Sky Journal of Agricultural Research Vol. 3(4), pp. 053 - 061, April, 2014
Available online http://www.skyjournals.org/SJAR
ISSN 2315-8751 ©2014 Sky Journals

Full Length Research Paper

Virulence diversity of *Colletotrichum lindemuthianum* in Uganda

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Accepted 3 April, 2014

Colletotrichum lindemuthianum **is a highly variable pathogen of common beans that easily defeats resistance in cultivars bred with single-gene resistance. To determine virulence variability of the pathogen in Uganda, samples of common bean tissues with anthracnose symptoms were collected in eight districts of Uganda, namely Kabarole, Sironko, Mbale, Oyam, Lira, Kapchorwa, Maracha and Kisoro. 51 isolates sporulated successfully on potato dextrose agar and Mathur's media and were used to inoculate 12 differential cultivars under controlled conditions. Five plants per isolate for each cultivar were inoculated and then evaluated for their reaction using the 1 – 5 severity scale of Drifjhout and Davis (1989). Data was subjected to analysis of variance and races were classified using the binary nomenclature system proposed by Pastor Corrales (1991). Variation due to cultivar and isolate effects was highly significant (P ≤ 0.001) for severity. The 51 isolates from eight districts grouped into 27 different races. Sironko district had the highest number of races followed by Mbale and Kabarole. Races 2047 and 4095 were the most frequently found, each with 10 isolates grouped under them. Race 4095 had the broadest spectrum of virulence since it caused a susceptible (S) reaction on all 12 differential cultivars and the susceptible check. This was followed by races 2479, 2047 and 2045 respectively. Two races, 4094 and 2479, caused a susceptible reaction on the highly resistant differential cultivar G2333, which nevertheless, showed the broadest resistance followed by Cornell, TU, and AB136 respectively. These cultivars are recommended for use in breeding programs aimed at breeding for broad resistance to bean anthracnose in Uganda.**

Key words: Bean anthracnose, resistance genes, races.

INTRODUCTION

Bean anthracnose, caused by *Colletotrichum lindemuthianum* (Sacc. and Magn.) Lams.-Scrib, is one of the most important diseases of common beans in Uganda (Opio et al., 2001) devastating farmers' fields causing up to 70% marketable yield losses on susceptible cultivars (Nkalubo et al., 2007). The pathogen is mainly seed borne (del Rio and Bradley, 2004) and, therefore, easily spread as farmers highly depend on farm saved seed and exchange of seed is common (Opio et al., 2001).

The pathogen has a wide pathogenic variation with various races reported in major beans producing countries such as Mexico and Brazil (Balardin and Kelly, 1998). High virulence diversity was also detected in Latin America (Pastor-Corrales et al., 1995). Mahuku and Riascos (2004) assessed virulence and molecular diversity of 200 *C. lindemuthianum* isolates collected from Andean and Mesoamerican bean cultivars and regions. They reported high levels of pathotypic (90 pathotypes) diversity among the 200 isolates. Bigirimana et al. (2000)

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characterized nine *C. lindemuthianum* races using 12 isolates collected from major bean growing areas in Burundi and12 standard differential cultivars. Races 9, 69, 87, 384, 385, 401, 448, 449 and 485 were identified. Seven of these races were reported for the first time in Burundi.

In Uganda, Leaky and Simbwa-Bunnya (1972) using differential cultivars from Shreiber and Hubberling, identified races 17, 19, 23, 102, 130, and 453 with isolates collected from Central, Western and South Western regions of Uganda. More recently, Nkalubo (2006) reported races 23, 55,102, 130, 227, 375, 511 and 767 from Kabale, Kisoro, Bushenyi and Mpigi, districts with race 767 reported as the most widespread and virulent. Three races of Nkalubo (2006) namely 23, 102 and 130 were similar to those of Leaky and Simbwa-Bunnya (1972). Mwesigwa (2008) reported 21 races (0, 2, 3, 4, 6, 14, 128, 262, 264, 268, 320, 452, 481, 1024, 1536, 1538, 1856, 1857, 1989, 3086 and 4033) out of 47 isolates collected from Kabale, Apac, Mbale, Mpigi and Wakiso districts. None of his races were similar to those of Leaky and Simbwa-Bunnya (1972) and Nkalubo (2006). Two highly virulent races 3086 and 4033 caused symptoms on the highly resistant differential cultivar G2333. Mwesigwa (2008) further reported that nine of the races were virulent to the Mesoamerican cultivars, three races were virulent to the Andean cultivars and seven races were virulent to both groups of cultivars.

There is a wide gap in time from the work of Leakey and Simbwa-Bunnya (1972) to that of Nkalubo (2006) and Mwesigwa (2008) to suspect tremendous change in diversity of the pathogen as a result of increase in bean production, introduction of new varieties from different gene pools and movement of bean seed within and outside the country and region. The differences in physiological races from the earlier studies could be as a result of use of different set of differential cultivars for characterization, sampling differences and emergence or introduction of new races that overcome previously stable resistances among the bean differential set. The aim of this study was to determine the current virulence diversity of *C. lindemuthianum* in Uganda including bean growing districts that earlier studies had not covered.

MATERIALS AND METHODS

Sample collection and pathogen isolation

Common bean pods with symptoms of anthracnose disease were collected in 2009 from different common bean cultivars from eight districts of Uganda namely Kabarole, Kapchorwa, Kisoro, Lira, Maracha, Mbale, Oyam and Sironko (Figure 1). Infected tissues from the bean pods were cut into small pieces of up to 5 cm long. The tissues were placed into a small beaker and 10 ml of Sodium hypochlorite (Jik) bleach was added. After 2 min, the bleach was removed and 10 ml of ethanol was added. After 2min the ethanol was removed and the tissues were rinsed with sterilized water. A pair of forceps was flamed under a sterile laminar flow hood. The tissues were placed on filter paper to remove excess water and dry them. The tissues were placed on PDA media and incubated in darkness at 22 - 24°C. After four days, the different cultures showed growth and were sub-cultured onto modified Mathur's Agar media (500 g) made up of 4 g of Dextrose, 1.25 g of Magnesium Sulfate, 1.35 g of Potassium Phosphate, 1.2 g of Neopeptone, 1 g of Yeast extract and 8 g of Agar, to get pure isolates and increase sporulation.

Inoculum preparation

Single-spore isolates were placed on fresh Mathur's agar medium in a petri dish and incubated at 22 - 24°C for 4 - 7 days to allow the fungus enough time to produce conidial spores. For inoculation purposes, conidial spores were scrapped off the growth medium into a small amount of water to make a suspension. Using a hemocytometer the concentration was adjusted to 1.2 x 10^6 conidia ml^{-1} 0.1% Tween 20 was added as a surfactant.

Inoculation and disease evaluation

Seed of the 12 differential cultivars were pre-germinated and later soaked for 30 min into the inoculum before transplanting into sterilized soil mixed with saw dust, in a controlled screen house at the National Crops Resources research Institute, NaCRRI. Five seeds of each of the 12 differential cultivars were sowed in a tray plus a known susceptible check K132. The screen house conditions were maintained at 95 - 100% relative humidity and temperature of 22 ± 2°C. Disease severity was scored 10 – 14 days after planting using a 1 – 5 scale (Drifjhout and Davis, 1989) Where; 1 = no symptoms (resistant), 2 = very small lesions mostly on primary leaves (resistant), 3 – 5 = numerous enlarged lesions or sunken cankers on the lower side of the leaves or hypocotyls (susceptible).

Race characterization and data analysis

To identify races, the binary system was used based on the sum of binary values assigned to each of the 12 differential cultivars proposed by Pastor-Corrales (1991) to characterize anthracnose races. Each differential cultivar had an assigned number 2^n where n corresponds to the place occupied by the cultivar within the differential series. The designation of a race number was obtained by summing the numerical values of all differential cultivars exhibiting susceptible (S) reactions to the isolate used for inoculation. Isolates with similar reactions on the differentials were grouped to form a race. Data was

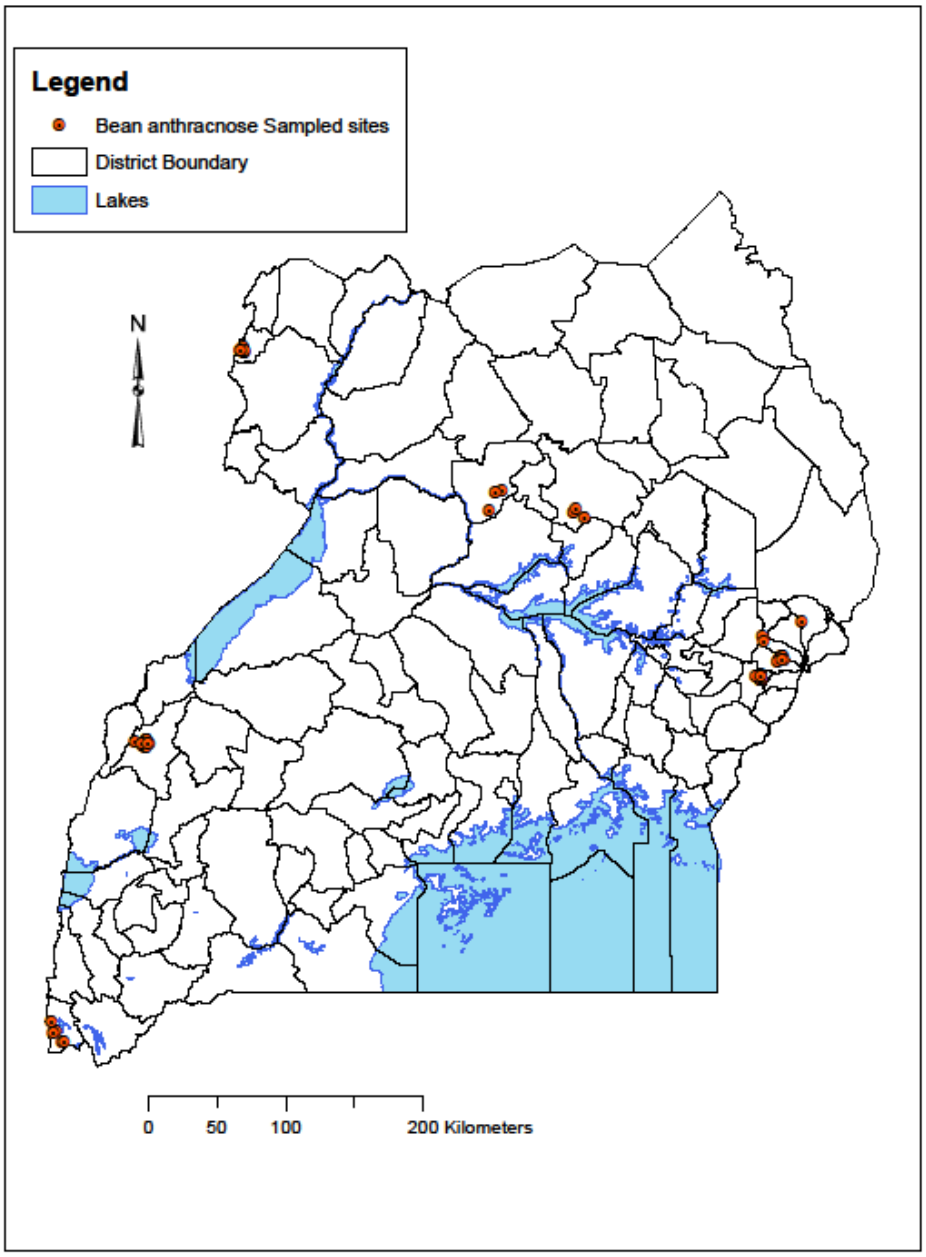

Figure 1. Map of Uganda showing sites where anthracnose diseased bean samples were collected.

subjected to two-way analysis of variance using GenStat 13th Edition (Payne et al., 2010).

RESULTS AND DISCUSSION

Analysis of variance (Table 1) revealed highly significant differences (P ≤ 0.001) among the cultivars and isolates. Variation due to the interaction between cultivars and isolates was also highly significant (P ≤ 0.001). Isolate contributed most (49.8%) to the total variation followed by cultivars (46.2%). Using the binary nomenclature system, the 51 isolates screened for virulence were grouped into 27 races (Table 2). Races 2047 and 4095 were the most abundant with each occurring 10 times. Race 4095 had the broadest virulence spectrum since it caused a susceptible (S) reaction on all of the 12 differential cultivars and the susceptible check. This was followed by races 2047, 2045 2039 and 2023. Two races, 4095 and 2479, representing a total of 11 isolates caused susceptible reactions on G2333, the most important source of resistance to bean anthracnose. Race 2047

Table 1. Analysis of variance for bean anthracnose severity on the 12 differential cultivars.

Source of variation	Severity		
	d.f	Mean square	% Contribution[a]
Rep	4	1.10	1.51
Cultivar	12	33.78*	46.20
Isolate	50	36.41*	49.79
Cultivar x Isolate	600	1.33*	1.82
Residual	2647	0.50	0.69

[a] Percentage contribution of each source of variation to the total variation.
* Significant at P ≤ 0.001.

Table 2. Race designation, isolate groupings and susceptible and resistant reactions of common bean differentials to 51 *C. lindemuthianum* isolates collected from Eastern, Northern and South Western Uganda.

	Differential cultivars and their respective resistance genes[a]												
	1	2	3	4	5	6	7	8	9	10	11	12	
Race*	Co-11	Co-1	Co-1^3	Co-2	Co-1^5	Co-1^2	Co-3	Co-4^3, Co-9	Co-4	Co-5	Co-6, co-8	Co-4^2, Co-5^2, Co-7	Isolates
0	R	R	R	R	R	R	R	R	R	R	R	R	38A, 59A, 57A, 25A
1	S	R	R	R	R	R	R	R	R	R	R	R	82A
42	R	S	R	S	R	S	R	R	R	R	R	R	85A
81	S	R	R	R	S	R	S	R	R	R	R	R	92A
128	R	R	R	R	R	R	R	S	R	R	R	R	36A
352	R	R	R	R	R	S	S	R	S	R	R	R	84A
386	R	S	R	R	R	R	R	S	S	R	R	R	61A
503	S	S	S	R	S	S	S	S	S	R	R	R	88A
704	R	R	R	R	R	R	S	S	R	S	R	R	34A
713	S	R	R	S	R	R	S	S	R	S	R	R	86A
767	S	S	S	S	S	S	S	S	R	S	R	R	69A
784	R	R	R	R	S	R	R	R	S	S	R	R	56A
1023	S	S	S	S	S	S	S	S	S	S	R	R	62A
1094	R	S	S	R	R	R	S	R	R	R	S	R	12A
1169	S	R	R	R	S	R	R	S	R	R	S	R	72A
1175	S	S	S	R	S	R	R	S	R	R	S	R	91A
1334	R	S	S	R	S	S	R	R	S	R	S	R	94A
1471	S	S	S	S	S	S	R	S	S	R	S	R	90A, 100A
1527	S	S	S	R	S	S	S	S	S	R	S	R	16A, 99A
1791	S	S	S	S	S	S	S	S	R	S	S	R	41A
1834	R	S	R	S	R	S	R	R	S	S	S	R	81A
2023	S	S	S	R	R	S	S	S	S	S	S	R	007A

2039	S	S	S	R	S	S	S	S	S	S	S	R	97A
2045	S	R	S	S	S	S	S	S	S	S	S	R	95A, 40A
2047	S	S	S	S	S	S	S	S	S	S	S	R	65A, 08A, 64A, 75A, 98A, 52A, 55A, 37A, 46A,71A
2479	S	S	S	S	R	S	R	S	S	R	R	S	83A
4095	S	S	S	S	S	S	S	S	S	S	S	S	66A, 63A, 44A, 67A, 76A, 28A, 001A, 77A, 73A, 96A

*Races characterized using the binary system (Pastor Coralles, 1991); Resistant reaction (R); Susceptible reaction (S).
[a] Differential cultivars: 1 = Michelite; 2 = MDRK; 3 = Perry Marrow; 4 = Cornell 49-242; 5 = Widusa; 6 = Kaboon; 7 = Mexico 222; 8 = PI 207262; 9 = TO; 10 = TU; 11 = AB136; and 12 = G 2333.
^Susceptible check K132: -/+ Cultivar (only K132) showed both (-) and (+) reaction to isolates with in a race.

was comprised of 10 isolates distributed in five districts of Kabarole, Kisoro, Maracha, Mbale and Sironko. Race 2479 was comprised of one isolate from Sironko district, while race 4095 was comprised of 10 isolates distributed in seven districts of Kabarole, Kapchorwa, Kisoro, Oyam, Mbale, Maracha and Sironko. This makes race 4095 the most widely distributed followed by race 4027. Sironko district had the highest number of races followed by Mbale and Kabarole (Figure 2). Only three races in this study were similar to races reported in earlier studies in Uganda namely race 0 and 128 (Mwesigwa, 2008) and race 767 (Nkalubo, 2006). This lack of consistency may be attributed to the different methodological/sampling approaches employed.

The differential cultivar G2333 showed the highest number of resistant (R) reactions followed by cultivars Cornell 49-242, TU and AB 136 (Figure 3). This implies that the cultivars in their respective order confer broad resistance to the anthracnose races in Uganda. The cultivars TU, AB136 and G2333 were also reported to be resistant against 12 isolates collected in major bean-growing areas in Burundi (Bigirimana et al., 2000). The similarity in trend with Ugandan isolates indicates that the four cultivars G2333, Cornell 49-242, TU and AB 136 are respectively the best parents to use in a hybridization program aiming at breeding for broad resistance against bean anthracnose. The highly effective resistance in cultivar G2333 is mostly attributed to its naturally occurring resistance gene pyramid comprising of Co-4^2, Co-5 and Co-7 genes, The allele Co-4^2 is recognized as being among the most effective resistance genes described in common beans (Balardin and Kelly, 1998; Silverio et al., 2002). Mwesigwa (2008), on the other hand, reported that the differential cultivar Widusa did not succumb to any isolate in his collection from which two races, 3086 and 4033, overcame the resistance in G2333. Widusa, however, ranked number five based on based on number of resistant (R) reactions. The cultivars K132 (check), PI 207262, MDRK and Michelite, respectively had the lowest number of Resistant (R) reactions, implying that their resistance is more specific in nature since they showed exceptionally effective resistance to certain isolates. They could still be useful in breeding programs targeting resistance against certain races or pyramiding genes to obtain broader resistance against a range of races. The cultivar Michelite, contrary to reports in the literature, was not the most susceptible and seemed to confer resistance in a more effective manner than the popularly known resistant cultivar PI207262. Mwesigwa (2008) observed the same about Michelite, which is usually reported as a universal susceptible differential cultivar. The resistance in this cultivar can be attributed to the *Co*-11 gene reported by Gonçalves-Vidigal et al. (2007).

Leaky and Simbwa-Bunnya (1972) observed that the immune nature of resistance in the cultivar Cornell 49-242, conferred by a single dominant gene Co-2 (Mastenbroek, 1960), reduced the disease to a status of no importance in Holland but when tested in Uganda it showed clear anthracnose symptoms with five Ugandan isolates. In our study the cultivar Cornell 49-242, which ranked second after G2333, showed clear anthracnose symptoms with 11 races. The cultivar PI207262 (Tlalnepantla 64), ranked the lowest of all the 12 differential cultivars. Bigirimana et al. (2000) also ranked it second lowest after Michelite. This cultivar has a naturally occurring resistance gene pyramid consisting of Co-4^3 and Co-9 genes. Its resistance is reported to have very specific breeding value against Andean races of bean anthracnose and is recommended for use only to diversify the resistance in gene pyramids because of its independence and potential value in controlling highly virulent Andean races (Kelly and Vallejo, 2004).

Conclusion

The results suggest a highly significant virulence diversity of *C. lindemuthianum* in Uganda which could be an outcome

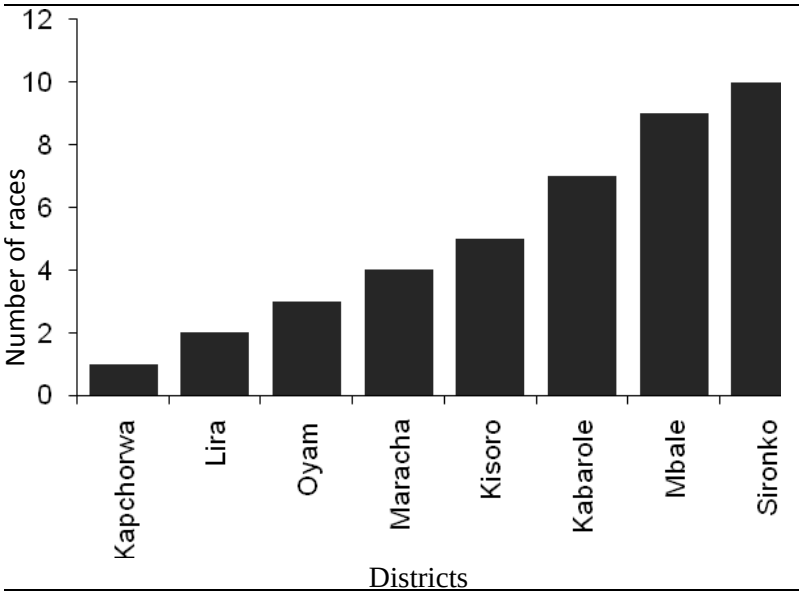

Figure 2. Number of races by district.

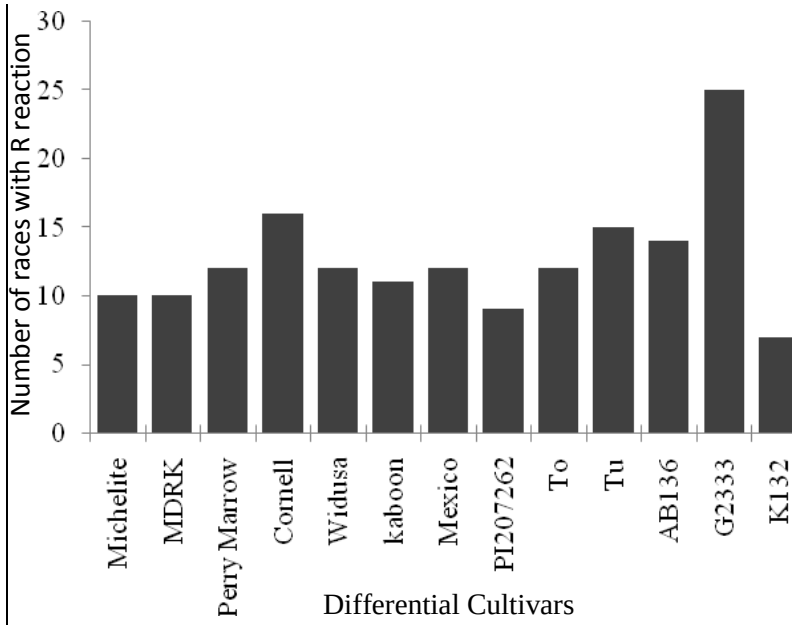

Figure 3. Pathogenicity of 27 races on 12 differential common bean cultivars and a susceptible check, K132 (CAL96).

of introduction into the country of already infected seed through illegal cross-border seed trade and/or the high genetic variability of the pathogen. The races 4095, 2479 and 2047 were the most virulent races in the sampled regions and probably in Uganda. The Eastern region of Uganda (Sironko and Mbale) had the highest number of races including the most virulent ones followed by the Western (Kabarole district) and South western (Kisoro district) regions. These are all high altitude bean growing regions ranging from 1429 – 1860 m above sea level. Such environments are known to have favorable conditions for the development and spread of bean anthracnose.

There is need to conduct regular disease surveillance and sampling for bean anthracnose covering a wider sampling area in Uganda. In addition, there is need to

initiate a gene pyramiding program using the resistance genes in cultivars G2333, Cornell 49-242, TU and AB136 to create new and favorable gene combinations using linked molecular markers. Some genes that could be used to create new combinations with genes in G2333 include Co-6 and Co-8 in AB136, Co-5 in TU, Co-3 in Mexico 222 and Co-1^5 in Widusa. These genes showed resistant (R) reaction where G2333 showed a susceptible (S) reaction with race 2479.

Acknowledgements

The study was funded by the Uganda National Council of Science and Technology (UNCST) through the Millennium Science Initiative (MSI) project. The Regional Universities Forum for Capacity Building (RUFORUM), NaCRRI and CIAT-Uganda are specially thanked for the support offered to the students.

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