternaria* include tomatoes, potatoes and eggplant (Pschedit, 1985). Other hosts, including nettle, red peppers and other vegetables like cabbage, cucumbers and flowers like Zinnia (Qusta, 2004). First report in Iran showed the disease in 1977 (Taheri et al., 2009).

Fungal diversity that exists in the country is due to variable weather conditions. In recent studies, it has been

identified in six species of *Alternaria* fungus causing a wave spot disease of potatoes (Taheri et al., 2007).

Evaluation of susceptibility or resistance exists in potato cultivars with some of these methods (Rodriguez et al., 2007). Evaluation of *in vitro* methods and spray the culture filtrated on the plant was introduced first by Locke (1949). Rodriguez et al. (2007) used direct filter extracts *in vitro* system. They were considered as absorption effects; the filter extracts from the roots, and extracts movement in the vessels within the seedlings. In these experiments, these two methods were used in order to better evaluate and compare the performance test methods, evaluating the greenhouse (Rodriguez et al., 2007).

Agria genotype has been reported as susceptible genotypes, isolates of *Alternaria solani* (Nasr, 2004), and Delta genotype were reported as resistant genotypes (Dita et al., 2006). This experiment made use of new techniques and conventional and on the basis of *in vitro*

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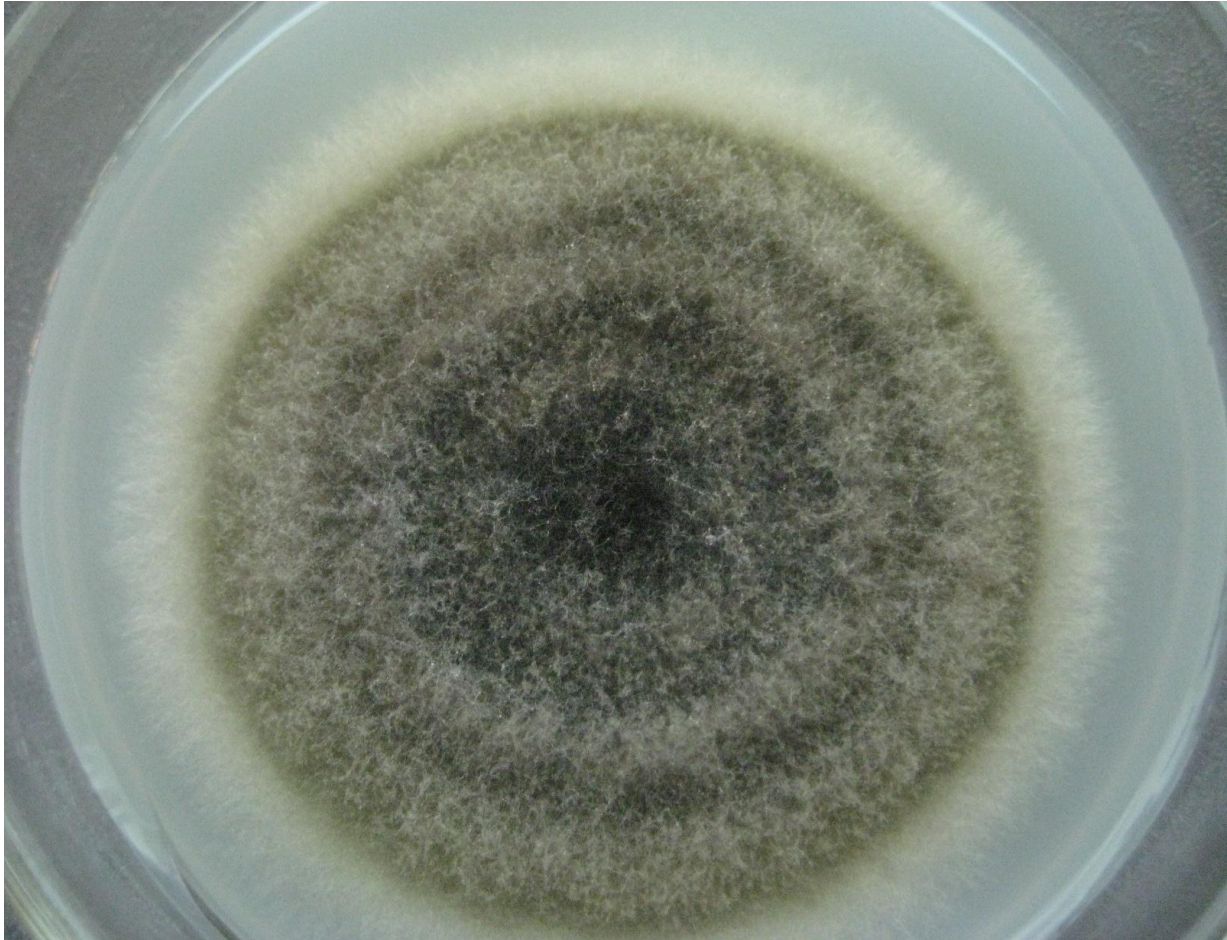


Figure 1. View grown pathogenic fungus *A. solani* in PDA

evaluation, measured the resistance levels of Atlantic genotype.

MATERIALS AND METHODS

Genetic samples were included in the Atlantic, Agria and Delta, which were obtained from the Department of Genetics and National Plant Gene Bank of Iran. The samples were kept as explants free viruses in the growth chamber under a temperature of $23 \pm 2^\circ\text{C}$ and the light / dark 8/16 h.

Tissue culture

Tissue culture was used as the basal MS medium without hormones and vitamins. Due to the increased efficiency of micro-propagation, we tried to use the lateral and terminal buds with a leaf. Accordingly, each seedling divided into several sections, and was placed in test tubes containing culture medium. Then, the test tubes were transferred to a growth chamber at the same

temperature (storage plants).

Fungal isolates culture

The fungal isolates were obtained from the Department of Genetics and National Plant Gene Bank of Iran under the temperature 20 to 25°C with white fluorescent light, cycle light / dark for 8/16 h (Figure 1). Then, we used a liquid medium PDB. Mixed broth cultures were passed through four layers of cloth (Tiffany), and put in a centrifuge for 20 to 15 min. Finally, we used the filter to syringes method (filters $0/2 \mu\text{m}$).

***In vitro* evaluation - using filtered yeast extract spread on the plant surface (droplet)**

In this method, the filtered extract from fungus grown in liquid medium drip was sprayed on the leaves of plants in test tubes. After inoculation, the seedlings were kept in the incubator under given temperature conditions.

Evaluation was based on observation of middle leaves



Figure 2. View of the *in vitro* evaluation, three genotypes tested with the pathogenic fungus *A. solani*.

Table 1. Analysis of variance assessed *in vitro* (drop - Direct) for fungal effects *A. solani*.

Source	Df	MS
Experimental methods	1	107.556**
Genetic samples	2	22.389**
Methods x sample	2	0.514**
Error	12	0.0730
Total	17	

from the first day to the sixth day (Rodriguez et al., 2007).

***In vitro* evaluation - a method of putting seedlings into the filtered extract (direct)**

In this way, 5 ml of the filtered extract was poured into the test tubes, and plants without roots removed and placed in a new tube containing filtered extract (Rodriguez et al., 2007).

Statistical analysis

The experiment was conducted as factorial based on Completely Randomized Design (CRD), and calculated

the area under the disease progress curve based on the scale (Pryor and Michalides 2002). The severity of the disease was based on the area under the disease progress curve with two factors genotype and experimental methods. Statistical analysis was performed using SPSS software.

RESULTS AND DISCUSSION

In vitro assessment of the drop method and symptoms were observed from the first day, and the direct method, apparently, were the second day (Figure 2).

Analysis of variance table shows experimental methods, samples of genetic and interactions between them are significant at the 1% level (Table 1).

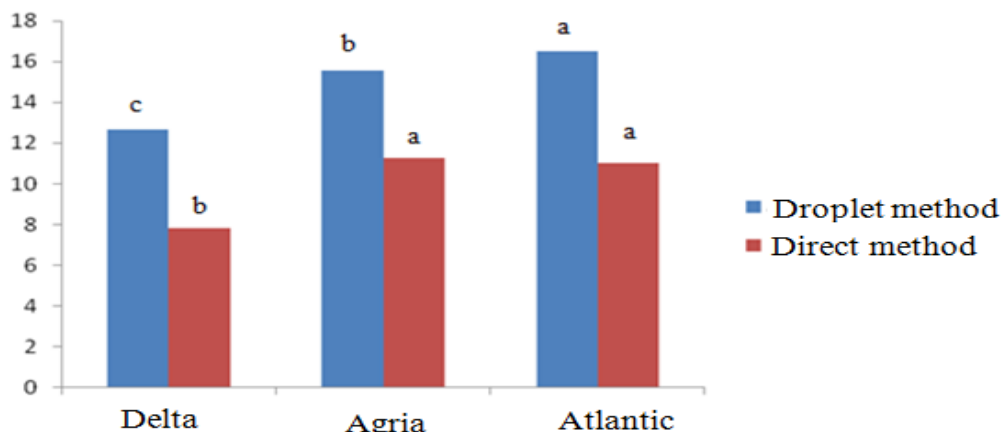


Figure 3. Comparison of genotypes tested out, based on area under the disease progress curve, due to the isolated fungus *A. solani*, *in vitro* condition.

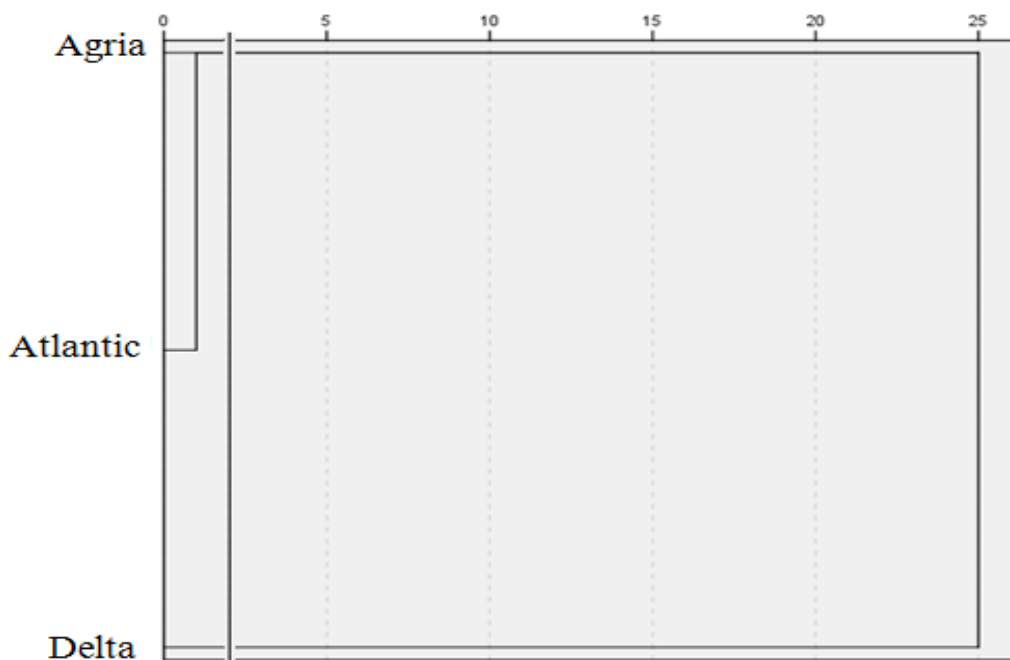


Figure 4. Cluster analysis of potato genotypes, based on *in vitro* assessment method and treatment of fungal isolates wave spot disease (*A. solani*).

According to the susceptible and resistant control in these experiments (Agria to Delta) were evaluated Atlantic genotypes.

Given the diversity of *Alternaria* species in the world, sources of resistance to the early blight disease is very small, and sometimes can be found in the wild plant (Mirkarimi et al., 2013). The transfer of genes from wild species is associated with many problems. Accordingly, resistant to diseases are considered an advantage (Rodriguez et al., 2007).

Comparison of chart showed the inside of the drop methods. Atlantic genotype had higher levels of genotype

Agria (susceptible) and the genotype of the Atlantic is a genotype susceptible to this disease. *In vitro* evaluation of the direct methods, Atlantic genotypes were similar to Agria and there is a susceptible genotype. The results are similar to the aforementioned method (Figure 3).

Cluster analysis

These analysis based on data were obtained from both genotypes tested *in vitro* evaluation of fungal isolates and can be categorized into three groups (Figure 4).

Genotype of Delta were included in the resistant genotypes of Agria and Atlantic was a susceptible group. The results of our experiments confirmed the results of the fungal isolates.

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