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Cultural and morphological variability of *Alternaria* species isolates causing early blight of potato

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Abstract

Twenty *Alternaria* species were collected from different locations from rabi season of 2021 and 2022. Out of these 20 isolates, 15 were of *Alternaria alternata* designated as, AA 1, AA 2, AA 3, AA 4, AA 5, AA 6, AA 7, AA 8, AA 9, AA 10, AA 11, AA 12, AA 13, AA 14, AA 15 and 5 were of *Alternaria solani* designated as, AS 1, AS 2, AS 3, AS 4, AS 5. Cultural characteristics based on colony colour and mycelial width were observed at different time intervals. The colony colour of *Alternaria alternata* was dark green in colour whereas, *Alternaria solani* is black and colony diameter of every isolate varies from each at different time interval. Morphological characteristics of *Alternaria alternata* conidia were septated by 1-2 vertical and 5-6 horizontal septa, while *Alternaria solani* conidia were septated by 3-4 vertical and 2-10 horizontal septa. Conidia in *Alternaria solani* range in length from 90 to 160 m, while conidia in *Alternaria alternata* range in length from 60 to 90 m. *Alternaria solani* has a beak length range of 18 to 22 mm, whereas *Alternaria alternata* has a beak length range of 8 to 11 mm. *Alternaria solani* and *Alternaria alternata* both have mycelial widths that span roughly from 9 to 12 metres.

Keywords: *Alternaria* species, *Alternaria solani*, *Alternaria alternata*, Variability

Introduction

Potato (*Solanum tuberosum*) is most important vegetable crop in the world, it is also known as king of vegetables and originated from Peru (South America). It is most important cash crop in the world and also forth most produced crop after rice, wheat and maize.

The potato tuber is primarily composed of water (75-80%), carbohydrates (6-20%), a small amount of fat (0.1-0.2%), and some amino acids. The mineral level of potatoes is good, ranging from 0.8 to 2.0 percent, and they also have 0.6 percent fiber. Additionally, it has vitamin C, B, and B₂ content, increasing its nutritional worth value (Walt and Merrill, 1963) [18].

Potatoes by product are primarily used for animal feeding but they have a wide range of uses and can be used as a source of nutrition and pharmaceutical importance. Potato peels are used in muffin preparation where they replace wheat and prevalent muffin oxidation is caused by oxidation of the potato peel (Hung *et al.*, 2004) [4].

Potato crop is infested by a variety of fungal, bacterial and viral diseases. *Alternaria* is a ubiquitous genus of Deuteromycetes fungi. They are air borne and mainly contaminate the aerial parts of the plants and tubers also. Some *Alternaria spp.* have shown promise as biocontrol agents invasive plant species and some also as highly bioactive metabolites.

There are majorly two species of *Alternaria* which affects potato crop i.e. *Alternaria alternata* and *Alternaria solani*. The *Alternaria alternata* infects the solanaceous crops mainly. It forms lesions similar to *Alternaria solani*. Spores are smaller (20-63×9-18 μm) and in form of chains and do not bear long beak. Conidia produced on synthetic media is smaller than conidia formed on plant tissue. It can cause brown spotting of leaves and black pitting in tuber tissue. *Alternaria solani* affects solanaceous crops majorly and cucurbits as well (Ellis & Gibson, 1975) [1]. Its conidia are longer than that of *Alternaria alternata* (15-19×150-300 μm), the size varies in different isolates mostly. The mycelium is usually dark grey or black in color. *Alternaria* bears dense colonies and possess hairy like appearance on media. Mycelium occurs on temperature ranging 1-45 °C but optimum temperature for growth on media is 28 °C.

In the present investigation, the extent of cultural and morphological variability, studied among 15 isolates of *A. alternata* and 5 isolates of *A. solani* collected from different locations.

Material and Methods

The present investigation was carried out at the Division of Plant Protection, ICAR- Central Potato Research Institute, Regional Station, Modipuram, Meerut, U.P., India.

Isolation and purification of *Alternaria* isolates was done

from fresh infected leaf, collected from year 2021 and 2022 from different locations of Uttar Pradesh (Table 1) and purified by the hyphal tip method. They were then incubated at 26 ± 1 °C temperature for observation (Kumar *et al.*, 2020) [7].

Table 1: Fungal isolates with their place of collection, year of collection and crop

S. No.	Isolate Name	Place of collection	Collection Year	Crop
1	AA 1	Uttar Pradesh	Rabi 2020-21	Potato
2	AA 2	Uttar Pradesh	Rabi 2020-21	Potato
3	AA 3	Uttar Pradesh	Rabi 2020-21	Potato
4	AA 4	Uttar Pradesh	Rabi 2020-21	Potato
5	AA 5	Uttar Pradesh	Rabi 2020-21	Potato
6	AA 6	Uttar Pradesh	Rabi 2020-21	Potato
7	AA 7	Uttar Pradesh	Rabi 2020-21	Potato
8	AA 8	Uttar Pradesh	Rabi 2020-21	Potato
9	AA 9	Uttar Pradesh	Rabi 2020-21	Potato
10	AA 10	Uttar Pradesh	Rabi 2020-21	Potato
11	AA 11	Uttar Pradesh	Rabi 2020-21	Potato
12	AA 12	Uttar Pradesh	Rabi 2020-21	Potato
13	AA 13	Uttar Pradesh	Rabi 2020-21	Potato
14	AA 14	Uttar Pradesh	Rabi 2020-21	Potato
15	AA 15	Uttar Pradesh	Rabi 2021-22	Potato
16	AS 1	Uttar Pradesh	Rabi 2021-22	Potato
17	AS 2	Uttar Pradesh	Rabi 2021-22	Potato
18	AS 3	Uttar Pradesh	Rabi 2021-22	Potato
19	AS 4	Uttar Pradesh	Rabi 2021-22	Potato
20	AS 5	Uttar Pradesh	Rabi 2021-22	Potato

*AA denotes the isolates of *Alternaria alternata* and AS denotes the isolates of *Alternaria solani*

Cultural variability of different isolates of *Alternaria spp.*

Twenty isolates from various locations and different years were isolated as pure cultures and kept under observation until the petri plate was fully filled, or 9 cm, in order to observe the cultural diversity among *Alternaria*. For a few days, growth was noticed and noted every 24 hours. When the fungal colony covered the plate, colour and difference in growth was noticed and recorded.

Morphological variability of different isolates of *Alternaria spp.*

Spores of *Alternaria spp.* were collected from the pure culture and put on the transparent glass slide to examine morphological variability. In order to provide a uniform distribution, spores were well mixed with lactophenol or distilled water before being dispersed over a cover slip. The fungus hyphae and spores were examined and photographed with a camera lucida coupled to a compound microscope.

Results and discussion

Cultural characters of different isolates of *Alternaria spp.*

Twenty isolates of *Alternaria* were grown on PDA to know the diversity among them. The bit of *Alternaria* culture was placed on the media and incubated at 26 ± 1 °C for 15 days. The colony characters and measurements were recorded after incubation at every 24 hours till 10 days. The results of colony growth of every isolate at different temperature are presented in Table 2. The colony colour of isolates of *Alternaria alternata* was dark green in colour whereas colony colour of *Alternaria solani* is black in colour. Different isolates shown different growth at different interval. At 48

hours, AA 13 has the highest colony diameter (34.00 mm), whereas AA 10 has the lowest (26.00 mm). At 72 hours, AS 3 has the highest colony diameter (44.25 mm), whereas AA 10 has the lowest (35.00 mm). At 96 hours, AS 1 has the highest colony diameter (51.00 mm), whereas AS4 has the lowest (43.50 mm). At 120 hours, AA 13 has the highest colony diameter (61.00 mm), whereas AS4 has the lowest (51.50 mm). At 144 hours, AA 13 has the highest colony diameter (70.25 mm), whereas AA 10 has the lowest (59.25 mm). At 168 hours, AA 13 has the highest colony diameter (77.75 mm), whereas AA 7 and AA 10 has the lowest (67.25 mm). At 192 hours, AA 13 has the highest colony diameter (86.25 mm), whereas AA 1 and AA 15 has the lowest (75.25 mm). At 216 hours, AA 13 and AS 2 has the highest colony diameter (90.00 mm), whereas AA 14 has the lowest (83.25 mm). At 240 hours, all plates get full by 90.00 mm growth of mycelium. The result obtained in the present study at par with the finding of (Najibullah *et al.*, 2016) [9]. They reported variation in colony diameter among the two isolates of the *Alternaria solani* were identified and both of the test isolates showed a wide range of variability in respect of their mycelial and conidial dimensions and septation. (Kaul and Saxena 1988) [5] observed differences in cultural characters like growth rate, type of growth, colony colour of the substrate and sporulation of the isolates. Some workers, (Rath and Padhi 1973) [12]; (Gupta and Nikharaj 1972) [2]; (Prasad *et al.*, 1973) [10]; (Sodlauskienė 2003) [15]; (Rodriguez and Santana 1991) [13]; (Jadhav *et al.*, 2011) [4] and (Ramegowda 2007) [11] examined the isolate of *A. spp.* at a specific temperature to look at its cultural traits and sporulation.

Table 2: Cultural variability of *Alternaria spp.*

Isolates	Colony Color	Colony Diameter (mm) At the interval of every 24 hours								
		48	72	96	120	144	168	192	216	240
AA 1	Dark Green	28.25	36.00	43.75	52.00	60.00	68.00	75.25	83.50	90.00
AA 2	Dark Green	29.00	37.25	45.25	53.50	60.50	67.75	75.50	83.50	90.00
AA 3	Dark Green	29.50	37.25	44.25	52.25	59.50	67.75	76.25	84.75	90.00
AA 4	Dark Green	29.75	37.50	46.50	54.75	62.50	70.25	78.25	86.00	90.00
AA 5	Dark Green	30.25	37.50	46.25	54.25	61.75	69.50	77.00	85.25	90.00
AA 6	Dark Green	33.00	36.25	44.00	52.00	61.25	70.50	78.50	85.75	90.00
AA 7	Dark Green	28.00	35.75	45.25	52.75	59.75	67.25	75.75	84.75	90.00
AA 8	Dark Green	33.25	41.75	50.00	58.00	65.25	75.25	79.75	86.00	90.00
AA 9	Dark Green	31.50	39.75	47.25	55.25	63.50	71.25	79.75	86.75	90.00
AA 10	Dark Green	26.00	35.00	43.75	52.00	59.25	67.25	76.00	84.75	90.00
AA 11	Dark Green	27.25	36.50	44.50	54.00	62.00	70.75	79.00	86.75	90.00
AA 12	Dark Green	30.75	39.00	44.90	53.75	61.75	70.00	77.25	85.00	90.00
AA 13	Dark Green	34.00	42.75	52.00	61.25	70.25	77.75	86.25	90.00	90.00
AA 14	Dark Green	29.50	37.00	45.25	53.00	61.00	68.75	75.50	83.25	90.00
AA 15	Dark Green	31.00	39.75	50.00	57.00	63.25	69.75	75.25	84.25	90.00
AS 1	Black	33.25	42.50	51.00	59.75	68.25	75.75	82.25	88.50	90.00
AS 2	Black	32.00	40.25	49.25	58.75	67.25	76.00	84.25	90.00	90.00
AS 3	Black	31.25	44.25	46.50	54.75	63.00	70.75	77.75	85.75	90.00
AS 4	Black	27.75	35.00	43.50	51.50	59.50	68.00	76.25	84.75	90.00
AS 5	Black	30.25	38.00	46.25	54.50	63.25	71.00	78.50	86.00	90.00

Morphological characters of different isolates of *Alternaria spp.*

The different isolates collected during survey were subjected to morphological variability tests and the results are presented in the Table 3. The study showed that, conidia of different isolates were septated by 1-2 vertical and 5-6 horizontal septa in *Alternaria alternata* and conidia were septated by 3-4 vertical and 2-10 horizontal septa in *Alternaria solani*. The length of conidia in *Alternaria alternata* ranges from 60-90 μm and in *Alternaria solani* conidial length ranges from 90-160 μm . The maximum length of conidia is recorded in AS 15 (127.27 μm) and the minimum is recorded in AA 12 (66.21 μm), whereas in case of width of conidia, the maximum width is recorded in AS 5 (44.43 μm) and minimum width is recorded in AA 2 (24.50 μm). Beak length in case of

Alternaria alternata is in range of 8-11 μm and in case of *Alternaria solani* it ranges between 18-22 μm . The maximum beak length is recorded in AS 4 (23.69 μm) and minimum beak length was recorded in AA 2 (9.11 μm), whereas, in case of mycelial width, the maximum mycelial width was recorded in AS 1 (12.53 μm) and minimum was recorded in AA 8 (9.11 μm). Mycelial width of both *Alternaria solani* and *Alternaria alternata* ranges from 9 μm to 12 μm approximately. These results are in similarity with earlier workers (Varma *et al.*, 2006) [17]; (Meena *et al.*, 2005) [8]; (Kaur *et al.*, 2007) [6]; (Singh *et al.*, 2007) [14]; (Stevenson and Pennypacker 1988) [16], who discovered morphological variation among *Alternaria spp.* from various geographic isolates.

Table 3: Morphological variability (number of septa, length and width of conidia, length of beak and mycelia width) of *Alternaria spp.* isolates

Isolates	*No. of Septa		*Conidia (μm)				*Beak Length (μm)	*Mycelial Width (μm)
	Horizontal	Vertical	Length (range)	Length (mean)	Width (range)	Width (mean)		
AA 1	2	5	68-83	71.50	24-35	27.65	10.66	11.21
AA 2	1	4	60-85	73.00	22-34	24.50	9.11	9.50
AA 3	2	4	69-100	78.00	24-37	31.25	9.72	10.20
AA 4	2	5	61-78	67.62	25-35	30.25	10.62	9.56
AA 5	2	6	61-98	75.66	23-38	29.13	10.71	9.68
AA 6	1	4	60-83	70.67	24-38	31.76	9.15	10.60
AA 7	1	5	63-87	68.63	22-35	27.47	11.31	9.48
AA 8	1	4	63-88	74.11	24-33	27.68	9.42	9.11
AA 9	1	4	60-87	66.60	23-36	28.32	9.33	10.28
AA 10	1	5	72-92	77.42	25-37	29.43	10.52	9.56
AA 11	2	5	58-76	69.10	23-39	30.68	11.21	10.83
AA 12	1	4	57-83	66.21	25-33	27.43	10.56	10.44
AA 13	1	5	63-79	68.20	23-35	29.65	9.43	11.23
AA 14	1	5	67-86	70.68	22-34	29.88	10.26	9.49
AA 15	2	4	61-81	67.52	22-37	30.52	9.57	10.82
AS 1	2	8	87-162	103.56	37-54	43.87	18.80	12.53
AS 2	2	10	90-158	112.52	28-55	40.27	23.51	11.89
AS 3	3	7	90-170	125.54	29-53	41.79	21.59	9.86
AS 4	4	8	89-165	117.63	29-54	43.25	23.69	9.79
AS 5	4	8	94-169	127.27	31-57	44.43	22.96	10.56

*Average mean of 8 replications for each isolate

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