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**COLLETOTRICHUM LINDEMUTHIANUM, THE CAUSAL AGENT OF BEAN ANTHRACNOSE**

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**SUMMARY**

Common bean (*Phaseolus vulgaris* L.) is an important constituent of people’s diets especially in developing countries. Dry beans find a unique position in the culinary items because of their high nutritional value. For instance, rice and bean recipe (Rajmah Chawal) is famous in the northern part of India. Many fungal, viral and bacterial diseases affect the crop and cause heavy losses worldwide. Among the various fungal diseases, bean anthracnose caused by *Colletotrichum lindemuthianum* is a serious disease under cool and humid environments. Under favorable conditions, the yield losses may be up to 100 percent. The scientific community across the world has been studying the bean-anthracnose interaction for over 100 years and the information has helped to understand the pathosystem and devise better disease management strategies. Many excellent reviews on anthracnose resistance genes, marker aided breeding and R gene signatures highlight different tactics for disease management. Assembling the substantial literature available on the pathogen is necessary for better understanding of the pathogen biology. The present review consolidates this information and provides a comprehensive outline about the detection, pathogenicity genes, pathogenic variability and molecular diversity of *C. lindemuthianum*. The importance of the bean genome and availability of SNP markers to dissect the bean-anthracnose interface is also addressed.

**Keywords**: Detection; pathogenic variability; molecular diversity; pathogenicity genes

**INTRODUCTION**

Common bean (*Phaseolus vulgaris* L.) belongs to the genus *Phaseolus*, subtribe *Phaseolinae*, tribe *Phaseoleae*, subfamily *Papilionoideae* in the family *Fabaceae* (Debouck, 1991; Singh et al., 1991, Singh, 2001; McClean et al., 2008). The genus includes many types of beans (like dry beans, green beans, shelling beans), and contains 50 different species (Delgado-Salinas et al., 1999), with most of them from Mesoamerica and South America. Among these, only five, i.e. common bean (*P. vulgaris*), runner bean (*P. coccineus*), year bean (*P. dumosus*), tepary bean (*P. acutifolius*) and lima bean (*P. lunatus*) are cultivated, bred and widely used for human consumption worldwide (Gepts et al., 2008; Aragão et al., 2011). *Phaseolus* species are diploid with 2n = 2x = 22 (Mercado-Ruaro and Delgado-Salinas, 1998).

Recent studies based on the molecular analysis of wild relatives of the crop suggest central Mexico as the centre of origin of *P. vulgaris* (Bitocchi et al., 2012). The crop was independently domesticated in both the Andean and Mesoamerican regions (Bitocchi et al., 2013; Bellucci et al., 2014) and was transported to Africa and Europe in the 1600s (Pathania et al., 2014; De Ron et al., 2016). Common beans grow at different altitudes ranging from 50 to 3000 meters above sea level and can withstand extreme environments too. The crop prefers temperatures ranging from 14 to 26°C, an annual precipitation between 400 and 1600 mm per year, a slightly acid soil pH (average 5-6), show a wide range for days to maturity (70-200) and seed yield potential (400-5000 kg ha⁻¹) (Wortmann et al., 1998; Debouck, 1999). Consumers eat edible dry seed and fresh (green) pods. The dry seeds are source of calories, minerals, fiber and are rich in dietary protein (18-40% of seed weight), which humans need on daily basis.

The low productivity of commercially cultivated bean varieties is due to many biotic and abiotic factors. Among the biotic factors such as anthracnose, angular leaf spot, *Bean common mosaic virus*, common bacterial blight and halo blight, bean anthracnose caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Lams.-Scrib, is a serious seed borne pathogen throughout the world. Infection of susceptible cultivars in cool and humid environmental

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conditions can result in yield losses as high as 100% (Sharma et al., 1994; Fernandez et al., 2000; Padder et al., 2007; Sharma et al., 2008). Despite availability of management practices like seed and foliar treatment with fungicides, crop rotation, certified seed and genetic resistance, the crop is vulnerable to the disease because of prevalence of diverse pathogen races. Though the disease can be managed successfully through the use of resistant cultivars, the high pathogenic variability renders the majority of the cultivars susceptible (Pastor-Corrales et al., 1995; Balardin et al., 1997; Balardin et al., 1999; Mahuku and Riascos, 2004; Pathania et al., 2006; Sharma et al., 2007). The international bean differential set and the corresponding binary codes (Drijfhout, 1978; Pastor-Corralas, 1991) for anthracnose race designation have allowed consistent comparison of virulence among different research groups. Anthracnose resistance genes present in different cultivars were comprehensively documented (Kelly and Vallejo, 2004; Ferreira et al., 2013) and the pathogenic variability was first summarized by Melotto et al. (2000). Bean genome availability was recently used to dissect the anthracnose resistance genes in terms of their R gene signatures (Meziadi et al., 2016). Similarly marker assisted backcross breeding has resulted in development of anthracnose resistant cultivars in different parts of the world (Alzate-Marin et al., 2004; Faleiro et al., 2004; Ferreira et al., 2012; Madakbas et al., 2013; Hegay et al., 2014).

Comprehensive information on anastomosis, pathogenesis, pathogenic and molecular variability is available for C. lindemuthianum, but needs to be consolidated into a comprehensive review. The focus of the present review is to discuss the various features of pathogen biology so that the information may be helpful to research groups working on bean anthracnose around the world.

THE PATHOGEN

The fungus Colletotrichum lindemuthianum taxonomically belongs to: Fungi, Ascomycota, Pezizomycotina, Sordariomycetes, Hypocreomycetidae, Glomerellales, Glomerellaceae; Colletotrichum lindemuthianum as reported by Mycobank (http://www.mycobank.org/). The fungus was discovered by Lindemuth in 1875 (Tiffany and Gilman, 1954) and first described by Saccardo (1878). Then the disease was reported not only on P. vulgaris but also on other Phaseolus species in different parts of the world (Sicard et al., 1997b). The infectious nature of the pathogen was established by Frank in 1883 (Dey, 1919). Since then, anthracnose has occurred widely in Europe and North America. In 1891, the bean crop was completely destroyed in certain parts of Italy and in 1915 and 1916 serious epidemic occurred in Germany. The pathogen overwinters in seed and crop residues (primary source of infection) and infects all aerial parts of the bean plant. Typical symptoms are deep, shrunken lesions containing flesh-colored spores on bean pods that are the most distinctive symptoms of anthracnose. Lesions also commonly appear on stems, hypocotyls and leaf veins of seedling plants, with more advanced disease resulting in wilting and flagging of chlorotic leaves similar to that of other foliar pathogens. Further advancement of the disease leads to complete girdling and eventually death of the plant. Infection of the bean pods results in rust-colored lesions that develop into sunken cankers with black ring borders. Severely infected premature pods abort and fall early, while pods that mature produce infected seed with dark cankers that make the seed unmarketable to consumers (Pastor-Corrales and Tu, 1989). The anamorph stage produces acervuli that erupts through epidermis. It has short crowded conidiophores at the bottom with conidia and the spores escape through an opening at the top. Water splashes disperse spores and result in the secondary spread of disease (Fig. 1).

Sexual form (teleomorph) of the pathogen was reported more than 100 years ago by Shear and Wood (1913) in the laboratory grown cultures. These cultures produced perithecia and asci, a typical characteristic of ascomycetes and named the perfect stage as Glomerella lindemuthiana. Later, Kimati and Galli (1970) rediscovered the perfect stage of the fungus by mating two different isolates. Since the ascospores were pathogenic to beans only, they named it to G. cingulata f. sp. phaseoli. The teleomorph stage rarely occurs under field conditions, however since 1970 many attempts to develop perithecia under laboratory conditions have been successful in Brazil (Ishikawa et al., 2010a; Barcelos et al., 2011, 2014). Recently G. cingulata f. sp. phaseoli or G. lindemuthiana have been detected on bean lesions exhibiting typical anthracnose symptoms on pods, leaves or stems (Camargo et al., 2007; Ishikawa et al., 2010a; Souza et al., 2010; Barcelos et al., 2014). Sexual compatibility studies performed in the laboratory have revealed both homo- and heterothallism in different isolates.
of *G. cingulata* f. sp *phaseoli* (Mendes Costa, 1996; Rodriguez-Guerra et al., 2005; Camargo et al., 2007; Souza et al., 2010). Heterothallism investigation in *C. lindemuthianum* lead to characterization of MAT1-2-1 (García-Serrano et al., 2008). Both parent strains contained a single copy of this gene encoding high mobility group motif (HMG). Recently, Barcelos et al. (2014) conducted a comprehensive study of *Glomerella* species isolated from anthracnose infected bean lesions. The authors rejected the theory of *Glomerella* being the perfect stage of *C. lindemuthianum*. They suggested that *Glomerella* species present in the lesions are epiphytes that grow opportunistically like *C. gloeosporioides* species complex that are weak pathogens of beans and take the advantage of lesions produced by infection of aggressive *C. lindemuthianum*. A recent study by Mota et al. (2016) showed pathogenic nature of *Glomerella* species group II of Barcelos et al. (2014). The isolates caused mild symptoms on the host surface after 10 days post inoculation and at a later stage it is difficult to distinguish between the *C. lindemuthianum* and *Glomerella* spp. based on the symptoms. The study reflects the anthracnose/scab complex on *P. vulgaris* and warrants a detailed investigation because isolates belonging to *Glomerella* spp. group I (Mota et al., 2016) failed to cause disease.

**PATHOGEN DETECTION**

Although bean anthracnose is caused by *C. lindemuthianum*, the occurrence of other *Colletotrichum* and *Glomerella* species (Mota et al., 2016) from infected host tissues warrants accurate detection of the pathogen. Identification of the fungus based on morphology, symptoms on host surface and pathogenicity is time-consuming and needs a specialist in taxonomy. The detection becomes strenuous in case of seed and donor plant certification, therefore an accurate, sensitive and effective diagnostic tool is necessary. Among the various methods for fungal identification, PCR or qPCR is a robust, quick and sensitive method. Pathogen specific primers exist for a particular pathogen and *C. lindemuthianum* is not an exception. A sensitive and specific PCR based anthracnose pathogen detection method was developed by Chen et al. (2007). They developed a forward primer within the vicinity of ITS region. This primer, when used with universal ITS4 primer (White et al., 1990), amplifies a 461 bp rDNA region specifically in *C. lindemuthianum* with a detection limit as low as 10 fg. Wang et al. (2008) used two primer pairs (CY1, CY2 and CD1, CD2) for accurate evidence of anthracnose infection in bean tissues and seeds. Detection using CY1/CY2 primer pair was not specific as the 442 bp amplicon was also amplified in *C. orbiculare* but amplification using CD1/CD2 primer pair distinguished between *C. lindemuthianum* and *C. orbiculare*. Unfortunately, the authors did not sequence the 638 bp DNA segment amplified using CD1/CD2 primer pair. However, recently Gutierrez et al. (2014) sequenced a *C. lindemuthianum* isolate (A83) through pyrosequencing and local BLASTX search against de novo assembly resulted in identification of a contig carrying CD1 and CD2 specific sequences. The sequence encodes for an iron permease (*Frl1*) pseudogene flanked by a gene encoding for a polyhydroxyproline-rich protein in *Colletotrichum*. A primer pair specific to *C. lindemuthianum* HMG domain developed by García-Serrano et al. (2008) was used by Pinto et al. (2012) and Mota et al. (2016) for a more accurate detection of anthracnose pathogen. Among the primers used for detection of anthracnose pathogen, HMG primer proved to be the best for PCR detection. Among the two mating idiomorphs (MAT1-1 and MAT1-2) present in the genome of ascomycetes, all *Colletotrichum* species carry the MAT1-2 idiomorph, while MAT1-1 idiomorph has never been reported from the genus *Glomerella* despite several attempts to amplify the alpha domain (Vallancourt et al., 2000; Rodriguez-Guerra et al., 2005; Menat et al., 2012). Sequence analysis of MAT1-2-1 suggests a huge difference among different *Colletotrichum* species (García-Serrano et al., 2008). Because of sequence variation (above 45%) in the MAT1-2-1 locus, this region seems a better choice to develop species specific markers than the universal ITS region. Recently Chen et al. (2013) developed a qPCR based detection of *C. lindemuthianum* with a detection limit up to 5 fg of *C. lindemuthianum* genomic DNA. Primer pair for qPCR detection was developed within the ITS region of fungus.

**ANASTOMOSIS**

Heterokaryon formation between compatible strains of the same fungus (anastomosis) is an important and common component in the life cycle of many fungi. Strains that form a stable heterokaryon are referred to as vegetative compatible and the resulting groups are ascribed to a particular vegetative compatible group (VCG). Vegetative incompatibility is a genetic mechanism that restricts the heterokaryosis between individuals that differ in one or more bet or vic loci (Glass et al., 2000, 2004; Xiang and Glass, 2004). Mating of genetically identical strains leads to death because of presence of different bet loci (Hall et al., 2010) and similar cell death occurs in *C. lindemuthianum* (Carvalho and Mendes-Costa, 2011). Based on nitrate metabolism, Carvalho and Mendes-Costa (2011) identified 18 nit mutants in *C. lindemuthianum* and classified them into four groups (*nit1, nit2, nit3 and nitM*) with high frequency of *nit2* group. Confrontation among different *nit* mutants resulted in hyphal anastomosis in 32 cases whereas 23 confrontations were incompatible. Castro-Prado et al. (2007) reported the occurrence of successful parasexuality between five races of *C. lindemuthianum* under laboratory, however, a few race confrontations were vegetative incompatible. Confrontations within 13 isolates of race 65 suggest that anastomosis also happens within race isolates.
(Ishikawa et al., 2008). In addition to hyphal anastomosis, *C. lindemuthianum* shows conidial anastomosis (Roca et al., 2003) and later this phenomenon was reported to occur in various fungal species (Roca et al., 2004). Conidial anastomosis in the fungus occurs on the host surface (Ishikawa et al., 2010a) and within the acervulus either on the host or in the culture (Roca et al., 2003). Conidial anastomosis occurs between a specialised hypha called as conidial anastomosis tube (CAT) that allows fusion between conidia and conidial germlings (Read et al., 2009, 2010). This fusion between germlings acts as a single coordinating unit for the exchange of nutrients, water, signal molecules, nuclei and other organelles (Read et al., 2009, 2010). In asexually reproducing fungi, CATs may contribute to high level of variability through parasexual recombination that occurs within and between *C. lindemuthianum* and *C. gossypii* (Roca et al., 2004; Read and Roca, 2006). Detailed studies on CATs using live cell imaging over a time course showed that it depends on culture age, media and the strain used (Ishikawa et al., 2010b). High numbers of CATs take place in water after 72 h of incubation whereas nutrient media inhibited its formation. The blending takes about 60 h to complete and at 56 h, cytoplasm moves between the conidia (Ishikawa et al., 2010b, 2013). Labelling nuclei with green fluorescent protein (GFP) and tracking with confocal microscopy revealed that the nuclei move after 40 to 41 h after CATs formation (Fig. 2). Labelling nuclei of two strains of *C. lindemuthianum* with green and red fluorescent proteins, respectively, revealed that vegetative incompatibility was suppressed by the CATs formation (Ishikawa et al., 2012).

**PATHOGENESIS AND PATHOGENICITY GENES**

Based on the feeding habit, *C. lindemuthianum* is a hemibiotroph (Ferreira et al., 2013) that relies on the common bean for nutrients before causing cell death. A number of reviews on how the pathogen infects the host has been published (O’Connell et al., 1985; Pastor-Corrales and Tu, 1989; Bailey and Jeger, 1992; Ishikawa et al., 2010a). Conidia germinate on the host surface and the germ tube differentiates into a specialized penetration structure known as appressorium. Melanized appressorium produces high turgor pressure to penetrate the host surface directly. An infection peg emerges from the appressorium and afterwards the fungus forms infection vesicles and primary hyphae (biotrophy). Biotrophic hyphae spread to a few adjacent cells and then the fungus switches to necrotrophy by producing secondary hyphae. The expression of genes during biotrophic and necrotrophic phases is indispensable for successful infection in order to prevent pathogen triggered and effector triggered immunity defences (O’Connell et al., 2012). Forward fungal genetics, and particularly insertion mutation studies, have provided valuable insights on the molecular mechanism of *Colletotrichum* infection process. Traditionally, mutants in fungi were developed using transposons, polyethylene glycol (PEG) and restriction enzyme–mediated integration (REMI). However, recently *Agrobacterium tumefaciens* mediated transformation (ATMT) has gained popularity because of its simplicity. Several *Colletotrichum* mutants deficient in the pathogenicity at a particular stage of infection have revealed key pathogenicity genes. Expression of these genes is necessary for successful establishment and colonization of host tissue (Munch et al., 2011; Nakamura et al., 2012; Liu et al., 2013a; Korn et al., 2015). For instance, studies on *Colletotrichum* species have shown that melanization of appressorium is indispensable for virulence (Rasmussen and Hanau, 1989; Lin et al., 2012; Takahara et al., 2012; Liu et al., 2013b). Unlike other *Colletotrichum* species, there is a lack of literature on pathogenicity genes in *C. lindemuthianum* (Table 1). Most of these studies have shown key genes and transcriptional factors necessary for causing infection of bean. Random insertion mutagenesis first carried out by Dufresne et al. (1998) in *C. lindemuthianum* identified a mutant that showed inability to penetrate bean leaves. Sequence analysis showed that the gene named *clkl* is a member of serine/threonine protein kinases. In another study, Dufresne et al. (2000) identified a transcriptional factor (*CLTA1*) belonging to the zinc cluster (Zn[[II]]2Cys6) family essential for regulating the switch between biotrophic and necrotrophic phases. Another gene (*CLNR1*) responsible for causing necrotrophy in bean tissue induces nitrogen starvation *in planta* (Pellicer et al., 2003). Nitrogen starvation genes are expressed during the early phases of infection in many fungi (Talbot et al., 1993; Stephenson et al., 1997; Stephenson et al., 2000). Formation of a penetration peg in *C. lindemuthianum* is necessary for successful infection of bean tissues. This specialized structure is under the control of several genes (Xu et al., 1997, 1998; Balhadere and Talbot, 2001; Clergeot et al., 2001; Kim et al., 2002; Park et al., 2002). Among these, PLS1 (punchless) gene is crucial for the peg formation in *Magnaporthe grisea*, the rice blast fungus (Clergeot et al., 2001). The gene encodes for a protein tetraspanin that plays an important role in animals.
and fungi (Boucheix and Rubinstein, 2001; Gourgues et al., 2002; Hemler, 2003; Stipp et al., 2003; Gourgues et al., 2004). Insertional mutagenesis in *C. lindemuthianum* led to the discovery of *CIPLS1* pathogenicity gene that is similar in function to *M. grisea PLS1* gene (Veneault-Fourrey et al., 2005).

**PATHOGENIC VARIABILITY**

Variability in *C. lindemuthianum* was first described by Barrus (1911) when he noticed differences between virulence of two races of anthracnose against 139 bean cultivars. These first two races were identified as α and β, and laid the foundation for the discovery of greater pathogenic variability. Prior to 1988, fourteen bean anthracnose races were reported using different sets of differential cultivars (Burkholder, 1923; Frandsen, 1953; Oliari et al., 1973; Wallen, 1979). However, many researchers in various countries used local codes instead of the Greek letters to identify anthracnose races (Melotto et al., 2000). No standardized system limited the knowledge of the global variability of *C. lindemuthianum*. Recently an international differential set comprising of 12 bean cultivars (Pastor-Corrales, 1991) was established to determine isolate race(s) (Table 2). Each differential has a binary number and the sum of the cultivars with susceptible reactions gives the binary number of each cultivar used to characterize races of anthracnose in common bean. Table 2. Anthracnose differential series, host gene pool, resistance genes, and the binary number of each cultivar used to characterize races of anthracnose in common bean.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Annotation</th>
<th>Infection process</th>
<th>Strategy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>clh1</td>
<td>serine/threonine protein kinases</td>
<td>appressorium functionality</td>
<td>REMI&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Dufresne et al., 1998</td>
</tr>
<tr>
<td>CLTA1</td>
<td>Transcriptional activator belonging to the fungal zinc cluster (Zn&lt;II&gt;Cl2) family</td>
<td>Switch between the biotrophy and the necrotrophy</td>
<td>REMI</td>
<td>Dufresne et al., 2000</td>
</tr>
<tr>
<td>CH1</td>
<td>Prolin-rich glycoprotein</td>
<td>Biotrophy</td>
<td>Immunofluorescence</td>
<td>Perfect et al., 2000</td>
</tr>
<tr>
<td>clp1</td>
<td>Copper-transporting ATPase</td>
<td>Fewer appressoria with less melanin</td>
<td>REMI</td>
<td>Parisot et al., 2002</td>
</tr>
<tr>
<td>CLNR1</td>
<td>AREA and NIT2 global fungal nitrogen regulator</td>
<td>Few anthracnose lesions seldom occur and the mutant is impaired in causing necrotrophy</td>
<td>REMI</td>
<td>Pellier et al., 2003</td>
</tr>
<tr>
<td>CIPLS1</td>
<td>Tetraspanin super family</td>
<td>Infection vesicles and primary hyphae</td>
<td>REMI</td>
<td>Veneault-Fourrey et al., 2005</td>
</tr>
<tr>
<td>PacCl</td>
<td>pH-responsive transcriptional regulator</td>
<td>Maceration on the infected plant tissue</td>
<td>DNA hybridization to single plaques</td>
<td>Soares et al., 2014</td>
</tr>
</tbody>
</table>

<sup>a</sup> Restriction Enzyme Mediated Insertion (REMI)
gene. Other studies suggest high pathogenic variability within the isolates of a race, for instance, high variability exists within isolates of race 65 in Brazil (Ishikawa et al., 2008; Davide and Souza, 2009; Ishikawa et al., 2011). A few races are present in a particular region, whereas some are prevalent in many countries. For instance, race 73 is prevalent in the USA, Canada and Mexico (Kelly et al., 2008; Balardin and Kelly, 1997; Kelly et al., 1994; Sicard et al., 1997b; Pastor-Corrales et al., 1993) whereas races 903, 931, 935 are only present in some countries. For instance, race 65 in Brazil (Ishikawa et al., 2011; Pinto et al., 2012; Silva et al., 2007; Goncalves-Vidigal et al., 2008; Thomazella, 2002; Somavilla and Prestes, 1999; Ribeiro et al., 2016).

Table 3. Colletotrichum lindemuthianum races present in various countries across the world.

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of isolates used</th>
<th>Races</th>
<th>Total number of races</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>474</td>
<td>0,1, 4, 5, 7, 8, 17, 21, 23, 31, 38, 52, 55, 64, 65, 66, 67, 69, 71, 72, 73, 75, 77, 79, 81, 83, 85, 86, 87, 89, 93, 95, 96, 97, 101, 102, 103, 105, 109, 111, 137, 139, 121, 123, 125, 127, 131, 193, 217, 249, 302, 321, 337, 339, 343, 351, 453, 581, 585, 2047</td>
<td>60</td>
<td>Pinto et al., 2012; Silva et al., 2007; Goncalves-Vidigal et al., 2008; Thomazella, 2002; Somavilla and Prestes, 1999; Ribeiro et al., 2016</td>
</tr>
<tr>
<td>Turkey</td>
<td>51</td>
<td>1, 2, 6, 8, 17, 32, 47, 54, 55, 64, 96, 102, 110, 141, 231, 233, 515, 520, 585, 641, 833, 1032, 1153, 1165, 1344, 1472, 1929, 1993, 2047, 2560, 2690, 2816, 3195, 3632, Alfa, beta, Gamma, Delta</td>
<td>39</td>
<td>Madakbas et al., 2013; Alam and Rudolph, 1993</td>
</tr>
<tr>
<td>USA</td>
<td>491</td>
<td>0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 16, 17, 18, 19, 23, 38, 39, 65, 73, 87, 89, 129, 130, 131, 133, 134, 136, 137, 183, 192, 201, 290, 295, 393, 513, 589, 1032, 1153, 1161, 1481, 1993, 65-Epsilon</td>
<td>117</td>
<td>Goswami et al., 2011; Awale et al., 2008; Ansari et al., 2004; Balardin and Kelly, 1997; Kelly et al., 1994; Sicard et al., 1997b; Pastor-Corrales et al., 1993</td>
</tr>
<tr>
<td>Greece</td>
<td>35</td>
<td>2, 6, 22</td>
<td>3</td>
<td>Bardas et al., 2007</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>34</td>
<td>2, 6, 22, 54, 81</td>
<td>3</td>
<td>Kryakov, 2000; Kryakov and Genchev, 2004</td>
</tr>
<tr>
<td>Spain</td>
<td>115</td>
<td>3, 6, 7, 19, 38, 102</td>
<td>6</td>
<td>Ferreira et al., 2008; Fernández et al., 2000; Ferreira et al., 1998</td>
</tr>
<tr>
<td>India</td>
<td>175</td>
<td>0, 1, 2, 3, 17, 39, 73, 83, 101, 103, 115, 119, 130, 131, 195, 513, 915, 521, 529, 537, 547, 571, 581, 585, 591, 598, 613, 615, 631, 639, 643, 647, 707, 775, 903, 931, 953, Alfa Brazil, Beta, Gamma, IndI, IndII, IndIII, IndIV, IndV, IndVI (Alfa-Brazil), IndVII, IndVIII, IndIX</td>
<td>49</td>
<td>Sharma et al., 2007; Sharma et al., 1999</td>
</tr>
<tr>
<td>Mexico</td>
<td>284</td>
<td>0, 1, 2, 7, 8, 9, 17, 19, 55, 64, 65, 73, 81, 89, 96, 128, 193, 201, 209, 256, 257, 264, 272, 288, 292, 298, 304, 320, 321, 328, 336, 337, 357, 384, 392, 448, 449, 453, 457, 465, 467, 469, 521, 833, 1033, 1088, 1163, 1344, 1431, 1472, 1545, 1600, 1601, 1673, 1677, 1741, 1929, 1993</td>
<td>57</td>
<td>Gonzalez-Chavira et al., 2004; Gonzalez et al., 1998; Balardin et al., 1997, 1997a; Rodriguez-Guerra et al., 2003</td>
</tr>
<tr>
<td>Kenya</td>
<td>4</td>
<td>485</td>
<td>1</td>
<td>Ombiri et al., 2002</td>
</tr>
<tr>
<td>Africa</td>
<td>12</td>
<td>9, 69, 87, 384, 385, 401, 448, 449, 485</td>
<td>9</td>
<td>Bigirimana et al., 1999, 2000</td>
</tr>
<tr>
<td>Canada</td>
<td>44</td>
<td>Alfa Brazil</td>
<td>1</td>
<td>Tu, 1994</td>
</tr>
</tbody>
</table>

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5 Total number of isolates. Predicted the presence of additional virulence factors in their respective regions.

Among the 12 differential cultivars, eight are from the Mesoamerican gene pool, while four belong to the Andean gene pool. Andean differential cultivars contain Co-1 loci whereas Mesoamerican lines contain Co-2 to Co-11 genes (Table 2), with majority of them dominant except Co-8. Comprehensive information is available on resistance genes present in the differential set (Kelly and Vallejo, 2004; Ferreira et al., 2013). Besides the genes present in the differential set, there are major resistance genes such as Co-12, Co-13, Co-14, Co-15, Co-16 and Co-17 present in different Andean and Mesoamerican genotypes (Vidal Filho et al., 2007; Goncalves-Vidigal et al., 2008, 2009, 2012; Coelho et al., 2013; Sousa et al., 2015). In addition to numbered Co genes, there are also Co-Te, Co-x, Co-y and Co-z genes present in different cultivars (Geefroy et al., 1999, 2008). Since not all the anthracnose resistance genes are present in the differential set, there is compelling evidence to revise the current differential set and include all major anthracnose resistance genes. An effort to develop
near isogenic lines (NIL) carrying all resistance genes in a susceptible background would be helpful in devising management strategies for different countries.

MOLECULAR DIVERSITY AND POPULATION GENETICS

Disease management is proportional to population dynamics and understanding different causes of pathogen variability and population genetics is essential. Phenotypic markers do not infer how pathogen population is structured in a particular region because of their low abundance and many other disadvantages. To overcome these problems, molecular markers offer better insights about the population genetics and various evolutionary forces that are shaping a pathogen population in a particular region. DNA-based markers have many advantages over phenotypic markers and prove valuable to understand the population biology of almost all phytopathogenic fungi. Many excellent reviews on the role of molecular markers in deducing diversity in phytopathogens exist and are useful for further readings (Michelmore and Hulbert, 1987; Majer et al., 1996; McDonald, 1997). RAPDs, ISSR, RFLP, PCR-RFLP and ITS sequencing were used among various markers for deducing variability in *C. lindemuthianum* (Balardin et al., 1997; Balardin and Kelly, 1998; Mahuku and Riascos, 2004; Bardas et al., 2007; Padder et al., 2007; Sharma et al., 2007; Silva et al., 2007; Bardas et al., 2009; Kachapulula et al., 2010). The first molecular analysis of *C. lindemuthianum* was carried out by Fabre et al. (1995). They used three different markers (RAPD, RFLP and PCR-RFLP) to infer the genetic variability among different pathogen isolates collected from diverse regions. Most of the *C. lindemuthianum* diversity studies have shown high variability in the pathogen without congruence of phenogram with pathogenicity of the region, suggesting the pathogen has not evolved in a specific gene pool. However, a few studies have shown positive correlation of dendrogram with pathogenicity traits. Comparing regional studies to build up an international perspective of *C. lindemuthianum* population dynamics would be worthwhile, but unfortunately has not proved possible. Population genetic study of *C. lindemuthianum* suggested Mesoamerica as the origin of pathogen (Sicard et al., 1997a, 1997b; Ansari et al., 2004).

All the diversity and population genetic studies in *C. lindemuthianum* are based on RAPD as dominant marker. Numerous disadvantages with RAPD technique have made it obsolete and new markers, especially SSRs, are being preferred as the marker of choice. Unfortunately, to date no SSR marker system is available for *C. lindemuthianum*. However, SSR markers developed for many Colletotrichum species (Ranathunge et al., 2009; Ciampi et al., 2011; Moges et al., 2016) have proved valuable in understanding their population genetics. With the advancement of new sequencing tools, particularly next gene sequencing, at low costs, it is easy to develop SSR markers for *C. lindemuthianum* that may be used to infer population structure at regional and global level. These SSR markers will certainly help in deducing the population genetics of pathogen.

ROLE OF GENOME WIDE ASSOCIATION STUDIES (GWAS) AND BEAN SNP CHIP IN DISSECTING BEAN-ANTHRACNOSE PATHOSYSTEM

Plant disease resistance is either simple (major gene) or complex (quantitative) in inheritance. The former provides complete or near complete resistance through direct or indirect interaction between the pathogen effector and R gene encoding proteins, but the loss of the pathogen recognition target makes the resistance ineffective. Conversely, quantitative resistance is more durable, as a pathogen strain that overcomes a single allele with minor effect does not leave the host completely susceptible. Various biparental populations used for inheritance and allelism tests against anthracnose have shown presence of more than 20 major resistance genes in different bean genotypes. The major anthracnose resistance loci have been mapped to Pv01, Pv02, Pv03, Pv04, Pv07, Pv08, Pv09 and Pv11 bean chromosomes (Geffroy et al., 2008; Ferreira et al., 2013; Meziadi et al., 2016; Zuiderveen et al., 2016). In addition to these major genes, nine anthracnose QTLs were identified in nuna bean PHA1037 against races 23 and 1545 of the pathogen (Oblessuc et al., 2014). The identification of additional resistance specificities in anthracnose resistant genotypes through classical bi-parental population analysis and afterwards allelism tests is time-consuming. GWAS are complementary to bi-parental analysis and can identify traits of economic importance in the crops. GWAS take advantage of natural variation in the population accumulated during historic recombination. GWAS provided comprehensive insights to identify complex traits in both model and non-model plants. The availability of bean genome sequence (Schmutz et al., 2014) and SNP markers in BARCBean6K_3 BeadChip (Hyten et al., 2010; Song et al., 2015) has given fresh impetus to the bean scientific community to map R genes. The SNP chip has resulted in the fine mapping of many resistance sources including: *Co-x* (Richard et al., 2014), *Co-1* (Zuiderveen et al., 2016) *Co-1p* (Vazin, 2015) and the *Co-4* (Oblessuc et al., 2015) and the discovery of new genomic regions and candidate genes associated with anthracnose resistance (Gonzalez et al., 2015). The *Co-x* gene was fine mapped to Pv01, independent of the *Co-1* locus, and to a syntenic region, located at one end of soybean (*Glycine max*) chromosome 18 that carries *Rbg1*, a major gene conditioning resistance to soybean cyst nematode (Richard et al., 2014). Fine mapping of the *Co-4* (COK-4) locus to Pv08 revealed 18 copies of the COK-4 gene in a 325 kbp segment of that chromosome (Oblessuc et al., 2015). Andean bean diversity panel (Cichy et al., 2015) was recently used to identify extra anthracnose
resistance specificities and new genomic regions involved in bean anthracnose resistance (Zuiderveen et al., 2016). The SNP chip currently available to the bean consortium has resulted in its use in the US, but the recent publication of a second bean genome by an international consortium (Vlasova et al., 2016) should hasten additional research on the P. vulgaris-anthracnose pathosystem. In addition to the SNP chip, RNAseq based transcriptome analysis has been used to study the interaction between common bean NIL pair that differs at Co-1 locus following infection with race 73 of C. lindemuthianum (Padder et al., 2016). Differentially expressed transcripts adjacent to the Co-1 locus suggest the global reprogramming in the host.

CONCLUSION AND FUTURE THRUST

Studies on pathogen biology and variability at morphological and molecular level have certainly improved our understanding about the fungus structure and its population genetics. Similarly, host-pathogen dialogue between bean and Colletotrichum advanced our understanding about defence mechanism, resistance genes that are expressed during the interaction with the host. The identification of 182 bean anthracnose races following inoculation of 12-member differential set with more than 1500 isolates illustrates the high pathogenic variability of C. lindemuthianum. Many differential genotypes carry more than one anthracnose resistance gene. There is a need to monitor the prevalence of new isolates for virulence and improve the differential set by developing NIL lines carrying a single anthracnose resistance gene similar to the rice blast pathogen (Telebancovyanoria et al., 2010; Telebanco-Yanoria et al., 2011).

We know fungi secrete a plethora of effector proteins for successful colonization of the host tissue. Studies showed that Colletotrichum species also produce effectors, but how many of them are secreted by bean anthracnose fungus is not known (Tang et al., 2006; O’Connell et al., 2012; Alkan et al., 2013; Schliebner et al., 2014). Effectors produced by Colletotrichum species are cysteine-rich but many of the effectors reported do not share homology with known effectors. The use of next generation sequencing platforms has resulted in genome sequencing at affordable costs and has increased the genomic resources available to study Colletotrichum (Gan et al., 2013; Baroncelli et al., 2014, 2016; Han et al., 2016). In Brazil, genome sequencing of races 83 and 89 is in progress (http://www.colletotrichum.org/genomics/) and access to this information should boost bean/anthracnose research in the years to come. Availability of these resources to the scientific community (http://www.colletotrichum.org/genomics/) needs to be mined for secretome and development of better marker systems such as SSRs and SNPs for discerning the population structure and for identification of evolutionary forces that shape the trajectory of the fungus at both the international and regional level.

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