

# TAXONOMIC STUDIES ON CULTURAL AND MORPHOLOGICAL CHARACTERS FOR RE-EVALUATION IN *HELMINTHOSPORIUM* SPECIES COMPLEX

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## ABSTRACT

Forty-two sporulating isolates of *Drechslera*, *Helminthosporium*, *Bipolaris* and *Exserohilum* obtained from Indian Type Culture Collection, New Delhi were used in this study. Macroscopic and microscopic studies revealed that, the isolates were assembled into two major groups and three sub-groups; there was a lot of variation in the cultural characters. In group I (28 isolates) aerial mycelium was fluffy, cottony and whitish gray in colour and there was wide variation in radial growth of the isolates [8.45 cm in D19 to 2.2 cm in D7]. In group II (14 isolates), most of the cultures were black in colour and texture was smooth in which radial growth was highest in E7 (8.65 cm) and lowest in D42 (6.25 cm). It was found that germination from two or more cells of conidia separates *Drechslera* from *Bipolaris* and *Exserohilum*. Whereas, presence of strongly protuberant hilum structure differentiates between *Exserohilum* and *Bipolaris* isolates. Thirteen of the isolates having strongly protuberant hilum structure were grouped into the genus *Exserohilum*. Remaining 29 isolates were considered as the genus *Bipolaris* group with no protuberant hilum.

## INTRODUCTION

Species of *Bipolaris*, *Drechslera* and *Exserohilum* constitute a group of taxonomically related and ecologically similar Deuteromycetes (mitosporic fungi) that are important plant pathogens or common saprophytes throughout the world. This group of pathogens, belonging to the Helminthosporia, is particularly important since it includes many fungi that cause considerable losses to different cereal crops, such as barley, maize, rice, oats, wheat and sorghum (Alcorn, 1982, Drechsler, 1923; Kwasna, 1995; Sivanesan, 1987) and produces some of the most powerful phytotoxins known (Chelkowski 1995; Shurtleff 1980). The pathogenic species of *Bipolaris*, *Drechslera* and *Exserohilum* can cause diseases that include leafspots, seedling blights, foot and root rot, blotches (Kwasna 1995), necrosis and chlorosis (due to host specific toxins). Serious diseases caused by these fungi are Brown spot of rice (*B. oryzae* (Breda de Haan) Shoemaker), Brown stripe of sugarcane (*B. stenospila* (Drechslera) Shoemaker), Northern leaf blight (*E. turcicum* (Pass.) Leonard and Suggs) and Southern leaf blight (*B. maydis* (Nisikado and Miyake) Shoemaker) of maize (Luttrell 1978).

Genus *Helminthosporium* was one large group of fungi when it was first named by Link in 1809. Then *Helminthosporium* has gone through frequent refinement in taxonomy over the past 50 years leading to establishment of new genera *Drechslera* (Ito 1930, Manamgoda et al., 2011), *Bipolaris* (Shoemaker 1959) and *Exserohilum* (Leonard and Sugg, 1974). Genera and species of hyphomycetes are identified largely

on the basis of conidial morphology. *Drechslera*, *Bipolaris* and *Exserohilum* were segregated from *Helminthosporium* in several revisions from 1930 to 1974 based on morphology. Conidial features used in taxonomy include germination of conidia, shape, size, colour, septation, and the presence of protruded hila in detached conidia. However, there is no common agreement among mycologists, regarding the correct identification of these important genera.

Ellis (1971) regarded *Helminthosporium* and *Drechslera* as synonyms as he used the two generic names interchangeably, e.g. *Drechslera maydis* and *Helminthosporium maydis*. Subramanian and Jain (1966) did not agree with the grouping of *Helminthosporium* species in the two genera i.e. *Drechslera* and *Bipolaris* and have amended the description of *Drechslera* to include all the species under *Drechslera* and *Bipolaris*. "*Exserohilum turcicum*" may be reported under anyone of four anamorphic genera (i.e. *Bipolaris*, *Drechslera*, *Helminthosporium* and *Luttrellia* Khokhr.) or three teleomorphic genera (i.e. *Keissleriella* Hbhn., *Setosphaeria* Leonard and Suggs and *Trichometasphaeria* Munk) (Sivanesan 1987).

Thus, literature defines that these genera were established based on very few and inadequate characters. For the casual observer these three genera are similar and therefore they have been used as synonyms frequently. Thus, the present study investigates the ability of morphological characters to define the establishment of three genera *Drechslera*, *Bipolaris* and *Exserohilum*.

## MATERIALS AND METHODS

### Collection of *Helminthosporium* group isolates

Fifty-five isolates of *Drechslera*, *Helminthosporium*, *Bipolaris* and *Exserohilum* from Indian Type Culture Collection (ITCC), New Delhi and six isolates of *Drechslera* from Microbial Type Culture Collection, Chandigarh collected from different places and different sources in India were obtained and used in this study. Out of 61 isolates, only 42 were found to be sporulating and were used for morphological examination.

### Morphological examination

#### Macroscopic studies

The cultural characteristics of sporulating isolates of three genera were studied on potato dextrose agar (PDA). Mycelial discs (6mm) of young growing cultures of respective isolates of three genera were kept at the centre of petri plate containing potato dextrose agar medium and incubated at  $28 \pm 2^\circ\text{C}$  for eight days. Three replications for each isolate were maintained. Radial growth was recorded at two days interval. Other parameters such as colony colour, texture, margin and form were recorded at the end of incubation period.

#### Microscopy studies

The collected isolates were cultured on PDA slants at  $25^\circ\text{C}$  under ambient laboratory conditions. After eight days of inoculation, slides were made to study the microscopic features of isolates such as shape, colour, septation, thickness of septa and the presence of protruded hila in detached conidia (Alcorn, 1988). The photographs were taken under 100X magnification using Olympus digital camera (Aneja, 2005).

#### Germination test

Two percent water agar was prepared and autoclaved at  $1.1 \text{ kg/cm}^2$  ( $121.6^\circ\text{C}$ ) for 15 minutes in an autoclave. About 1 mL of molten agar was poured on the sterilized slides uniformly and left to solidify in laminar air flow. Spore suspension was prepared using distilled water and  $100\text{-}200\mu\text{l}$  was spread uniformly on two per cent water agar slides. The slides were kept at room temperature in moist condition for germination of spores. Observations on germination of conidia, germ tube direction and septum ontogeny were taken under microscope after 24-48 h of inoculation. The photographs were taken under 100X magnification using Olympus digital camera. (Alcorn, 1988).

## RESULTS

### Morphology Examination

#### Macroscopic studies

On the basis of observations, the isolates were made into two

groups. In group I (28 isolates) aerial mycelium was fluffy, cottony and whitish gray in colour. There was wide variation in radial growth of the isolates [8.45 cm in D19 to 2.2 cm in D7]. According to the texture, margin and form of the cultures, these isolates were sub-grouped (Fig. 1) into Sub-group A (smooth, entire and circular), Sub-group B (smooth, undulate and irregular) and Sub-group C (rough, undulate and irregular). In group II (14 isolates), most of the cultures were black in colour and texture was smooth. Radial growth was highest in E7 (8.65 cm) and lowest in D42 (6.25 cm). All the cultures were entire except that of isolate D42. Further, group II was sub-grouped into two: Sub-group A (smooth, undulate and irregular) and Sub-group B (smooth, entire and circular) based on texture, margin and form (Fig. 1).

Although the isolates were made into two major groups and three minor groups there was a lot of variation in the colony characters. Therefore, differentiating the genera based on colony characters was not possible.

#### Microscopy studies

Eight days old cultures of the above 42 isolates were morphologically characterized using conidial (shape, colour and hilum) and conidial germination. Conidial shape varied from fusoid, navicular, oblong, ovoid and curved to straight. Conidial colour was pale brown, olivaceous brown, golden brown, hilum either flat or prominent (Table 1, Fig. 2). In some isolates the end cells were cut-off by dark septa (Table 2).

It was observed that, all the isolates were germinating principally from one or both polar cells and there was no germ tube development from intermediate cells (Table 2, Fig. 3). The basal germ tube was emerging adjacent to the hilum and growing in the direction of the long-axis of the conidium (semi axial) in all the isolates.

Except for hilum and septal characters, no other conidial morphology or conidial germination was able to separate the genera. A strongly protuberant hilum structure and pale end cells having thick septa were found in 13 isolates, which were grouped into the genus *Exserohilum* according to the reported literature (Alcorn, 1988). The remaining 29 isolates having no visible hilum and thick septa were considered as the genus *Bipolaris*. None of them were found belonging to the genus *Drechslera*. Thus, the 42 isolates kept under four different genera at ITCC were made into only two groups *viz.*, *Exserohilum* and *Bipolaris* based on morphological characterization.

#### Accepted species in *Bipolaris* from results

##### *Bipolarismaydis*

(Y. Nisik. & C. Miyake) Shoemaker, Can. J. Bot. **33**: 882 (1959) = *Ophiobolus heterostrophus* Drechsler, J. Agric. Res. **31**: 701 (1925)

**Table 2: Grouping of isolates based on morphology into *Exserohilum* and *Bipolaris***

Genera	Characters	Isolates
<i>Exserohilum</i>	A strongly protuberant hilum structure and end cells cut off by dark septa	D12, D39, D40, D41, D42, D43, D44, E3, E4, E5, E6, E7, E8
<i>Bipolaris</i>	Flat hilum and thick septa	D1, D2, D3, D4, D5, D6, D7, D9, D12, D13, D14, D15, D16, D17, D18, D19, D20, D21, D22, D23, D24, D26, D27, D29, D30, D32, D45, H2, E2

**Table 3: Revised identification characters of *Bipolaris* isolates**

Isolate No.	ITCC No.	EarlierNo.	Conidial Shape	Colour	Hilum	Germination
B1	6658	D1	Oblong-elliptical	Pale to mid brown	Flat	Unipolar-bipolar
B2	6321	D2	Oblong-elliptical	Pale to mid brown	Flat	Unipolar-bipolar
B3	6710	D3	Oblong-elliptical	Pale to mid brown	Flat	Unipolar-bipolar
B4	6943	D4	Oblong-elliptical	Golden brown	Flat	Bipolar
B5	5069	D5	Oblong-elliptical	Mid brown	Flat	Bipolar
B6	2445	D6	Oblong-elliptical	mid brown	Flat	Bipolar
B7	2769	D7	Fusiform-elliptical	Olivaceousbrown	Flat	Bipolar
B8	5504	D9	Oblong-elliptical	Mid brown	Flat	Mostly Unipolar
B9	6774	D32	Navicular	Olivaceousbrown	Flat	Bipolar
B10	3543	D45	Fusiform-elliptical	Brown	Flat and protruding	Bipolar
B11	3544	D13	Fusiform-elliptical	Olivaceousbrown	Flat and protruding	Bipolar
B12	3771	D14	Fusiform-elliptical	Olivaceousbrown	Flat and protruding	Bipolar
B13	4700	D15	Fusiform-elliptical	Brown	Flat and protruding	Bipolar
B14	5439	D16	Fusiform-elliptical	Olivaceousbrown	Flat and protruding	Bipolar
B15	1942	D17	Fusiform-elliptical	Golden brown	Flat and protruding	Bipolar
B16	2466	D18	Oblong-elliptical	Pale to golden brown	Flat	Unipolar -Bipolar
B17	3250	D19	Oblong-elliptical	Mid brown	Flat	Unipolar - Bipolar
B18	3578	D20	Oblong-elliptical	Mid brown	Flat	Unipolar - Bipolar
B19	4620	D21	Oblong-elliptical	Mid brown	Flat	Unipolar - Bipolar
B20	4922	D22	Oblong-elliptical	Mid brown	Flat	Unipolar -Bipolar
B21	5137	D23	Oblong-elliptical	Mid brown	Flat	Unipolar - Bipolar
B22	5495	D24	Oblong-elliptical	Pale brown	Flat	Unipolar - Bipolar
B23	3446	D26	Oblong-elliptical	Pale brown	Flat	Unipolar and Bipolar
B24	1590	D27	Curved and fusiform	Mid brown	Flat	Bipolar
B25	6863	E2	Curved and fusiform	Mid brown	Flat	Bipolar
B26	3449	D29	Curved and fusiform	Mid brown	Flat	Bipolar
B27	3294	H1	Oblong-elliptical	Pale to mid brown	Flat	Bipolar
B28	1646	D30	Oblong-elliptical	Mid brown	Flat	Bipolar
B29	MTCC-507	H2	Oval	Olivaceous brown	Flat	Unipolar

**Table 4: Revised identification characters of *Exserohilum* isolates**

Isolate No.	ITCC no.	EarlierNo.	Conidial Shape	Colour	Hilum	End cells	Germination
E1	72	D39	Curved -ellpsoidal	Golden brown	Protuberant	Cut off by dark septa	Bipolar
E2	73	D40	Curved -ellpsoidal	Golden brown	Protuberant		Bipolar
E3	112	D41	Curved -ellpsoidal	Golden brown	Protuberant		Bipolar
E4	6959	D42	Curved -ellpsoidal	Golden brown	Protuberant		Bipolar
E5	2483	D43	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E6	6555	D44	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E7	4686	E3	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E8	4813	E4	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E9	2048	D12	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E10	4839	E5	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E11	5438	E6	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E12	2200	E7	Fusiform-elliptical	Golden brown	Protuberant		Bipolar
E13	3465	E8	Fusiform-elliptical	Golden brown	Protuberant		Bipolar

= *Helminthosporium maydis* Y. Nisik. & C. Miyake Sci.Res. Alumni Assoc. Mirioka agric. Col. Japan 3: 46 (1926)

= *Cochliobolus heterostrophus* (Drechsler) Drechsler, Phytopathology 24: 973 (1934)

= *Drechslera maydis* (Y. Nisik. & C. Miyake) Subram.& B.L. Jain, Curr. Sci. 35: 354 (1966)

#### ***Bipolarissorokiniana***

(Sacc.) Shoemaker, Can. J. Bot. 37: 884 (1959)

= *Helminthosporium sorokinianum* Sacc. InSorok,Trans. Soc. Nat. Univ. Kazan 22: 15 (1890)

= *Helminthosporium sativum* Pammel, C. M. King & Bakke,

B. Iowa. State. Coll. 116: 180 (1910)

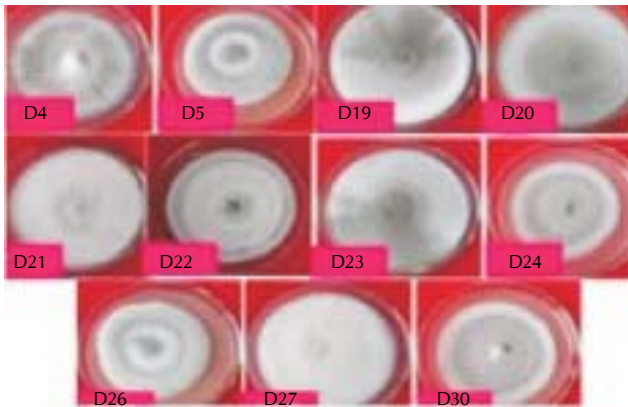
= *Helminthosporium acrothecioides* Lindf, Svenskbot. Tidskr. 12: 562 (1918)

= *Helminthosporium californicum* Mackie & G.E. Paxton, Phytopathology 13: 562 (1923)

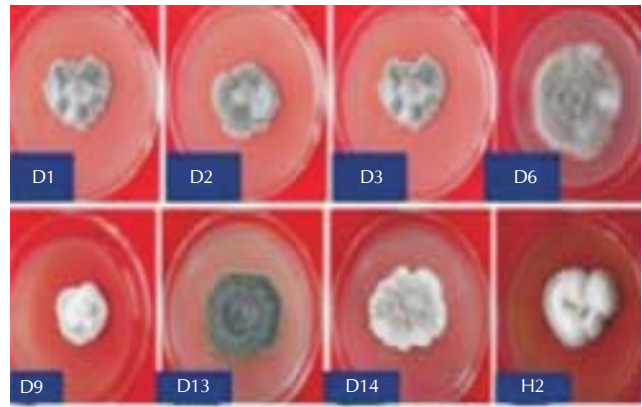
= *Ophiobolus sativus* S. Ito & Kurib, Trans. Sapporonat. Hist. Soc. 10: 138 (1929)

= *Cochliobolus sativus* (S. Ito & Kurib.) Drechslerex Dastur, Indian J. Agr Res 12: 733 (1942)

= *Drechslera sorokiniana* (Sacc.) Subram. & Jain, Curr.Sci. 35: 354 (1966).



Group A: (smooth, entire and circular)



Group B: (smooth, undulate and irregular)



Group C (rough, undulate and irregular)

Group A (Smooth, Undulate, Irregular) Group B (Smooth, Entire, Circular)

### **Bipolarisoryzae**

- (Breda de Haan) Shoemaker, Can. J. Bot. 37: 883 (1959)  
 = *Helminthosporium macrocarpum* Grev, Scott. crypt. fl., 2: 148 (1824) (1825)  
 = *Helminthosporium oryzae* Breda de Haan, Bulletin Inst. Bot. Buitenzorg 6: 11 (1900)  
 = *Ophiobolus miyabeanus* S. Ito & Kurib, Proc. Imp. Acad. Hokkaido Imp. Univ 6 (1927)  
 = *Cochliobolus miyabeanus* (S. Ito & Kurib.) Drechsler ex Dastur, Indian. J. Agri. Res 12: 733 (1942)  
 = *Drechslera oryzae* (Breda de Haan) Subram. & B. L. Jain, Can. J. Bot. 37: 883 (1959).

### **Bipolaris hawaiiensis**

- (M.B. Ellis) J.Y. Uchida & Aragaki, Phytopathology 69: 1115 (1979)  
 = *Drechslera hawaiiensis* Bugnic. ex M.B. Ellis, Dematiaceous Hyphomycetes: 415 (1971)  
 = *Cochliobolus hawaiiensis* Alcorn, Trans. Br. Mycol. Soc. 70: 64 (1978)  
 = *Bipolaris hawaiiensis* (M.B. Ellis) J.Y. Uchida & Aragaki, Phytopathology 69: 1115 (1979)

- = *Pseudocochliobolus hawaiiensis* (Alcorn) Tsuda & Ueyama, Mycologia 73: 92 (1981)

### **Bipolaris spicifera**

- (Bainier) Boedijn, Bull. Jard. bot. Buitenz, 3 Sér. 13(1): 127 (1933)  
 = *Dendryphion spiciferum* (Bainier) Sacc. & Traverso, Syll. fung. (Abellini) 19: 560 (1910)  
 = *Helminthosporium tetramera* McKinney, Bull. U. S. Department of Agriculture 1347: 33 (1925)  
 = *Curvularia tetramera* (McKinney) Boedijn ex J.C. Gilman, Manual of Soil Fungi: 303 (1945)  
 = *Helminthosporium spiciferum* (Bainier) Nicot, Öst. bot. Z. 100: 482 (1953)  
 = *Bipolaris tetramera* (McKinney) Shoemaker, Can. J. Bot. 37(5): 884 (1959)  
 = *Drechslera tetramera* (McKinney) Subram. & B. L. Jain, Curr. Sci. 35: 355 (1966)  
 = *Bipolaris spicifera* (Bainier) Subram., Hyphomycetes (New Delhi): 756 (1971)

## **DISCUSSION**

Distinction between genera of *Helminthosporium* group has always been a problem due to the subtle inherent variability found in the genera of the hyphomycetes (Pitt, 1985, Premkumar *et al.*, 2015, Adhikary *et al.*, 2013). However, two important characters *i.e.* conidial characters (size, shape, hilum and colour) and germination has been used by various scientists around the world to group the three genera of *Helminthosporium* complex. The morphological features observed in the experiment such as conidial and germination characters, suggest that the isolates belong to the genera *Bipolaris* and *Exserohilum*. Additionally, the morphology observed is quite similar to that described by Alcorn (1983). All the germinating conidia produced germ tube from one or both the end cells. This result is in conformity with the studies of Alcorn (1983) who confirmed that polarity, the position and the direction of the germ tube from the basal cells *per se* is a reliable indicator of genera. Thirteen isolates having strongly protuberant hilum with thick dark septa at the end pale cells of the conidia were grouped under *Exserohilum*. The protuberant hilum is considered as one of the major distinguishing characters for



Figure 1: Sub-grouping of group I and group II isolates of *Drechslera*, *Helminthosporium*, *Exserohilum* species (8 days old)



Figure 2: Grouping of different isolates of *Drechslera*, *Helminthosporium* and *Exserohilum* species (100X) on the basis of conidial characters

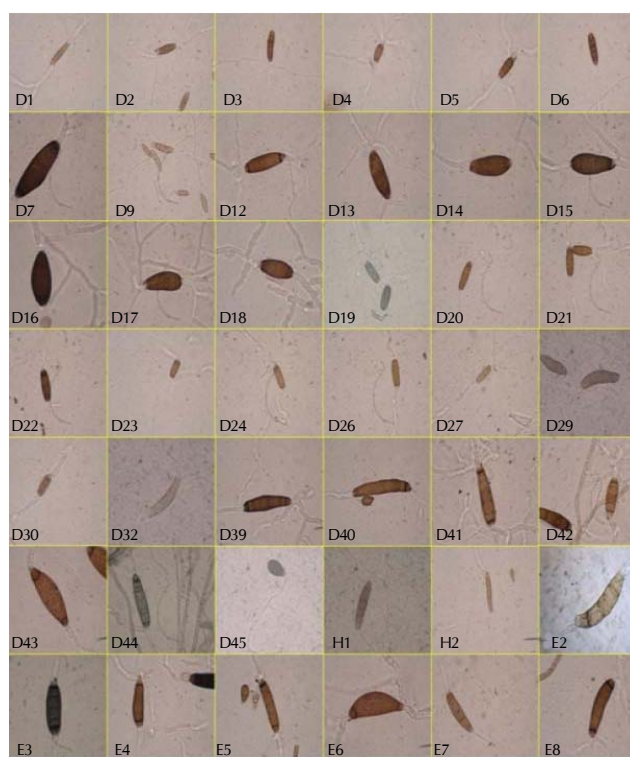


Figure 3: Germination studies of different isolates of *Drechslera*, *Helminthosporium* and *Exserohilum* species (100X)

the genus *Exserohilum* (Alcorn 1988; Sivanesan 1987). Hilum is the scar or mark, where the conidium attaches to conidiophore (Hawksworth et al., 1995). Structure of hilum is very important character which leads to erection of new genera and separation of *Exserohilum* from *Bipolaris*. Motlagh and Kaviani (2008) carried out an experiment in order to identify the genus and species of rice brown spot agent in Guilan, north of Iran. In order to identify the isolates collected from rice fields, conidium, conidiophore morphology, process of conidium formation and pattern of its germination were

studied. According to the results, isolates were belonging to *Bipolaris oryzae*, *B. victoriae*, *B. indica* and *B. bicolor*. The total isolates include of 85% *B. victoriae*, 10% *B. oryzae*, 2% *B. indica* and 3% *B. bicolor*.

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