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PATHOGENIC VARIABILITY OF THE FUNGUS *COLLETOTRICHUM LINDEMUTHIANUM* ON DRY BEAN IN SOUTH AFRICA

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ABSTRACT

Dry bean (*Phaseolus vulgaris* L) anthracnose is an economically important seed-borne fungal disease caused by the fungus *Colletotrichum lindemuthianum.* The pathogenic variability of *C. lindemuthianum* was evaluated in a glasshouse study. A total of 32 isolates were collected in three provinces, namely KwaZulu-Natal, Mpumalanga and North-West. The isolates were collected from different fields of dry bean at research stations and also from small-scale farmers' fields. Inoculum developed from the different isolates was sprayed onto 12 CIAT differential dry bean cultivars that were used to identify pathogen races. The inoculation was carried out during the trifoliate developmental stage of the dry bean seedlings raised in pots 14 days post-sowing. Using the CIAT binomial system, eight pathogenic races of *C. lindemuthianum* were identified, namely, 3, 6, 7, 81, 83, 89, 263 and 323 out of the 32 isolates evaluated. Only pathogenic races 7, 81, 83 and 89 were found in the more humid locations of the province of KwaZulu-Natal. Races 7, 81 and 89 are internationally recognized and show characteristics reported of races in Brazil. Race 6 was identified in Mpumalanga and North west provinces and this was important as it has been reported in other Southern African countries. The races populations were distinct between locations as they infected both the Andean and the Meso-American bean landraces. The most important dry bean landraces were AB 136, G 2333, Kaboon, TU and PI 207262 as they showed complete resistance from the isolates. The study findings suggests that these six landraces can be successfully used to improve anthracnose resistance, especially G 2333 because of its horizontal resistance that can be used to improve the current cultivars used for the control of anthracnose in South Africa. Additionally, Cornell 49242 was one of the landraces of importance, as it showed glimpses of anthracnose that faded overtime under controlled suitable environmental conditions. Use of these landraces will ensure stability in the long-term control of dry bean anthracnose since the pathogen *C. lindemuthianum* is highly variable and widely distributed in South Africa.

Key words: Anthracnose, *Colletotrichum lindemuthianum,* dry bean, disease, pathogenic races, seed, differential cultivars, resistance

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INTRODUCTION

Anthracnose disease of dry bean (*Phaseolus vulgaris* L) caused by *Colletotrichum lindemuthianum* is an economically important seed-borne fungal disease [1]. Anthracnose causes seed impurity rendering the seed unmarketable and under favourable conditions can directly reduce yield [1, 2]. Anthracnose infects all the above parts of the plant including the seed. Due to the seed-borne nature of the disease and its capability to heavily infest the foliage part of the plant under favourable climatic conditions, challenges such as poor seed germination, seed emergence, stand and poor seed quality are common [3]. This can cause heavy losses that can reach as far as 100% [4, 5, 6, 7], especially when a susceptible cultivar is planted in places with high humidity [8].

Anthracnose symptoms include the abaxial vein blackening that extends to the petiole and stem [9]. On the pod, the pathogen causes brick-red to a purple circular lesion that appears as the pod desiccates. The raised halo edge has many acervuli containing masses of conidia [10]. Since the initial report of the disease in 1875 by Lindemuth, documented and supported losses have been reported and intensive research has over the years been conducted on the disease, especially in the Americas and Europe [11, 12]. The most momentous discovery on the disease was its ability to develop variable pathogenic races that are adapted to different production regions of the world. Identification of C. *lindemuthianum* pathogenic variability is of great importance to circumvent cultivar resistance in a given location or season [13, 14]. The first report on the pathogenic variability of *C. lindemuthianum* was in 1911 [15]. The classification started with the use of a set of different differential cultivars by different researchers around the world [16]. Several pathogenic races were identified and were designated by the Greek alphabet [16]. However, the increasing pathogenic variability proved to be difficult for the different differential sets, and the issue of consistency and comparison in the work of different researchers was impossible with this system [16]. This resulted in 12 set differential cultivars with nine genes introduced to better estimate and differentiate the pathogenic races by the International Centre for Tropical Agriculture [17]. Since the establishment of this method, over 180 pathogenic races have been identified throughout the globe [18]. The occurrence of anthracnose is common in production areas with cool and humid conditions. The South African climate is divided into the Mediterranean, arid, semi-arid, temperate, tropical, and subtropical, and the production of dry beans is distributed throughout the country. However, major dry beans production is limited in provinces with temperate, tropical climates and dry winter, this includes Free State, Mpumalanga, KwaZulu-Natal and North-West. In 1994, Edington [19] generally confined the significance of anthracnose in KwaZulu-Natal province due to the prevalence of cool humid environmental conditions. However, the variability of the pathogen is certainly of significance, considering its occurrence and favourable environmental conditions in the majority of dry bean production localities in the four main producing provinces within South Africa. Therefore, understanding the pathogenic variation of anthracnose in South Africa will greatly contribute to the dry bean industry, especially breeding to ensure genetic gains achieved are sustained, improved and resources are allocated as required according to anthracnose distribution. For instance, some races of anthracnose are limited to either

Meso American or Andean group, consequently the resistant group can be selected for breeding against the identified races in a particular region. Better understating of anthracnose variability will further enable researchers to devise management strategies that enables optimal management of anthracnose according to the distribution of the pathogen or pathogen races in the country. Furthermore, to this challenge, there has not been a study that evaluates pathogen distribution throughout South Africa except for selected locations that were presented by Mohammed [19, 20]. Therefore, the purpose of this study was to identify the pathogenic variability of selected *C. lindemuthianum* isolates from major dry bean production regions in South Africa.

MATERIALS AND METHODS

Pathogen collection and isolation

Thirty-two isolates were collected from various dry bean production fields in South Africa, where incidences of the anthracnose disease were observed. Isolates were collected in the 2018 and 2019 dry bean growing seasons. The various fields were spread-out in four major dry bean producing provinces. The provinces and locations included Mpumalanga (Delmas, Balfour and Ermelo), KwaZulu-Natal (Cedara and Greytown), Free State (Bethlehem) and North-West (Potchefstroom). The isolates were sampled from a wide range of commercial cultivars and populations of recombinant inbred lines (RINL). The isolates were sourced from various plant tissues such as leaves pods and seeds.

The isolation of pathogens from diseased plant tissue was conducted under sterile conditions at the Agricultural Research Council Grain Crops (ARC-GC) laboratory. Small pieces of tissue showing typical anthracnose lesions were cut out using a scapula, rinsed with 70% ethanol and hydrated in distilled water for two minutes. The isolates were plated and cultivated in Petri-dishes of potato dextrose agar (PDA). The plates with isolates were placed in an incubator at a temperature of 23^{o}C for conidia germination, and a waiting period of seven days was allowed. Pure colonies grown on plates were identified by their morphological characteristics as described by Sutton [21]. Purification of the colonies was achieved by repeating the plating of the pathogen conidia with pure characteristics of *C. lindemuthianum*, of which after 24 h single conidia were transferred to PDA plates and incubated at room temperature for 10 to 12 days [22, 23].

Plant materials

The 12 set of anthracnose differential cultivars recommended by CIAT [17] were used for the study (Table 1). The material was sourced from the ARC-GC dry bean breeding and germplasm maintenance program.

Inoculum, inoculation and evaluation

The inoculum was prepared by extricating spores from single conidial isolates by gently scraping the culture colonies using a sterile spatula. The detached spores were mixed with distilled water and filtered through cheesecloth. The final concentration was adjusted to 1.2 x 10^{6} spore/ml with the aid of a haemocytometer.

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Seeds of the 12 differential cultivars were planted in five cm-diameter plastic pots and placed in trays. The pots were filled with Culterra soil mix and a cover of Hygrotech medium-grade vermiculite was added for regulated aeration. The plants were grown in a glasshouse with a temperature of 18°C – 26°C regulated by an air condition. Ten sterilized trays with a set of 12 differential cultivars were kept and maintained in the glasshouse. The trays with pots were arranged using a randomised block design and each differential cultivar was replicated three times in a single pot and tray for each distinguished inoculum based on location and unique morphological characteristics. The seedlings were inoculated 14 days after planting using an atomizer with an adjusted inoculum solution of 1.2 x 10^6 spore/ml until runoff. The inoculated seedlings were placed and raised in a dew chamber for three days at ± two degrees Celsius with a relative humidity greater than 90% and a room temperature of 20°C. The plants were then transferred to the glasshouse for evaluation. Disease severity rating was done seven days after inoculation using a scale of 1-9, and a rating of 1-3 was rated as resistant and 4-9 was rated as susceptible.

RESULTS AND DISCUSSION

All 32 *C. lindemuthianum* isolates sourced in the 2018 and 2019 seasons from four provinces (North-West, KwaZulu-Natal, Mpumalanga and Free State) (Table 2) and seven major dry bean production locations within these provinces showed signs of variability. Eight pathogenic races (3, 6, 7, 81, 83, 89, 263 and 323) were identified from the 32 isolates (Table 3).

Races 81 (56.25%) and 89 (9.38%) were both identified from the total isolates sourced from locations in the four provinces in South Africa. Both races were identified from the two locations (Cedara and Greytown) in KwaZulu-Natal and in Mpumalanga, races 3, 6, and 263 were identified. Races 6, and 263 were identified from three different locations (Delmas, Balfour and Ermelo), except for race 3 that was only identified in Balfour. In the North-West and Free State provinces, only one race in each was identified, race 6 and race 323 respectively. Michelite was the most susceptible genotype infected by all races except for race 6. Only AB 136, G 2333, Kaboon, TU and PI 207262 exhibited complete resistance. Cornell 49242 was only mildly susceptible to race 89 in both Cedara and Greytown.

Based on the study findings, it was clear that the existence of anthracnose in South Africa is variable. The presence of variable anthracnose in various locations in South Africa is significant as it confirms the adaptability of *C. lindemuthianum* in various environmental conditions around the world as previously documented by Ishikawa [25] and Mota [26]. With eight pathogenic races identified, the populations found in the more humid areas of KwaZulu-Natal proved to be pathogenic against the Mesoamerican genotypes. Races 81 and 89 were both found in the two locations (Cedara and Greytown) based in KwaZulu-Natal. Their prevalence in two separate locations within the province and over 65% of the total isolates indicates their importance, especially race 81 that further affects the Andean type. The importance of race 81 in South Africa was first reported by Koch [27] and emphasized by Muth [20] over its prevalence in three different surveys over a decade. However, race 89

resembled characteristics previously reported by Muth [20], the race virulence on Cornell 49242 lapse overtime when culture is purified further. In Potchefstroom, where the environmental conditions are less humid and warmer only race 6 was identified, which was first identified by Mohammed [19]. Interestingly race 6 was also identified in Delmas where the environmental conditions are more humid. Race 6 was one of the important pathogenic races as it was reported in other African countries such as Tanzania and Zambia [28].

It has been previously reported that anthracnose races have circumvented multiple resistance from different sources because of its variability as documented by Fouilloux [29] and Menezes [30]. In South Africa, there is sufficient genetic material that can be used to provide resistance to anthracnose. The most important landraces being AB 136, G 2333, Kaboon, TU and PI 207262, since they showed complete resistance in all production regions including KwaZulu-Natal. Another germplasm of importance is Cornell 49242 that proved to be resistant over repeated cycles in sub-culturing against race 89. G 2333 is one genotype that has been documented as the most effective material in controlling anthracnose because of its dominant gene that can easily be transferred to susceptible varieties [31].

It was, therefore, concluded that in South Africa there are six cultivars (AB 136, G 2333, Kaboon, TU and PI 207262 and Cornell 49242) identified in the study that can be successfully used to improve anthracnose resistance. Special interest should focus on landraces that consist of the *Co-4 gene* since it accounts for 97% resistance to anthracnose pathogenic races [32]. In addition, G 2333 should be exploited to improve the current cultivars used for the control of anthracnose in South Africa, as it is associated with a high resistance level [33]. This will ensure stability in the long term since the pathogen *C. lindemuthianum* is highly variable and widely distributed in South Africa. The presence of different resistance genes and alleles in the different germplasm makes it possible to breed for race specific resistance and genetic pyramiding (vertical resistance).

CONCLUSION

Anthracnose of dry bean is widely distributed and variable in South Africa. However, the present genetic materials are sufficient to provide durable resistance against the races identified. Going forward, the transition of *C. lindemuthianum* should be monitored and the available resistant genes incorporated in the national breeding programme.

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Table 1: CIAT Dry Bean Anthracnose differential cultivars

Differential cultivar	Origin	Resistance Gene	Binary value	Seed type/Colour	Growth habit
AB 136	Meso American	*Co-6, co-8*	1024	Small red	IV
Michelite	Meso American	*Co-11*	1	Small white	II
Michigan Dark Red Kidney	Andean	*Co-1*	2	Large dark red kidney	I
Perry marrow	Andean	*Co-1*^{3}	4	Medium white	II
Cornell 49242	Meso American	*Co-2*	8	Small black	II
Widusa	Meso American	*Co-1*^{5}*, Co-9*	16	Medium white	I
Kaboon	Andean	*Co-1*^{2}	32	Large crème	II
Mexico 222	Meso American	*Co-3*	64	Medium white	I
PI 207262	Meso American	*Co-4*^{3}*, Co-9*	128	Small tan	III
To	Meso American	*Co-4*	256	Medium carioca	I
Tu	Meso American	*Co-5*	512	Small black	III
G 2333	Meso American	*Co-4*^{2}*, Co-5, Co-7*	2048	Small red	IV

Growth habit: I= determinate; II= indeterminate bush, erect stem; III= indeterminate bush, weak stem and prostrate branches; IV= indeterminate climbing habit [17]. Adapted from Mohammed [19] and Kelly [24]

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Table 2: **Collection of *C. lindemuthianum* isolates from different South African geographical locations**

Province	Location	GPS Coordinates		
		Latitude	Longitude	Altitude (m)
KwaZulu-Natal	Cedara	29°32'51,70″ S	30°16'00,03″ E	1115
	Greytown	29°02'38,33″ S	30°35'53,17″ E	1092
North-West	Potchefstroom	26°44'04,21″ S	27°04'43,52″ E	1350
Free State	Bethlehem	28°13'41,70″ S	28°16'59,77″ E	1717
Mpumalanga	Balfour	26°37'31,40″ S	28°35'27,24″E	1639
	Delmas	26°08'06,62″ S	28°40'29,53″ E	1569
	Ermelo	26°30'37,37″ S	29°59'02,82″ E	1697

Table 3: *C. lindemuthianum* races identified and disease ratings in 32 isolates from 7 locations in South Africa

	Isolates from different locations										
Differential cultivars	**CI1**	**CI2**	**CI3**	**CI4**	**GI1**	**GI2**	**PI**	**BI**	**BAI**	**EI1**	**DI1**
AB 136	R	R	R	R	R	R	R	R	R	R	R
Michelite	S	S	S	S	S	S	R	S	S	R	S
Michigan Dark Red Kidney	S	R	S	R	R	R	S	S	S	S	S
Perry marrow	S	R	S	R	R	R	S	R	R	S	S
Cornell 49242	R	R	R	S	S	R	R	R	R	R	R
Widusa	R	S	S	S	S	S	R	R	R	R	R
Kaboon	R	R	R	R	R	R	R	R	R	R	R
Mexico 222	R	S	S	S	S	S	R	S	R	R	R
PI 207262	R	R	R	R	R	R	R	R	R	R	R
To	R	R	R	R	R	R	R	S	R	R	S
Tu	R	R	R	R	R	R	S	R	R	R	R
G 2333	R	R	R	R	R	R	R	R	R	R	R
Total binary value	7	81	83	89	89	81	6	323	3	6	263
No of Isolates	2	9	5	2	1	9	1	1	3	1	1

CI1=Cedara isolate 1, CI2=Cedara isolate 2, CI3=Cedara isolate 3 and CI4=Cedara isolate 4. GI1=Greytown isolate 1 and GI2=Greytown Isolate 2. PI=Potchefstroom Isolate. BI=Bethlehem isolate. BAI=Balfour isolate. EI=Ermelo isolate. DI=Delmas isolate. R= Resistant. S=Susceptible

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