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STUDIES ON VARIABILITY IN *ALTERNARIA ALTERNATA* (KESSLER) CAUSING LEAF BLIGHT OF ISABGOL (*PLANTAGO OVATA*)

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ABSTRACT
All the five isolates differed in colony characters i.e. dark black colourd and very fast mycelial growth (90.00mm), light black with white at centre and fast growing(80.00mm), dark brown and mycelial growth (75.00mm) with smooth margin, black colourd, flat mycelial growth (68.00mm)and white with slightly black in colour with slow mycelial growth (65.00mm) were observed in Aa-1, Aa-2, Aa-3, Aa-4 and Aa-5 respectively.Similarly spore (conidial) morphology also varied in terms of length and width with beak and without beak in all five isolates.The isolates Aa-1 was found to be highly pathogenicwhich showing 52.12% disease intensity followed by Aa-3 (47.56%), Aa-2 (41.40%) and Aa-4 (38.20%) isolates.However in toxin production, where, Aa-1 was very severely toxic followed by Aa-2 and so on. This suggests that isolates differed in toxin production and their role in aggravating of the disease.

INTRODUCTION

Isabgol, *Plantago ovata*, belongs to a large genus of herbs distributed mostly in the temperate regions and a few in the tropics. Plantago in commerce is important for its seeds and husk which have been used in the indigenous medicine for many centuries. Seed is the important plant part which has medicinal properties. The husk, which is separated from the seeds, has the property of absorbing and retaining water and therefore it works as an anti-diarrhoea drug. It comprises about 800 species of which 10-14 are natives of India.In India, it is mainly cultivated in Mehsana and Banaskantha district of Gujarat and adjoining districts of Rajasthan and to a limited extent in Haryana. Presently Rajasthan is a dominating state in Isabgol production.Isabgol is cultivated in about 2,27,705 hectares in Rajasthan with the production of 1,39,998 tones (Govt. of Rajasthan, 2009-10). Isabgol growing districts of Rajasthan are Jalore, Barmer, Jodhpur, Bikaner, Pali, Sirohi, Chittorgarh and Udaipur. However, *Plantago ovata*, commonly known in English as Blonde Psyllium

Mandal (2010) reported that a number of pathogens from Isabgol*viz.Fusarium wilt* (*Fusarium oxysporum*), damping off (*Pythium ultimum*), leaf blight (*Alternaria alternata* (Fr.) Keissler), downy mildews (*Peronospora plantaginis* Underwood, *Peronospora alta* Fuckel and *Pseudopernospora plantaginis*) and powdery mildew (*Erysiphe cichoracearum* D.C.). Leaf blight of Isabgol *(Plantago ovata)* has become a serious problem in recent years. It has been found that downy mildew affected crop is more prone to be attacked by *Alternaria alternata*. It causes considerable damage every year and sometimes become very severe which results in total loss of yield.

Variability studies are important to document the changes occurring in populations and individuals as variability in morphological and physiological traits indicate the existence of different pathotypes. The variability is a well known phenomenon in genus Alternaria and may be noticed as changes in spore shape and size, growth and sporulation, pathogenicity, etc. Pathogen population structure and mechanisms by which variation arises within a population is of paramount importance for devising a successful disease management strategy. This requires continuous monitoring of the development of pathogen variability more so for the breeding programme aimed at developing resistant genotypes to the given set of pathogenic races (Sartorato, 2002). Variation in pathogen populations can generally be detected with methods like cultural, spore morphology, pathogenic and toxin variability. Hence, present investigations were carried out to study the variability in *Alternaria alternata* (Kessler) causing leaf blight of Isabgol (*Plantago ovata*).

MATERIALS AND METHODS

Isolation, Purification and Identification of the pathogen

The fungal isolates were collected from five different Agro climate zone of Rajasthan *i.e.* R.C.A. farm (Udaipur), Kapasan (Chittorgarh), Mandore (Jodhpur), Sumerpur (Pali) and Keshwana (Jalore). On the basis of morphological, cultural and pathogenic characteristics, the isolates were identified as

Alteraria alternata (Fr.) Keissler. Pathogenicity test was done according Koch's postulates for all the five isolates. The identity of R.C.A. farm (Udaipur) isolate was confirmed by Indian Type Culture Collection (ITCC), Division of Plant Pathology, IARI, New Delhi-110012 (The ITCC Code no. 6317, 2008).The culture was purified by single spore technique.The single spore technique was conducted as per the method described by Sofi *et al*. (2013).

Cultural variability

For cultural variability, five isolates of the pathogen were grown on PDA to observe their growth pattern. The 5mm discs of pure culture of isolates were inoculated at the center of the pre poured Petri plates from 10 days old actively growing culture.All inoculated plates were incubated attemperatureof 25ºC ± 1ºC in BOD (Biological oxygen demand) incubator. Each isolate was replicated thrice. The growth rate was measured and colony characters, pigmentation, growth habit and sporulation were measured after 24 hrs of incubation till the growth of the pathogen in Petri plate completes.

Variability in spore morphology

To purify *Alternaria* culture, a conidial suspension was prepared in sterilized distilled water from 10 days old culture on PDA and flooded on 2% plain agar in Petri plates. The excess suspension was drained out and the Petri plates were then incubated in inverted position at 25 ±1ºC. After eight hours a single germinating spore was marked with the help of dummy objective and then transferred individually with a piece of plane agar medium to PDA slants by inoculating needle under aseptic conditions. These monoconidial isolates maintained on PDA slants were used to study spore morphology as described by Boedo *et al*. (2012). The observations on variation in conidial morphology of five isolates of *A. alternata* were recorded with the help of Ocular and Stage Micrometer.

Pathogenic variability

Pathogenic variability is the genetic character of fungi which may vary amongst isolates. Healthy seeds of Isabgol variety RI-89 were surface sterilized with 0.1% $HgCl_2$ and were sown @ 10 seeds per pot and replicated thrice. Leaves stem and branches of six weeks old plants were randomly selected, and these were injured gently by delicate brush and ten days old culture suspensions of isolates were sprayed with hand automizer in early morning hours, when dew deposition on leaves is there. Simultaneously, un-inoculated check was maintained by spraying sterilized distilled water on plants. The inoculated plants were observed daily to record the incubation period for the disease development. The disease intensity was calculated with the help of disease rating scale (1-5).The details of the rating scale are as follows.

Proposed percent disease incidence rating scale for individual plant (1-5)

1-20% infection or 1-20% leaves of the plant are infected.(2) 21-40% infection or 21-40% leaves of the plant are infected.(3) 41-60% infection or 41-60% leaves of the plant.(4) 61-80% infection or 61-80% leaves of the plant are infected.(5) 81-100% infection or 81-100% leaves of the plant are infected.

Numbers of plants in each score were recorded.

Per cent disease index was calculated from these data following standard formula byWheeler (1969), as given:

$$PDI = \frac{\sum 1xn + 2xn + 3xn + 4xn + 5xn}{N} X \frac{100}{\text{Maximum score (5)}}$$

Where

n = Number of plants in each score

N= Total number of plant

Toxin variability

Several phytopathogenic species of Alternaria have been reported to produce phytotoxic metabolites, which play a significant role in pathogenesis and many of them have been chemically characterized (Devi *et al.,* 2010a). For toxin variability, 25 mL Richards' medium (pH 6.5) in 100 ml sterilized flasks were inoculated with 5 mm diameter fungal bits of 10 days old culture of different isolates of *Alternaria alternata* grown on PDA and incubated at 25 ± 1ºC for 15 days. The culture filtrate was obtained by filtration through Whatman No.42 filter paper. The culture filtrates which were obtained from 15day old cultures of *Alternaria alternata* were centrifuged at 600 rpm for 20 min. The clear supernatant solutions were collected in clean sterilized conical flasks and pellet sedimented at the bottom of the centrifuge tube was discarded. The clear supernatant solutions served as samples of crude toxin produced by different isolates of *Alternaria alternata* used to study toxin variability, by using detached plant twigs dip method. Observations were recorded regarding toxicity symptom expressions like necrosis, leaf drooping, wrinkling and drying of leaves at regular intervals (6 hr, 12 hr 18 hr 20 hr and 24 hrs).

RESULTS AND DISCUSSION

Cultural variability

All the five isolates showeddifferences in colony characters *i.e.* dark black colored and very fast mycelial growth with smooth margin., light black with white at centre and fast growing., dark brown and mycelial growth with smooth margin., black colored, flat mycelial growth with smooth margin, and white with slightly black in colored with slow mycelial growth were observed in Aa-1, Aa-2, Aa-3, Aa-4 and Aa-5 respectively. The average radial growth of isolate Aa-1 was highest *i.e.* 90.00 mm while, in isolate Aa-2, Aa-3, Aa-4 and Aa-5, it was comparatively less *i.e.* 80.00 mm, 75.00 mm, 68.00 mm and 65.00 mm respectively on 7th day of incubation under uniform environments and medium. Sporulation was recorded in all five isolates but very good sporulation was observed in Aa-1. In view of the results obtained for cultural variation, it is clear that all the five isolates differed with respect to mycelial growth of *Alternaria alternata* attained after 7th day for sporulation and colony characters (Table 1).

These results are in agreement with Pandey *et al.* (2005) where, they concluded that variability in *Alternaria solani* exists only with respect to cultural characters. Eleven isolates of *A. solani* causing early blight of tomato showed cultural variability in respect of radial growth, growth rate per day and pigmentation on potato dextrose agar.These results are also in similarity with the results obtained by Verma *et al.* (2007), Raja and Reddy (2007) and Tetarwal *et al.* (2008).

Table 1: Cultural variability among five isolates of *Alternariaalternata* on PDA

S.no.	Isolates	Location of isolates	Radial mycelialgrowthin (mm)	Sporulation	Colony characters
1.	Aa-1	Udaipur	90.00	+ + + +	Dark black colourd, very fast mycelial growth with smooth margin.
2.	Aa-2	Chittorgarh	80.00	+ + +	Light black with white at centre and fast growing.
3.	Aa-3	Jodhpur	75.00	+ + +	Dark brown and medium mycelial growth with smooth margin.
4.	Aa-4	Pali	68.00	+ +	Black colourd, flat mycelial growth with smooth margin.
5.	Aa-5	Jalore	65.00	+ +	White with slightly black in colour and slow mycelial growth.
SEm ±			0.865		
CD at 5%			2.821		
CV%			2.46		

*Average of three replications; Note: + + = Fair, + + + = Good, + + + + = very good.

Table 2: Variation in conidial morphology of five isolates of *Alternariaalternata*

S. no.	Isolates	Conidial morphology with beak(μm)				Conidial morphology without beak(μm)			
		Length		Width		Length		Width	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range
1.	Aa-1	28.05 ± 1.68	23-31	17.18 ± 1.00	14-19	12.93 ± 1.76	6-18	8.73 ± 0.69	6-11
2.	Aa-2	24.84 ± 1.26	22-28	15.23 ± 0.76	13-16	10.22 ± 0.51	9-11	8.47 ± 0.42	7-10
3.'	Aa-3	32.19 ± 1.69	29-36	20.08 ± 1.48	14-23	16.67 ± 4.02	7-21	9.55 ± 0.46	8-11
4.	Aa-4	22.22 ± 0.99	20-24	15.23 ± 0.76	14-17	9.30 ± 0.72	8-12	7.88 ± 0.44	6-9
5.	Aa-5	28.01 ± 2.90	18-31	18.95 ± 1.91	12-22	12.07 ± 1.00	10-15	8.43 ± 0.44	7-9
SEm ±			0.26		0.19		0.34		0.05
CD at 5%			0.74		0.55		0.97		0.16
CV%			9.80		11.35		8.40		6.84

* Mean no. of 25 conidia and ± S.D. of mean value

Table 3: Pathogenic variability of five isolates *Alternariaalternata* under artificial inoculation conditions.

S.no.	Isolates	Disease Intensity (%)*	Incubation periods (days)*
1.	Aa-1	52.12(52.12)	4-5 days
2.	Aa-2	41.40(41.39)	4-6 days
3.'	Aa-3	47.56(47.56)	4-6 days
4.	Aa-4	38.20(38.19)	5-7 days
5.	Aa-5	35.48(35.48)	5-7 days
6.	Spray with water (Control)	0.00(0.00)	-
SEm ±	0.569		
CD at 5%	1.792		
CV%	2.41		

*Average of three replications; Figures in parentheses are angular transformed values

Variability in spore morphology

The perusal of data presented in Table 2 shows that Aa-1: Conidia were simple, obclavate, pale to dark brown formed in chains. Conidia have both transverse and vertical septa measuring 23-31 x 14-19μm (with beak) and 6-18 x 6-11μm (without beak). Aa-2: Conidia were light brown to dark brown, obclavate, measuring 22-28 x 13-16μm (with beak) and 9-11 x 7-10μm (without beak). Aa-3: Conidia dark brown colourd, long beak and both transverse and vertical septa were present. The size of conidia measuring 29-36 x 14-23μm (with beak) and 7-21 x 8-11μm (without beak). Aa-4: Conidia obclavate, shorten beak and light brown to dark brown in colour measuring 20-24 x 14-17μm (with beak) and 8-12 x 6-9μm (without beak).Aa-5: Conidia were light brown and measuring 18-31 x 12-22μm (with beak) and 10-15 x 7-9μm (without beak).

The present result was agreement with the results of Raja and Reddy (2007) whocollected the samples of leaf spot and fruit rot caused by *Alternaria alternata* from brinjal growing areas and it was found that the size of conidia varied from 35.2 - 43.5 μm and 12.4-13.9 μm wide, with average beak length of 9.6 -12.4 μm. Horizontal and vertical septations of conidia varied form 1.8 and 0.3, respectively and conidia were produced in chain. Similar results was also observed by Wagh *et al*. (2013) that seven days old culture of *Alternaria alternata* revealed hyaline, septate and branched mycelia, conidiophores with 30.0-80.2 μ length and 3-6 μ width and obclavate to obpyriform conidia (23-30 x 9.2-12.7 μ) with short conical beak arranged in acropetal fashion.

Pathogenic variability

The isolates of same pathogen collected from different geographical areas may show difference in virulence. The isolates Aa-1 was found to be highly pathogenic on Isabgol cv. RI-89 under artificial inoculation conditions, showing 52.12% disease intensity followed by Aa-3 (47.56%), Aa-2 (41.40%), Aa-4 (38.20%) and Aa-5 (35.48%). However, 4-5 days of incubation was recorded in Aa-1 followed by Aa-3 (4-6), Aa-2 (4-6), Aa-4 (5-7) and Aa-5 (5-7). The seedlings grown

Table 4: Toxin variability among five isolates based on their culture filtrates (crude toxin) toxicity symptoms on Isabgol leaves

S.No.	Isolates	Toxicity symptoms observed	Grade
1.	Un-inoculated broth	Did not show any toxicity.	Non toxic
2.	Aa-1	Initial toxicity symptom expression in six hours, leading to complete and severe necrosis of leaves with distinct black colourations.	Very Severely Toxic
3.	Aa-2	Initial toxicity symptom expression in twelve hours, leading to complete leaf drooping, wrinkling, drying and brittling of leaves.	Severely Toxic
4.	Aa-3	Initial toxicity symptoms expression in eighteen hours, leading to complete wrinkling and necrosis.	Moderately Toxic
5.	Aa-4	Initial toxicity symptoms expression in twenty hours, leading to slight necrosis.	Slight Toxic
6.	Aa-5	Initial toxicity symptoms expression after twenty-four hours, leading to least toxic.	Least toxic

applying sterilized distilled water without inoculation did not produce any blighted symptoms and grew healthy. (Table 3)

The pathogenic variability studies have also been carried out by Verma *et al.* (2007), Kumar *et al.* (2008) on *A. solani*. They recorded pathogenic variability among different isolates of *A. solani*. Tetarwal *et al.* (2008) observed variability among six isolates of *A. alternata* infecting Senna (*Cassia angustifolia*)

Toxin variability

The details of the experimental results are presented in Table 4. The culture filtrate is assumed as 100 per cent toxin concentration.This was simply an indicator test for toxin production. The symptoms like drooping of leaves, blackening of leaves was initiated at 6 hours and continued up to twenty-four hours, finally leading to wilting and necrosis, thus revealing the existence of variation among the isolates in producing toxic metabolites in the culture medium, which was reflected in terms of inducing wilting of Isabgol cuttings. The results indicated that Aa-1 isolate showed very severe toxic effect where initial toxicity symptom expression was within six hours, leading to complete and severe necrosis of leaves with distinct black colourations. Similarly, severely toxic , moderately toxic, slight toxic and least toxic effect were observed in cultural filtrate toxin of Aa-2, Aa-3, Aa-4 and Aa-5 isolates, respectively. This suggests that the toxin has active role in causing disease as well as mortality.

Such phytotoxic effects produced by culture filtrate were also reported by Reddy and Chaudhary (1990) where, they observed that, when pigeon pea seeds were soaked in culture filtrate of six *Fusarium udum* isolates for 6, 12, 24 h, no germination occurred after 24 h and radial length was also decreased with increasing in soaking time. Maiero *et al.* (1991) stated that *Alternaria solani* produced phytotoxic metabolites, and tomato seedlings exposed to culture filtrates for 20 h exhibited marginal and inter veinal leaf necrosis and subsequently wilting.

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