

## Activity 1. Characterization of *Pythium* spp prevalent in Eastern Africa

**Rationale:** *Pythium* spp are fungal pathogens frequently associated with severe outbreaks of root rots of bean in eastern and central Africa, often resulting in crop failures. Some bean varieties have been observed to be resistant in one region but not in another. At least four *Pythium* spp. are known to cause bean root rots, but their distribution and relative importance in the region is unknown. Identification of *Pythium* spp. is both difficult and slow using morphological or pathogenic characteristics and when extracted from soil, is complicated by the presence of a wide range of other pathogens and saprophytes. Characterization of *Pythium* species and their distribution is therefore a necessary pre-requisite for the identification and development of host resistance, in developing or adapting some of the rapid detection and characterization techniques, (e.g. Reverse Dot Blot Hybridisation) or in assessing the effect of other management practices on soil inoculum levels. We therefore continued with studies initiated last year to characterize the *Pythium* spp prevalent in the region.

**Materials and Methods:** Studies conducted last year showed that RFLP groupings of most (but not all) *Pythium* isolates based on PCR/RFLP analysis using restriction enzymes was comparable to results obtained using sequence analysis. We therefore adopted an approach where we initially group *Pythium* isolates using PCR/RFLPs analysis and then use this as the basis to select candidates for sequencing. Using a *Pythium* selective medium, 450 isolates were recovered from sample collected in Rwanda (250) and Kenya (220) and their DNA extracted. Restriction analysis was done on 206 *Pythium* samples by digesting PCR products using *Cfo* I, *Hinf* I and *Mbo* I endonucleases and separated on 2% agarose gels. Five previously characterized *Pythium* spp isolates were included for comparison.

**Results:** Restriction analysis of PCR products of 206 *Pythium* isolates using restriction enzymes *Cfo* I, *Hinf* I, *Mbo* I grouped the isolates into 24 RFLP groups (**Table 1**). Nine isolates were not grouped into any particular group. The number of isolates in groups varied between 2 and 74. *Pythium salpingophorum* isolate was grouped together with the largest group implying that the group probably consisted of isolates that might belong to this species. The group associated with *P. ultimum* was the second largest with 20 isolates. Isolates representing different RFLP groups have been selected for sequence analysis. More PCR/RFLPs analysis is underway to facilitate selection of isolates for sequencing.

**Table 1. RFLP groups for *Pythium* spp. isolated from beans with root rot disease from various locations in Kenya and Rwanda.**

RFLP Group	Number of isolates	Reference Species
1	12	-
2	10	-
3	2	-
4	4	-
5	74	<i>P.salpingophorum</i>
6	3	-
7	2	-
8	8	<i>P. torulosum</i>
9	20	<i>P. ultimum</i>
10	4	-
11	2	-
12	3	<i>P. echinulatum</i>
13	3	<i>P. pachycaule</i>
14	5	<i>P. oligandrum</i>
15	6	-
16	3	-
17	4	-
18	2	-
19	3	-
20	5	-
21	12	-
22	4	-
23	2	-
24	3	-
Ungrouped	9	-

**Contributors:** R. Buruchara, S. Mayanja, and G. Mahuku

## Activity 2. Pathogenicity of identified *Pythium* spp on bean

**Rationale:** Despite the large number of *Pythium* species occurring in the soil (about 100), four (*Pythium ultimum*, *P. irregulare*, *P. aphanidermatum*, and *P. myriotylum*) have been reported to be pathogenic to beans (Abawi, 1985). To determine the role and possible contribution of some of the species associated with bean root rots in Africa, pathogenicity studies were conducted on isolates representing different species that have been characterized using PCR/RFLP and rDNA sequencing

**Materials and Methods:** Isolates representing *Pythium* species, previously identified on the basis of rDNA sequencing of ITS1 region were artificially inoculated on susceptible cultivars CAL 96 and K20 and on the resistant cultivar RWR 719 in the greenhouse. Seed of these cultivars were planted on trays containing soil infested with each of the isolates represented and under conditions, which favor pathogen establishment and disease development. Four weeks after emergence, plants were evaluated for root rot damage based on and the CIAT scale of 1 – 9 where 1 = no root rot symptoms and 9 root system completely necrotic and/or plant dead.

**Results:** All isolates belonging to *P. ultimum var ultimum* (6) and *P. spinosum* (4) tested, caused severe root rot on susceptible bean varieties CAL 96 and K20 (**Table 2**). Similarly, isolates belonging to *P. torulosum*, *P. nodosum* and *P. pachycaule* were pathogenic on CAL 96 and K20 (**Table 2**). Pathogenicity of isolates belonging to *P. spinosum*, *P. nodosum*, *P. pachycaule*, *P. torulosum* and *P. salpingophorum* species is interesting, demonstrating that more than four previously reported *Pythium* species are prevalent, pathogenic and with the potential to cause *Pythium* root rots on beans. Our ultimate goal is however to establish the relative importance and contribution of these species in the root rot problem in east and central Africa. Isolates belonging to *P. torulosum* and *P. salpingophorum* showed variation in pathogenicity. This might indicate pathogenic variation within the species. Further investigations are underway to compare pathogenic variation with possible molecular variation based on AFLP markers.

Resistant and intermediate reactions observed on RWR 719 following inoculation with isolates representing all species used in this study demonstrates the potential value of resistance in the variety. Representative isolates of the different species will also be used to evaluate potential sources of resistance (e.g. Root Rot Nursery) so as to identify parents that may be used for genetic improvement.

**Table 2. Pathogenicity of *Pythium* species (identified using sequence analysis) on bean varieties CAL 96, K20 and RWR 719.**

Species	Isolate / Strain	Disease severity			
		CAL 96	K 20	RWR 719	
<i>P. spinosum</i>	JM 70H1(KAB 4)	8.9	8.6	6.3	
	JM 70H2 (KAB 5)	6.6	5.7	4.1	
	A 672-4 (KIS 10)	8.3	8.3	3.7	
	MS 34	8.7	8.6	3.8	
<i>P. torulosum</i>	JM 41B (KAB 10)	6.4	6.3	4.5	
	JM 67A (KAB 15)	7.8	8.0	3.7	
	JM 12A (KIS 5)	8.2	8.1	3.9	
	JM 5 (KIS 14)	9.0	7.7	3.8	
	JM 91 (KAB 28)	8.8	8.2	4.7	
<i>P. salpingophorum</i>	JM 59H (KAB2)	7.8	7.3	6.0	
	JM 84(KAB6)	8.3	8.5	5.2	
	JM 41A (KAB9)	6.7	5.1	4.5	
	JM 65A (KAB13)	8.8	8.2	6.1	
	JM 71 (KAB26)	4.9	3.9	4.1	
	JM 92 (KAB29)	6.5	6.8	5.5	
	JM 6A (KIS 2)	5.0	3.8	2.0	
	JM 19B MB (MB5)	5.6	5.1	3.3	
	<i>P. ultimum var ultimum</i>	JM 85A (KAB8)	9.0	9.0	2.7
		JM 90S (KAB22)	7.3	7.6	4.0
JM 8 MB (MB1)		6.6	7.2	3.7	
MS 1		9.0	8.0	5.8	
MS 50		7.5	8.1	4.0	
MS 61		9.0	9.0	6.3	
JM 29A (KIS 6)		8.2	7.8	3.7	
<i>P. pachycaule</i>	JM 29A (KIS 6)	8.2	7.8	3.7	
<i>P. nodosum</i>	JM 7H1 (KIS 3)	7.4	6.6	4.3	
<i>P. echinulatum</i>	JM 8H (KIS 4)	5.4	3.9	4.3	
	Control	1.0	1.0	1.0	
LSD p= 0.05			1.6		
CV			4.4%		

Disease severity based on the CIAT scale of 1-9

**Contributors:** J. Mukalazi, R. Buruchara, F. Opio (NARO)

### Activity 3. Developing a quantification method for *Fusarium solani f.sp phaseoli* using a bean bioassay

**Rationale:** Management practices for soilborne diseases have the potential to directly or indirectly influence inoculum levels of the responsible pathogens. Evaluating the effect such practices is commonly based on disease incidence and / or severity both of which are unsatisfactory under unfavorable climatic conditions during disease development. To understand the contribution of management practices on inoculum potential (both in the short and long term) there is a need to develop easy but reliable methods, which may be used to either directly or indirectly quantify inoculum levels. In preliminary studies we observed that the slow growing nature of *Fusarium solani f.sp phaseoli* (FSP) pathogenic to beans, makes the use of dilution plating technique difficult because it is easily overtaken in growth by fast species. This study was therefore meant to evaluate the potential use of bean bioassay in quantification of FSP.

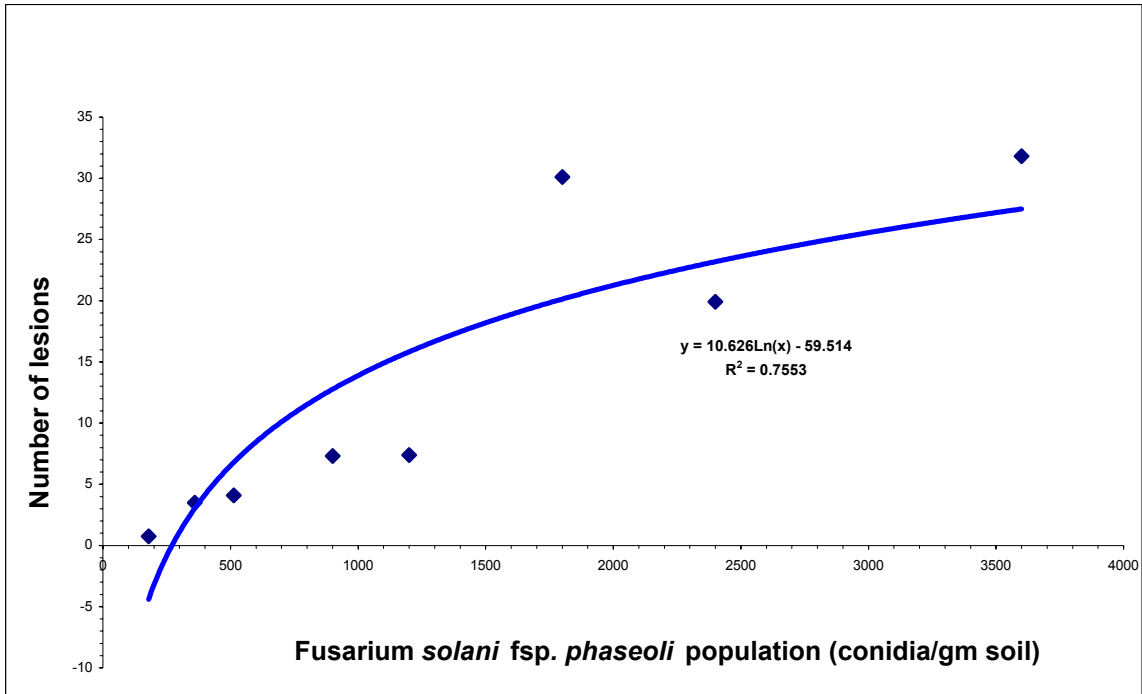
**Materials and Methods:** Soil from fields that had not grown beans was artificially infested with *F. solani f.sp phaseoli* so as to generate varying levels of the pathogen population ranging from 200 – 4000 conidia per gram of soil. Several six-cm, 1-week old hypocotyl pieces of bean cultivar K20 were placed in these soils and incubated at room temperature for 1 week. Thereafter, lesions of *F. solani f.sp phaseoli* were counted and plotted against soil population.

**Results and Discussion:** There was a positive relationship (**Figure 1**) between soil population and the number of lesions that developed on the bean hypocotyls as described by a non-linear equation developed:

$$Y = 10.626\ln(x) - 59.5; R^2 = 0.75$$

where Y= soil population; x = number of lesions per 6-cm piece of bean (cv K20) hypocotyls.

The equation was used to assess the population of the pathogen in a susceptible (K20) and resistant (RWR 719) bean crop growing in soils amended with farmyard manure and green manure (*Calliandra* spp.). The development of the bean assays method and its application in quantifying of *F. solani f.sp phaseoli* was a significant achievement in efforts to better understand epidemiological principles of the disease and will be useful for evaluating and developing management options. The method is reasonably simple, cheap and relatively fast.



**Figure 1. Relationship between *Fusarium solani* f sp. *phaseoli* populations in soil and number of lesions per 6-cm length bean hypocotyl**

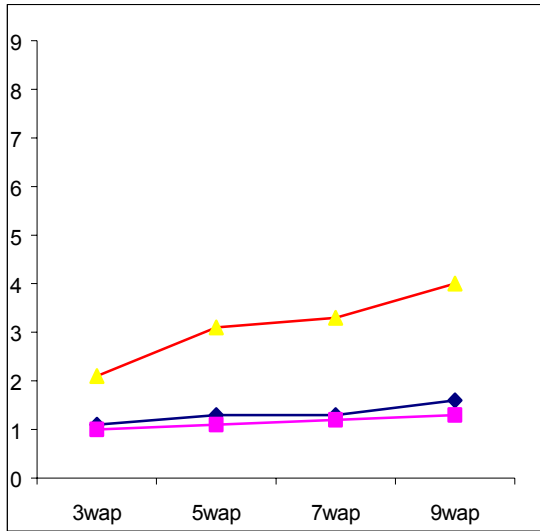
**Contributors:** G. Tusiime, J., R. Buruchara (CIAT) F. Opio (NARO).

**Activity 4. Effect of organic amendments (farmyard manure or green manure (*Calliandra* spp.) on severity of root rots and soil population of *Fusarium solani* f.sp *phaseoli***

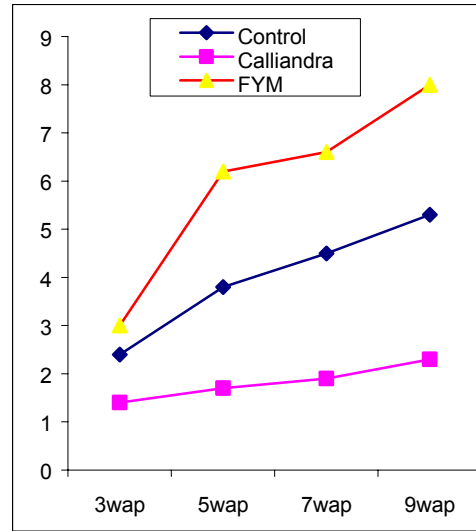
**Rationale:** Soilborne pathogens associated with bean root rots are common soil inhabitants and facultative saprophytes (Hillocks and Waller, 1997) therefore able to obtain their nutritional requirements from organic residues, as well as from living hosts. Members of the bean root rot complex differ in their ability to compete with other saprophytic microorganisms, as well as in the type of substrates they are able to exploit. Organic amendments are likely to have different effects on soilborne pathogens and may suppress or enhance pathogen populations. Previous studies have shown that some organic amendment reduce disease severity of bean root rots and enhance plant growth possibly due to improvement in soil structure, nutrient or the suppressive effect on the pathogen population. Following the development of quantification method for *F. solani* f.sp *phaseoli*, studies were carried out to evaluate the effect of organic amendments on disease severity, pathogen population and plant growth.

**Material and Methods:** Bean cvs K20 and RWR 719 were grown in the greenhouse in wooden trays containing soil infested with *F. solani* f.sp *phaseoli* at an inoculum concentration of 3000 conidia per gram of soil. Root rot severity and dry matter production were assessed fortnightly beginning 3 weeks after planting until crop maturity. At maturity, soils from different treatments were sampled and pathogen population therein determined using the method described in 3.1.3 above. A similar trial was set up in the field at two sites; Rubaya and Kicumbi, in Kabale district southwestern Uganda

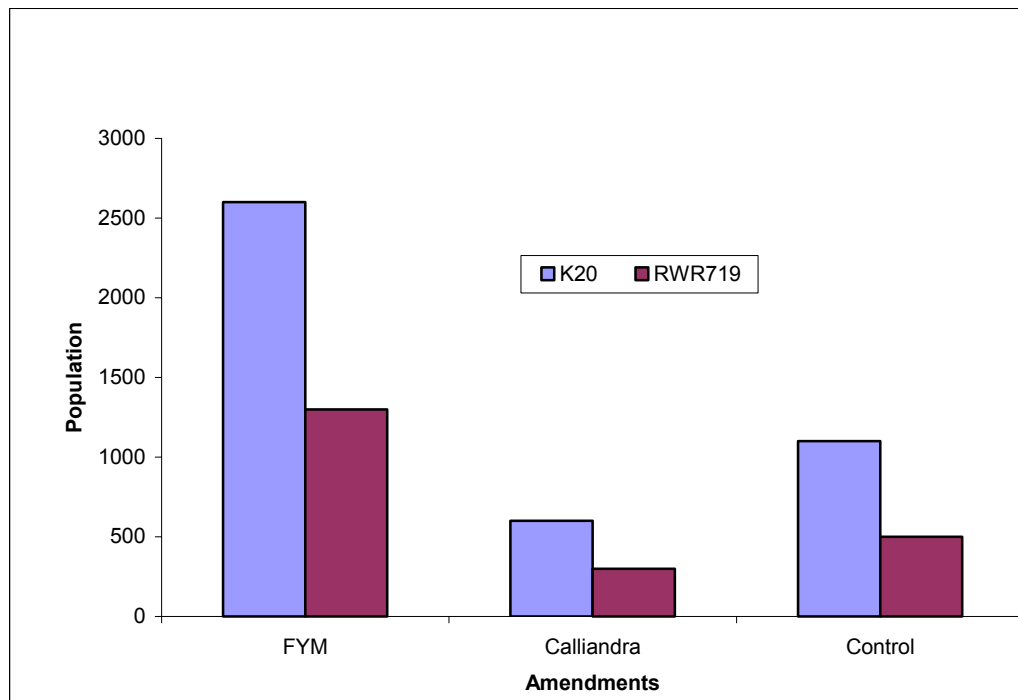
**Results and Discussion:** Both bean cultivars showed variable levels of fusarium root rot in screenhouse trials although disease progress was slower on RWR 719 than on K20. Whereas *Calliandra* suppressed disease severity on both varieties, farmyard manure enhanced it (**Figure 2a and b**). Soil population of the pathogen at harvest showed a similar trend; soils amended with farmyard manure maintained pathogen population at near original level, while that amended with *Calliandra* spp was significantly reduced (**Figure 3**). Similar observations were made in field studies except that the effects of amendments on disease severity were more apparent in the later stages of crop growth (**Table 3**). Surprisingly, in both greenhouse and field trials, the highest dry matter and grain yields were obtained from the crop grown in soils amended with farmyard manure, followed by *Calliandra* spp. and least in unamended soils. Although FYM increased both dry matter and grain yield, the pathogen population remained just below the original level by the end of the season. FYM seemed to overcompensate for the negative effects of increased disease severity possibly through nutrient supply. This study shows that despite the positive contributions to plant growth, organic amendments have differential effects on pathogen population and therefore the need to assess these effects when considering their long-term contribution as management options. Further studies are planned to assess and compare alternative organic amendments sources.



**Figure 2a: Effect of organic amendment on severity of fusarium root rot – cv. RWR 719, greenhouse**



**Figure 2b: Effect of organic amendment on severity of fusarium root rot – cv. K20, greenhouse**



**Figure 3. Effect of FYM and Calliandra green manure on the population of *Fusarium solani* f.sp *phaseoli* in soil having a bean crop in the screenhouse**



**Table 3. Effect of amending field soil with farmyard or green manure on Fusarium root rot severity in 2 sites in southwestern Uganda - 2002A.**

Variety	Soil amendment	Kicumbi			Rubaya		
		Weeks after Planting			Weeks after Planting		
		6	8	10	6	8	10
K20	Farm yard manure	4	7.8	8.8	4.0	5.7	7.3
	Green manure (Calliandra spp)	3	5.0	5.9	4.2	5.1	5.3
	Control	3	8.2	5.7	3.8	8.0	8.7
RWR 719	Farm yard manure	1.2	1.7	2.33	1.6	2.0	3.3
	Green manure	1.1	1.3	2.0	1.9	2.2	2.8
	Control	1.1	1.3	2.5	2.3	2.5	3.4
LSD		2.08 NS	0.78***	1.96**	1.6 NS	1.4*	0.68***
CV(%)		49.1	8.5	11.2	34	17.8	7.2

**Contributors:** G. Tusiime, J. R. Buruchara (CIAT) F. Opio and M. Ugen (NARO).

## **Activity 5. Participatory variety selection of root rot resistant varieties in southwest Uganda**

**Rationale:** In southwest Uganda farmers grow beans as varietal mixtures. In recent years bean root rots have had an effect on the types and composition of varietal mixtures grown. The response by farmers to this situation has been limited to varietal manipulation leading to reduction or elimination of large and introduction of small seeded components in their varietal mixtures. Consequently, there is a great demand for varieties that are resistant to bean root rots. Farmer participation in selecting in varieties is considered important as it incorporates farmer's diverse criteria, their indigenous knowledge, and heterogeneous environments to meet their preferences.

**Materials and Methods:** Materials selected (from the Root Rot Nursery consisting of 68 entries) from evaluations made last year by four farmer groups were grown in communal plots of the groups. At different stages of plant growth, the groups visited their communal plots and assessed the materials with the final assessment and selections made at harvest. Seed increase of some of the selected materials was done for wider evaluations in individual plots of the group members and other interested farmers. In addition 55 new lines derived from crosses for multiple constraint resistance and selected for their resistance or tolerance to root rots were evaluated in communal plots of the four farmer groups.

**Results.** A total of 44 (out of 68) entries were selected by the four farmer groups. Selection criteria included adaptation to their conditions, resistance to root rots, vigor, yield and seed characteristics. For the 30 entries among those selected, each entry occurred only once in any one of the 4 groups. However, eight entries (FEB 181, VAX2, CC 814, MLB-40-89A, A 686, G 2858, MLB-68-89A) were common in at least 2 groups, four (SCAM-80CM/5, AB 136, Ihumure, MLB-22-89A) in at least three groups and two (MLB-48-89A and CC 906) in all the four groups. A number of entries selected will be further grown in communal plots for seed increase but several will be grown in individual plots. The relatively large number selected (44) illustrates the interest for large and diverse types of materials by the groups and the potential value using this approach to incorporate and take advantage of farmer selection criteria.

**Contributors:** R. Buruchara, G Manzi, S. David, F. Opiro (NARO).

## **Activity 6. Publications, book chapters, workshops, conferences, trips, meetings, training, courses, and thesis**

### **Publications**

George.S. Mahuku, María Antonia Henríquez, Jaime Munõz and Robin A. Buruchara. 2002. Molecular Markers Dispute the Existence of the “Afro-Andean” group of the Bean Angular Leaf Spot Pathogen, *Phaeoisariopsis griseola*. *Phytopathology* 92: 580-589.

### **Book chapter**

Buruchara, R.A. L. Sperling, P. Ewell and R. Kirkby, 2002. The Role of Research Institutions in Seed-Related Disaster Relief: Seeds of Hope Experiences in Rwanda. In Louise Sperling and Catherine Longley (eds). Pp 193-206. *Beyond Seeds and Tools: Effective Support to Farmers in Emergencies*. DISASTERS Special Issue: Volume 26, Number 4, Blackwell Publishers, London.

### **Workshop and Conferences.**

Buruchara Robin, R. Otsyula, F. Opio, A. Musoni & P. Kimani, Potential for host resistance in developing integrated management strategies for *Pythium* root rot of beans (*Phaseolus vulgaris* L.) in east and central Africa. Paper presented in the International Conference on Integrated Pest Management for Sub-Saharan Africa. 8-12 Sept 2002, Kampala, Uganda.

Buruchara R. A., G. Mahuku, K. Ampofo, 2002. Integrated Disease, Pest and Soil Fertility Management for Sustainable Soil Health. Paper presented at Workshop on Soil Fertility Degradation in Africa: Leveraging Lasting Solutions to a Long-Term Problem. March 4 - 8, 2002, Rockefeller Foundation Bellagio Study and Conference Centre, Italy.

Tusiime, G. R. Buruchara, E. Adipala, J. Carder, F. Opio and N. Spence. 2002. Variation and detection of *Fusarium solani* from beans exhibiting *Fusarium* root rots disease symptoms. Paper presented in the International Conference on Integrated Pest Management for Sub-Saharan Africa. 8-12 Sept 2002, Kampala, Uganda.

Mukalazi, J, R. Buruchara, E Adipala J. Carder, F. Opio, T. Pettitt & N. J. Spence. 2002. Characterization of *Pythium* species pathogenic to common beans in Uganda. Paper presented in the International Conference on Integrated Pest Management for Sub-Saharan Africa. 8-12 Sept 2002, Kampala, Uganda.

Opio, F. P. M. Kimani, S. M. Musaana and R. Buruchara, 2002. Research on Common Bacterial Blight and Halo blight of common bean in East and Central Africa *BIC* 45: 160-161.

Kimani, Paul, R.Chirwa, R. Buruchara and M. Pyndji, 2002. Breeding Strategy in PABRA countries. Paper presented at the Breeders’ and Pathologists’ Working Group Meeting and Monitoring tour, Nazareth, Ethiopia, 25 August - 1 September 2002. P.M. Kimani, H.Assefa, G. Rakotomalala and A. Rabakoarihanta. 2002. Research on bean rust in East and Central Africa: status and future directions *BIC* 45: 134 –135.

Kimani, P.M., R.A. Buruchara, R. Otsyula and G. Rachier. 2002. Breeding bean cultivars resistant to angular leaf spot and root rots in Eastern and Central Africa. Paper presented in workshop on 'Biotechnology, Breeding and Seed Systems for African Crops: 'Research and product development that reaches Farmers'. 4-7 November, Entebbe, Uganda. The Rockefeller Foundation.

Musoni, A., R. A. Buruchara and P.M. Kimani. 2002. Breeding climbing beans resistant to angular leaf spot, Pythium root rots and fusarium wilt. Paper presented in a workshop on 'Biotechnology, Breeding and Seed Systems for African Crops: 'Research and product development that reaches Farmers'. 4-7 November, Entebbe, Uganda. The Rockefeller Foundation.

Namayanja, A., Buruchara, R.A and P. M. Kimani. 2002. Inheritance of angular leaf spot and marker assisted selection for disease resistance in common bean. Paper presented in a workshop on 'Biotechnology, Breeding and Seed Systems for African Crops: 'Research and product development that reaches Farmers'. 4-7 November, Entebbe, Uganda. The Rockefeller Foundation.

Buruchara, R. A., G. Mahuku, S. Mayanja J. Mukalazi and A. Levesque. 2002. Adaptation of specific PCR based markers to characterize and differentiate *Pythium* spp associated with Pythium root rot of beans (*Phaseolus vulgaris* L). Paper presented in a workshop on 'Biotechnology, Breeding and Seed Systems for African Crops: 'Research and product development that reaches Farmers'. 4-7 November, Entebbe, Uganda. The Rockefeller Foundation.

Otsyula Reuben. Robin Buruchara Paul Kimani George Mahuku Patrick Rubaihayo 2002. Identification and inheritance of pythium root rot resistance in major market class bean (*phaseolus vulgaris*) varieties.

### **Proposal/concept notes developed**

"Achieving wide impact with climbing bean and agroforestry interventions in the Eastern and Central Africa Highlands: a win-win combination" submitted to USAID.

### **Project submitted**

"Supporting Improved Nutrition, Food Security And Community Empowerment For Poverty Alleviation" a PABRA project submitted to CIDA.

### **Trips and attendance at Meetings**

- Visited trials and diseases nurseries at Bembeki and Chitedze, Malawi.
- Attended the CRISP-Africa planning meeting at Bunda, College, Malawi.
- Attended breeders' and pathologists' workshop and monitoring tour in Ethiopia.
- Attended the all Africa staff meeting at Kawanda, Uganda.
- Participated in CIDA organized Results-Based Management training workshop held at Kawanda for PABRA staff.

- Participated in ECABREN steering committee meeting in Nairobi, Kenya.
- Participated in ISAR/ATDT/ASSS Stakeholders' Meeting in Rwanda.
- Participated in PABRA steering committee meeting in Malawi.
- Attended in the International IPM Conference at Makerere University, Kampala, Uganda.
- Gave technical support to collaborative research activities and research planning of the national bean program of Rwanda.
- Visited root rot research under the Rockefeller Foundation project in Western Kenya.
- Visited root rot activities, participatory variety selection activities and graduate student research in South West Uganda.
- Visited to support coordination of the ATDT project.
- Attended a donor meeting on strategic alliance between CIAT, TSBF and ICRAF Bellagio, Italy.
- Attended the Quality of Science meeting in Participatory Plant Breeding Rome Italy.

### **Training and courses**

- Two scientists from Rwanda (ISAR and University of Rwanda) and a technician (from ISAR) received individual training at Kawanda, Uganda on research method on bean pathology.
- Organized a training course for fifteen assistants, technician and graduate students on data management and analysis at Kawanda, Uganda.

### **Thesis**

#### **MSc Supervision**

- An MSc Ugandan student from Makerere University conducting her thesis on “Inheritance And Marker Assisted Selection For Angular Leaf Spot (*Phaeoisariopsis griseola*) Resistance in Common Bean” at Kawanda, Uganda.
- An MSc Ugandan student from Makerere University initiating thesis research at Kawanda, Uganda on “Characterization of pathogenic diversity of *Phaeoisariopsis griseola* in Uganda.
- An MSc Rwandan student in Nairobi University is conducting thesis research at the University on “Breeding for resistance to Fusarium wilt.”

#### **PhD Supervision**

- A PhD Ugandan student registered at Makerere University conducting research at CIAT-Kawanda on “Variation and detection of *Fusarium solani* f.sp *phaseoli* and quantification of soil inoculum in common beans.”
- A PhD Kenyan student registered at the University of Nairobi conducting research at the University on “Molecular and virulence characterization of *Phaeoisariopsis griseola* and reaction of bean germplasm to races of the ALS pathogen.”

- A PhD Ugandan student registered at Makerere University conducting research at CIAT-Kawanda on “Pathogenic variation and quantification of Pythium species of beans in Uganda.”
- A PhD Kenyan student registered at Makerere University conducting research at CIAT-Kawanda on “Inheritance and transfer of root rot (Pythium) resistance to bean varieties.”