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First report of race 485 of *Colletotrichum lindemuthianum* in Kenya and its implication in bean resistance breeding

Erstnachweis der physiologischen Rasse 485 von *Colletotrichum lindemuthianum* in Kenya und ihre Bedeutung für die Resistenzzüchtung von Bohnen

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Summary

Four isolates of *Colletotrichum lindemuthianum* were collected in 1997 from bean seeds produced in Rongai in Nakuru District. Characterisation of the isolates using the twelve internationally recommended differential bean cultivars and the Binary System of classification demonstrated that the four isolates were similar and were classified as race 485.

The characterisation of race 485 is yet the first report of such a race in Kenya. It lacks virulence genes against the *Are* gene evidenced by incompatible reaction with cultivar 'Cornell 49-242'. The new race therefore poses no threat to the bean genotypes with *Are* gene. However it poses a great threat to the popularly grown Kenyan beans which have no *Are* gene. It seems to have more virulence genes than races so far reported in Kenya. This implies that the host may require more resistant genes to stand the increased virulence genes and therefore maintain a long-term durable resistance.

Zusammenfassung

1997 wurden von Bohnensamen aus Rongai, Nakuru-District, vier Isolate von *Colletotrichum lindemuthianum* gewonnen. Mit Hilfe des international empfohlenen Differentialsortiments von Bohnen und dem binären Klassifikationssystem wurde nachgewiesen, dass die vier Isolate einheitlich und als Rasse 485 zu bezeichnen waren.

Die Prüfung der Isolate ergab den Erstnachweis der Rasse 485 in Kenya. Ihr fehlen Virulenzgene gegen die *Are*-Resistenzgene, wie aus der inkompatiblen Reaktion mit der Sorte 'Cornell 49-242' ersichtlich wurde. Die neue Rasse ist demnach nicht gefährlich für Bohnengenotypen mit dem *Are*-Gen. Sie ist jedoch eine große Bedrohung für die allgemein angebauten Bohnensorten in Kenya, denen ohnehin das *Are*-Resistenzgen fehlt. Immerhin hat sie mehr Virulenzgene als alle bisher in Kenya bekannten Rassen. Dies bedeutet, dass der Wirt eine größere Zahl von Resistenzgenen braucht, um der erhöhten Zahl von Virulenzgenen zu widerstehen und damit eine längerdauernde Resistenz zu gewährleisten.

Introduction

Pathogenic variability of *Colletotrichum lindemuthianum* (SACC. & MAGNUS) Lams.-Scrib., the causal agent of bean anthracnose, was first reported by BARRUS (1911). Since then, several races of this fungus have been described (MENEZES and DIANESE 1988; GATHURU and MWANGI 1991; KELLY et al. 1994; TU 1994; MESQUITA et al. 1998). This variability of the fungus has been cited as the primary reason for the economic importance of anthracnose in many areas of Central and Eastern Africa, as well as Latin America (GARRIDO and COVA 1989).

Studies on pathogenic variation in *C. lindemuthianum* in Kenya have been conducted (GATHURU and MWANGI 1991) where thirty six isolates collected from nine districts of Kenya were cultured and inoculated on bean differentials: 'Michelite', 'Perry Marrow', 'Michigan Dark Red Kidney', 'Emerson 847', 'Kaboon', 'Cornell 49-242', 'Processor' and 'Canadian Wonder'. Eleven isolates were grouped as beta, eight as gamma, five as epsilon, two as delta and one as alpha. The system proposed by HUBBELING (1957) was used. Nine isolates did not fit in any of the known races. The cultivar 'Cornell 49-242' was found resistant to all isolates. The cultivar 'Kaboon' was found susceptible to the majority of isolates. So far six races namely alpha, beta, delta, epsilon, gamma and lambda have been reported in Kenya (KINYUA 1976; MWANGI 1986; GATHURU and MWANGI 1991). The system of characterising the isolates used by the researchers was limited in that some isolates could not be identified.

Researchers at CIAT (1987) came up with a system where they defined a group of twelve common bean differentials to be used internationally in order to facilitate the exchange of information and of resistant germplasm. At the same time, a binary system of classification was proposed. With the utilisation of the twelve differential cultivars coupled with binary system for race designation, it is possible to characterise all to the existing races by designating them a number. In this study, this system was utilised to identify Kenyan isolates which would have not been characterised using the method formerly used by researchers.

Materials and Methods

Isolation of C. lindemuthianum

Anthracnose-infected bean seeds of variety 'Rosecoco' were obtained from Rongai in Nakuru District. These were surface sterilised by immersing them in 0.5 % NaOCl for 3 minutes. After which they were rinsed in sterile distilled water, blotted dry on sterile filter paper, and placed on Glucose peptone agar (GPA). Streptomycin sulphate (0.1 g l^{-1}) was added to suppress bacterial growth (DILLARD and COBB 1993). The cultures were incubated for 14 days in darkness at 20–21 °C. *C. lindemuthianum* was identified based on descriptions by Mordue (1971). Single spore isolates were made from the isolates by preparing a highly diluted conidial suspension in sterile water. Two to three millilitre conidial suspension was spread on Water Agar (WA) in petridish under sterile conditions and incubated in darkness at 20–21 °C for 24 hours. The cultures were examined under microscope for germination of conidia on WA. Under sterile conditions, four germinated conidia per culture-plate were removed using a thin isolation needle and plated separately on GPA plates. After 4 to 5 days the cultures were subcultured on GPA plates and incubated for 14 days.

Inoculum preparation

Inocula of isolates A, B, C, and D of *C. lindemuthianum* were produced by growing them in culture bottles with GPA. The isolates were incubated for 2 weeks in darkness at 20–21 °C. Spore suspensions were prepared by flooding the cultures with 5ml distilled water. Spores were dislodged by scrapping the culture surface using a glass rod. Suspensions for inoculation of germinated bean seeds were filtered through cheese cloth, and the concentration adjusted to 10^6 spores per millilitre using a microscope and a haemacytometer.

The following formula was used to adjust the inocula to the desired concentrations (BESHIR 1991):

$V_0 \times C_0 = V_g \times C_g$
where V_0 = initial volume
C_0 = initial concentration obtained by counting on haemacytometer
V_g = final volume for inoculation
C_g = final concentration
$V_g = V_0 \times C_0 / C_g$

Raising of bean seedlings and inoculation

Twelve standard differential cultivars used were the following:

1. 'Michelite'; 2. 'Michigan Dark Red Kidney' ('MDRK'); 3. 'Perry Marrow'; 4. 'Cornell 49-242'; 5. 'Widusa'; 6. 'Kaboon'; 7. 'Mexico 222'; 8. 'PI207262'; 9. 'TO'; 10. 'TU'; 11. 'AB136'; and 12. 'G2333'.

A slight modification of the method used by MENEZES and DIANESE (1988) was utilised in germination of the seeds and inoculation of the germinated seeds. Seeds were germinated in petridishes containing moist vermiculite. After 3 days of germination, the seedcoats of the germinated seeds were removed in order to expose the cotyledons, when dipped in conidial suspension for 2 minutes. The inoculated germinated seeds were sown in 10 cm diameter pots filled with sand. Ten seeds were inoculated per differential cultivar per isolate. Each pot containing 10 seeds were replicated twice. The plants were kept in a growth chamber at 20–21 ° C.

The infection severity of the hypocotyl was assessed by a modification of classification by DRIJFHOUT and DAVIS (1989) as follows:

		Severity scale
Healthy	– no symptom	1.0
Light infection	– pin-point lesions	1.5
Moderate infection	– up to 5 small to large sunken lesions (about 3 mm)	3.0
Severe infection	– large deep lesions (larger than 3 mm) and /or death of seedlings.	5.0

Disease severity index (DSI) was computed using the following formula:

$$DSI = (1 \times n_1 + 1.5 \times n_2 + 3 \times n_3 + 5 \times n_4) / N,$$

where 1.0, 1.5, 3.0 and 5.0 are the four severity scales and n_1 to n_4 refer to the number of seedlings with the respective severity scales. N refers to the total number of seedlings examined per variety per isolate.

The cultivar disease reactions were categorised based on DSI scores as follows:

Resistant = 1.0 – 1.5
Moderate = 1.6 – 3.0
Susceptible = 3.1 – 5.0

Based on the reaction of differential cultivars to the isolates, the races were identified by designating them values in accordance to binary system where a value is given to a cultivar when it gives a susceptible reaction to a race (CIAT 1987). The race is then identified by a number, which is the sum of all values associated with a cultivar when it gives a susceptible reaction to the race.

To monitor the accuracy of the inoculation and scoring techniques used in race characterisation, the bean differential series were inoculated with two known races lambda and kappa. The methods used were the same as those already outlined.

Results

Identification of the isolates using differential cultivars and Binary system

The cultural growth of the four isolates on GPA were characteristic of the bean anthracnose fungus. All the four isolates exhibited similar responses to the differential cultivars (Table 1) suggesting that they are of the same physiological race. Clear-cut race-specific reactions of the cultivars were observed. The cultivars tested were either resistant or susceptible to the isolates. None of the cultivars exhibited moderate resistance. This indicates a high degree of specificity with *C. lindemuthianum*.

Based on the reaction of the differential bean cultivars to the isolates, the race was identified by designating them values according to Binary system (CIAT 1987; BURUCHARA 1991).

The isolates were therefore identified as race 485. From the reaction of the differential cultivars to race 485, it was noted that the race has a fairly broad pathogenicity with the virulence genes for six of the differential cultivars. However, it lacks virulence genes against the *Are* gene evidenced by incompatible reaction with the cultivar 'Cornell 49-242'.

Comparison of race 485 to races lambda and kappa

The reaction of the 12 differential bean cultivars to races lambda and kappa (Table 2) was in agreement to what has already been reported by other workers (TU 1994; BALARDIN et al 1997). It was also noted that race 485 has more virulence genes against the differential cultivars than lambda and kappa.

Discussion

All the four isolates obtained from infected seeds of 'Nakuru' exhibited more or less similar response to the differential cultivars, suggesting that they are of the same physiologic race. Based on the reaction of the differential cultivars to the isolates, the race could be

Table 1. Reactions[a] of differential bean cultivars to isolates of *C. lindemuthianum*.
Reaktion der Testsorten von Bohnen gegen Isolate von Colletotrichum lindemuthianum.

Variety	Binary number	Isolate A	Isolate B	Isolate C	Isolate D
'Michelite'	1	S (5.0)[b]	S (4.4)	S (4.1)	S (4.3)
'MDRK'	2	R (1.0)	R (1.0)	R (1.0)	R (1.3)
'Perry Marrow'	4	S (5.0)	S (5.0)	S (4.7)	S (4.4)
'Cornell 49-242'	8	R (1.0)	R (1.0)	R (1.0)	R (1.0)
'Widusa'	16	R (1.0)	R (1.0)	R (1.0)	R (1.0)
'Kaboon'	32	S (4.8)	S (5.0)	S (5.0)	S (4.9)
'Mexico 222'	64	S (3.2)	S (3.4)	S (3.1)	S (3.0)
'PI 207262'	128	S (5.0)	S (5.0)	S (5.0)	S (5.0)
'TO'	256	S (5.0)	S (5.0)	S (5.0)	S (5.0)
'TU'	512	R (1.0)	R (1.0)	R (1.0)	R (1.0)
'AB 136'	1024	R (1.0)	R (1.0)	R (1.0)	R (1.0)
'G 2333'	2048	R (1.0)	R (1.0)	R (1.0)	R (1.0)
Race number		485	485	485	485

[a] R = resistant S = susceptible
[b] Numbers in parentheses indicate disease severity index

Table. 2. Disease reactions[a] of race 485 of *C. lindemuthianum* on twelve differential bean cultivars compared to races lambda and kappa.
Reaktion der Testsorten von Bohnen gegen Rasse 485 von C. lindemuthianum im Vergleich zu den Rassen lambda und kappa.

Variety	Binary number	Race		
		Race 485	kappa	lambda
'Michelite'	1	S	S (5.0)[b]	S (4.7)
'MDRK'	2	R	S (5.0)	S (4.5)
'Perry Marrow'	4	S	S (5.0)	S (5.0)
'Cornell 49-242'	8	R	S (5.0)	R (1.0)
'Widusa'	16	R	S (4.8)	S (4.8)
'Kaboon'	32	S	R (1.1)	S (3.5)
'Mexico 222'	64	S	R (1.0)	R (1.0)
'PI 207262'	128	S	R (1.0)	R (1.0)
'TO'	256	S	R (1.0)	R (1.0)
'TU'	512	R	R (1.0)	R (1.0)
'AB 136'	1024	R	R (1.0)	R (1.0)
'G 2333'	2048	R	R (1.0)	R (1.0)
Race number		485	31	55

[a] R = resistant S = susceptible
[b] Numbers in parentheses indicate disease severity index

Table 3. Comparison of race 485 with the other 6 characterised races of *C. lindemuthianum* in Kenya based on the reaction[a] of the 12 differential bean cultivars.
Vergleich der Rasse 485 mit den sechs in Kenya nachgewiesenen Rassen von C. lindemuthianum anhand der Reaktion des Testsortiments.

Cultivar	Binary value	Race						
		Race 485	alpha	beta	gamma	delta	epsilon	lambda
'Michelite'	1	S	S	R	R	S	S	S
'MDRK'	2	R	R	S	S	S	R	S
'Perry Marrow'	4	S	R	R	S	S	R	S
'Cornell 49-242'	8	R	R	R	R	R	R	R
'Widusa'	16	R	S	R	R	S	R	S
'Kaboon'	32	S	R	R	S	R	R	S
'Mexico 222'	64	S	R	R	R	R	R	R
'PI 207262'	128	S	R	R	R	R	R	R
'TO'	256	S	R	R	R	R	R	R
'TU'	512	R	R	R	R	R	R	R
'AB 136'	1024	R	R	R	R	R	R	R
'G 2333'	2048	R	R	R	R	R	R	R
Race number		485	17	2	38	23	1	55

[a] R = resistant S = susceptible

identified as race 485 by designating it values according to Binary system (CIAT 1987; BURUCHARA 1991). This is the first report of this race in Kenya. The fungus has also been reported in Burundi without consideration of physiologic races (BIGIRIMANA et al. 2000). Previously, six races in addition to some uncharacterised ones have been reported in Kenya namely alpha, beta, delta, epsilon, gamma and lambda (KINYUA 1976; MWANGI 1986; GATHURU and MWANGI 1991). The six races have been designated as race 17, 2, 38, 23, 1 and 55 respectively (as shown in Table 3) according to the Binary system of classification (BURUCHARA 1991; TU 1992 and 1994). This implies that more resistance genes are needed to overcome the virulence genes of race 485 compared to other reported races in Kenya.

The reaction of the 12 differential cultivars to races lambda and kappa was in agreement to what has already been reported by other workers (TU 1994, BALARDIN et al 1997). This confirmed that the test procedures were accurate and the results internationally comparable. It was also noted that race 485 has more virulence genes against the differential cultivars than lambda and kappa.

The control of bean anthracnose in Kenya by means of genetic resistance has relied primarily on deployment of the *Are* gene (GATHURU and MWANGI 1991). The *Are* gene condition resistance to six distinct physiologic races, namely alpha, beta, delta, epsilon, gamma and lambda (TU 1992).

From the reaction of the differential cultivars to race 485, it was noted that the race has a fairly broad pathogenicity with the virulence genes for six of the differential cultivars. However, it lacks virulence genes against the *Are* gene evidenced by incompatible reaction with the cultivar 'Cornell 49-242'. This implies that the new race poses no threat to the bean genotypes with *Are* gene. However, it poses a great threat to the popularly grown beans which have no *Are* gene. It seems to have more virulence genes than the races so far reported in Kenya. This implies that the host may require more resistance genes to overcome the increased virulence genes and therefore maintain a long-term durable resistance (VANDERPLANK 1968; NELSON 1978; SCHAFER and ROELFS 1985).

The occurrence of the new race in Kenya is a challenge to the bean breeders to incorporate more resistance genes to the popularly grown bean cultivars which succumb to the anthracnose fungus. This will enable the host to overcome increased virulence genes of the fungus and therefore delay any possibility of break down of resistance.

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Literature

BARLARDIN, R. S., A. M. JAROSZ and J. D. KELLY 1997: Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central and North America. Phytopathology **87**, 1184–1191.

BARRUS, M. F. 1911: Variation of varieties of beans in their susceptibility to anthracnose. Phytopathology **1**, 190–195.

BESHIR, T. 1991: Some research techniques of bean anthracnose. In: BURUCHARA, R. A. (Ed.): Proceedings of the first Pan-African working group meeting on anthracnose of beans. Ambo, Ethiopia. February 17–23, 1991. CIAT African Workshop Series No. **15**, 17–20.

BIGIRIMANA, J., R. FONTAINE and M. HÖFTE 2000: Virulence of *Colletotrichum lindemuthianum* isolates from Burundi, Central Africa. Plant Disease **84**, 491.

BURUCHARA, R. A. 1991: Differential cultivars in *Colletotrichum lindemuthianum* race determination and nomenclature. In: BURUCHARA, R. A. (Ed.): Proceedings of the first Pan-African working group meeting on anthracnose of beans. Ambo, Ethiopia. February 17–23, 1991. CIAT African Workshop Series No. **15**, 24–29.

CIAT 1987: Standard System for the evaluation of bean germplasm. SCHOONHOVEN, A. VAN and M. A. PASTOR-CORRALES (compilers). Cali, Collombia.

DILLARD, H. R. and A. C. COBB 1993: Survival of *Colletotrichum lindemuthianum* bean debris in New York State. Plant Dis. **77**, 1238.

DRIJFHOUT, E. and J. H. C. DAVIS 1989: Selection of a new set of homogeneously reacting bean (*Phaseolus vulgaris*) differentials to differentiate races of *C. lindemuthianum*. Plant Pathol. **38**, 391–396.

GARRIDO, R. E. R. and R. S. COVA 1989: Identification of physiological races of *Colletotrichum lindemuthianum* (Sacc. & Magn.) Scrib. Agrociencia **7**, 139–156.

GATHURU, E. M. 1991: Bean Anthracnose: Importance of the disease and the Genus *Colletotrichum*. In: BURUCHARA, R. A. (Ed.): Proceedings of the first Pan-African working group meeting on anthracnose of beans. Ambo, Ethiopia. February 17–23, 1991. CIAT African Workshop Series No. **15**, 5–11.

GATHURU, E. M. and S. F. M. MWANGI 1991: Bean Anthracnose Research in Kenya. . In: BURUCHARA, R. A. (Ed.): Proceedings of the first Pan-African working group meeting on anthracnose of beans. Ambo, Ethiopia. February 17–23, 1991. CIAT African Workshop Series No. **15**, 43–50.

HUBBELING, N. 1957: New aspects of breeding for disease resistance in beans. Euphytica **6**, 111–141.

KELLY, J. D., L. AFANADOR and L. S CAMERON 1994: New races of Colletotrichum lindemuthianum in Michigan and implications in dry bean resistance breeding. Plant Dis. 78, 892–894.

KINYUA, G. K. 1976: The occurrence of race alpha of Colletotrichum lindemuthianum in Kisii district of Kenya. Ministry of Agriculture (NHRS-Thika) report. Pp. 3.

MENEZES, J. R. and J. C. DIANESE 1988: Race characterisation of Brazilian isolates of Colletotrichum lindemuthianum and detection of resistance to anthracnose in Phaseolus vulgaris. Phytopathology 78, 650–655.

MESQUITA, A. G. G., T. J. PAULA, M. A. MOREIRA and E. G. DE BARROS 1998: Identification of races of Colletotrichum lindemuthianum with the aid of PCR-based molecular markers. Plant Dis. 82, 1084–1087.

MORDUE, J. E. M. 1971: Colletotrichum lindemuthianum (Sacc. & Magn.) Br. & Cav. C.M.I. Descriptions of plant pathogenic fungi and bacteria No. 316, 2.

MWANGI, S. F. M. 1986: Pathogenic variation in Colletotrichum lindemuthianum (Sacc. and Magn.) Scrib., the cause of bean anthracnose and its implication to Kenya. Msc. Thesis. University of Nairobi, pp. 96.

NELSON, R. R. 1978: Genetics of horizontal resistance to plant diseases. Annu. Rev. Phytopathol. 16, 359–378.

SCHAFER, J. F. and A. P ROELFS 1985: Estimated relation between numbers of urediniospores of Puccinia graminis f. sp. tritici and rates of occurrence of virulence. Phytopathology 75, 749–750.

TU, J. C. 1992: Colletotrichum lindemuthianum on bean. Population dynamics of the pathogen and breeding for resistance. In: BAILEY, J. A. and M. J. JEGER (Eds.): Colletotrichum Biology, Pathology and Control. C.A.B. International, Wallingford, UK, 203–224.

TU, J. C. 1994: Occurrence and characterisation of alpha-Brazil race of bean anthracnose (Colletotrichum lindemuthianum) in Ontario. Can. J. Plant Pathol. 16, 129–131.

VANDERPLANK, J. E. 1968: Disease resistance in plants. Academic Press, New York.

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