

STUDIES ON MORPHOLOGICAL AND CULTURAL VARIABILITY OF *ALTERNARIA* SPP. ISOLATES CAUSING LEAF BLIGHT OF COTTON

SANGEETHA K. D.*, S. A. ASHTAPUTRE AND M. S. L. RAO

Department of Plant Pathology,
University of Agricultural Sciences, Dharwad - 580 005, Karnataka, INDIA
e-mail: sangu931@gmail.com

KEYWORDS

Alternaria macrospora
Alternaria alternata
morphological
Cultural variability
Bt cotton

Received on :
28.01.2015

Accepted on :
17.03.2016

***Corresponding author**

ABSTRACT

A. macrospora and *A. alternata* causing leaf spot in Bt cotton were isolated and purified from diseased leaf tissues collected from the affected fields during survey. Morphological variations in length and breadth of conidia, number of horizontal (1-2) and vertical (4-6) septations were observed in the test isolates. The conidial size varied from 21.5 x 6.87 μ m in Haveri and 49.38 x 12.82 μ m in Karlakatti isolates. Variability with respect to cultural characters varied from grey to black, with white to black colony margin either irregular or smooth, raised to flat mycelial growth and sectoring was observed in few isolates in all the tested different solid media. Out of ten isolates, two resembled *A. macrospora* and three resembled *A. alternata*. Maximum dry mycelial weight of *A. macrospora* was observed after sixteen days of incubation. Existence of variation among different *Alternaria* isolates is evident from the results obtained.

INTRODUCTION

Cotton is one of the most important commercial crop which is known as the 'King of fiber' and 'White Gold'. In India, leaf spot of cotton (*Alternaria macrospora* Zimm,) was reported for the first time by Uppal et al. (1935) which is a major contributing factor from the recent past for low productivity of cotton in Karnataka. The characteristic symptoms of the disease are dark brown to black concentric rings, which produce target board effect. Variability is a well known phenomenon in *A. spp.* and variability in morphological and cultural criteria indicate the existence of different patho-types. The existence of the high level of variability was reported by many workers. The variants within population may affect the rate of disease development and induce infection in more host lines, which might have implication for stability of cultivar resistance (Naik et al., 2010). However, little is known about the variability of *A. alternata* and *A. macrospora* isolates in India as well as in Karnataka. Analysis of pathogenic variation within pathogen populations is helpful in understanding host-pathogen interaction, epidemiology and developing strategies for resistance management. Understanding the pathogen variability will help in developing the effective management strategies. So, the present investigation was carried out to find a comprehensive understanding of this causal organism with reference to morphological and cultural variability within the four different host cultivars from North Karnataka region.

MATERIALS AND METHODS

Alternaria infected leaves were collected from infected fields and used for isolation of the fungus *in vitro*. The isolation of the fungus was made by following standard tissue isolation technique. Identification of the fungus was carried out based on the morphological characters of the isolated fungus. Selection of basal medium for growth and sporulation of the fungus was done by using potato dextrose agar and studied morphological characters like length and breadth of conidia, number of horizontal and vertical septa and beak length were measured under 40x using DIC microscope. Later the measurements were compared with the standard descriptions given by Ellis (1971) regarding *Alternaria macrospora* and *Alternaria alternata* for identification of *Alternaria* spp. Later cultural study was carried out by inoculating the pathogen on Potato Dextrose Broth and dry mycelial weight was recorded at regular intervals in order to know the number of days required for maximum growth of the fungus. The isolates were grown on 8 different solid media to select best media for growth viz., Potato Dextrose Agar (PDA), Potato Carrot Agar (PCA), Czapek's Dox Agar (CDA), Host Extract Agar (HEA), Oat Meal Agar (OMA), Corn Meal Agar (CMA), Sabouraud's-Dextrose Agar (SDA) and V8 Juice Agar (V8JA) and to find the difference in colony characters such as radial growth, type of colony margin, colour of margin, mycelial growth, sectoring and sporulation. The best media was found and used as a basal media for further studies.

Radial growth (mm) = (length + breadth of grown mycelium) / 2

RESULTS AND DISCUSSION

Cultural variability

The different *Alternaria* spp. isolates produced different morphological characters. Isolates collected during survey viz., Karlakatti (A_1), Amminbhavi (A_2), Yamkanmardi (A_3), Marewada (A_4), Unkal (A_5), Chandanamatti (A_6), Haveri (A_7), Saundatti (A_8), Gadag (A_9) and Jagalur (A_{10}) were isolated and pure culture was maintained and were stored in the refrigerator at 5°C for further studies (Table 1). The isolates exhibited variability in cultural characters when grown on 8 different solid media. Among ten isolates, A_4 and A_9 showed maximum radial growth (90 mm) on many of the media viz., PDA, PCA, HEA, OMA and SDA tested. Whereas, Isolate, A_2 showed least mean radial growth (61.98 mm).

Among the eight solid media, HEA (90 mm) and OMA (89.25 mm) showed maximum radial growth in all isolates. Majority of the isolates showed moderate to excellent sporulation (Table 1). SDA (80.43 mm) and PCA (79.17 mm) were on par with each other. Whereas, PDA recorded mean radial growth of 77.49 mm and V8JA (43.81 mm) showed least radial growth. The results are in agreement with Tanya *et al.* (2014).

The isolates grown on different media showed varied colony characters (Table 2). A_1 , A_2 , A_5 , A_7 and A_9 isolates showed grey colony on most of the media, whereas isolates, A_3 , A_6 , A_8 and A_{10} showed grey and black colour colonies. The colony margin varied from grey to black in all isolates. Irregular margin was seen predominantly in the isolates viz., A_1 , A_3 , A_5 , A_6 , A_7 and A_8 , whereas isolates, A_9 and A_{10} showed smooth margin among the media tested. Several workers observed diversity in cultural characteristics such as growth rate, type of growth, colony colour and sporulation among different isolates of *Alternaria* spp. infecting sesame, sunflower and cotton (Ramegowda, 2007 and Pushpanajaliet *al.*, 2013).

Morphological variability

Conidiophores of *Alternaria macrospora* arise singly or in groups, straight or flexuous, tapering towards the apex and septate. They are pale brown in colour, 4-9 μ m thick and upto 180 μ m in length. Conidia are solitary or in chains of two, straight or curved with the body of the conidium ellipsoidal tapering to a narrow beak and equal in length or upto twice as long as body. They are reddish brown in colour with four to nine transverse septa and several longitudinal septa (Ellis, 1971). Several attempts are made to classify *Alternaria* genera, several re-descriptions and revised criteria of these genera (Joly, 1964) resulted in a growing number of new species. Though several cotton varieties and hybrids are being released from time to time, none of them has shown complete resistance to the disease. This indicates the existence of variability among the pathogens. Hence the present study helps in understanding different aspects of *Alternaria* spp. with respect to its morphological and cultural variability since not much work has been done on these aspects in the past. In addition, it helps in comprehensive understanding of the causal organism. So, it can be concluded from this study that *Alternaria* spp. exhibits high morphological and cultural variability among

Table 1: Growth and sporulation of the isolates of *Alternaria* spp. on different solid media

| Sl. No. | Isolates | Radial growth (mm) | | | | | | | | Mean |
|----------|---------------|--------------------|-------|-------|-----------|-------|-------|-------|-------|-------|
| | | PDA | PCA | CDA | HEA | OMA | CMA | SDA | V8JA | |
| A_1 | Karlakatti | 77.50 | 63.77 | 71.23 | 90.00 | 82.50 | 43.60 | 82.50 | 42.50 | 69.20 |
| A_2 | Amminbhavi | 60.33 | 82.50 | 65.00 | 90.00 | 90.00 | 25.00 | 45.47 | 37.50 | 61.98 |
| A_3 | Yamkanmardi | 75.00 | 61.00 | 73.37 | 90.00 | 90.00 | 34.27 | 86.00 | 35.00 | 68.08 |
| A_4 | Marewada | 90.00 | 90.00 | 90.00 | 90.00 | 90.00 | 31.34 | 90.00 | 56.27 | 78.45 |
| A_5 | Unkal | 77.17 | 73.33 | 83.33 | 90.00 | 90.00 | 36.17 | 71.27 | 34.60 | 69.48 |
| A_6 | Chandanamatti | 76.77 | 84.83 | 77.83 | 90.00 | 90.00 | 65.83 | 85.00 | 82.30 | 81.57 |
| A_7 | Haveri | 81.27 | 90.00 | 75.43 | 90.00 | 90.00 | 83.83 | 82.43 | 39.92 | 79.11 |
| A_8 | Saundatti | 73.33 | 69.83 | 75.33 | 90.00 | 90.00 | 44.33 | 81.67 | 40.17 | 70.58 |
| A_9 | Gadag | 90.00 | 90.00 | 71.42 | 90.00 | 90.00 | 90.00 | 90.00 | 36.08 | 80.94 |
| A_{10} | Jagalur | 73.50 | 86.38 | 56.46 | 90.00 | 90.00 | 67.67 | 90.00 | 33.77 | 73.47 |
| | Mean | 77.49 | 79.16 | 73.94 | 90.00 | 89.25 | 52.20 | 80.43 | 43.81 | 73.29 |
| | Isolates (I) | | | | Media (M) | | | 1 x M | | |
| | S. Em. \pm | 0.27 | | | 0.24 | | | 0.78 | | |
| | CD at 1% | 1.09 | | | 0.97 | | | 3.09 | | |

++: Moderate sporulation; +++: Good sporulation; ++++: Excellent sporulation; PDA – Potato dextrose agar; PCA – Potato carrot agar; CDA – Czapeck's dextrose agar; HEA – Host extract agar; OMA – Oat meal agar; CMA – Corn meal agar; SDA – Sabouraud's dextrose agar; V8JA – V-8 Juice agar

Table 2: Growth and cultural characteristics of the isolates of *Alternaria* spp. from Bt cotton.

| Sl. Isolates Media | Colony characters – colour, margin, type of margin, mycelia growth | PCA | HEA | OMA | CMA | SDA | V8/A |
|------------------------------|--|---|---|--|---|--|---|
| A ₁ Karfakatti | Dark grey, irregular margin, raised mycelium | Whitish grey, irregular margin, raised mycelium | Grayish black, smooth margin, raised mycelium | Grey white, irregular margin, raised mycelium | Black, irregular margin, distorted mycelium | Dark grey, irregular margin, flat mycelium | Black, irregular margin, raised mycelium |
| A ₂ Aminbhavi | Grey, irregular margin, raised mycelium | Grey, irregular margin, flat mycelium | Grayish black, smooth margin, raised mycelium | Grayish black, irregular margin, raised mycelium | Black, irregular margin, distorted mycelium | Grey, irregular margin, raised mycelium | Grey, smooth margin, flat mycelium |
| A ₃ Yamkanardi | Grey white, irregular margin, raised mycelium | Grey, irregular margin, raised mycelium | Black, irregular margin, raised mycelium | Grey, irregular margin, raised mycelium | Black, irregular margin, distorted mycelium | Grayish black, irregular margin, raised mycelium | Black, irregular margin, flat mycelium |
| A ₄ Marewada | Grayish black, smooth margin, flat mycelium | Grey, smooth margin, flat mycelium | Black, smooth margin, raised mycelium | Black, smooth margin, raised mycelium | Black, irregular margin, distorted mycelium | Black, irregular margin, raised mycelium | Black, irregular margin, raised mycelium |
| A ₅ Unkal | Black white, irregular margin, raised mycelium | Grey, smooth margin, raised mycelium | Grey, irregular margin, raised mycelium | Grey, irregular margin, raised mycelium | Grey, distorted margin, raised mycelium | Grayish black, irregular margin, raised mycelium | Black, irregular margin, raised mycelium |
| A ₆ Chandanamatti | Black grey, smooth margin, flat mycelium | Whitish grey, irregular margin, raised mycelium | White, smooth margin, flat mycelium | Black, irregular margin, raised mycelium | Grey, irregular margin, distorted mycelium | White grey, irregular margin, raised mycelium | Grey black, irregular margin, raised mycelium |
| A ₇ Haveri | Black, irregular margin, raised mycelium | Grey, irregular margin, flat mycelium | Grey, smooth margin, raised mycelium | Grey, irregular margin, raised mycelium | Distorted mycelium | Black, irregular margin, raised mycelium | Black, irregular margin, raised mycelium |
| A ₈ Saundatti | Grey, irregular margin, raised mycelium | Grey, irregular margin, raised mycelium | Grayish black, smooth margin, raised mycelium | Grey white, irregular margin, raised mycelium | Black, irregular margin, raised mycelium | Black grey, irregular margin, raised mycelium | Black, irregular margin, raised mycelium |
| A ₉ Gadag | White grey, smooth margin, flat mycelium | Grey, smooth margin, flat mycelium | White, smooth margin, flat mycelium | Grey black, smooth margin, flat mycelium | Grey, smooth margin, distorted mycelium | White grey, smooth margin, flat mycelium | White grey, irregular margin, flat mycelium |
| A ₁₀ Jagalur | Grayish black, irregular margin, raised mycelium | Grayish black, irregular margin, flat mycelium | Black, smooth margin, raised mycelium | Grey, smooth margin, flat mycelium | Black, irregular margin, distorted mycelium | Black, smooth margin, flat mycelium | Grey, smooth margin, flat mycelium |

PDA - Potato dextrose agar, PCA - Potato carrot agar, HEA - Host extract agar, OMA - Oat meal agar, CMA - Corn meal agar, SDA - Sabouraud's dextrose agar, V8/A - V-8 Juice agar

the isolates themselves. The present investigation opens up the scope for further studies in detail regarding physiological, ecological, genetic and pathogenic variation of strains within the hosts and their DNA analysis for molecular variation of the pathogen which will help in developing an effective breeding programme for disease resistance of the crops and also helpful for designing integrated disease management strategies.

REFERENCES

- Ellis, M. B. 1971. *Dematiaceous Hypomycetes*, Commonwealth mycological institute, Kew, Surrey, England, pp. 495-496.
- Joly, P. 1964. *Legenre Alternaria*. Encyclopédiemycologique XXXIII, P. Lechevalier, Paris, France.
- Pushpanjali, W., Sandeep, S., harivinder, K. S. and Khare, U. K. 2013. Pathogenic behavior of *Alternaria alternata* and phytotoxicity of its culture filtrates on *Lepidumsativum*: A medicinal herb of immense phormocological potential. *The Bioscan*. **8(2)**: 643-647.
- Naik, M. K., Prasad, Y., Bhat, K. V. and Devika Rani, G. S. 2010. Morphological, physiological, pathogenic and molecular variability among isolates of *Alternaria solani* from tomato. *Indian Phytopath.* **63(2)**: 168-173.
- Ramegowda, G. 2007. Disease scenario in *Bt*cotton with special reference to *Alternaria* leaf spot. *M. Sc. (Agri.) Thesis*, Univ. Agric. Sci., Dharwad, Karnataka (India).
- Uppal, B. N., Patel, M. K., Kamat, M. M. 1935. The fungi of Bombay Dept. of Agric., *Bull. No. 176*, p. 28.
- Tanya, R. M., Bharat, S. A. and Srikanta, D. 2014. Cultural, morphological and biochemical variations of *Alternaria solani* causing diseases on solanaceous crops. *The Bioscan*. **9(3)**: 1295-1300.

