



Identification of Bean Rust (*Uromyces appendiculatus*) Races on Isolates Collected from Nyamira County and Narok South Sub County, Kenya

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Authors' contributions

This work was carried out in collaboration between all authors. Author EKN designed the study, wrote the protocol and interpreted the data. All authors managed the literature searches. Author EKN produced the initial draft. All authors read and approved the final manuscript

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ABSTRACT

Common bean rust (*Uromyces appendiculatus*) is an etiological agent of bean rust disease of common beans (*Phaseolus vulgaris* L.) that causes damage and consequent yield losses. The high and wide virulence variability of the rust pathogen poses a major challenge to bean breeding programmes in Kenya with an objective of developing resistant varieties to the disease. This study was undertaken to identify *Uromyces appendiculatus* races and evaluate the virulence variability of *Uromyces appendiculatus* races occurring in common beans. The isolates were collected from Nyamira county and Narok South sub county, Kenya using transect line sampling method. Seven single pustule isolates obtained from the two counties were used to inoculate standard differential cultivars in a rust free green house to determine the physiological races of *U. appendiculatus*. The application of a set of 12 standard differential cultivars and the international classification and binary nomenclature system grouped the seven isolates into four different *U. appendiculatus* races;

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29-1, 29-3, 31-3 and 31-11. The race 31-11 was only prevalent in Nyamira county. The races affected the differential cultivars attributed to the Andean gene pool. The Meso american differential cultivars were resistant to most races of the rust pathogen. The races were found to vary in virulence based on their reaction with the standard differential cultivars. The findings from this study informs on the need for regular screening of bean genotypes for tolerance to bean rust as a strategy of effectively developing common bean varieties with broad resistance to manage bean rust in Nyamira and Narok south, Kenya.

Keywords: Resistance; races; common beans; virulence; urediniospores.

1. INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) form a major income earning crop as well as a key source of protein, especially for the poor people in Kenya and Eastern Africa. Production of common beans requires an optimum temperature range of between 16 and 25°C, grown under crumbly loam soils with relatively high levels of organic matter and pH of about 6.5-7.5 [1]. *Uromyces appendiculatus* forms an etiological agent of common bean rust disease that causes yield losses of about 25-100% [2]. The fungus attack common bean plant and exhibit symptoms mainly on the upper and lower surface of the leaf, which manifest as pustules bearing urediniospores having a rusty appearance that easily rub off onto the fingers [3]. In the field, different forms of uredinal pustules can be observed on the same host plant, suggestive of the existence of different diverse races of the pathogen [3]. *Uromyces appendiculatus* possesses different diverse races which are highly variable in virulence [4,5]. The virulence diversity of *U. appendiculatus* races can be measured by use of host differential cultivars having single or multiple resistance genes [6].

Use of common bean varieties with resistance gene combinations against *U. appendiculatus*, has been shown to be harmless to the environment and is economically viable in preventing and managing common bean rust disease when compared to the heavy dependence on fungicides as chemical control measures [7]. Over reliance of fungicides poses environmental problems including destroying non target organisms in the soil. The presence of a wide virulence diversity of *U. appendiculatus* coupled with limited information on prevalent races of the rust pathogen in many locations including eastern Africa, presents a challenge to breeding strategies aimed at developing common bean cultivars with broad resistant to the rust pathogen [8,9].

In any common bean breeding for resistance program, the identification of common bean rust races forms a foundation for resistance breeding since the identified races would help in monitoring and evaluating the effectiveness of rust resistance genes as a strategy of developing bean cultivars with broad resistance to the rust pathogen [6]. The knowledge of virulence diversity of *U. appendiculatus* races in Kenya as well as East Africa as a whole is important in the deployment of genes for effectively developing common bean varieties with broad resistance to the rust pathogen [10].

2. MATERIALS AND METHODS

2.1 Collection of *U. appendiculatus* Spores and Description of Study Area

Transect line sampling method was used in the collection of diseased common bean leaves bearing urediniospores in Nyamira county and Narok South sub county [6]. Along the transect line, sampling was done after every 5 km whereby a single farm of small holder farmer growing common beans was randomly selected for collection of rust samples. On each farm, 10 samples from 300 randomly selected common bean plants were sampled, giving a total of 80 samples that were used in this study. In Nyamira county, rust samples were collected from Ikonge, Ekerenyo, Kebirigo and Tinga whereas in Narok South sub county, rust collections were done from Olulung'a, Olashapani, Ereteti and Nkareta.

Nyamira county lies between longitudes 340 581'E and 350 05° E and latitudes 00.351° S and 00.883° S. It covers an area of 899.4 km² and borders Homa bay county to the North, Bomet county to the South East, Kericho county to the East and Kisii county to the west [11]. Nyamira county has a population of 598,252 [11]. The annual rainfall ranges between 1200 to 2100 mm per annum with an average rainfall of 1600 mm per annum. The mean temperatures range

from 10 to 28.7°C. Long rains are experienced between March and May and short rains between August and September. The soils found in the region are the red volcanic soils which are deep, fertile and well drained, accounting for 75% and clay soils are found in the valley bottoms and swampy areas accounting for 25% suitable for brick making. The major cash crops are; coffee, tea, bananas and pyrethrum. The major food crops in the region are; maize, beans, sweet potatoes and horticultural crops such as tomatoes, kales and indigenous vegetables [11].

Narok South is one of the six constituencies in Narok county lying between longitudes 35.37°E and 35.6°E and latitudes of between 1.15°S and 1.25°S. Narok South covers an area of 4,959.2 km² with a population of 176,764 [12]. The region experiences rainfall ranging from 500 to 1800 mm per annum. The mean temperature ranges from 12 to 28°C [12]. The type of soil found in the region is the clay loams. The main farming activities in the region are livestock rearing and crop farming. The main crops are; wheat, barley, Irish potatoes, maize and beans.

2.2 Standard Differential Cultivars for Identifying *U. appendiculatus* Races

The seeds of a set of 12 standard differential cultivars used for identifying *U. appendiculatus* races were sourced from Center for International and Tropical Agriculture (CIAT). The seeds were multiplied in a rust free green house. Six of the differential cultivars (Early Gallatin, Redlands Pioneer, Montcalm, Pompaour Checa-50, Golden Gate Wax, and PI 260418) were of the Andean gene pool whereas six other cultivars (Great Northern 1140, Aurora, Mexico 309, Mexico 235, Compuesto Negro Chimaltenango and PI 181996) were of the Meso american gene pool.

2.3 Single Pustule Isolation

Uromyces appendiculatus single pustule isolates from the two counties were obtained according to a procedure adopted by Shaik [13]. Ten day seedlings of GLPX92, a local common bean susceptible variety were inoculated with *U. appendiculatus* spores using a painter's brush no. 1 moistened by use of sterile water. After inoculation, the seedlings were incubated for two days to hasten disease development. In order to increase chances of obtaining single pustule isolates, concentration of 1.0×10^4

urediniospores/ml was used. This concentration is lower than the usual concentration that promotes development of widely spaced pustules. Different pustule sizes were collected from the leaf surfaces showing differences in pustule reaction and size caused by the diverse races of the rust pathogen. The pustules containing urediniospores were opened up using a sharp needle and the spores were tapped onto a clean piece of paper (Whatman paper no. 1). The procedure for multiplying single pustule spores was done three times in order to obtain enough spores for use in identification as well as evaluating the variability of *U. appendiculatus* races.

2.4 Single Pustule Isolate Inoculations

Single pustule isolate inoculations were done by inoculating a set of 12 standard differential cultivars that are used in identifying *U. appendiculatus* races. Individual assays were conducted to classify the pustule races. A set of the 12 standard differential cultivars were planted in plastic containers with a soil mixture of sand, farm yard manure and loam soil in the ratio of 1:1:2 in rust free green house. Ten day old seedlings were inoculated with *U. appendiculatus* spores suspended in tween 20 (0.05% v/v) at a concentration of 2.0×10^4 urediniospores/ml. After inoculation, the plants were incubated for 2 days. Evaluation of disease scores was done after 14 days from the day of inoculation.

2.5 Evaluation of Disease Scores

The plant reactions were evaluated for disease scores based on a rust grading scale of 1-6 adopted by Steadman et al. [14]. The scale considers six types of infection levels where, 1- no pustules (cultivar considered immune to the rust pathogen); 2- presence of necrotic patches with no sporulation (cultivar considered resistant to the rust pathogen); 3- pustules exhibiting sporulation of a diameter of less than 300 µm (cultivar considered resistant to the rust pathogen); 4- pustules exhibiting sporulation of a diameter spanning between 300-499 µm (cultivar considered susceptible to the rust pathogen); 5- pustules exhibiting sporulation with a diameter spanning between 500 to 800 µm (cultivar considered susceptible to the rust pathogen); and 6- pustules eliciting sporulation with a diameter above 800 µm (cultivar considered susceptible to the rust pathogen).

2.6 Identification of *U. appendiculatus* Races

The identification of isolate races was determined by binary nomenclature system based on a reaction between the 12 standard differential cultivars and the *U. appendiculatus* isolates. The procedure for identification of bean rust races was adopted from Steadman et al. [7].

The isolate races were identified through binary nomenclature system, designated by two digits that are separated by a hyphen, where the first digit represents the sum of the binary values of the susceptible Andean cultivars and the second digit the sum of the binary values of the susceptible Meso american cultivars.

2.7 Data Analysis

Analysis of data was done using general statistics package (Genstat) 12th edition, 2009. Analysis of variance (ANOVA) was done to determine the effect of *U. appendiculatus* races on standard the differential cultivars.

3. RESULTS

The use of standard differential cultivars and the binary nomenclature system grouped the seven isolates collected from Nyamira county and Narok South sub county into four different *U. appendiculatus* races (Table 1). The most prevalent races were 29-1, 29-3, and 31-3 which were found in both Nyamira county and Narok South sub county. The race 31-11 was only

found in Nyamira county. The reaction between the standard differential cultivars and the four *U. appendiculatus* races resulted in clear differences in reaction between the Andean cultivars and the Meso american cultivars. It was observed that the races were more virulent on the Andean cultivars compared to the Mesoamerican cultivars. From this study, five of the Andean host differential cultivars; Early Gallatin, GN 1140, Golden gate Wax, Montcalm and PC-50 were susceptible to all the races. The Mesoamerican cultivars, CNC, Mexico 309 and P1181996 were resistance to all the races identified in this study. The Mesoamerican cultivar Mexico 235 was resistant to all the races tested in this study except race 31-11. The cultivar Redlands pioneer was only susceptible to two races (31-11 and 31-3).

The reaction between *U. appendiculatus* races and the standard differential cultivars resulted in clear and wide resistance spectrum among the differential cultivars towards *U. appendiculatus* races (Table 3).

Analysis of variance showed that there was significant difference in resistance among the differential cultivars against *U. appendiculatus* races. Early Gallatin, Montcalm and PC-50 were the most susceptible cultivars to all the races tested in this study (Table 1). This was closely followed by the cultivars; Golden Gate wax, GN1140 and Aurora. The cultivars; PI 181996, CNC and Mexico 309 were the most resistant cultivars to the races tested in this study (Table 3).

Table 1. Reaction of 12 standard differential cultivars to seven *U. appendiculatus* isolates collected from Nyamira county and Narok South sub county

Differential cultivar	Reaction to the <i>U. appendiculatus</i> isolates						
	Nya 1	Nya 2	Nya 3	Nya 4	Nks 1	Nks 2	Nks 3
Early Gallatin	5	6	6	6	5	4	4
Redlands pioneer	2	2	5	5	3	2	6
Montcalm	6	6	5	4	5	5	4
PC-50	5	6	5	4	6	5	6
GGwax	5	6	5	4	5	5	4
PI 260418	1	1	2	5	2	2	1
GN1140	4	5	6	4	5	4	5
Aurora	3	4	5	4	5	3	4
Mexico 309	2	1	1	1	2	2	1
Mexico 235	1	1	1	4	1	1	1
CNC	1	1	1	1	2	1	1
PI 181996	1	1	2	1	1	2	1

Isolates designated as 'Nya' were collected from Nyamira county and those designated as 'Nks' were from Narok South sub county. Cultivars that represented reaction grade of 3 and lower were considered resistant and those that represented grade 4 and higher susceptible

Table 2. Designation of *U. appendiculatus* races using the binary nomenclature system adopted from Steadman et al. [14]

Gene pool	Binary value	Differential cultivar	Reaction to the seven <i>U. appendiculatus</i> isolates						
			Nya 1	Nya 2	Nya 3	Nya 4	Nks 1	Nks 2	Nks 3
Andean	1	Early Gallatin	-	-	-	-	-	-	-
	2	Redlands pioneer	+	+	-	-	+	+	-
	4	Montcalm	-	-	-	-	-	-	-
	8	PC-50	-	-	-	-	-	-	-
	16	GGwax	-	-	-	-	-	-	-
	32	P1260418	+	+	+	+	+	+	+
Mesoamerican	1	GN1140	-	-	-	-	-	-	-
	2	Aurora	+	-	-	-	+	-	-
	4	Mexico 309	+	+	+	+	+	+	+
	8	Mexico 235	+	+	+	-	+	+	+
	16	CNC	+	+	+	+	+	+	+
	32	P1181996	+	+	+	+	+	+	+
Nomenclature of the races			29-1	29-3	31-3	31-11	29-1	29-3	31-3

NB (+) resistant, (-) susceptible

Table 3. Means of disease scores of *U. appendiculatus* races on standard differential cultivars

Differential cultivar	Means of disease scores (± standard deviation)
Aurora	4.1±0.52
CNC	1.3±1.64
Early Gallatin	5.5±0.27
GN1140	4.8±0.25
Golden gate wax	4.8±0.25
Mexico 235	2.0±0.50
Mexico 309	1.4±0.18
Montcalm	5.1±0.30
PI 181996	1.1±0.13
P1260418	1.9±0.48
PC-50	5.0±0.27
Redlands pioneer	3.8±0.53
P- value	<0.001

Table 4. Means of virulence of *U. appendiculatus* races on standard differential cultivars

Races	Means of virulence (± standard deviation)
29-1	3.0±0.4
29-3	3.2±0.4
31-3	3.8±0.3
31-11	3.6±0.4

The plant reactions between the standard differential cultivars and the four *U. appendiculatus* races exhibited clear pathogenic variability among the races. There was significant difference in virulence among the

U. appendiculatus races on the differential cultivars. The race 31-3 was the most virulent on the standard differential cultivars. It was followed by races 31-11 and 29-3 (Table 3). The race 29-1 was the least virulent on the standard differential cultivars (Table 3).

4. DISCUSSION

The four races obtained from Nyamira county and the three races obtained from Narok South sub county provide evidence of differences in prevalence and distribution of *U. appendiculatus* races in various regions of different agro ecologies.

Climatic conditions and the different weather conditions seem to act on the biology of the common bean rust pathogen [15]. This results in different races being produced in different regions of the country, which indicates the need for regular screening of bean germplasm in highland areas for bean rust disease as a strategy of effectively developing resistant varieties to the common bean rust pathogen.

Effective management of bean rust requires that plant breeders employ a combination of genes from various resistance materials [6]. The Mesoamerican cultivars CNC, Mexico 309 and P1181996 were the most resistance to the races identified in the two areas of study. These cultivars provide broad resistance to the rust pathogen that can be used in bean breeding programmes aimed at developing rust resistant varieties. The cultivar Mexico 235 was found to be susceptible to race 31-11 that was identified

in Nyamira county. This cultivar is unsuitable for use in development of rust resistant varieties without protection by genes from other resistant materials. Redland pioneer only provide resistance to races 29-1 and 29-3, but was susceptible to race 31-3 and 31-11. This cultivar can only be used in bean breeding programme to control races 29-1 and 29-3. Environmental conditions may influence the genetic composition of bean rust. Temperatures between 16 and 24°C favour development of rust infection [15]. Such conditions explain why more races were found in Nyamira county as compared to Narok South sub county.

The identification of *U. appendiculatus* is crucial in resistance breeding since the identified races help in monitoring the effectiveness of resistance genes as a strategy of developing resistant varieties to the rust pathogen [6]. The combination of genes from various resistant materials promotes development of bean varieties that elicit broad resistance to the rust pathogen.

Evaluation of virulence variability was based on a reaction between the differential cultivars and the four *U. appendiculatus* races. Based on a scale of 1-6, the reactions ranged from immunity to susceptibility. The plant reaction classification was as a result of combining pustule size and infection intensity. The four races used in the experiment were found to be virulent to the entire differential cultivars both Andean and Mesoamerican cultivars. The differences observed in plant reactions suggest that the gene for gene theory has been shown to occur in *U. appendiculatus* races. This explains differences experienced in the host pathogen interactions between the differential cultivars and the *U. appendiculatus* races.

The reaction expressed by the cultivars was dependant on whether or not the races involved possessed genes for pathogenicity which overcame the genes for resistance present in the host. The virulence variability among the *U. appendiculatus* races, suggest that a cultivar may be resistant to one race and be susceptible to another race. There is need to identify physiological races of the rust pathogen which is crucial in identification and development of rust resistant bean varieties as an effective strategy of managing bean rust disease in the country.

The differences in means of virulence of *U. appendiculatus* races on standard differential cultivars provide evidence of virulence variability

of the common bean rust pathogen. High and wide virulence variability of the rust pathogen has been observed ranging from few selected pathotypes to large numbers of isolates [5]. In addition high virulence variability of bean rust pathogen has been reported in Africa suggesting high rust incidences in the region [14]. High virulence variability of the bean rust pathogen can be attributed to mutation, heterosis and hybridization occurring in the asexual stages of the bean rust pathogen as well as the wide range of varieties grown in Africa [16].

5. CONCLUSION

From this study, it is clear that common bean rust pathogen (*U. appendiculatus*) produces different diverse races some of which were only prevalent in certain regions and not others. It was also found out that there is wide virulence variability among *U. appendiculatus* races in the rust isolates collected from the two areas of study.

Identification of *U. appendiculatus* races is a pre requisite in any bean breeding programme with an objective of developing bean varieties with broad resistance to the rust pathogen as an effective strategy of managing bean rust disease.

The study findings inform on the need for regular screening of bean genotypes for resistance or susceptibility to bean rust as a strategy of developing rust resistant varieties with broad resistance to common bean rust.

6. RECOMMENDATION

Following the findings obtained, the following can be suggested from the current research;

- a) There is need for periodic surveys to determine the distribution and prevalence of *U. appendiculatus* races in the dry bean growing regions of the country as a strategy of developing bean varieties with broad resistance to the bean rust pathogen.
- b) There is need to screen more bean germplasm as a strategy of identifying more bean cultivars with broad resistance to the rust pathogen.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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