Documenting variation in *Alternaria brassicae* isolates based on conidial morphology, fungicidal sensitivity and molecular profile

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Abstract

Alternaria black spot (*Alternaria brassicae*) is an economically devastating disease of rapeseed-mustard in India. Losses in yield can exceed 50 percent depending on *Brassica* species, cultivar planted and prevailing weather. There is no known source of resistance to alternaria black spot in crop *Brassica* germplasm. In the absence of appropriate documentation on pathotype variation in the causal organism the past attempts at germplasm screening had at the best been tentative and had poor repeatability, possibly due to yearly variation in prevalent pathogenic profile. This communication documents the results of our attempt to collect and catalogue pathogenic variability in *Alternaria brassicae*. 322 isolations were made from alternaria black spot infested *Brassica* leaves collected from a wide geographic spread of north-west India. Detached leaf technique was used to test pathogencity of the isolates. 114 proved pathogenic. Among the pathogenic isolates 31, 24, 48 and 11 each were collected from *B. rapa*, *B. napus*, *B. juncea* and *B. carinata* respectively. These were purified using single spore culture and characterized on the basis of conidial morphology, colony diameter and sporulation patterns. Variation was recorded with range of 51.4-481.2 µm, 6.9-36.0 µm and 16.3 - 266.9 µm for conidia length, breadth and beak length respectively. Average number of horizontal, vertical septa in conidia and beak were 9.7, 0.8 and 3.7 respectively. Colony diameter and sporulation were in range of 13.0-77.5 mm and 1.0-4.0×10^6 spores / ml respectively. Colonies were brown, white, and olevaceous green with smooth or wavy margins and thick velvety to sparse growth. Morphological data was subjected to diversity analysis. Seven broad groups could be inferred, representing a very wide range of diversity. A representative isolate from each such morphological grouping was tested for relative sensitivity to six fungicides; dithiocarbamate, chlorothalonil, copper oxychloride, tebuconazole, difenoconazole and propiconazole by poison food technique at five concentrations each. Seven groups characterized on the basis of fungicidal reaction were concordant with those obtained on the basis of conidial morphology. Data on molecular diversity of pathogen isolates will be presented.

Key words: Oilseed rape, *Alternaria brassicae*, genetic diversity, conidial morphology, fungicidal sensitivity, molecular profile

Introduction

The black spot caused by *Alternaria brassicae* (Berk.) Sacc. is the most important disease and it occurs every year in all the rapeseed mustard growing states of India. The pathogen infects all aerial plant parts, reducing photosynthetic area and accelerating senescence and defoliation. Fruit infection results in premature shattering, leading to seed losses during harvesting. On average, yield loss of 46-47 percent in yellow sarson and 35-38 percent in mustard have been reported (Kolte 1985, Kolte *et.al,* 1987). Yield losses can go up to over 70 percent in highly susceptible cultivers. In addition to direct yield losses, the disease adversely affects the seed quality by reducing seed size, causing seed discolouration, and reduction in oil content (Kaushik *et.al,* 1984). All current oilseed *Brassica* cultivars are essentially susceptible to this pathogen. Variable economic losses may be construed as indicative of variability among the isolates and variability in resistance level of prevalent cultivars. Further, variation in effectiveness and efficiency of current fungicides to control alternaria blight has also been recorded. This may stem from variation in fungicidal sensitivity of common pathogen races. Development of resistant cultivars can be hastened by the knowledge of the pathogen variation. Present communication documents an attempt to assess diversity among *Alternaria brassicae* isolates in northwest India. Morphological variation, sensitivity to fungicides and polymorphism generated by random amplified polymorphic DNA (RAPD) markers were used to characterize variability of highly purified isolates.

Material and methods

322 isolations were made from alternaria black spot infested *Brassica* leaves collected from a wide geographic spread of northwest India. Pure cultures were prepared from single condia. Detached leaf technique was used to test the pathogenecity of the isolates. 144 proved pathogenic. These comprised 31, 24, 48 and 11 isolates from *B. rapa*, *B napus*, *B juncea* and *B carinata,* respectively.

Petriplates containing Potato Dextrose Agar (PDA) were inoculated in the center of each plate with a 5mm diameter plug obtained from the margin of 5 to 7 day old cultures grown on PDA. Colony diameter was recorded 7 days after inoculation while colony characteristics (colour and texture) were recorded after 15 days. For conidia morphology and sporulation, condia were sampled randomly from 15 days old colonies. Maximum length, width (at middle of spore), number of horizontal and vertical septa, beak length and beak septa were recorded from 50 conidia of each isolate. Sporulation was recorded using haemocytometer. Isolates were also tested for relative sensitivity to prevalent fungicides used for checking alternaria black

spot disease commercially. Three contact fungicides, dithiocarbamate, copper oxychloride and chlorothalonil and three systemic fungicides tebuconazole, difenocanazole, propiconazole were used. For dithiocarbamate, copper oxychloride and chlorothalonil; 50, 100, 500 ppm concentrations were used and for tebuconazole, difenocanazole, propiconazole concentrations used were 5, 10, 50 ppm. The effect of various fungicides on radial growth of isolates was studied by poison food technique. The radial mycelial growth was recorded seven days after inoculation. Genomic DNA was extracted from mycelium using protocol of Raeder and Broda, 1985. Thirty random decamer DNA primers were used in the study to generate polymorphism (Sharma and Tiwari, 1998).

Results

Morphological investigations indicated extensive variation with range of 51.4-481.2 µm, 6.9-36.0 µm and 16.3-266.9 µm for conidia length, breadth and beak length respectively. Average number of horizontal, vertical septa in conidia and beak were 9.7, 0.8 and 3.7 respectively. Colony diameter and sporulation were in range of 13.0-77.5 mm and 1.0-4.0×10^6 spores/ml respectively. Colonies were brown, white or olevaceous green with smooth or wavy margins and thick velvety to sparse growth. Reverse side of colonies were black, brown, white or olevaceous green. Analysis of these morphological descriptors allowed clustering of isolates in seven groups. Group five comprised maximum number of isolates. This was followed by group seven that included 36 isolates. The grouping was not consistent with geographic or species diversity.

Table 1. Grouping of *Alternaria brassicae* isolates based on conidial morphology, growth and sporulation.

Group	Isolate(s)*
1.	27R, 47R
2.	22G, 23G
3.	1C, 41G, 38R, 39R, 79R, 35T, 36T
4.	10C, 12G, 26G, 29G, 30G, 48G, 66R, 67R, 128R, 15T, 54T
5.	6C, 7C, 6G, 13G, 18G, 28G, 36G, 38G, 39G, 49G, 50G, 51G, 12R, 14R, 20R, 21R, 26R, 42R, 44R, 48R, 64R, 69R, 88R, 103R, 117R, 120R, 125R, 126R, 127R, 131R, 10T, 14T, 17T, 18T, 26T, 34T, 50T, 51T, 53T, 56T
6.	9C, 2R, 3R, 28R, 30R, 31R, 32R, 52R, 53R, 90R, 1T, 19T, 22T, 23T, 24T
7.	2C, 3C, 4C, 5C, 8C, 11C, 15G, 25G, 31G, 40G, 47G, 1R, 4R, 6R, 18R, 41R, 49R, 54R, 62R, 81R, 87R, 102R, 109R, 123R, 124R, 7T, 8T, 9T, 12T, 16T, 20T, 25T, 28T, 29T, 30T, 33T, 60T

* R: Brassica juncea ; T: Brassica rapa ; G: Brassica napus ; C: Brassica carinata

At discriminatory dose for contact and systemic fungicides viz 50, 100, 500 ppm and 5, 10, 50 ppm respectively, the isolates showed relative sensitive reaction to tested fungicides. Twenty four groups were characterized on the basis of fungicidal reaction (Table 2). These groups were not exactly consisted with the grouping based on conidia morphology, growth and sporulation.

Table 2. Relative sensitivity of isolates to different fungicides

Group	Isolate(s)*
1	38G
2	11C
3	3C, 81R, 7T, 8T, 56T, 60T
4	2C, 4C, 8C,9C, 6G, 38R, 123R, 50T
5	12G, 23T
6	25G, 2R, 28T
7	30T
8	26G, 62R
9	16T
10	13G, 41G, 25T
11	1T, 17T
12	90R, 9T, 20T
13	47G, 4R, 103R
14	48G, 14T
15	15T
16	3R, 29T
17	54R, 124R
18	20R, 21R, 49R
19	5C, 127R
20	4C, 10C, 23G, 39G, 50G, 51G, 30R, 39R, 41R, 66R, 69R, 88R, 12T, 18T, 24T, 26T
21	7C, 18G, 30G, 36G, 12R, 53R, 120R, 126R, 128R, 131R, 35T, 36T, 34T, 48R, 53T, 10T
22	40G
23	28G, 27R, 33T
24	1C, 15G, 22G, 29G, 31G, 49G, 1R, 6R, 14R, 18R, 26R, 28R, 31R, 32R, 42R, 44R, 47R, 52R, 64R, 67R, 79R, 87R, 102R, 109R, 117R, 125R, 19T, 22T, 51T, 54T

* R: *Brassica juncea* ; T: *Brassica rapa* ; G: *Brassica napus* ; C: *Brassica carinata*

Out of 24 classes, identified on the basis of fungicidal sensitivity, seven isolates were further characterized on the basis of polymorphism generated by RAPD primers. The clustering allowed grouping of the test isolates into four groups (Figure 1). Isolate 3R appeared to be the most distinct. Group 2 had 128R. It was associated with 3R with similarity coefficient of 0.61. Group 3 had two isolates 27R and 1C. The group 4 contained three isolates 22G, 64R and 1R. Last named 64R and 1R had a

similarity coefficient of 0.93. The grouping though preliminary basis on RAPD markers appeared more consistent with that based on relative sensitivity to the test fungicides. Molecular characterization of other isolates is underway.

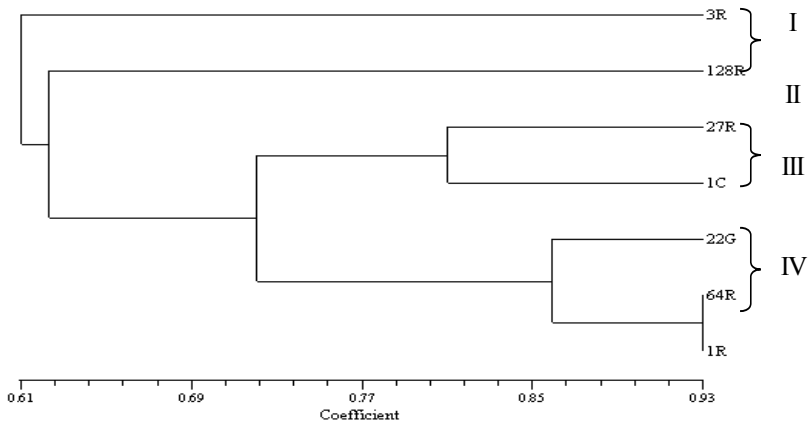

Figure 1: Clustering based on polymorphism generated by RAPD primers.

Discussion

A major lacunae in resistance breeding against alternaria blight has been the absence of sufficient documentation of pathogen variability for alternaria blight pathogen, although three isolates have been reported in the past (Vishwanath and Kolte, 1997). In present context, 114 pathogenic isolates could be characterized based on morphological parameters, fungicide sensitivity and molecular markers. No species/geographic specificity could be documented in the pathogenic isolates investigated.

Conclusion

Extensive variation was recorded for purified isolates collected from a geographic spread of northwest India. The grouping based on morphology, geographic, species and fungicide reaction were largely inconsistent.It may thus be imperative to attempt grouping based on differential and DNA based primers.

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