Plant Pathology Journal

ISSN 1812-5387

science alert

ANSInet
an open access publisher
http://ansinet.com

Plant Pathology Journal 13 (3): 167-172, 2014
ISSN 1812-5387 / DOI: 10.3923/ppj.2014.167.172

Cultural, Morphological and Pathogenic Variability of *Alternaria solani* Causing Early Blight in Tomato

Abhishek Singh, Vineeta Singh and S.M. Yadav
Department of Mycology and Plant Pathology, Institute of Agriculture Science,
Banaras Hindu University, Varanasi, 221005, India

Abstract: Experiment was conducted at BHU in the year 2012-13. Radial growth was not significantly different for most of the isolates. Seven DAI (days after inoculation) highest radial growth has obtained in isolate RF-1 (35.50 mm), Ten DAI maximum growth was observed in isolate EC-1 (52.00 mm) and Thirteen DAI maximum radial growth was same observed in two isolates IIVR and BHU-1 (88.75 mm). The maximum mean mycelial growth was observed in isolate IIVR (57.83 mm) followed by MF-4 (57.66 mm) and BHU-1 (56.83 mm). Isolates of *A. solani* depicted high variability in pigment production on PDA medium. Mycelial growth patterns were observed on PDA where BG RF-1 SF-1 MF-4 BHU-1 grew with circular margin with smooth surfaced colony and AF-2, PN-4, EC-1, BX-2 and IIVR isolates grew with irregular margin and rough surface. Five varieties of tomato were screened with 10 different isolates. The variety Selection-7 and H-86 were highly susceptible and susceptible, respectively with all the isolates tested. The variety Feb-2 was resistant while, the variety Flora Dade and Swarna Naveen were highly resistant with all the isolates. There was no significant difference between Flora Dade and Swarna Naveen. Out of 10 isolates, only three major groups were recorded on the basis of SAS analysis likewise Group-A isolates were highly virulent (MF-4 and PN-4), Group-C isolates indicate virulent (BG, AF-2, EC-1 and RF-1) and Group- E isolates were less virulent (BHU-1, IIVR, SF-1 and BX-2) with all the five varieties.

Key words: *Alternaria solani*, early blight, variability, tomato

INTRODUCTION

Alternaria solani (Ellis and Martin) Sorauer is an important pathogen causing early blight disease in tomato. It is very difficult to manage, due to its broad host range, extreme variability in pathogenic isolates and prolonged active phase of the disease cycle. Early blight on tomato was characterized by the appearance of brown to dark leathery necrotic spots first on leaflets producing target board effect (Locke, 1949). Symptoms produced by an aqueous solution of crystalline alternaric acid when introduced into the plants are identical to symptoms produced by crude fungus filters (Pound and Stahmann, 1951). *A. solani* belongs to muriform, beaked and large-spored group within the genus *Alternaria*, which is characterized by separate conidia borne singly on simple conidiophores (Neergaard, 1945). Potato dextrose agar and lima bean agar were the best media for growth and sporulation of *A. solani* (Barksdale, 1969). The optimum temperature for fungal growth was 23-28°C and pH 6-8 was found to be optimum (Tong *et al*., 1994). Penetration can occur at temperatures between 10 and 25°C (Sherf and MacNab, 1986). Early blight epidemics initially progress slowly but accelerate as plants mature, resulting in a typical sigmoidal disease progress curve (Nash and Gardner, 1988). In the present investigation, the extent of cultural, morphological diversity and pathogenic variability were studied among ten isolates of *A. solani* collected from different region in India.

MATERIALS AND METHODS

For investigation amongst all ten isolates, isolation and purification of *A. solani* cultures was done from fresh infected leaf, stem and fruit of tomato collected from different regions of India and purified by the hyphal tip method. They were stored at 4°C on PDA slants for further study.

Cultural variability of amongst isolates of *A. solani*: The cultural characters were recorded on 7th, 10th and 13th days after inoculation of all isolates of *A. solani*. Characters like mycelium growth diameter and growth characters, pigmentation on medium were recorded by

Corresponding Author: S.M. Yadav, Department of Mycology and Plant Pathology, Institute of Agriculture Science, Banaras Hindu University, Varanasi, 221005, India

direct observation of culture-grown in petri plates and sporulation was recorded on PDA medium. For this purpose ten selected isolates of *A. solani* were taken, representing Ara (AF-2), Raichur (RF-1), Banglore (BG), Varanasi (PN-4), Varanasi (BHU-1), Mirzapur (MF-4), Sonbhadra (SF-1), Buxer (BX-1), Adalpura (IIVR-2) and Exotic collection (EC-1). All these isolates were tested for their cultural and morphological variations on PDA. For each isolate five petri plates were poured with PDA medium. After solidification of the agar 5 mm culture bits of each isolates were inoculated onto the above-mentioned medium. These inoculated petri plates were kept in BOD at 25±2°C for growth. The data were recorded after 3 days from inoculation and radial growth was measured on 7th, 10th and 13th DAI on PDA medium. Morphological variability of different isolates of *A. solani* Slides were prepared from culture of all isolates of *A. solani* separately in 9 day old cultures of PDA and examined under the light microscope to record the width of the conidiogenous hyphae. The calibration was done with the help of ocular and stage micrometer.

Pathogenic variability: In order to confirm them identification of the disease and its causal agent, the pathogenic test was conducted under poly house conditions in pot experiments using five different tomato; varieties two highly resistant Flora Date and Swarna Naveen; one resistance Feb-2; one susceptible H-86 and one highly susceptible Selection-7. Seedlings were raised in pots filled with the mixture of soil: sand: FYM in a ratio of 1:1:1 were taken. Fifty nine days old plants were used for inoculation. For assessment of disease severity of different isolates on these five different type of varieties, 0-9 rating scale was fallowed (Ghosh *et al*., 2009). The data were taken six times at periodical intervals to see the disease progress of early blight. Symptoms expressed were studied and reisolated from the infected stem. The pathogenicity test as above was repeated twice to confirm the results.

Artificial inoculation technique: All the plants ranges from highly susceptible to highly resistant were artificially inoculated with a pure culture of *A. solani*. An inoculation technique was developed and standardized for inoculation. A 15 days old culture of ten isolates with known cultural and morphological variability grown on Potato Dextrose Agar (PDA) was scraped and macerated together by pestle and mortar. The optimum inoculum concentration was standardized at 126 CFU mL^{-1} for non sporulating isolates and 10^4 spore concentration for sporulating isolates. Minute, pinprick, inconspicuous necrosis of leaf tissues was observed 4-5 days after inoculation in susceptible and highly susceptible varieties.

Table 1: Description of disease scale (0-9) (Ghosh *et al*., 2009)

Scale	Description
0	No infection
1	0-10% leaf area infected
2	10-20% leaf area infected
3	20-30% leaf area infected
4	30-40% leaf area infected
5	40-50% leaf area infected
6	50-60% leaf area infected
7	60-70% leaf area infected
8	70-80% leaf area infected
9	80-90% or more leaf area infected

Calculation of disease parameters: The inoculated plants were regularly examined for appearance of symptoms starting from 24 h after inoculation. The data on PDI were recorded on four different dates at 7 days intervals i.e., 7th, 14th, 21th, 28th, 35th and 42nd days after inoculation (DAI).

Disease severity was scored on 0-9 scale and assessment of varieties was done (Table 1).

The percentage disease index (PDI) and area under disease progress curve (AUDPC) (Campbell and Madden, 1990; Johnson and Wilcoxson, 1982) were calculated as follows:

$$PDI = \frac{\text{Sum of all ratings}}{\text{Total No. of observations} \times \text{Maximum scale rating}} \times 100$$

Percent Disease Index (PDI) was worked out by using formula given by Wheeler (1969):

$$AUPC = \sum_{i=12} [(n-1X_{i+1} + X_i)^*(t_{i+1} - t_i)]$$

where, Xi is the disease index expressed as a proportion at the ith observation; ti is the time (days after inoculation) at the ith observations and n is the total number of observations.

Statistical analysis: The experiment was laid out in Completely Randomize Block Design (CRBD) with six replications. The values of data obtained from the poly house were subjected to following statistical analysis Duncan New Multiple Range Test analysis by SAS software version-9.2.

RESULTS AND DISCUSSION

Radial growth: Radial growth observed for ten isolates presented in Table 2 were significantly not different for

Table 2: Width of conidiogenous hyphae and mycelial growth of *Alternaria solani* on potato dextrose agar medium

Isolates	Width of conidiogenous hyphae (µm)	Mycelial growth (mm)			Mean mycelial growth (mm)
		7 DAI	10 DAI	13 DAI	
AF-2	1.18	35.250	46.250	77.000	52.83
BG	2.60	34.500	42.500	73.500	50.16
RF-1	4.80	35.500	42.500	74.000	50.66
PN-4	1.17	33.750	50.500	80.250	54.83
EC-1	9.40	30.750	52.000	84.000	55.58
SF-1	2.70	31.750	50.750	87.000	56.50
MF-4	1.16	32.750	51.750	88.500	57.66
BHU-1	2.50	31.500	50.250	88.750	56.83
BX-2	1.19	33.250	48.500	87.750	56.50
IIVR	2.12	33.000	51.750	88.750	57.83
CD	-	1.665	2.538	2.938	N/A
SE (m)	-	0.574	0.874	1.012	14.86
SE (d)	-	0.811	1.237	1.432	21.016
CV	-	3.456	3.593	2.441	46.847

Table 3: Cultural variability of different isolates of *Alternaria solani* on PDA medium at 25±2°C after 13 days

Isolates	Pigmentation	Sporulation on PDA media	Mycelial growth/Colony character		
			Circular/irregular	Smooth/ rough	Zonation
AF-2	Brownish black	No	Irregular	Rough	Without zonation
BG	Greenish black	No	Circular	Smooth	Without zonation
RF-1	Greenish black	No	Circular	Smooth	Without zonation
PN-4	Brownish black	No	Irregular	Rough	Zonation
EC-1	Redish black	2×10^3	Irregular	Rough	Zonation
SF-1	Redish black	0.5×10^3	Circular	Rough	Zonation
MF-4	Black	No	Circular	Smooth	Zonation
BHU-1	Brownish black	No	Circular	Smooth	Zonation
BX-2	Black	No	Irregular	Rough	Without zonation
IIVR	Whitish black	No	Irregular	Rough	Zonation

most of the isolates with highest radial growth was observed at 7 days after inoculation in isolate RF-1 (35.50 mm) which was in growth rate at par with isolates AF-2 (35.25 mm) and BG (34.05 mm). Maximum radial growth was also observed at 10 DAI in isolate EC-1 (52.00 mm) that was at par with isolates MF-4 (51.75 mm), IIVR (51.75 mm), SF-1 (50.75 mm), PN-4 (50.50 mm) and BHU-1 (50.25 mm). Maximum radial growth was also observed in two isolates IIVR and BHU-1 (88.75 mm) that was at par with isolates MF-4 (88.50 mm), BX-2 (87.75 mm) and SF-1 (87.00 mm) at 13 DAI. But the maximum mean mycelial growth was observed in isolate IIVR (57.83 mm) followed by MF-4 (57.66 mm) and BHU-1 (56.83 mm) and minimum mean mycelial growth was observed in isolate BG (50.16 mm) followed by RF-1 (50.66 mm) and AF-2 (52.83 mm). During the studies of cultural variability amongst isolates of *Alternaria solani*, most of the isolates of *Alternaria solani* were not significantly different in radial growth. But some of the isolates depicted variation in the growth on PDA medium. Some workers, (Rath and Padhi, 1973; Gupta and Nikharaj, 1972; Prasad *et al*., 1973; Stevenson and Pennypacker, 1988; Sodlauskiene *et al*., 2003; Rodriquez and Santana, 1991) tested the isolate of *A. solani* on particular temperature for observing the cultural characters.

Pigmentation: Isolates of *A. solani* depicted great variability in pigment production on PDA medium (Table 3). Two isolates (AF-2 and BHU-1) produced brownish black, two (BG and RF-1) greenish black pigment, two isolates (EC-1 and SF-1) reddish black pigment, two isolates (MF-4 and BX-2) were black pigmented while remaining two isolates (IIVR and PN-4) whitish black pigmented on PDA after 7 days of inoculation at 25±2°C. These isolates exhibited significant variation for their pigmentation. The cultural characteristics (colour, growth and sporulation) also differed in various isolates, making it possible to find almost as many races as the number of isolates tested (Rotem, 1966).

Sporulation: Conidia production was observed only in two isolates (EC-1 and SF-1) with low quantity i.e., 2×10^3 and 0.5×10^3, respectively (Table 3). Remaining eight isolate produced only mycelial growth in petri plates on PDA medium. Highest number of conidia were produced by isolate EC-1. In the study on sporulation of ten isolates of *Alternaria solani* only two isolates depicted sporulation on PDA medium. Some researchers such as (Kuangai and Oda, 1969; Kaoru and Mitsuo, 1970; Fourtouni *et al*., 1998; Lukens, 1962; Douglas and

Table 4: Mean AUDPC obtained after inoculating 5 tomato varieties with 10 different isolates of *Alternaria solani*

	Isolates										
Varieties	AF-2	BG	RF-1	PN-4	EC-1	SF-1	MF-4	BHU-1	BX-2	IIVR	Mean A
H-86	1383.37	1335.85	1373.99	1126.01	1051.33	983.78	1461.42	1115.32	1119.51	1083.74	1203.43
Sel-7	1367.88	1476.85	1484.90	1691.36	1278.21	1,303.29	1723.54	1325.26	1170.38	1353.96	1417.56
Feb-2	856.38	800.79	773.91	847.73	815.39	900.33	1044.90	695.12	657.42	672.28	806.42
Flora date	540.90	318.03	198.13	438.30	487.52	129.98	496.73	314.24	11.35	152.70	308.79
Swarna Naveen	113.58	350.84	45.43	455.59	289.00	159.01	371.41	68.15	136.29	254.92	224.42
Mean B	852.42	856.47	775.27	911.80	784.29	695.28	1019.60	703.61	618.99	703.52	792.13

Table 5: Grouping of varieties on the basis of mean AUDPC

Varieties	Mean AUDPC	Reaction
Sel-7	1417.560	Highly susceptible
H-86	1203.430	Susceptible
Feb-2	796.440	Resistant
Flora date	308.790	Highly resistant
Swarna Naveen	224.430	Highly resistant
LSD	86.623	

Table 6: Grouping of isolates on the basis of mean AUDPC

Group	Isolates	AUDPC	Pathogen reaction
A	MF-4	1019.60	HV
AB	PN-4	911.80	HV
BC	BG	856.47	V
BC	AF-2	852.42	V
CD	EC-1	784.29	V
CD	RF-1	775.27	V
DE	BHU-1	703.62	LV
DE	IIVR	703.52	LV
DE	SF-1	675.30	LV
E	BX-2	618.99	LV
LSD	122.5		

Pavek, 1972; Cotty, 1987) have done work on the effect of light, blue light, U.V. light for the sporulation of *A. solani* and also its other species as *A. tagetica*, *A. alternata* and *A. kikuchiana*.

Mycelial growth/colony character: Mycelial growth patterns were observed on PDA where BG, RF-1, SF-1, MF-4 and BHU-1 grew with circular margin with smooth surfaced colony and AF-2, PN-4, EC-1, BX-2 and IIVR isolates were growing with irregular margin and rough surface (Table 3).

Zonation: Out of 10 isolates, six isolates (PN-4, EC-1, SF-1, MF-4, BHU-1 and IIVR) depicted zonation and remaining four isolates (AF-2, BG, RF-1 and bx-2) were by without zonation (Table 3). In the study of colony character of isolates of *A. solani*, the mycelial growth pattern and zonation were found significantly different. So it is concluded that the mycelial growth pattern and zonation on PDA depends on nature of isolates. Similar experimemt was conducted by Kumar *et al.* (2008).

Pathogenic variability of *A. solani*

Area Under Disease Progress Curve (AUDPC): Five varieties of tomato were screened with 10 different isolates. The variety Selection-7 was observed highly susceptible with all the isolates (Mean AUDPC-1417.56) and variety H-86 was observed susceptible with all the isolates (Mean AUDPC-1203.43). Variety Feb-2 was observed resistant with all the isolates (Mean AUDPC-796.44) and variety Flora Dade and Swarna Naveen was observed highly resistant with all the isolates (Mean AUDPC-308.79 and Mean AUDPC-224.43), respectively. There was no significant difference between Flora Dade and Swarna Naveen (Table 4 and 5, Fig. 1).

Out of 10 isolates, only three groups were recorded on the basis of SAS analysis likewise Group-A isolates indicate highly virulent (MF-4 and PN-4) with Mean AUDPC 1019.60 and 911.80, Group-C isolates indicate virulence (BG, AF-2, EC-1 and RF-1) with mean AUDPC 856.47, 852.42, 784.29 and 775.27 and Group-E isolates indicate less virulence (BHU-1, IIVR, SF-1 and BX-2) with mean AUDPC 703.62, 703.52, 675.30 and 618.99, respectively with all five varieties (Table 4 and 6; Fig. 1). These isolates were collected from different geographical area. Shahbazi *et al.* (2010) observed varying degrees of virulence among *A. solani* isolates collected from different geographical locations. Disease severity was scored on 0-9 rating scale and phenotyping of variety was done (Ghosh *et al*., 2009).

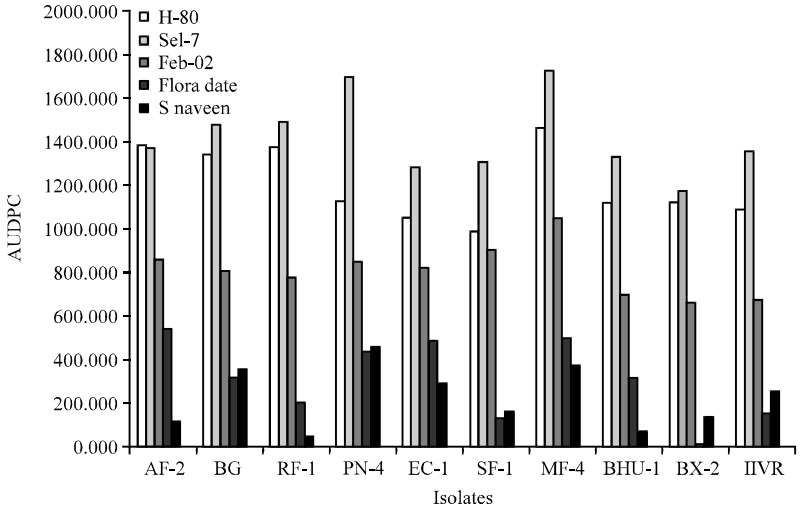

Fig. 1: Effect of *Alternaria solani* isolates on tomato varieties for disease development

REFERENCES

Barksdale, T.H., 1969. Resistance of tomato seedlings to early blight. Phytopathology, 59: 443-446.

Campbell, C.L. and L.V. Madden, 1990. Introduction to Plant Disease Epidemiology. Wiley, New York, ISBN-13: 9780471832362, Pages: 532.

Cotty, P.J., 1987. Modulation of sporulation of *Alternaria tagetica* by carbon dioxide. Mycologia, 79: 508-513.

Douglas, D.R. and J.J. Pavek, 1972. Screening potatoes for field resistance to early blight. Am. J. Potato Res., 49: 1-6.

Fourtouni, A., Y. Manetas and C. Christias, 1998. Effect of UV-B radiation on growth, pigmentation and spore production in the phytopathogenic fungus *Alternaria solani*. Can. J. Bot., 76: 2093-2099.

Ghosh, P.P., D. Mandal, S. Laha and M.K. Dasgupta, 2009. Dynamics and severity model in managing fungal diseases. J. Plant Prot. Sci., 1: 55-59.

Gupta, D.P. and N.S. Nikhiraj, 1972. Host relations in *Alternaria* blight of potato: Germination of spore. J. Bihar Bot. Soc., 1: 22-26.

Johnson, D.A. and R.D. Wilcoxson, 1982. A table of areas under disease progress curves. Technical Bulletin, Texas Agricultural Experiment Station, Texas, USA.

Kaoru, O. and N. Mitsuo, 1970. Effect of light on sporulation of *Alternaria kikuchina* Tanaka. Ann. Phytopathol. Soc. Jpn., 36: 11-16.

Kuangai, T. and Y. Oda, 1969. Blue and near ultraviolet reversible photoreaction in conidial development of the fungus, *Alternaria* tomato. Dev. Growth Differ., 11: 130-142.

Kumar, V., S. Haldar, K.K. Pandey, R.P. Singh, A.K. Singh and P.C. Singh, 2008. Cultural, morphological, pathogenic and molecular variability amongst tomato isolates of *Alternaria solani* in India. World J. Microbiol. Biotechnol., 24: 1003-1009.

Locke, S.B., 1949. Resistance to early blight and *Septoria* leaf spot in the genus *Lycopersicon*. Phytopathology, 39: 824-836.

Lukens, R.J., 1962. Photo-inhibition of sporulation in *Alternaria solani*. Am. J. Bot., 20: 720-723.

Nash, A.F. and R.G. Gardner, 1988. Heritability of tomato early blight resistance derived from *Lycopersicon hirsutum* PI 126445. J. Am. Soc. Hort. Sci., 113: 264-268.

Neergaard, P., 1945. Danish Species of *Alternaria* and *Stemphylium*: Taxonomy, Parasitism, Economic Significance. Oxford University Press, London, pp: 260-287.

Pound, G.S. and M.A. Stahmann, 1951. The production of toxic material by *Alternaria solani* and its relation to the early blight disease of tomato. Phytopathogy, 41: 1104-1114.

Prasad, B., B.L. Dutt and B.B. Nagaich, 1973. Inducing sporulation in *Alternaria solani* I. Effect of water treatment. Mycopathologia Mycologia Applicata, 49: 141-146.

Rath, G.C. and N.N. Padhi, 1973. Sporulation of *Alternaria solani* in pure culture. Indian Phytopathol., 26: 495-501.

Rodriquez, A.C.M. and R. Santana, 1991. Effect of new culture media and temperature on the growth *in vitro* of *Alternaria solani*. Centro Agricola, 18: 86-88.

Rotem, J., 1966. Variability in *Alternaria porri* f. sp. *Solani*. Israel J. Bot., 15: 48-57.

Shahbazi, H., H. Aminian, N. Sahebani and D.A. Halterman, 2010. Biochemical evaluation of resistance responses of potato to different isolates of *Alternaria solani*. Phytopathology, 100: 454-459.

Sherf, A.F. and A.A. MacNab, 1986. Vegetable Diseases and their Control. John Wiley and Sons, New York, pp: 634-640.

Sodlauskiene, A., A. Rasinskiene and E. Surviliene, 2003. Influence of environmental conditions upon the development of *Alternaria* genus fungi *in vitro*. Sodininkyste Darzininkyste, 22: 160-166.

Stevenson, R.E. and S.P. Pennypacker, 1988. Effect of radiation, temperature and moisture on conidial germination of *Alternaria solani*. Phytopathology, 78: 926-930.

Tong, Y.H., J.N. Liang and J.Y. Xu, 1994. Study on the biology and pathogenicity of *Alternaria solani* on tomato. J. Jiangsu Agric. Coll., 15: 29-31.

Wheeler, B.E.J., 1969. An Introduction to Plant Diseases. 2nd Edn., John Wiley and Sons Limited, London, Pages: 301.