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 serious diseases caused by these fungi are Brown spot of rice (E. turcicum (Drechslera) Shoemaker), Northern leaf blight (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 (Niskado 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 Helminthusporium maydis. Subramaniam 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 one of four anamorphic genera (i.e. Bipolaris, Drechslera, Helminthus porum and Luttrella Khokhr.) or three teleomorp genera (i.e. Keissleriella Hbhn., Setospheeria 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 ± 2°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°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 kg/cm² (121.6ºC) 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 100-200µl 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 microscopeafter 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

Bipolaris maydis

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

<table>
<thead>
<tr>
<th>Genera</th>
<th>Characters</th>
<th>Isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exserohilum</td>
<td>A strongly protuberant hilum structure</td>
<td>D12, D39, D40, D41, D42, D43, D44, E3, E4, E5, E6, E7, E8</td>
</tr>
<tr>
<td></td>
<td>and end cells cut off by dark septa</td>
<td></td>
</tr>
<tr>
<td>Bipolaris</td>
<td>Flat hilum and thick septa</td>
<td>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</td>
</tr>
</tbody>
</table>
TAXONOMIC STUDIES ON CULTURAL AND MORPHOLOGICAL CHARACTERS

Table 3: Revised identification characters of Bipolaris isolates

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>ITCC No.</th>
<th>Earlier No.</th>
<th>Conidial Shape</th>
<th>Colour</th>
<th>Hilum</th>
<th>End cells</th>
<th>Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>6658</td>
<td>D1</td>
<td>Oblong-elliptical</td>
<td>Pale to mid brown</td>
<td>Flat</td>
<td>Cut off by dark septa</td>
<td>Bipolar</td>
</tr>
<tr>
<td>B2</td>
<td>6321</td>
<td>D2</td>
<td>Oblong-elliptical</td>
<td>Pale to mid brown</td>
<td>Flat</td>
<td>Unipolar-bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>B3</td>
<td>6710</td>
<td>D3</td>
<td>Oblong-elliptical</td>
<td>Pale to mid brown</td>
<td>Flat</td>
<td>Unipolar-bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>B4</td>
<td>6943</td>
<td>D4</td>
<td>Oblong-elliptical</td>
<td>Golden brown</td>
<td>Flat</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>B5</td>
<td>5069</td>
<td>D5</td>
<td>Oblong-elliptical</td>
<td>Mid brown</td>
<td>Flat</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>B6</td>
<td>2445</td>
<td>D6</td>
<td>Oblong-elliptical</td>
<td>Mid brown</td>
<td>Flat</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
</tbody>
</table>

Table 4: Revised identification characters of Exserohilum isolates

<table>
<thead>
<tr>
<th>Isolate No.</th>
<th>ITCC No.</th>
<th>Earlier No.</th>
<th>Conidial Shape</th>
<th>Colour</th>
<th>Hilum</th>
<th>End cells</th>
<th>Germination</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>72</td>
<td>D39</td>
<td>Curved-ellipsoidal</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Cut off by dark septa</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E2</td>
<td>73</td>
<td>D40</td>
<td>Curved-ellipsoidal</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E3</td>
<td>112</td>
<td>D41</td>
<td>Curved-ellipsoidal</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E4</td>
<td>6959</td>
<td>D42</td>
<td>Curved-ellipsoidal</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E5</td>
<td>2483</td>
<td>D43</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E6</td>
<td>6555</td>
<td>D44</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E7</td>
<td>4686</td>
<td>E3</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E8</td>
<td>4813</td>
<td>E4</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E9</td>
<td>2048</td>
<td>D12</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E10</td>
<td>4839</td>
<td>E5</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E11</td>
<td>3438</td>
<td>E6</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E12</td>
<td>2200</td>
<td>E7</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
<tr>
<td>E13</td>
<td>3465</td>
<td>E8</td>
<td>Fusiform-elliptical</td>
<td>Golden brown</td>
<td>Protuberant</td>
<td>Bipolar</td>
<td>Bipolar</td>
</tr>
</tbody>
</table>

= Cochliobulus heterostrophus (Drechsler) Drechsler, Phytopathology 24: 973 (1934)

Bipolarissorokiniana
= Helminthosporium acrothecoides Lindf, Sevenskbot. Tidskr. 12: 562 (1918)
= Helminthosporium californicum Mackie & G.E. Paxton, Phytopathology 13: 562 (1923)
= Cochliobulus sativus (S. Ito & Kurib.) Drechsler ex Dastur, Indian J. Agr Res 12: 733 (1942)
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**Bipolarisoryzae**

- Helminthosporium macrocarpum Grev, Scott. crypt. fl., 2: 148 (1824) (1825)
- Ophiobolus miyabeanus S. Ito & Kurib, Proc. Imp. Acad. Hokkaido Imp. Univ. 6 (1927)

**Bipolaris hawaiiensis**

- Drezslera hawaiiensis Bugnic. ex M.B. Ellis, Dematiaceae Hyphomycetes: 415 (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
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 a 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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REFERENCES


