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DNA Microsatellite Analysis of Kenyan Isolates of *Rhizoctonia solani* from Common Bean (*Phaseolus vulgaris* L.)

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Abstract: Seven hundred and fifty bean plant samples with root rot symptoms were collected from farmers' fields during two surreys carried out in Embu district, Kenya. Various fungal pathogens were isolated in the laboratory from these samples; among them were 50 isolates of *Rhizoctonia solani*, which were subjected to pathogenicity tests in a glasshouse. Thirty-six isolates of *R. solani* obtained from beans with root rots were subjected to DNA microsatellite analysis. Five isolates of *R. solani* that cause black scarf of potatoes (*Solanum tuberosum* L.) were also analysed alongside those from the beans. A total of 50 alleles were detected when six microsatellite loci were typed in the 41 samples, with the mean of 8.33 and a range of 3 at locus RB23 to 19 at locus AF513014. The smallest allele size was 129 basepair at locus RE102 and the largest was 297 basepair at locus AY212027. Microsatellite analysis showed a moderate variation among the isolates from different agro-ecological zones and administrative boundaries (divisions). Phylogenetic analysis revealed 3 major clusters within the population of 41 isolates of R. solani from Kenya. Clusters 1, 2 and 3 had 15, 10 and 75% isolates, respectively. However, cluster 3 had 4 sub-clusters and cluster 1 had 2 sub-clusters, while cluster 2 did not have a sub-cluster. There was no relationship between microsatellites and geographical origin of the isolates. This is the first study on the genetic diversity of *R. solani* using DNA microsatellite analysis in Kenya.

Key words: DNA microsatellite analysis, Kenya, *Phaseolus vulgaris*, *Rhizoctonia solani*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is the most widely grown legume in Kenya and second only to maize (*Zea mays* L.) in importance as a food crop (Wortmann and Allen, 1994; Gethi *et al*., 1997). Beans are mainly grown by small-scale farmers, 65% of which are intercropped with other crops, while 35% are grown as pure crop (Wortmann *et al*., 1998). Bean root rot disease is caused by a number of soil-borne pathogenic fungi including *Rhizoctonia solani, Macrophomina phaseolina*, *Pythium* sp. and *F. solani* f.sp. *phaseoli* and is one of the major causes of low yields (Nderitu *et al*., 1997).

The genus *Rhizoctonia* represents taxonomically diverse groups of fungi that vary in many significant features, which include their sexual stages (teleomorph), asexual stages (anarmoph) and other characters (Sneh *et al*., 1991; Talbolt, 1970). Genetically diverse groups of *Rhizoctonia* exist at several levels of organisation. These include higher taxonomic diversity based on absence of distinctive taxonomic features. In this case the *Rhizoctonia* species may belong to different orders of basidiomycetes and ascomycetes (Parmeter, 1970). Examples of plant pathogenic *Rhizoctonia* species are *R. solani*, R. zeae and *R. oryzae*. Species level diversity based on morphology, pathology and hyphal anastomosis behaviour attempt to describe genetic variations that exist within *Rhizoctonia* species. Isolates belonging to different Anastomosis Group (AG) do not undergo hyphal fusion with each other (Parmeter *et al*., 1969). Due to generality of AG, the concept of intraspesific group (ISG) was introduced as a more specific category of intraspecific variation that recognizes groupings based on combined evidence from anastomosis behaviour, pathogenicity and morphology (Ogoshi, 1987). Morphological and cultural characters have immensely contributed in the development of species concepts. However, few species have been recognized (Talbolt, 1970). Many bottlenecks associated with studying different levels of diversity in Rhizoctonia species are best handled through the use of molecular genetic markers

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such as restriction amplified polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP) and Simple Sequence Repeats (SSR)/ microsatellites (Talbolt, 1970).

Efforts to breed for disease resistance to root rot diseases must take into account the genetic diversity of the pathogen. It is necessary to understand the genetic relationships among different isolates of a plant pathogen, as it is a prerequisite to understanding the interaction between that pathogen and the host plant. Conventionally, identification of fungal pathogens is based on morphology. Use of host differentials to carry out race identification on a new isolate is time consuming, scoring of symptoms is at times subjective and the infection process which is a function of temperature, light intensity and humidity can be quite variable even with the same race and cultivar combination (Carling *et al*., 1994). Molecular biology techniques, offer a better alternative especially where morphological characteristics are too few to distinguish between isolates of pathogen. Microsatellite allele commonly mutate either by gain or loss of a single repeat unit (Schlotterer and Tautz, 1992; Levinson and Gutman., 1987). However the mutation process of microsatellite is biased towards longer alleles (Rubinsnztein *et al*., 1995). This would affect the interpretation of the observed allele frequencies. The allelic state of microsatellite is determined by Polymerase Chain Reaction (PCR) analysis which are aided by primers that flank the repeat sequence and are used for PCR amplification (Tautz, 1989). Many investigators have used microsatellite DNA loci to study the polymorphism and evolutionary relationships of closely related species (Takezaki and Nei, 1996). Microsatellite have been used to study polymorphism of plant pathogenic fungi including *Botrytis cinerea* (Fournier *et al*., 2002) and *S. sclerotionum* (Sirjusingh and Kohn, 2001). The use of microsatellite to characterize *R. solani* could greatly enhance in the understanding the variability within the fungus that could be directly useful in breeding programmes aimed at developing resistance to this fungus.

MATERIALS AND METHODS

Fungal isolates: Thirty-six isolates of *R. solani* were obtained from root or stem samples of common bean that were sampled from Embu district and five isolates from potatoes sampled from Tigoni, Kiambu district. The isolates from Embu were sampled from five administrative divisions namely Runyenjes, Manyatta, Kyeni and Central; and it covered five agro-ecological zones (AEZs) namely, lower highland 5 (LH5), upper midland 1 (UM1), UM2, UM3 and UM4. One centimetre portions of lower stem or root samples were cut and washed in running tap water for 30 sec, 1% sodium hypochlorite was used to surface sterilize the portions for 3-5 min, then rinsed five times in sterile distilled water and aseptically placed onto a plate containing agar and incubated at room temperature for 3 days. Hyphal tip transfer was done onto plates of Potato Dextrose Agar (PDA) and incubated at room temperature for 7-14 days and these were maintained as pure cultures. Identification of the pathogen was done using cultural and morphological characteristics and pathogenicity tests using a susceptible Kenyan bean variety Rosecoco (GLP-2).

DNA extraction: DNA was extracted from 41 isolates of *R. solani*. Erlenmeyer flasks (500 mL) containing 200 mL of Czapek Dox (Oxoid) liquid medium (Coddington and Gould, 1992) were inoculated with three 5 mm^2 agar blocks exercised from margins of 7 day old cultures on PDA medium, then placed on a rotary shaker at 120 rpm and incubated at 24-25°C in darkness for 7 days. The mycelia were harvested by filtration through two layers of cheesecloth, then freeze dried using liquid nitrogen and ground to fine powder using a mortar and pestle. DNA extraction was done according to CTAB (Cetyltrimethyl-Ammonium Bromide) method (Doyle and Doyle, 1990). The DNA pellets were dissolved in 500 μL of TE buffer and stored at -4°C. The DNA was quantified by running 10 μL of each sample on 1% agarose gel along with undigested λ DNA (Roche Diagnostics) 20 ng μL^{-1} and making comparisons of their relative fluorescence in the presence of UV light and 0.1 μL mL^{-1} ethidium bromide solution.

DNA Microsatellite analysis: Six polymorphic microsatellite primers were used to determine polymorphism among 41 isolates of *R. solani*. The primer sets were synthesized using an Applied Biosystems 394 DNA/RNA synthesizer. Primer oligonucleotides were extracted from the synthesis columns with three 1 mL volumes of ammonia using 5 and 1 mL syringes. Ammonia solutions containing the oligonucleotides were incubated for at least 8 h at 55°C to remove the base-protecting groups. The primer solutions were then vacuum-dried in microcentrifuges in a SVC 200 Speed Vac (Savant). Primers were then re-suspended in 200 μL of 2.5 M ammonium acetate (pH 7.0) and ethanol precipitated for 30 min at -20°C. The precipitated oligonucleotides were pelleted in a vacuum microcentrifuge at full speed for 6 h. A 100 μL distilled water was used to resuspend the primers. Quantification of the primer concentrations was done as described by Sambrook *et al*. (1989).

PCR were done on 20 ng templates DNA in a 10 μL reaction volume. The DNA samples were mixed with fluorescent-labelled oligonucleotide primers, deoxyribonucleoside triphosphates (dNTPs) and thermostable Taq Polymerase in 10x PCR buffer. The amplifications were performed in a GeneAmp 9700 (Applied Biosystems Inc.) using 96-well plates. In one reaction 1.5 μL PCR buffer (100 mL tris, 500 mM KCl, 1.5 mM $MgCl_2$, PH 8.3), 3.2 μL dNTPs, 0.16 μ: Taq polymerase, 0.5 μL of each primer pair (5 pMol μL^{-1}) and 1.0 μL DNA (20 ng μL^{-1}) was mixed with 2.64 μL of deionized water to make a volume of 9 μL. The PCR cycle was 95°C for 5 min, 95°C for 1 min, to 55°C primer annealing for 1 min, then to 72°C for 1 min, at 35 cycles then to 72°C for 10 min and to 6°C.

Following the PCR, the resultant products were electrophoresed on the ABI Prism™ 377 automated DNA sequencer, to separate the alleles. The acrylamide solution was filtered through a 0.2 μm cellulose nitrate filter and then degassed for 2-5 min, 2.5 mL of filtered 10X TBE buffer was added and the volume was adjusted to 25 mL by adding deionized water (dH_2O). TEMED (17.5 μL) was added to the gel solution gently and allowed to polymerize for 2 h. Preparation of the electrophoresis buffer, loading and setting of electrophoresis run was done as described in the ABI Prism™ Users Manual (Applied Biosystems, 1995). The blue, green and yellow-labelled DNA fragments (electropherograms) representing different alleles at the microsatellite loci studied were assigned sizes (in base pairs). This data were than tabulated and exported into the Microsoft Excel software application program.

Data analysis: The Genescam™ 672 analysis software (version 1.2.2.) was used to size the resultant DNA fragment following electrophoresis. The software operated with the Perkin Elmer ABI Prism™ 377 DNA sequencer to automatically size and quantify DNA fragments by automated fluorescence detection. The observed heterozygosity (Ho) was calculated manually by directly counting the number of individual heterozygous at each locus, while the expected hetrozygosity (He) was computed from individual allele frequencies using the DISPAN computer programmes. The genetic distance estimation was determined to reveal the extent of genetic differences within *R. solani*, based on allele frequencies. The DISPAN computer programme was used to calculate standard genetic distances (DS) and genetic distances (DA) with their standard errors for the pair-wise comparisons (Nei, 1978; Nei *et al*., 1983). DA distances were used to evaluate the phylogenies.

The DISPAN computer programme was used to construct phylogenetic trees by the Unweighted Pair Group Method Arithmetic (UPGMA) and Neighbour-Joining (NJ) methods using matrices of DA genetic distances (Felsenstein, University of Washington, Washington DC, USA). Bootstrapping of 1000 replications was done to provide confidence statements about the groupings of the samples as revealed in the dendrograms. An individual specific analysis was done using microsat (version 1.5d) and phylip (version 3.57c). The matrix created by microsat was used by PHYLIP to plot a phylogenetic tree.

RESULTS

A total of 50 alleles were detected when six microsatellite loci were typed into the 41 individual isolates of *R. solani* (Table 1). The mean number of alleles detected at each locus was 8.33. The actual number of observable alleles at each locus ranged from 3 at locus RB23 to 19 at locus AF513014. Different allele sizes were recorded in all the loci, with the allele sizes varying at each locus; the smallest allele size detected being 129 base pairs (bp) at locus RE102 and the largest being 297 bp at locus AY212027. The expected heterozygosity obtained was 0.699 while the observed heterozygosity was 0.606. The gene diversity for the six microsatellite loci expressed as heterozygosity ranged from 0.493-0.909 (Table 1). There was a considerable deficiency of heterozygotes at

Table 1: Number of alleles sampled per locus typed into 41 isolates of *R. solani* isolates and heterozygosity

Primer	Primer sequence (5'-3')	Annealing temperature (°C)	No. of alleles	Size range (bp)	Observed heterozygosity (H_o)	Expected heterozygosity (H_e)
RB23	CAG CCG TCT TTC TCT CTC C GCC TTG AAT CACTACCTCCA	55	3	184-194	0.399	0.493
RE14	TAC CCA TTG CCT TGT TTC C ACT CCG CGT TCT GCT AGA G	55	7	175-191	0.564	0.658
RE102	GGA CTT GTC AGC GTC AAG TCA ACC ATC TCA AGG TAT GTC	58	5	129-145	0.628	0.722
BC5	CGT TTT CCA GCA TTT CAA GT CAT CTC ATA TTC GTT CCT CA	58	12	142-180	0.732	0.826
AF513014	GGCATATTGAGTATGGTATGGATG CTC CCG AGA TCT TGT TCA	58	19	188-238	0.815	0.909
AY212027	GAG CTG TGC GCG AGT CTG TG ACT GCT CCT TCGA GTC GTC A	55	4	285-297	0.499	0.593

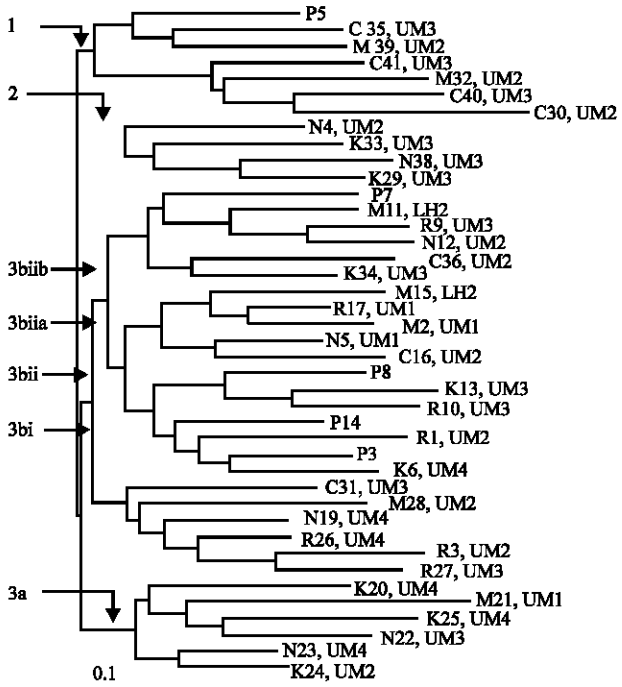

Fig. 1: NJ rectangular cladogram showing genetic relationship of 41 isolates of *Rhizoctonia solani* obtained by strict consensus tree (unrooted) using UPGMA method from 7 primer combinations in the PHYLIP 3.5c program. M-Manyatta, N-Nembure, R-Runyenjes, K-Kyeni, P-Potato, UM-Upper midland Zone, LH-Lower highland zone

RB23 and AY2120 loci, with 0.493 and 0.593 heterozygosity, besides having 3 and 4 alleles, respectively, both are indicators of lower levels of genetic variability at these particular two loci.

Phylogenetic analysis revealed 3 major clusters within the 41 isolates of *R. solani* (Fig. 1). Clusters 1, 2 and 3 had 15, 10 and 75% isolates, respectively. However, cluster 3 had 4 sub-clusters and cluster 1 had 2 sub-clusters, while cluster 2 did not have a sub-cluster. Cluster 1 had 29% of Manyatta samples while cluster 3 had 71%, cluster 2 had 29% of Nembure samples while cluster 3 had 71%, while all the samples obtained from Runyenjes were in cluster 3. In Kyeni, 25% of the samples were in cluster 2 while 75% were in cluster 3. Most of the samples (57%) in Central division were found in cluster 1 while 43% were in cluster 3. Twenty percent isolates from potatoes were in cluster 1 and 80% in cluster 3. The phylogenetic tree analysis established that isolates from any particular division were found to be in at least two different clusters except for Runyenjes, which were all found in cluster 3. Runyenjes isolates were closely related to 71, 71, 43, 75 and 80% of the isolates in Manyatta, Nembure, Central, Kyeni and Tigoni potato respectively. Similarity levels of isolates from different agro-ecological zones were determined. It was observed that 25% of isolates from UM2 were in cluster 1 while 8.7% were in cluster 2, 33.7% were in cluster 3.All the isolates from UM1 were in cluster 3 and the same applies to isolates from UM4 and LH5. However, 25% of UM2 were in cluster 1 and another 25% were in cluster 2 while 50% were in cluster 3.

DISCUSSION

Microsatellite analysis was chosen because of its precise alleles designation and ability to resolve PCR products differing in size by one base on polyacrylamide gels. DNA microsatellite loci selected had simple repeat units with dinucleotide repeat motifs, selected from microsatellites obtained from closely related species and were found to be highly polymorphic. These are in agreement with Moore *et al.* (1991), who reported successful microsatellite polymorphism with use of loci from closely related species, this has been explained to be due to the unique flanking sequence which are conserved in closely related species (Stallings *et al.*, 1991).

In the present study the loci revealed variable levels of polymorphism with different allele sizes. The variable number of alleles revealed per loci is an indication of high level of polymorphism, which is in agreement with the findings of Nei (1978) who reported the use of the number of alleles to measure the genetic diversity. There was little overlap of allele size frequencies distribution across the microsatellite loci between the isolates of *R. solani* indicating moderate levels of genetic differentiation. The

expected and observed heterozygosity was 0.699 and 0.606, respectively, while the different loci revealed heterozygosity range of 0.493 and 0.909. This range shows moderate heterozygosity of the *R. solani* isolates, which is in conformity with observations made by Bjorstad and Roed (2001) who reported moderate genetic variability of 0.6 and 0.8. The relationship was moderate and may be due to the fact that farmers exchange planting materials thereby leading to exchange of the pathogen types across farms and divisions. Microsatellites have been used to study polymorphism of other plant pathogenic fungi including *Botrytis cinerea* (Fournier *et al*., 2002) and *Sclerotinia sclerotiorum* (Sirjusingh and Kohn, 2001).

Isolates analysis by the phylogenetic tree revealed three distinct clusters showing intraspecific variation. The groupings observed from the phylogenetic tree analysis could be due to genetic variation arising typically by slow, gradual accumulation of mutational changes resulting in novel phenotypes upon which natural selection took place resulting to gradual shifting of allele frequencies as has been reported by Schardl and Craven (2003). Microevolution could be linked as has been explained by Brasier (1995), who suggested microevolution particularly on ascomycetes due to combination of haploid-based genetic systems; the fact that *R. solani* is a heterogeneous assemblage of basidiomycetes, ascomycetes and deuteromycetes (Parmeter, 1970) thus can explain the present results. Other researchers reported analoguos events in the ancestry of filamentous fungi because of their numerous nuclei within cells (Kristler *et al*., 1995; O'Donnell and Cigelnik, 1997); this has also been reported in *Phytophthora* (Oomycota) (Brasier, 1995). Other suggestions include the parasexual hybridization, which has been reported in ascomycetes (Endophytes) Giovanetti *et al*., 2001). Sanders (2002) suggested that most of the polymorphisms represent divergence of alleles within clonal lineages, with formal possibility of polymorphism due to nuclear exchange between individual species. Nuclear exchange has also been reported in members of the same species (Schardl and Craven, 2003), which could be the case in this study. Polymorphism has been observed in other fungi as a direct record of genetic evolution (Schardl and Craven, 2003).

There was no relationship between microsatellites and geographical origin of the isolates. There is need to establish the extent of relationship of the *R. solani* isolates from a large geographic area using microsatellites, as well as other molecular marker techniques such as AFLP to provide a comparison to the present results.

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REFERENCES

Applied Biosystems, 1995. Gene Amplification and DNA/RNA synthesizer, Pretoria. South Africa.

Bjorstad, G. and K.H. Roed, 2001. Breed darmacation and potential breed allocation of Horses assessed by microsatellite markers. Animal Gene., 32: 59-65.

Brasier, C.M., 1995. Episodic selection as a force in fungal microevolution, with special reference to clonal speciation and hybrid introgression. Can. J. Bot., 73: S1213-S1221.

Carling, D.E., C.S. Rothrock, G.C. MacNish and M.W. Sweetingham *et al*., 1994. Characterization of anastomosis group 11 (AG11) of *Rhizoctonia solani*. Phytopathol., 84: 1387-1393.

Coddington, A., D.S. Gould, 1992. Use of RFLPs to Identify Races of Fungal Pathogens. In: Techniques for Rapid Detection of Plant Pathogens. Duncan, J.M. and L. Torrance (Eds.), Blackwell scientific publications, pp: 162-176.

Doyle, J.J. and J.L. Doyle, 1990. Isolation of plant DNA from fresh tissue. FOCUS, 12: 13-15.

Fournier, E., T. Giraud, A. Loiseau and D. Vautrin *et al*., 2002. Characterization of nine polymorphic microsatellite loci in the fungus *Botrytis cinerea*. Mol. Mycol. Notes, 2: 253-255.

Gethi, M., F.M. Mwithi, N. Macharia and K. Njoroge, 1997. Maize bean intercropping system in medium potential area of Kenya. Farmer Practice and Research Challenges, Afri. Crop Sci. Conference Proceedings, 3: 756-770.

Giovanetti, M., P. Fortuna, A.S. Citernesi, S. Morini and M.P. Nuti, 2001. The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phythol., 151: 717-724.

Kristler, H.C., U. Benny, E.W.A. Boehm and T. Katan, 1995. Genetic duplication in *Fusarium oxysporum*. Curr. Genet., 28: 173-176.

Moore, B.J., S.P. Kwan, N.T. Bech-Hansen, 1991. A polymorphic dinucleotide repeat at the DXS7 locus. Nucl. Acid Res., 20: 929.

Nderitu, J.H., R.A. Buruchara and K.O. Ampofo, 1997. Integrated pest management of beans. African Highland Initiative, pp: 11.

Nei, M., 1978. Estimation of average heterozygosity and genetic distance from a small amount of individuals. Genetics, 89: 583-590.

Nei, M., F. Tajima and Y. Tateno, 1983. Accuracy of estimated phylogenetic trees from molecular data. J. Mol. Evol., 19: 153-170.

O'Donnell, K. and E. Cigelnik, 1997. Two divergent intagenomic rDNA ITS2 types within a monophyletic lineage of he fungus Fusarium. Mol. Phylogenet. Evol., 7: 103-116.

Ogoshi, A., 1987. Ecology and pathogenicity of anastomosis intraspecific groups of *R. solani* Kuhn. Ann. Rev. Phytopathol., 25: 125-143.

Parmeter, J.R., R.T. Sherwood and W.D. Platt, 1969. Anastomosis groupings among isolates of *T. cucumeris*. Phytopathol., 59: 1270-1278.

Parmeter, Jr. and J.R., 1970. *R. solani*: Biology and Pathology. Univ California Press Berkeley, pp: 255.

Sambrook, J., E.F. Fritsch and T.M. Maniatis, 1989. Molecular Cloning: A Laboratory Manual, 2nd Edn., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanders, I.R., 2002. Ecology and evolution of multigenomic arbuscular mycorrhizal fungi. Am. Natur., 160: S128-S141.

Schardl, C.L. and K.D. Craven, 2003. Interspecific hybridization in plant associated fungi and Oomycetes: A review. University of Kentucky, Lexington, Kentucky, USA., pp: 40546-0091.

Sirjusingh, C. and L.M. Kohn, 2001. Characterization of microsatellite in the fungal plant pathogen *S. sclerotiorum*. Mol. Ecol. Notes, 1: 267-269.

Sneh, B., I. Burpee and A. Ogoshi, 1991. Identification of *Rhizoctonia* species. St Paul, Minnesota: APS Press.

Stallings, R.L., A.F. Ford, D. Nelson, D.C. Torney, C.E. Hildebr and R.K. Moyzis, 1991. Evolution and distribution of (GT) n repetitive sequence in mammalian genomes. Genomics, 10: 807-815.

Takezaki, N. and M. Nei, 1996. Genetic distance reconstruction of phylogenetic tree from microsatellite DNA. Institute of molecular evolutionary genetics and dept. of Biology. The Pennsylvania State University. Genetics 144, 389-99.

Talbolt, P.H.B., 1970. Taxonomy and Nomenclature of the Perfect State of *Rhizoctonia solani*. In: Biology and Pathology. Parmeter, J.R. Jr. (Ed.), *Rhizoctonia solani*: Univ. California Press, Berkeley, pp: 255.

Tautz, D., 1989. Nucl. Acid Res., 17: 6463.

Wortmann, C.S., D.J. Allen, 1994. African bean production environment, their definition and constraints. Network on beans research in Africa. Occasional paper series No. 11 CIAT Daresalaam.

Wortmann, C.S., R.A. Kirby, C.A. Eledi and D.J. Allen, 1998. Atlas of common bean (*Phaseolus vulgaris*) production in Africa. Cali. Colombia CIAT, pp: 33.