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Full Length Research Paper

Characterization of *Uromyces appendiculatus* isolates collected from snap bean growing areas in Kenya

E. E. Arunga[1]*, J. O. Ochuodho[2], M. G. Kinyua[1] and J. O. Owuoche[3]

[1]Department of Biotechnology,Chepkoilel University College, Box 1125-30100 Eldoret, Kenya.
[2]Department of Seed, Crops and Horticultural Sciences, Chepkoilel University College, Box 1125-30100 Eldoret, Kenya.
[3]Department of Crops, Horticultural and Soil Science, Egerton University, Box 536, Egerton, Kenya.

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Bean rust (*Uromyces appendiculatus* (Pers.:Pers.) Unger var. *appendiculatus*) is one of the most devastating and variable pathogens of common bean (*Phaseolus vulgaris* L.) worldwide that can cause total crop loss. Characterization of bean rust races can help in screening resistant materials during gene pyramiding. The aims of the present research were first, to characterize bean rust isolates collected from snap bean growing areas in Kenya. Secondly, to identify which of the available rust resistance genes in common bean differentials are most effective to control rust in those areas. Snap bean leaf tissues with rust pustules were collected from different farms in eight locations in central and western Kenya during the years 2010 and 2011. Forty seven single pustule isolates were obtained and inoculated on 12 bean rust differential cultivars. For consistent results, the inoculation was repeated twice. The new international classification system and the binary nomenclature grouped the 47 single pustule isolates of *U. appendiculatus* into 9 different races, most of this affected the Andean gene pool. Hierarchical cluster analysis grouped the races into two major clusters depending on the virulence of the races on the host differential cultivars. The most resistant genes for pyramiding in Kenya were identified as *Ur*-5, *Ur-11* and *Ur-CNC*. An important output of this study was the identification of races with potential use during gene pyramiding process.

Key words: *Uromyces appendiculatus*, snap bean, races, genes, characterization.

INTRODUCTION

Bean rust caused by *Uromyces appendiculatus* (Pers.:Pers.) Unger var. *appendiculatus* is one of the most devastating fungal diseases of common bean (*Phaseolus vulgaris* L.) worldwide (Liebenberg and Pretorius, 2010; Souza et al., 2008). An effect of this rust is the 18 to 100% reduction of grain yield in dry beans and the reduction in pod quality in snap beans (De Jesus et al., 2001; Lindgren et al., 1995; Omunyin et al., 1984). Field experiments and surveys have ranked bean rust as a major disease in snap beans in Kenya (Monda et al., 2003; Wahome et al., 2011). Snap beans comprise a group of common bean that have been selected for succulent pods with reduced fibre whose immature pods and seeds are consumed as a green vegetable (Myers and Baggett, 1999). The rust pathogen is highly variable in virulence and it possesses many races (Mmbaga et al., 1996). Diversity of *U. appendiculatus* has been measured classically using host differential cultivars having different single or multiple rust resistance genes (Araya et al., 2004; Souza et al., 2007a). However, there is little information about the virulence diversity of this pathogen in Kenya and this has posed a major challenge to breeding programmes in East and central Africa (Kimani et al., 2002).

It is important to utilize available resistance genes in developing resistant cultivars since it is an economical way of managing bean rust as compared to application of fungicides, because of the significant losses caused by

*Corresponding author. E-mail: eea08ke@hotmail.com.

the disease (Miklas et al., 2006; Souza et al., 2007b). In addition, the knowledge of virulence diversity of bean rust races is of importance for effective introgression of resistance genes (Coyne et al., 2003).

The identification of these races is a basic stage for resistance breeding and the identified races will help in monitoring the effectiveness of resistance genes during the deployment process.

This research was therefore aimed at first, to characterize bean rust isolates collected from different snap bean growing regions in Kenya using a set of international differential cultivars. Secondly, to determine which of the available rust resistance genes (*Ur*) in the common bean differentials are the most effective to control rust in those regions.

MATERIALS AND METHODS

Rust collection

Rust pustules were collected from diseased leaves in 28 snap bean farms in the following localities in central Kenya {Embu (0° 25' S, 37° 28' E, 1711 meter above sea level (masl), Meru (0°07' N, 37° 40' E, 1491 masl), Mwea (0° 37' S, 37° 20' E, 1204 masl) and Thika (0° 59' S, 37°00' E, 1570 masl)} and in western Kenya {Eldoret (0° 34' N, 35° 18' E, 2153 masl), Kisii (0° 48' S, 34° 58' E, 2135 masl), Kitale (1° 01' N, 35° 05' E, 1878) and Naivasha (0° 40' S, 36° 22' E, 1900 masl)}. The field collections were done randomly between May 2010 and June 2011. The number of farms per locality ranged from one to five with one to two samples collected per farm. The farms were either planted with commercial cultivars or experimental plots. In total, forty seven samples were collected for this study. The leaf samples were air dried for three days at room temperature and stored at -20°C or inoculated immediately on two week old plants.

Bean rust differential cultivars

The seeds of the new set of 12 differential cultivars (Steadman et al., 2002): Early Gallatin (*Ur-4*), Golden Gate Wax (*Ur-6*), Aurora (*Ur-3*), Mexico 309 (*Ur-5*), Mexico 235 (*Ur-3+*) and Compuesto Negro Chimaltenango (*Ur-unknown*) were obtained from the National Gene Bank of Kenya. Cultivars PI 260418, PI 181996, Great Northern 1140 (*Ur-7*), Pompadour Checa 50 (*Ur-9* & *12*) and Montcalm (*Ur-unknown*) were supplied by Centro Internacional de Agricultura Tropical (CIAT), Uganda. Cultivar Redlands Pioneer (*Ur-13*) was provided by Dr. Merion Liebenberg, formerly of the Agricultural Research Council, South Africa. In order to prevent cross-pollination, seed increases were done under rust-free greenhouse environment.

Virulence tests

U. appendiculatus single-pustule isolates were obtained from the 47 collections by inoculating the cultivars from which the isolates were collected. In order to increase the chance of obtaining single pustules, a lower than usual inoculum concentration (1.0×10^4 urediniospores/ml) was used before spraying the urediniospores on the original cultivars. More than one pustule was collected from leaves showing differences in pustule reaction and size. The method of single-pustule isolation was adopted from Shaik (1985). To describe the method, sterilized scissors were used to isolate single and unopened pustules, including 25 mm^2 of the surrounding leaf tissue from the leaves. The cut leaf tissue and pustule were then transferred to Petri-dishes containing water agar and was incubated at 19 ± 2°C until it was open and well developed. After ensuring that only one pustule was present, spores were transferred to fresh seedlings of the susceptible cultivars using a wet brush. Thereafter, the plants were maintained at 19 ± 2°C temperatures and relative humidity of ≥ 95% under a 12-h light/dark regime for 48 h before transferring them to a rust free greenhouse maintained at 20 to 28°C until the pustules were well developed. The single-pustule isolates were multiplied for three cycles and were used for classification.

The 47 single-pustule isolates were used to inoculate the differential cultivars in the greenhouse. Individual assays were conducted in order to classify each pustule race. Seeds from 12 differential cultivars were grown in a rust free greenhouse at 20 to 28°C and were inoculated with rust spores as described by Stavely (1983). Plants were inoculated when primary leaves were ⅓ to ⅔ expanded, that is, 8 to 10 days after planting. The 12 differentials were sprayed with urediniospores suspended in Tween 20 (0.05%, v/v) at a concentration of 2.0×10^4 urediniospores/ml. After inoculation, the plants were transferred to a controlled chamber at 19 ± 2°C temperatures and relative humidity of ≥ 95% under a 12-h light/dark regime for two days. Thereafter, the plants were transferred to a glass house maintained at 20 to 28°C for 12 more days. The seedlings were evaluated for infection types when 50% of the pustules had sporulated approximately the 15th day after inoculation. Inoculation of the differential cultivars was repeated twice for consistent results.

Classification of the races

The reaction of the differential cultivars to each of the races of the rust pathogen used in this study was determined on the basis of the scale adopted from Steadman et al. (2002). This scale considers six infection types: 1, no pustules (immunity); 2, necrotic spots without sporulation (hypersensitivity); 3, pustules undergoing sporulation with a diameter of < 300 µm; 4, pustules undergoing sporulation with a diameter ranging from 300 to 499 µm; 5, pustules undergoing sporulation with a diameter ranging from 500 to 800 µm; and 6, pustules undergoing sporulation with a diameter of > 800 µm.

The cultivars that predominantly presented degrees 3 or lower were classified as resistant, whereas those with predominant degrees 4 or higher were considered to be susceptible. The race of each isolate was determined based on the binary nomenclature system suggested by Steadman et al. (2002) based on the 12 international differential cultivars. Each race was designated by two numbers separated by a hyphen.

The first number was obtained by the sum of the binary values attributed to the susceptible Andean cultivars of the set. The second number was obtained by the sum of the binary values of the susceptible Mesoamerican cultivars. Races were assigned numbers, prefaced by “Ua” and letters denoting the location and country of origin (e.g, Eldoret Kenya: ELDKEN-Ua1). The urediniospores were deposited in the Biotechnology Laboratory of Chepkoilel University College, Eldoret, Kenya.

Statistical data analysis

The virulence reactions of the standard differentials were recorded as disease scores based on the rust resistance scale. Hierarchical cluster analysis was performed using the unweighted pair group method average (UPGMA) (Sneath and Sokal, 1973) procedure based on Euclidean dissimilarity matrix using NTSYS version 2.11 statistical package (Rohlf, 1989). A dendrogram was derived illustrating similarities in the reaction of 12 differential cultivars to the 47 bean rust isolates.

RESULTS

The application of the 12 international differentials and the binary nomenclature system to the group of 47 isolates resulted in their classification into nine different races namely: 29-0, 29-1, 29-3, 31-1, 31-3, 31-11, 61-1, 61-3 and 63-3 (Table 1). The most predominant race was 29-1 which was found in all locations. Races 31-1, 61-1 and 63-3 were found only in Eldoret, while 31-11 was found only in Kisii. Another prevalent race was 31-3 that was found in Eldoret, Kisii, Mwea and Meru, while 29-3 was found in Eldoret, Kisii and Mwea. The races from western Kenya were more virulent to a large number of differentials and diverse than those from central Kenya based on the number of races per region. Seven different races were found in Eldoret, five in Kisii, three in Mwea and two in Naivasha and Meru. Single races were characterized from Embu, Kitale and Thika. In total, eight different races were characterized in western Kenya, while four different races were obtained in central Kenya.

There were clear differences in the effects of rust between the Andean and Mesoamerican differentials. It was observed from Table 1 that the collected races were virulent on *Ur* genes of Andean genepool as compared to the Mesoamerican genes. Three of the Andean host differentials (Early Gallatin, Montcalm and Pompadour Checa-50 [PC-50]) were susceptible to all the races used in this study, while Redlands Pioneer and PI 260418 showed resistance to 55 and 66% of the nine classified races, respectively. The Mesoamerican differential cultivars, Compuesto Negro Chimaltenango (CNC), Mexico 309 and PI 181996 were resistant to all the races included in the test. Great Northern 1140 (GN1140), a Mesoamerican differential was susceptible to all but one race (29-0), whereas Mexico 235 was resistant to all but one race (31-11).Cluster analysis of the 47 isolates based on the reaction of the 12 differentials grouped them into two main clusters distinguished majorly by the reaction of Redlands Pioneer (*Ur-13*) at 72% dissimilarity coefficient (Figure 1). Moreover, the isolates were separated into groups based on the predominance of virulence on the Andean or Mesoamerican differentials. The first cluster was made up of isolates belonging to three races: 31-3, 31-11 and 63-3 which were virulent on *Ur-13*, *Ur-3* and one on *Ur*-3+. However, race 63-3 and 31-11 were distantly related to the other member (31-3) of the same cluster, because they were virulent on PI 260418 and Mexico 235, respectively. KISKEN-Ua15 had the highest Euclidean distance from the other 47 isolates.

The second cluster was further divided into two sub-clusters, A and B. Sub-cluster A was made up of 90% of the isolates belonging to the Andean race 29-1 which were clustered together with races 31-1, 29-0 and 61-1 at 3.706 Euclidean distances.

The races that made up this sub-cluster were not virulent on *Ur-13*. Race 29-0 was virulent only on the Andean cultivars and thus considered to be truly 'Andean-specific'. Races 29-3 and 61-3 that were not virulent on Redlands Pioneer but virulent on Aurora made up sub-cluster 2B that was intermediate between clusters 1 and 2A.

DISCUSSION

The nine races obtained from the 47 *U. appendiculatus* isolates and the two groups obtained from the dendrogram provide evidence of virulence diversity of this pathogen. Wide variability of the pathogen has been observed ranging from few selected pathotypes to large numbers of isolates (Araya et al., 2004; Mmbaga et al., 1996; Sandlin et al., 1999). Stavely and Pastor-Corrales (1989) reported high virulence diversity in Latin America and Africa and this can partly explain the high rust incidences in these regions. The high virulence diversity in Africa can be attributed to mutation and hybridization occurring in asexual stages (Linde et al., 1990) and the wide diversity of beans grown in Africa. During this study, sexual spores were not observed. Although, teliospores have been observed in South Africa, they are rarely seen in eastern Africa (Kimani et al., 2002; Liebenberg, 2003). The diversity of the races collected in Kenya has implications for the release of snap bean cultivars. First, cultivars with single resistance genes might not be appropriate to create durable resistance and secondly, the large number of different races, some of which were unique for certain regions, requires the use of specific resistance genes in different regions.

Races 29-3 and 63-3 have been characterized in Brazil while races 31-11, 31-1, and 31-3 have been observed in South Africa based on the reaction to the new set of differential cultivars (Liebenberg, 2003; Souza et al., 2007a). The similarity in races will help in screening and identification of similar important genes especially in Africa and the rest of the world. In this study, more than one sample was collected per field simply because race variation had been reported earlier even from single leaf samples (Jochua et al., 2008). This was the case especially in western Kenya where five different isolates were collected from an experimental plot in Eldoret in one season.

Management of bean rust is most efficacious if producers employ a combination of resistant cultivars. From this study, Mesoamerican genes confer resistance to most of the Kenyan races and this suggests that they could be a valuable source of resistance. Similar trends have been observed in Mozambique, South Africa and Tanzania indicating the usefulness of Mesoamerican genes in Africa (Jochua et al., 2004; Liebenberg, 2003; Mmbaga et al., 1996).

The combination of certain genes is a sure way of creating broad resistance to this pathogen. Combinations of certain genes in control of multiple rust races have been effective in the world (Liebenberg and Pretorius, 2010). The summary of races identified so far in Kenya showed that important genes to use are *Ur-11*, *Ur-5*, and

Table 1. Reaction of 12 international rust differential cultivars inoculated with 47 *U. appendiculatus* isolates collected from French bean growing localities in Kenya in 2010 and 2011.

Isolate identity	Race	Early Gallatin	Redlands pioneer	Mont-calm	PC-50	GGWax	PI 260418	GN 1140	Aurora	Mex 309	Mex 235	CNC	PI 181996
MWEKEN-Ua36	29-0	5, 4[a]	1[b]	5, 4	6	6	1	3, 2	2, 1	1	1	1	1
ELDKEN-Ua4	29-1	6	1	5	6	6, 5	1	6	1	1	1	1	1
ELDKEN-Ua8	29-1	6	1	6	6	6, 5	1	6	1	1	1	1	1
EMBKEN-Ua9	29-1	6	1	6	4	6, 4	1	6	2	1	1	1	1
EMBKEN-Ua10	29-1	5	1	5	4	6, 4	1	6	2	1	1	1	1
EMBKEN-Ua11	29-1	4	1	6	5, 6	5, 6	1	5	1	1	1	2	1
EMBKEN-Ua12	29-1	4	1	5	4	6, 4	1	6	2	1	1	1	1
KISKEN-Ua13	29-1	5	1	6, 4	5	4	1	4	1	1	1	1	1
KISKEN-Ua18	29-1	6	1	6, 5	5	5	1	6	1	1	1	1	1
KISKEN-Ua22	29-1	6	1	5	6	6, 5	1	6	1	1	1	1	1
KISKEN-Ua23	29-1	6	1	5	6		1	6	1	1	1	1	1
KTLKEN-Ua25	29-1	6	1	6	4	5, 4	1	4, 3	2, 1	1	1	1	1
KTLKEN-Ua26	29-1	5	1	6	4	6	1	6	2	2	1	1	1
KTLKEN-Ua27	29-1	6, 3	1	6	4	5, 4	1	4, 3	2, 1	1	1	1	1
KTLKEN-Ua28	29-1	6	1	6	6, 5	6	2, 1	5	2	1	1	2	1
MERKEN-Ua29	29-1	6	1	6	6, 5	6	2, 1	5	2	1	1	2	1
MERKEN-Ua31	29-1	6	1	6	5	6	2, 1	6	2	1	1	2	1
MERKEN-Ua32	29-1	6	1	5	6	4	1	5	1	1	1	1	1
MERKEN-Ua33	29-1	6	1	6	5	4	1	6	1	1	1	2	1
MERKEN-Ua34	29-1	5	2	5	4	5	1	5	1	1	1	1	1
MWEKEN-Ua37	29-1	5	1	6	6, 5	6	2, 1	5	1	1	1	1	1
MWEKEN-Ua39	29-1	5	1	6	5	6	2, 1	5	1	1	1	1	1
MWEKEN-Ua40	29-1	4	2	6	5	5	1	6	2	1	1	1	1
MWEKEN-Ua41	29-1	5	1	6	6, 5	6	2, 1	5	1	1	1	1	1
NVSKEN-Ua43	29-1	6	1	6	6, 5	6	1	6	1,2	1	1	1	1
NVSKEN-Ua44	29-1	6	1	6	6	6	1	6	1	1	1	1	1
THIKEN-Ua45	29-1	4	1	4, 5	5	6, 4	1	5,4	2	1	1	2	1
THIKEN-Ua46	29-1	5	1	6	6	6	1	6	1	1	1	1	1
THIKEN-Ua47	29-1	5	1	6	6	6	1	5	2	1	1	1	1
ELDKEN-Ua1	29-3	6	1	5	5, 4	6	1	6	4, 3	1	2	1	1
KISKEN-Ua16	29-3	6	2	6	5	6	1	6	5	1	1	1	1
KISKEN-Ua21	29-3	5	2	6	6	5	1	5	4	1	1	2	1
KISKEN-Ua24	29-3	6	2	5	6	5	1	6	1	1	1	1	1
MWEKEN-Ua35	29-3	6	2	6, 5	5	6	1	6	5, 4	1	1	1	1

Table 1. Contd.

ELDKEN-Ua5	31-1	6	4	6, 5	6, 5	6, 5	1	5	2, 1	1	1	1	1
ELDKEN-Ua3	31-3	6	5	5	6	6, 5	1	6, 5	4	1	1	1	1
KISKEN-Ua14	31-3	6	6	5	5	6	1	6	6, 5	1	1	2	1
KISKEN-Ua17	31-3	6	5	6	5	6	1	6	6	1	1	1	1
KISKEN-Ua19	31-3	6	6	5	5	6	1	6	4	1	1	2	1
MERKEN-Ua30	31-3	6	4	5, 4	5	4	1	5	6, 4	1	1	1	1
MWEKEN-Ua38	31-3	6	6	5, 6	5	6	1	6	4, 5	1	1	1	1
NVSKEN-Ua42	31-3	6	6	6, 5	5	6	1	6	5, 4	1	1	1	1
KISKEN-Ua15	31-11	6	5, 4	5, 4	4	4	2	4	6, 4	1	4	1	1
ELDKEN-Ua2	61-1	6	2	5	5	5	4	6	1	1	1	1	1
ELDKEN-Ua6	61-3	6	2	6, 4	6, 5	6	4	5	4	1	1	1	1
KISKEN-Ua20	61-3	6	2	6	6	5	4	5	4	1	1	2	1
ELDKEN-Ua7	63-3	6	6	6	5	5	4	5	4	1	1	2	1

[a]When several infection grades are present, they are recorded according to their predominance, the most prevalent being listed first. [b]Varieties considered resistant: predominance of grade 3 or lower. Varieties considered susceptible: predominance of grade 4 or higher.

Ur-CNC and *Ur-3*+. The combination of *Ur-3*, *Ur 3+* and *Ur-11* or *Ur-5* or the resistance from PI 260418, may be sufficient to control African races. Although, CNC is susceptible to Tanzanian races (Mmbaga et al., 1996); this study has demonstrated that CNC, PI 18996, and Mexico 309 were the most resistant differentials to Kenyan races. It has been shown that CNC, Mexico 235, Mexico 309 and to some extent Redlands Pioneer have broad resistance to rust (Pastor-Corrales, 2002). On the contrary, Mexico 235 is susceptible to some races from South Africa and one race in this study indicating that it may be unsuitable for use without protection by other genes. *Ur-3* was overcome by some races during this research which was disparate from Brazil where the gene was overcomed by all isolates assessed (Souza et al., 2007a). Therefore, it can be of substance in local breeding programmes only when integrated with other resistance genes. The *Ur-11* gene has been reported to be resistant to a large number of rust races and it is resistant to 89 of the 90 races maintained at the United States Department of Agriculture, Beltsville, MD (Pastor-Corrales, 2002). The resistance in PI 260418 has not been characterized, but can give protection; although, the cultivar showed profuse sporulation even from relatively small pustules and therefore gave a moderate susceptible reaction to three races. Cultivars carrying *Ur-4*, *Ur-6*, *Ur-7*, *Ur-9* and *Ur-12* have been previously reported to be susceptible to many races from Africa (Bokosi et al., 1997; Stavely, 1999) and therefore they are not good sources of resistance to Kenyan races.

The dendrogram was divided majorly based on the reaction of Redlands Pioneer to the isolates. The reaction of Redlands Pioneer to the disease was an intermediate between Andean and Mesoamerican cultivars. Redlands Pioneer is listed as an Andean differential cultivar, although Liebenberg et al. (2004) proved that it belongs to the Mesoamerican origin. That could be the reason why races that were virulent on Redlands Pioneer seemed to have mixed virulence. Closely related isolates were grouped together in the dendrogram and this showed the similarity that exists between these races. Less virulent races were grouped together and the same trend was observed in the more virulent races. The race 29-0 was the only one that was found to be 'Andean-specific' and this shows that the races identified in this study are of mixed virulence, although mostly inclined to the Andean genepool since no true Mesoamerican race was found. The absence of true Mesoamerican races was also observed in Australia (McClean et al., 1995). Common beans exhibit high variability for morphological, biochemical, and genetic traits, and have been divided into the Andean and Mesoamerican genepools (Johnson and Gepts, 2002). Majority of the beans grown in Eastern African countries and in particular Kenya, are large-seeded which originated in the Andean region (Asfaw et al., 2009; Gepts and Bliss, 1988) and this could be the reason for the high susceptibility of the Andean

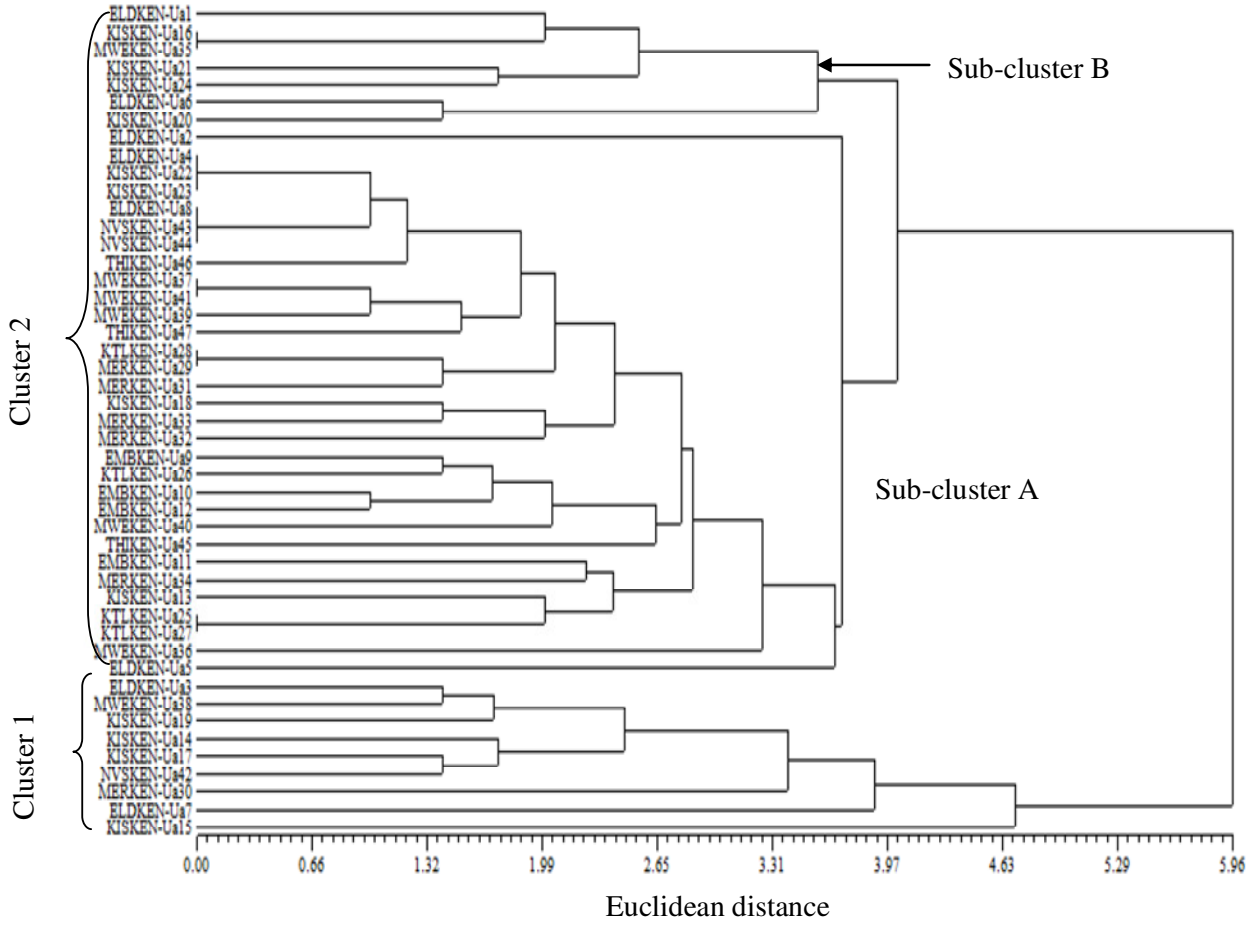

Figure 1. Dendrogram illustrating similarities 47 *U. appendiculatus* isolates using the unweighted paired group method average (UPGMA) based on the disease reaction of 12 differential cultivars.

cultivars. A number of plant pathogens including bean rust, powdery mildew of barley (*Erysiphe graminis* f. sp. *Hordei*) and bean anthracnose (*Colletotricum lindemuthianum*) have an evolutionary relationship with their hosts, resulting from continuous adaptation to changes in host morphology, biochemistry and ecology (McDermott et al., 1994; Melloto and Kelly, 2000; Pastor-Corrales, 1996). It is therefore probable that ongoing adaptation between the pathogen and host is responsible for the characterization of these major groups.

The races in Cluster 1 and sub-cluster 2B were virulent on *Ur-3* and to a lesser degree *Ur-13* and *Ur-3+* of Mesoamerican genepool and thus considered of mixed virulence. Mixed virulence groups of *U. appendiculatus* races have been reported and may provide further evidence of a transition area between these two genepools in both the common bean host and bean rust pathogen isolates (Araya et al., 2004; Sandlin et al., 1999). The race KISKEN-Ua15 appeared to be the most distant race in the dendrogram, because it attacked the Mesoamerican cultivar, Mexico 235. This particular race was collected from Kisii highlands where there was high and moderate virulence on the Mesoamerican cultivars Aurora and Mexico 235, respectively in the field. Environmental factors and the cultivars grown in an area may influence the genetic composition of bean rust. Temperatures between 16 and 24°C with moisture which is common in highland tropical areas favour development of rust infection (Liebenberg and Pretorius, 2010). Such conditions generally explain the high virulence diversity that was observed in western Kenya. The localities in western Kenya are therefore recommended for field screening for bean rust resistance.

CONCLUSIONS AND RECOMMENDATIONS

In conclusion, nine bean rust races were identified during the study which were mostly virulent on the Andean differential cultivars. More races were obtained from the Kenyan highlands (Eldoret and Kisii) as compared to central Kenya and therefore those places can be used for field screening of cultivars to the disease. The most effective rust resistance genes to the races from Kenya

were identified as *Ur-5*, *Ur-11* and *Ur-CNC.* The identification of Kenyan races is important for breeding and also adds information on the reaction of both races and cultivars. Presently, races that show virulence on resistant cultivars for instance *Ur-5*, *Ur-11* and *Ur-CNC* are not available in Kenya and therefore continued race characterization in Kenya over locations and time, in all common bean growing areas is recommended.

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