Morphological and molecular identification of *Pythium* species pathogenic to common beans in Uganda

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Background

In the last ten years, common bean yields in Uganda have declined by about 50%. The main causes of this reduction are declining soil fertility, insect pests and diseases, most importantly root rots caused by *Pythium*, *Rhizoctonia* and *Fusarium* spp. *Pythium* species pathogenic to beans in Uganda have not been well characterised, yet this is crucial for effective epidemiological studies.

Severe *Pythium* root rot on bean

Aims of this study

- To characterise *Pythium* isolates from infected bean plants using morphological and molecular markers.
- To examine the pathogenicity of representative isolates
- To examine the diversity within pathogenic *Pythium* populations.

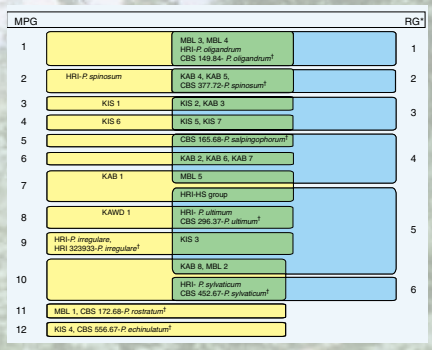
Materials and Methods

Twenty-one *Pythium* strains from Uganda and 14 from culture collections were compared morphologically (Plaats-Niterink, 1981) and by RFLPs of the ITS/5.8*S* rRNA gene region amplified using primers ITS1/4 (White *et al.*, 1990).

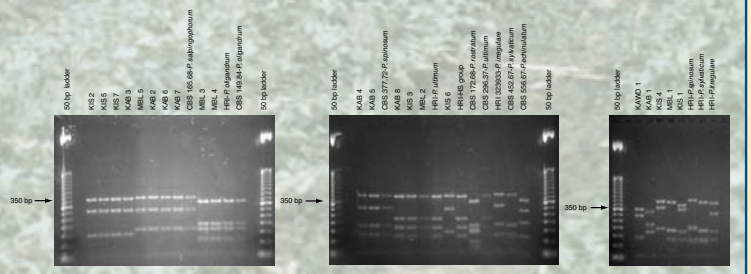
Map of Uganda showing the three regions where *Pythium* infected beans were sampled

Results

Morphology (MPG) and RFLPs by *Cfo*I, *Hin*f I & *Mbo*I (RG) groupings of the 35 *Pythium* strains



Pythium isolates source
Uganda: KIS=Kisoro; KAB=Kabale; MBL=Mbale; KAWD=Kawanda.
CBS=Centraalbureau voor Schimmelcultures; †Morphological identification not confirmed
*strains not in the intersection possessed unique RFLP patterns



Agarose gels showing restriction banding pattern of the amplified ITS products from 35 *Pythium* strains using restriction endonuclease *Hin*f I

Discussion

Morphological and molecular data both demonstrate the wide diversity of strains recovered from common bean plants with *Pythium* root rot. If morphological similarities within a collection of *Pythium* strains indicate genetic relatedness, then this is generally supported by our RFLP data. There was complete agreement between morphology and molecular groupings in the case of all isolates identified as *P. oligandrum* and all but one of those named as *P. spinosum*. Most other groups formed using morphology and RFLPs showed incomplete agreement although some RFLP groups (e.g. RG 3, 4 and 5) contained the majority of isolates from two or more morphological groups. It is difficult to interpret the relatedness among these groups mainly because the extent of intraspecific variation within *Pythium* spp. is still unknown. Very few isolates of named species have so far been sequenced (Wang and White, 1997; Matsumoto *et al.*, 1999), and it is important to expand on this work with morphologically well-characterised isolates before firm conclusions can be made.

Future work

The RFLP findings will be linked with results from pathogenicity studies currently in progress. This will provide the basic information needed to design specific primers for:

- Detecting pathogenic *Pythium* strains
- Quantification of inoculum levels in the soil

References

Matsumoto, C., Kageyama, K., Suga, H & Hyakumachi, M., 1999. Mycoscience 40: 321-331.

Plaats-Niterink, A.J., 1981. *Monograph of the genus Pythium*. Studies in Mycology No. 21. Baarn, Netherlands: Central Bureau Voor Schimmelcultures.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. In: Innis, M.A, Gelfand, D.H., Sninsky, J.J., White, T.J. eds. *PCR Protocals, A Guide to Materials and Applications*. San Diego: Accademic Press, 315-322.

Wang, P-H. & White, J.G., 1997. *Physiological and Molecular Plant Pathology* 51: 129-143.

Recent developments in the characterization of *Pythium* spp. causing bean root rots in Uganda

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Background

In the last ten years, common bean yields in Uganda have declined by about 50%. The main causes of this reduction are declining soil fertility, insect pests and diseases, most importantly root rots. One of the major genera causing severe bean root rots in Uganda is *Pythium*. Others include *Fusarium* and *Rhizoctonia.* The identification of *Pythium* species pathogenic to beans in this disease complex is critical for effective epidemiological studies leading to development of control strategies.

Beans infected with *Pythium* root rot

Aim of this study

- To characterise *Pythium* isolates from infected beans using DNA based molecular markers
- To examine the pathogenicity of representative isolates

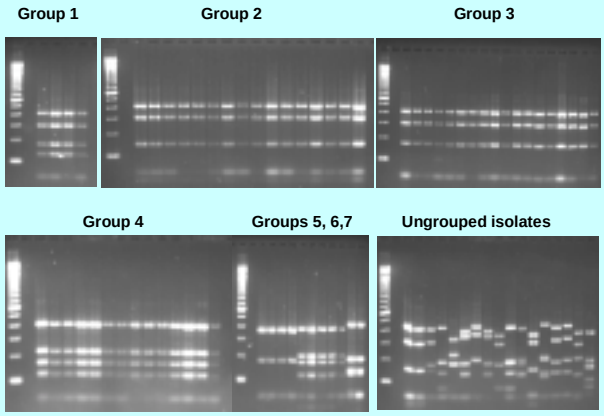
Materials and Methods

Sixty-six *Pythium* strains from Uganda identified and grouped using morphological structures (Plaat-Niterink, 1981), and 14 from culture Collection were considered for the study. The ITS/5.8*S* rRNA gene regions were amplified using ITS1/4 primers (White *et al*., 1990). The products were later digested with *Cfo*I, *Mbo*I and *Hinf* I enzymes.

Groupings arising from RFLP patterns were compared with results from sequence analysis of the ITS I of representative isolates and pathogenicity tests. *Pythium* ITS I sequences were compared with ITS I sequence data of *Pythium* species available in the database for identification.

Results

- **RFLP analysis and grouping of 66 *Pythium* isolates from Uganda and 14 from culture collection using *Cfo*I, *Hinf*I and *Mbo*I endonucleases**



Agarose gels showing restriction-banding patterns for 80 *Pythium* isolates basing on the ITS rDNA with *Hinf* I.

- **Relation between RFLP grouping, pathogenicity and *Pythium* identification of some isolates using ITS I sequence comparisons with known *Pythium* species**

Isolates & species identified	No of isolates	Disease level of some isolates
Group 1 (*P.spinosum*)	**4**	
KAB 4		3.3
KAB 5		3.2
GROUP 2 (*P. torulosum*)	**8**	
KAB 10		0.0
KAB 11		0.1
GROUP 3 (*P. salpingophorum*)	***8***	
KAB 2		2.0
KAB 12	.	0.8
Group 4 (*P. ultimum*)	**18**	
KAB 23		2.3
KAB 24		2.5
Group 5 (*P.oligandrum*)	**2**	
MBL 3		0.0
MBL 4		0.4

Scale: 0= no root symptoms; 1= 25% of root tip necrotic; 2= 25-50% of root tip necrotic; 3= 50-100% of root tip necrotic plus localised necrotic lesions on the tap root or crown; 4= extensive root rot with few or no white roots, crown rot extensive; 5= root system completely necrotic and plant dead or moribud (Chellemi *et al.*, 2000).

Conclusion and future work

The morphological and various molecular analyses revealed the wide diversity within the collected *Pythium* spp. Initial pathogenicity tests have identified pathogenic and non-pathogenic groups of *Pythium*. Further pathogenicity tests on *Pythium* species are in progress and pathogenic variation will be compared with molecular variation based on AFLP markers. The information will be useful in developing specific probes aimed at faster and simpler detection and possibly quantification of pathogenic *Pythium* spp. in soil.

Acknowledgement

This is a collaborative research by NARO, CIAT, HRI, NRI and Makerere University funded by DFID.

References

Chellemi, D.O., Mitchell, D.J., Kannwischer-Mitchell, M.E., Rayside, P.A. & Rosskopf, E.N., 2000. *Plant Disease* 84: 1271-1274

Plaats-Niterink, A.J., 1981. *Monograph of the genus Pythium. Studies in Mycology No. 21*. Baarn, Netherlands: Central Bureau Voor Schimmelcultures.

White, T. J., Bruns, T., Lee, S. & Taylor, J. 1990. In: Innis, M.A, Gelfand, D.H., Sninsky, J.J., White, T.J. eds. *PCR Protocals, A Guide to Materials and Applications*. San Diego: Accademic Press, 315-322.

Detection and diversity of *Fusarium solani* f.sp. *phaseoli* from common beans in south-western Uganda

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Background

***Fusarium solani* f. sp. *phaseoli* is one of a complex of organisms that can cause bean root rot disease, currently epidemic in south-western Uganda and other areas in the Great Lakes Region of Africa. Total crop losses as a result of this disease are now common in the region. However, the development of effective root rot control measures are hampered by (i) the lack of rapid methods for pathogen detection (ii) no knowledge of pathogen variation in south-western Uganda.**

Severe root rot caused by isolate P099

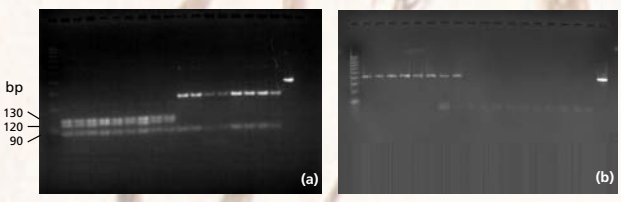
Objectives

(i) to develop specific detection systems for *F. solani* f. sp. *phaseoli*

(ii) to examine *F. solani* f. sp. *phaseoli* population diversity in south-western Uganda

Results

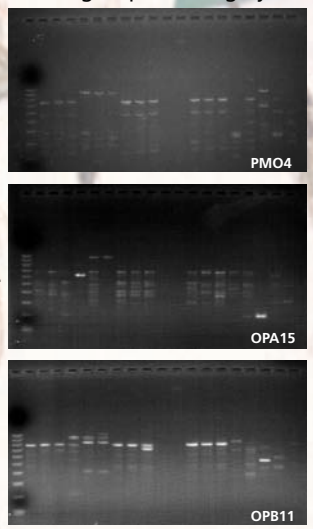
- *Hae* III digests of ITS3/4 (White *et al.*, 1990) amplicons produced 3 bands (ca. 90, 120 and 130bp) with 43 *F. solani* isolates studied. This pattern was not shared with any of the isolates from nine other genera studied (a).
- A primer pair developed from *F. solani* f.sp. *phaseoli* ITS sequence amplified a band of about 400 bp with *F. solani* isolates but not with the isolates listed above (b).



(a) **Lane 1:** Marker; 2-7 Ugandan *F. solani* isolates; 8: CBS 835.85 *F.s.* fsp. *phaseoli*; 9: MUCL 906 *F.s.* fsp. *pisi*; 10: IMI 172300 *F.s.* fsp. *fabae*; 11-15: Ugandan *F. oxysporum* isolates; 16: S. African *F. solani* isolates; 17: CBS 935.73 *F. o.* fsp. *phaseoli*; 18: *F. poae*; 19: undigested CBS 835.85 *F.s.* fsp. *phaseoli*.

(b) Same as (a): except Lane 19 water control. Lane 20: CBS 835.85 *F.s.* fsp. *phaseoli*.

- RAPD banding patterns revealed differences between isolates, dividing the Ugandan ones into two main groups.
  - One group composed slow growing isolates (1.4-1.8mm/day on PDA) with blue-purple tinted colonies producing large numbers of macroconidia. The two isolates tested from this group were highly virulent on beans.
  - The other group composed relatively fast growing isolates (6 – 8 mm/day on PDA) with mostly light yellow colonies producing both micro- and macroconidia. Two isolates tested from this group displayed lower virulence on beans.



RAPDs:
L-R: Marker; P099; P056; P083; TG13; TG87/3; P036; P015; P035; P030; Mb 08; Mb 10; P070; P078; CBS 835.85; CBS 190.35; DPO A/1.2/1; 835.85; IMI 172300 *F.s.* fsp. *fabae*; MUCL 906 *F.s.* fsp. *pisi*; *F. poae*; water control

Discussion and conclusions

RFLPs of ITS 3/4 amplicons and the specific primers used in these studies both grouped together all *F. solani* isolates irrespective of morphological or pathogenic variation. This was expected since both methods targeted the ITS, a region highly conserved within species (White *et al.*, 1990). However, RAPD analysis did reveal variation within the *F. solani* isolates studied. Two main groups were identified and these were correlated with colony morphology and virulence. This indicates a high probability of identifying DNA sequences that could be useful in designing pathogen specific-primers.

Future work:

(i) Examine larger numbers of isolates to validate these preliminary findings.

(ii) Employ either RAPD or AFLP techniques to identify DNA sequences that can be used to design pathogen specific primers.

Reference:
White, T. J., Bruns, T., Lee, S. and Taylor, J. 1990. In PCR Protocols: A guide to methods and applications, Innis, M. A., Gelfand, D. H., Sninsky Jand White, T. J. Eds., Academic Press, San Diego, pp 315-322.

Developing a method for quantification of population levels of *Pythium* species pathogenic to beans

J. Mukalazi R. Buruchara, E Adipala J. Carder, F. Opio, T. Pettitt & N. J. Spence.

Background

The population of soil-borne bean pathogens influences incidence and severity of bean root rots. Some management efforts target reduction in inoculum level with the objective of reducing it to below economic threshold levels. Disease severity is an indirect but not always a reliable indicator of soil inoculum level of the pathogen. There is need to develop a method to quantify the effect and relative value of different management practices and interactions on the inoculum levels of *Pythium* pathogens.

Objectives:

- To develop procedures for quantification of *Pythium* pathogen population in the soil
- To assess the effect of different organic amendments

Method:

Use of dilution plating combined with molecular detection

Screen house experiments: Soil is artificially inoculated with known amounts of inoculum (300-400 cfu).

Fig 1. Pythium quantifiifaction in the screen house

Green manures (Caliandra) and FYM is assessed on;

- *Pythium* population
- Severity
- Plant growth

Field experiments (Kabale): Based on natural infection.

Fig 2. Pythium quantification trial in Kabale; Fore=CAL96 +Caliandra, Middle: CAL96+FYM, Back: CAL96+no ammendments

Assessment of Pathogen population:

- Dilution-plating on CMA media amended with Pimaricin and rifamycine.
- Colony counts are recorded after 1-2 days.
- Computation of population (cfu/g of soil)

Fig 3. Colonies of *Pythium* from soil growing on selective media

Molecular detection:

- Samples of different colonies are sub-cultured for the further identification of *Pythium* spp. using molecular techniques

Molecular and pathogenic variation among *Fusarium solani* isolates from beans with root rot symptoms

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Background

The bean root rot disease is currently epidemic in the Great Lakes region of Africa. In south-western Uganda, total bean crop loss as a result of this disease is now common. A complex of organisms including *Fusarium solani*, is responsible for this epidemic. Some control measures have been put in place but their effectiveness vary with location and sometimes with seasons. Variation within the pathogen population may partly be responsible. This study was therefore set up to investigate molecular and pathogenic variation within the *F. solani* population recovered from beans with root rot disease mainly from SW Uganda.

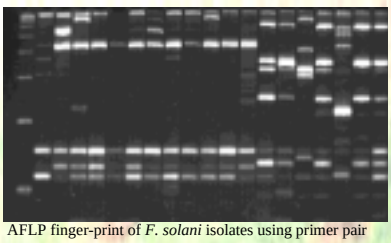
Bean crop with severe root rot disease (left) in Kabale Uganda.

Methods

Isolates were cultured from diseased beans sampled from mainly Kabale and Kisoro districts and a few from other Ugandan districts. Forty-six of these were inoculated on 15 bean varieties, which were assessed for root-rot damage after 5 weeks. DNA from these isolates was subjected to amplified fragment length polymorphism (AFLP) analysis to study their molecular variability using 15 pairs of primers (Vos *et al*., 1995).

Results

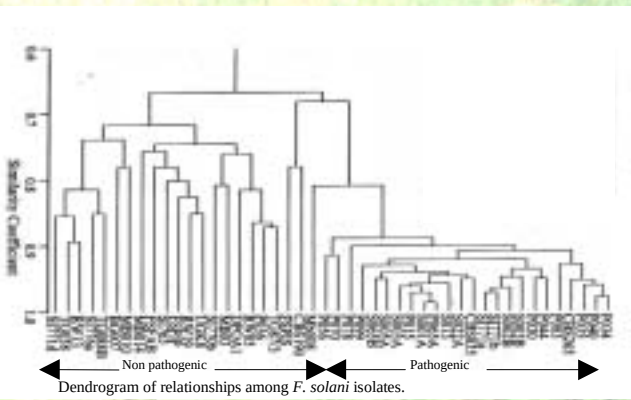
Two types of isolates were recovered. Group 1 was buff coloured, slow growing (<1.8mm day^{-1} on PDA), produced num,erous macroconidia and no microconidia. It later developed bluish colours in the centre. Group 2 grew fast (>6mm day^{-1} on PDA), was light yellow in colour and produced conidia numerous micro-conidia and no macroconidia Group 1 was pathogenic, while Group 2 was not. Their severity on bean cultivar K20 and pathogenicity of 2 sample isolates are shown in the Table and Picture below, respectively. At molecular level, Group 1 was highly uniform.



AFLP finger-print of *F. solani* isolates using primer pair *Eco*RI-AT/*Mse*I-CAT

Isolate	Severity*	Relative Dry Matter	
		Root	Shoot
Group 1			
P099	**7.4**	**0.39**	**0.67**
P056	**8.2**	**0.32**	**0.64**
S013	**7.0**	**0.32**	**0.65**
P078	**8.5**	**0.30**	**0.61**
Group 2			
P036	**1.0**	**0.73**	**0.77**
TG38	**1.5**	**0.88**	**0.99**
Mb14	**1.4**	**0.93**	**0.88**
Mb10	**1.0**	**1.01**	**1.06**
Control	**1.0**	**1.00**	**1.00**

*1=No disease; 9=Whole root system rotten.



Dendrogram of relationships among *F. solani* isolates.

Discussions, conclusions and recommendations

Two morphologically and molecular diverse populations were recovered from diseased beans. One of these was pathogenic and corresponded to the highly molecularly uniform group. All bean varieties succumbed to this group of isolates. The seasonal and locational variability in root rot occurrence and management success therefore, does not seem to be pathogen related. Environmental factors may be playing a significant role. Therefore, there is need to study the interaction between the environment and the pathogen. Control measure efforts should include resistance breeding since the pathogenic *F. solani* population is uniform.

Acknowledgement

This is a collaborative study by NARO, CIAT, HRI, NRI, MUK, and is funded by DFID.

Reference

Vos *et al*., 1995. AFLP: a new technique for DFNA fingerprinting. **Nucleic Acids Res**. 23:4407-4414.

Development of a quantification technique and the effect of soil amendments on soil populations of *Fusarium solani* f. sp. *phaseoli*

G. Tusiime, R. Buruchara, E. Adipala, J. Carder, F. Opio and N. Spence.

Background

- *Fusarium solani* fsp. *phaseoli* is a fungus among a complex of organisms that cause bean root rot diseases, currently epidemic in some regions of E. and Central Africa.
- Soil population of the pathogen plays a significant role
- The pathogen also acts synergistically with other pathogens like *Pythium* spp.
- Some of bean root rot control measures target reduction of inoculum levels.

Therefore,

Development of quantification techniques for assessing soil pathogen levels will strongly improve the basis for selecting efficient and effective Integrated Disease Management options for the disease.

The Project:

We are developing quantification techniques of soil population and investigating the effect of Farm yard manure (FYM) and Green (*Calliandra* spp.) manure on the soil population of *F. solani* f. sp. *phaseoli*.

Typical bean root rot disease in a field

Methods

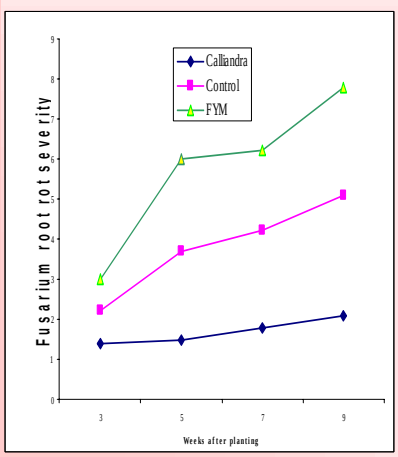
- Infest soil with *F. solani* f. sp. *phaseoli*
- Distribute infested soil and apply amendments
- Quantify soil pathogen population (using a bean tissue bioassay)

Bioassay of soil population of *F. solani* f sp. *phaseoli* using bean hypocotly

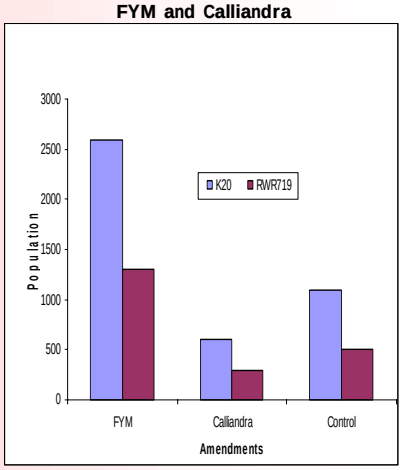
Results

Effect of amending soil with FYM and Calliandra on Fusarium root rot

Disease Progress on CV. K20



Soil population of *F. solani* after amending soil with FYM and Calliandra



Conclusions

- The bean bioassay was effective in assessing soil populations of *F. solani* f.sp. *phaseoli*.
- Green manure and FYM enhance bean crop growth
- Green manure reduces soil pathogen population and reduces Fusarium root rot disease severity
- FYM maintains *F. solani* fsp *phaseoli* spoil population high, increases root rot severity. It however, enhances crop growth and yields.

Recommendation

Judicious integration of both GM and FYM in the control strategy could improve root rot management through reduction of soil inoculum and improved soil fertility.

Characterisation of *Fusarium solani* isolates from root rot infected Phaseolus beans in Uganda

G. Tusiime, R. Buruchara, E. Adipala, J. Carder, F. Opio and N. Spence.

Background

- Bean root rot disease incited by a complex of microorganisms is currently devastating the crop in many parts of the great lakes region of Africa.
- The current epidemic is mostly associated with the fungi *Fusarium solani* and *Pythium* spp.
- Control measures advocated for this disease have not been consistent.....

.....Questions

1. Is the population of the causal organisms varied?

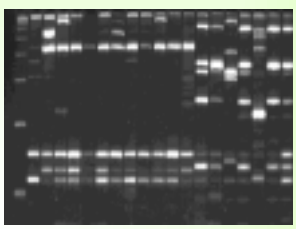
2. If varied; does the variation interact with different bean varieties resulting in the differences in disease severity in different locations?

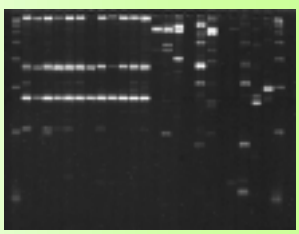
Aim

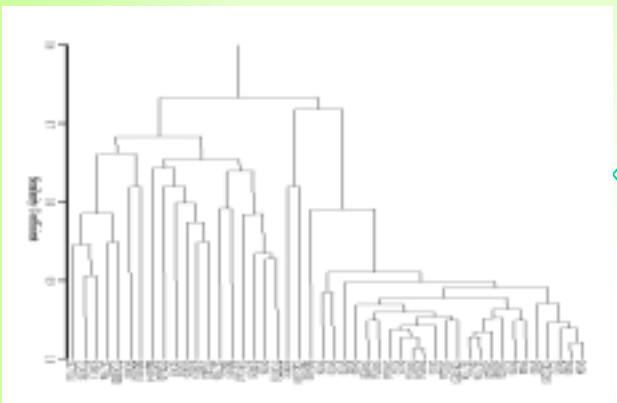
To characterise the diversity in the *Fusarium solani* population from beans with root rot disease in Uganda using molecular markers

A. Fungal isolates cultured on PDA

B. AFLP DNA analysis







Pathogenicity tests

Non-Pathogenic

Pathogenic

Outcomes

- A diversity of *F. solani* is found on beans with root rot symptoms.
- Some are pathogenic; some are not.
- There is a high diversity in the non-pathogenic population.
- Pathogenic isolates (*F. solani* f.sp. *phaseoli*) have high molecular uniformity.

Conclusion

- seasonal and locational differences in root rot occurrence and severity are not related to a variable pathogen population.
- Soil, environmental and possible interaction factors may be the most important.

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