Effect of *Alternaria solani* Exudates on Resistant and Susceptible Potato Cultivars from Two Different pathogen isolates

Hadis Shahbazi*, Heshmatollah Aminian2, Navazollah Sahebani2 and Dennis Halterman1

1Young Researchers Club, Arak Branch, Islamic Azad University, PO Box 38135/567, Arak, Iran
2Department of Plant Protection, Aboryhan Campus, Tehran University, P.O. Box 33955-159, Tehran, Iran
3U.S. Department of Agriculture-Agricultural Research Service, Vegetable Crops Research Unit, Madison, WI 53706, USA

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Early blight of potato, caused by *Alternaria solani*, is a ubiquitous disease in many countries around the world. Our previous screening of several Iranian potato cultivars found that significant variation in resistance phenotypes exists between two cultivars: resistant ‘Diamond’ and susceptible ‘Granula’. Our previous analysis of five different pathogen isolates also identified varying degrees of aggressiveness regardless of the host cultivar. Here, a bioassay was used to study the role of liquid culture exudates produced in vitro on pathogenicity and elicitation of disease symptomology in seedlings as well as detached leaves. Responses of host genotypes to the exudates of the two *A. solani* isolates were significantly different. Detached leaves of the resistant cultivar ‘Diamond’ elicited fewer symptoms to each isolate when compared to the susceptible cultivar ‘Granula’. Interestingly, the phytotoxicity effect of the culture filtrate from the more aggressive isolate A was higher than from isolate N suggesting an increased concentration or strength of the toxins produced. Our results are significant because they indicate a correlation between symptoms elicited by *A. solani* phytotoxins and their aggressiveness on the host.

*Keywords*: *Alternaria solani*, bioassay, early blight, pathogenicity, phytotoxicity.
sistance phenotypes of nine potato cultivars to five isolates of *Alternaria solani* (Shahbazi et al., 2010). Two potato cultivars, resistant ‘Diamond’ and susceptible ‘Granula’, and two pathogen isolates with significantly different pathogenicity phenotypes, named A and N, were selected for further analysis. Here, we monitored the effect of exudates from liquid grown cultures of *A. solani* isolates A and N on cultivars ‘Diamond’ and ‘Granula’ using whole seedlings and detached leaflets. We hypothesized that phytotoxins present in the liquid exudates of the two isolates would elicit distinct effects on the host plants. Our results indicate that isolates of *A. solani* exhibiting different degrees of pathogenicity produce phytotoxins with corresponding effects on potato.

### Materials and Methods

**Cultural conditions.** Potato (*Solanum tuberosum*) seeds were planted in plastic pots (30 cm diameter) containing soil and perlite under greenhouse conditions (18-25°C) at Tehran University. Potato seed consisted of small whole tubers weighing approximately 50 g each. One seed piece was planted per pot. Tubers were treated with thiabendazole (tektro WP60%, Golsam Gorgan Company, Tehran) before planting. Plants were irrigated once per day or as needed.

**Alternaria solani isolation and spore production.** Potato leaves infected with *Alternaria solani* were collected in 2008 from commercial potato fields in Iran. Two isolates of *A. solani* were recovered from sections of potato leaves with early-blight lesions. An isolate, named “A”, was previously characterized as being highly aggressive on potato and a second isolate, named “N”, was less aggressive (Shahbazi et al., 2010). The isolates were maintained on PDA at 20°C for 7 days. To induce sporulation, cultures were grown for 6 days at 23-25°C on PDA under near-ultraviolet light (310-400 nm) with 16 h/day light. Conidia were collected by washing plates with sterile water and the resulting spore suspension was adjusted to a concentration of 10⁶ spores/mL.

**Greenhouse test.** Four plants (45 days old) of *Solanum tuberosum* cvs. ‘Diamond’ (resistant) and ‘Granula’ (susceptible) were inoculated separately by spraying with each isolate of *A. solani* at a concentration of 10⁶ spores/mL or a sterile water control (Bokshi et al., 2003; Nadia et al., 2007). The plants were covered with clear plastic bags for 24 h to increase humidity and accelerate infection and then grown under normal conditions in the greenhouse at 26°C (Bokshi et al., 2003; Pelletier and Fry, 1989). The intensity of infection was recorded using the scale described in Table 1 (Rodriguez et al., 2007).

**Table 1. Scale for evaluation of the foliar damage caused by *Alternaria solani***

| Rating | Description of Symptoms |
|--------|-------------------------|
| 10     | Spots on lower leaves   |
| 20     | Spots on most of the lower leaves |
| 30     | Spots on all lower and some of the middle leaves |
| 40     | Clearly developed blight lesions in lower leaves |
| 50     | Blight lesions in lower leaves spread to some middle leaves |
| 60     | Blight lesions developed in all inferior and most of the middle leaves |
| 70     | Blight lesions developed in all lower and middle leaves |
| 80     | Blight lesions developed in all lower and middle leaves and spread to upper leaves |
| 100    | Total blight (death of the plant) |

**Culture filtrate.** Small disks (2 mm diameter) from 1-week old PDA cultures were placed in 25 ml of sterile liquid medium containing 1.0 g of KH₂PO₄, 0.5 g of MgSO₄, 6.0 g of casein hydrolysate, 100 g of sucrose, 1 mg of FeSO₄, 0.15 mg of CuSO₄, 0.10 mg of ZnSO₄, and 0.10 mg of Na₂MoO₄ per liter of distilled water. The medium was adjusted to pH 4.9 with 0.1 M HCl and autoclaved before inoculation. The pH remained at 4.9 after autoclaving. The cultures were incubated for 6 weeks at 24°C in the dark without shaking (Maiero et al., 1991). The contents of the flasks were filtered through the Whatman no. 4 filter paper. Culture filtrates were stored at 4°C in the dark up to 10 hours until used.

**Phytotoxicity tests of culture filtrates on potato leaves in vitro.** Aqueous dilutions of 2:1, 5:1, 10:1 v/v (culture filtrate: sterilized water) were prepared and autoclaved for 15 min. In earlier studies of *Alternaria* toxic metabolites, autoclaving did not affect phytotoxicity of culture filtrates (Pound and Stahmann, 1951). Control treatments included dilutions of noninoculated growth media with distilled water. A method similar to that described by (Hernandez et al., 1991) was used; however, without removing the roots. Plantlets from each cultivar were inoculated by placing 45 day-old whole *in vitro* plantlets in a 15 × 2 cm test tube containing 5 ml of the culture filtrate dilutions or control treatments containing corresponding dilutions of noninoculated media. Leaflets of 4-week old grown plants were excised and placed in plastic petri dishes on 9-cm discs of filter paper saturated with 3 ml of culture filtrate dilutions or control treatments containing corresponding dilutions of noninoculated media (Gilchrist and Grogan, 1976; Hanneke et al., 1988; Siler and Gilchrist, 1983). The petri dishes were sealed with parafilm. The test tubes and petri dishes were placed in a growth chamber for 72 h at 22 ± 2°C, with
a photosynthetic photon flow density of 100 lE/m/s and a day length of 16 h. To evaluate the individual response of in vitro plantlets to the culture filtrate in tubes, a scale of 0-7 degrees was used (Table 2; Rodríguez et al., 2007). The detached leaflets were rated for the degree of phytotoxicity in petri dishes on a scale of zero to three in which 0 = no symptoms, 1 = slight necrosis, 2 = moderate necrosis and wilting, and 3 = severe necrosis and wilting (Maiero et al., 1991).

**Analysis of results.** All experiments were carried out using a factorial model of a completely randomized experimental design in four replicates per treatment. In greenhouse evaluations, the factorial model was $2 \times 3$ (two potato cultivars $\times$ three treatments), in culture filtrate tests it was $2 \times 3 \times 3$ (two cultivars $\times$ three treatments $\times$ three dilutions). Analysis of variance was performed on data from whole plant inoculation experiments using SAS v9. A Kruskal-Wallis analysis of variance was performed on in vitro phytotoxicity tests due to the nonparametric data sets. All data are presented as means of at least four replications.

**Results**

**Greenhouse evaluation.** Greenhouse infection assays of two potato cultivars with different isolates of *A. solani* revealed significant differences between hosts. *S. tuberosum* cv. ‘Diamond’ exhibited lower disease severity with an average score ($\pm$ std. error) of 16 ($\pm$ 2) among the two *A. solani* isolates (Fig. 1). Cultivar ‘Granula’ showed higher disease severity with an average score of 51 ($\pm$ 2). Variance analysis indicated a significant difference between responses of these two cultivars to *A. solani* infection as well as a significant difference from the water-inoculated control plants, which exhibited no visible symptoms. Between two

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**Table 2. Scale for evaluation of the damage produced by *A. solani* culture filtrate in potato in vitro plantlets (in tubes)**

| Rating | Description of symptoms |
|--------|-------------------------|
| 0      | No visible symptoms     |
| 1      | Light chlorosis at basal zone of plantlet |
| 2      | Advanced chlorosis at basal zone of plantlet |
| 3      | Basal zone slightly necrotic and some leaves of the middle zone show light chlorosis |
| 4      | Accented necrosis at basal zone and advanced chlorosis in the middle zone of plantlet |
| 5      | Dead leaves at basal zone, middle zone slightly necrotic and apical zone with light chlorosis |
| 6      | Plantlet necrotic up to the middle zone and apical zone with advanced chlorosis |
| 7      | Total collapse of plantlet |

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**Fig. 1.** Disease severity scores of greenhouse-grown potato cultivars inoculated with *A. solani* isolates (N and A) or water (CO). Error bars show the standard error among four replications.

*Fig. 2.** Phytotoxicity tests of culture filtrates on plantlets in vitro. DCO: ‘Diamond’ in non-inoculated medium culture; GCO: ‘Granula’ in non-inoculated medium culture; GA: ‘Granula’ in culture filtrate of *A. solani* isolate A; DA: ‘Diamond’ in culture filtrate of *A. solani* isolate A; DN: ‘Diamond’ in culture filtrate of *A. solani* isolate N; GN: ‘Granula’ in culture filtrate of *A. solani* isolate N. Results are the means of four replications. Error bars denote standard error.
the two cultivars, but were not statistically significant. In both cultivars, symptom expression increased with a higher concentration of fungal culture filtrate and differences between dilutions were significant at P = 0.017.

Differences in host symptom expression were also observed when using isolates of A. solani with different aggressiveness phenotypes (Fig. 2). Culture exudate from A. solani isolate A, which is more aggressive, elicited more symptoms on both cvs. ‘Diamond’ and ‘Granula’ than the less aggressive isolate N and this difference was significant at P < 0.001. The symptoms elicited by the two exudates were also significantly different from those elicited by the control regardless of the dilution used. In an analysis of reactions between isolates, dilution of the exudate of isolate N at a ratio of 5:1 (80%) resulted in approximately the same symptoms as the exudate from isolate A diluted 2:1 (66.7%). Compared to the fungus-free control, both culture filtrates of A. solani isolates elicited significantly more symptoms regardless of the dilution.

**Culture filtrate in petri dishes.** We also assessed the reaction of mature detached leaves to exposure to fungal exudates. In these experiments, the degree of symptoms in leaves of resistant cv. ‘Diamond’ was significantly less when compared to leaves of cv. ‘Granula’ (P = 0.010). Differences in symptoms elicited by the two A. solani isolates were less pronounced in detached leaves compared to the whole-plant assays although the distinction was still significant at P < 0.001 (Fig. 3). Symptom expression was also higher than in the control-inoculated leaflets regardless of the isolate. Dilution of the fungal exudates also resulted in significant differences in symptoms in both ‘Diamond’ and ‘Granula’ leaflets with more symptoms observed in the most concentrated solution of fungal exudate (P < 0.001).

**Discussion**

We have previously shown that there are significant differences among resistance phenotypes of potato cultivars ‘Diamond’ and ‘Granula’, as well as multiple cultivars with intermediate phenotypes (Shahbazi et al., 2010). These differences are likely not monogenic traits since a gradient of resistance phenotypes were observed when multiple cultivars were tested. We have also observed significant differences among isolates of A. solani. Two of these isolates, A and N, produce significantly more and fewer, disease symptoms, respectively. The phenotypic differences in resistance correlate with an significant increase in phenolic compounds (Shahbazi et al., 2010) and expression of β-1,3 glucanase activity (Shahbazi et al., unpublished data), suggesting that these responses have a role in early blight resistance.

Here, we have shown that pathogenesis of A. solani in potato is also correlated with its ability to produce toxin metabolites that elicit disease symptoms in the absence of the pathogen. A. solani isolates with higher (A) and lower (N) degrees of aggressiveness were significantly separated by disease severity scores as well as the symptoms elicited by their toxins in detached leaflet assays. Rodriguez et al. (2007) showed that culture medium of A. solani causes leaf and stem necrosis in in vitro plantlets of Solanum sp. that are phenotypically very similar to those caused by infection by spores. This phenomenon has also been observed elsewhere can be used effectively to select for individuals with increased resistance to early blight (Hernandez et al., 1991; Martinez and Sinclair, 1994; Veitia et al., 2001). Our results support the conclusion that different cultivars of potato contain variable reactions to A. solani toxins. Additionally, we have shown that different isolates of A. solani can produce toxins that produce more or fewer symptoms on the host, suggesting variability in the quantity or effectiveness of the toxins that are produced.

Two A. solani isolates with significantly different degrees of aggressiveness were identified using greenhouse infection assays. The A isolate induced more disease and N induced less disease, regardless of the host cultivar used. Although genotypic differences have not been established for these isolates, the presence of multiple strains of A. solani in potato growing regions is not uncommon (Loureń et al., 2009; van der Waals et al., 2004). Isolates from the same host, such as potato, have been found to be more closely related to one another than those collected from another host, such as tomato (Weir et al., 1998). Therefore, among isolates collected from the same host, any genetic variability...
that translates to differences in aggressiveness is of great interest and warrants further study.

The two cultivars ‘Diamond’ and ‘Granula’ exhibited different levels of resistance to the two isolates using a detached leaflet assay. Together, differences in interaction between resistant ‘Diamond’ and susceptible ‘Granula’ with \( A.\ solani \) after 72 hours, suggest that their responses are closely associated with defense responses and that the differences in phenotypes are likely due to variability in sensitivity to metabolites produced during growth. The generation of phytotoxins has been demonstrated in several plant pathogens including individuals from the genera \( Aspergillus, Penicillium, Fusarium, Claviceps, \) and \( Verticillium \) (Svabova and Lebeda, 2005). To accelerate disease resistance evaluation, \textit{in vitro} selection of toxin sensitivity has been successfully applied in several crops such as banana, carnation, grapevine, strawberry and wheat (Svabova and Lebeda, 2005). Here we have shown that an increased sensitivity to toxins produced by \( A.\ solani \) correlates with increased susceptibility to the pathogen. Furthermore, this sensitivity could be assayed using seedlings as young as 45 days old. Further work is required to determine whether the correlation between toxin sensitivity and resistance can be expanded to other potato cultivars or wild species of potato. We expect that the use of seedlings for \textit{in vitro} evaluation of \( A.\ solani \) toxin sensitivity could provide a useful method for early generation selection of individuals in families segregating for resistance to early blight.

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**References**

Bokshi, A., Morris, S. and Deverall, B. 2003. Effects of benzo thiadiazole and acetylsalicylic acid on \( \beta\)-1,3-glucanase activity and disease resistance in potato. \textit{Plant Pathol.} 52:22–27.

Brian, P. W., Elson, G. W., Hemming, H. G. and Wright, J. M. 1952. The phytotoxic properties of alternateic acid in relation to the etiology of plant diseases caused by \( A.\ solani \) (Ell. & Mart.) Jones & Grout. \textit{Ann. Appl. Biol.} 39:308–321.

Ellis, M. G. and Gibson, I. A. S. 1975. \textit{Alternaria solani}. Commonwealth Mycological Institute, Kew, Surrey, UK.

Gilchrist, D. G. and Grogan, R. G. 1976. Production and nature of a host-specific toxin from \( A.\ solani \) f. sp. \textit{lycopersici}. \textit{Phytopathology} 66:165–171.

Hanneke, M. A., Witsenboer, C. E., Schak, V., Bino, R. J., Löffler, H. J. and Jackes, H. 1988. Effects of \( A.\ alternata \) f. sp. \textit{lycopersici} toxins at different levels of tomato plant cell development. \textit{Plant Sci.} 56:253–260.

Hernandez, M. M., Kowalski, B., Lorenzo, P. and Ortiz, U. 1991. Effectiveness of the filtrate use of \( A.\ solani \) (Ellis and Martin) (J and G) in the selection \textit{in vitro} of resistance forms. \textit{Cult. Trop.} 12:48–50.

Langsdorf, G., Park, P. and Nishimura, S. 1991. Investigations on \( A.\ solani \) infections: Effect of alternate acid on the ultrastructure of tomato cells. \textit{Ann. Phyto. Soc. Jap.} 57:32–41.

Lourenço, V., Moya, A., González-Candelas, F., Carbone, I., Maffia, L. A. and Mizubuti, E. S. G. 2009. Molecular diversity and evolutionary processes of \( A.\ solani \) in Brazil inferred using genealogical and coalescent approaches. \textit{Phytopathology} 99:765–774.

Lynch, D. R., Coleman, M. C. and Lyon, G. D. 1991. Effect of \( A.\ solani \) culture filtrate on adventitious shoot regeneration in potato. \textit{Plant Cell Rep.} 9:607–610.

Maiero, M., Bean, G. A. and Ng, T. J. 1991. Toxicity production by \( A.\ solani \) and its related phytotoxicity to tomato breeding lines. \textit{Phytopathology} 81:1030–1033.

Martinez, P. R. and Sinclair, M. 1994. \textit{Selection in vitro} of resistance to early blight (\( A.\ solani \) Sorauer) in creole potatoes (\textit{Solanum phureja Janz}). \textit{Fitopatol. Colomb.} 18:90–100.

Matern, U., Strobel, G. and Shepard, J. 1978. Reaction to phytotoxins in a potato population derived from mesophyll proto-plasts. \textit{Proc. Natl. Acad. Sci. USA.} 75:4935–4939.

Montemurro, N. and Visconti, A., 1992. \textit{Alternaria} metabolites – chemical and biological data. In: \textit{Alternaria biology: plant disease and metabolites}, ed. by J. Chelkowski and A. Visconti, pp. 449-558. Elsevier, Amsterdam, Netherland.

Nadia, G., El-Gamal, G., Abd-El-Kareem, F., Fotouh, Y. and El-Mougy, N. 2007. Induction of systemic resistance in potato plants against late and early blight diseases using chemical inducers under greenhouse and field conditions. \textit{Res. J. Ag. Bio. Sci.} 3:73–81.

Neergaard, P. 1945. Danish species of \textit{Alternaria} and \textit{Stemphylium}: taxonomy, parasitism, economical significance. Munksgaard, Copenhagen, Denmark, 560 pp.

Otani, H., Kohimoto, K. and Kodama, M. 1995. \textit{Alternaria} toxins and their effects on host plants. \textit{Can. J. Bot.} 73:453.

Pelletier, J. and Fry, W. 1989. Characterization of resistance to early blight in three potato cultivars: Incubation period, lesion expansion rate, and sporulation production. \textit{Phytopathology} 9:511–517.

Pound, G. S. and Stahmann, M. A. 1951. The production of a toxic material by \( A.\ solani \) and its relation to early blight disease of tomato. \textit{Phytopathology} 41:1104–1114.

Rodriguez, N. V., Kowalski, B., Rodríguez, L. G., Caraballoso, I. B., Suárez, M. A., Pérez, P. O., Quintana, C. R., González, N. and Ramos, R. Q. 2007. \textit{In vitro} and \textit{ex vitro} selection of potato plantlets for resistance to early blight. \textit{J. Phytopath.} 155:582–586.

Rotem, J. 1994. The genus \textit{Alternaria}: biology, epidemiology, and pathogenicity. APS Press, St. Paul, Minnesota, USA, 326 pp.

Scherf, A. F. and MacNab, A. A. 1986. Vegetable diseases and
their control. Wiley, New York, USA, 729 pp.
Shahbazi, H., Aminian, H., Sahebani, N. and Halterman, D. 2010. Biochemical evaluation of resistance responses of potato to different isolates of Alternaria solani. Phytopathology 100: 454–459.
Siler, D. J. and Gilchrist, D. G. 1983. Properties of host specific toxins produced by Alternaria alternata f. sp. lycopersici in culture and in tomato plants. Phys. Plant Pathol. 23:265–274.
Svabova, L. and Lebeda, A. 2005. In vitro selection for improved plant resistance to toxin-producing pathogens. J. Phytopath. 153:52–64.
van der Waals, J. E., Korsten, L. and Slippers, B. 2004. Genetic diversity among Alternaria solani isolates from potatoes in South Africa. Plant Dis. 88:959–964.
van Dyke, C. G. and Trigano, R. N. 1987. Light and scanning electron microscopy of the interaction of the biocontrol fungus Alternaria cassiae with sicklepod (Cassia obtusifolia). Can. J. Plant Pathol. 9:230–235.
Veitia, N., Dita, M. A., García, L., Herrera, L., Bermúdez, I., Acosta, M., Clavero, J., Orellana, P., Romero, C. and García, L. 2001. Use of tissue culture and the mutagenesis in vitro for the improvement of the resistance to Alternaria solani (Solanum tuberosum Lin.), to var Desirée. Biotechnol. Veg. 1:43–47.
Von Ramm, C. 1962. Histological studies of infection by Alternaria longipes on tobacco. Phytopath. Zeit. 45:391–398.
Weir, T. L., Huff, D. R., Christ, B. J. and Romaine, C. P. 1998. RAPD-PCR analysis of genetic variation among isolates of Alternaria solani and Alternaria alternata from potato and tomato. Mycologia 90:813–821.