PROCEEDINGS OF A PAN-AFRICAN WORKING GROUP MEETING ON BACTERIAL AND VIRUS DISEASES OF COMMON BEAN

Kampala, Uganda
13 – 16 June, 1994

CIAT African Workshop Series No. 34

Edited by David Allen and Robin Buruchara

Workshop Organizers: R.A. Buruchara, CIAT
F. Opio, NARO

PREFACE

This volume reports the proceedings of a Pan-African working group meeting on bacterial and virus diseases of the common bean, and is the 34th in a series of workshop proceedings amongst which it is a sequel particularly to No. 13 (that also addressed virus diseases) and to No. 20 (that reported proceedings of the first bean pathology workshop, including work on common bacterial blight).

Since this workshop was held, bean common mosaic virus (BCMV) has been separated into two distinct potyvirus species : the necrosis-inducing strains of serotype A are now named bean common mosaic necrosis virus (BCMNV), while serotype B isolates, that do not normally induce necrosis except at high temperature, retain the name BCMV that now embraces strains of blackeye cowpea mosaic and several other legume virus isolates (eds.).

These documents, which are designed to serve the bean research networks in Africa, are supported through funds provided by the Office of Agriculture, Bureau for Research and Development, U.S. Agency for International Development, under grant No. LAG-4111-G-00-2026-00, and the Canadian International Development Agency (CIDA). Activities of the bean research networks in Africa are further supported by the Swiss Agency for Development and Cooperation (SDC). The opinions expressed herein are those of the authors and do not necessarily reflect the views of these contributing donor organizations, nor of CIAT.

Further information on regional research activities on bean in Africa that are part of these projects is available from :

Pan-Africa Coordinator, CIAT, P.O. Box 6247, Kampala, Uganda.
Coordinator, SADC Bean Network, P.O. Box 2704, Arusha, Tanzania.
Coordinator, Eastern and Central Africa Bean Research Network (ECABREN), P.O. Box 2704, Arusha, Tanzania.

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OBJECTIVES OF THE MEETING

R.A. Buruchara

The First African Bean Pathology Workshop, which was held in Kigali in 1987, addressed "the most damaging diseases of common bean in Africa". Virus diseases were not covered, so that in due course a working group meeting on virus diseases was convened, in Kampala in 1990. In May 1992, the first Pan-Africa Bean Pathology Working Group met in Thika, where proceedings focussed only on fungal pathogens. In order to retain an appropriate balance between pathogen groups, the present meeting provides a forum at which to give adequate attention to bacterial and viral diseases of beans.

Research on bacterial and viral diseases of beans in Africa is conducted under the auspices of national bean programmes, universities and other regional and international research institutions in Africa. Collaborative research between universities or between advanced laboratories in the USA and Europe, and the national institutions consider certain themes to make use of comparative advantage of collaborators. The Bacterial and Viral Working Group comprises key scientists from the bean research networks in Africa who are actively working on bacterial and viral diseases of beans. The group has the responsibility of offering specialist advice to the Steering Committees of the bean networks on important bacterial and viral constraints, when considering research activities, priorities and strategies proposed by network scientiests. This particular meeting brings together scientists from some of the national programmes in Africa, universities and some of the collaborating research institutions in Europe working on viral and bacterial diseases.

The objectives of the meeting are :

a. To offer a forum for peer review of on-going research activities on bacterial and viral bean diseases and to facilitate exchange of information among scientists.

b. To review past and current research activities of regional (bacterial and viral) sub-projects and any other to be able to assess past achievements and on-going research on bacterial and viral diseases.

c. To develop future strategies and determine specific areas, and priorities to be considered by research (sub-projects).

d. To review the effects of bacterial and viral pathogens and the role of research on quarantine regulations and exchange of germplasm.

e. To identify ways for improved collaboration, sharing of responsibilities and exchange of information and research results.

The meeting will be run in two separate parts. The first will comprise presentation of research on virus diseases (Session 1) and on bacterial diseases (Session 2), with speakers drawing attention not only to their results but also to their failures and difficulties. These

will then be used as the basis for the second part, topics to be addressed in two working groups, one for bacterial and one for viral diseases. The working groups will employ the Project Planning by objectives (ZOPP) method that was used most effectively at the Thika meeting (see Workshop Procs No. 23) to identify problems, analyze solutions, and to develop areas for future research.

VARIATION FOR PATHOGENICITY AMONG ISOLATES OF BEAN COMMON MOSAIC VIRUS IN AFRICA

Nicola J. Spence and David G.A. Walkey

Horticulture Research International, Wellesbourne, Warwick, UK

ABSTRACT

BCMV isolates were collected from crops of Phaseolus vulgaris (bean) and from wild legume species in Burundi, Ethiopia, Kenya, Lesotho, Malawi, Morocco, Rwanda, Swaziland, Tanzania, Uganda, Zaire, Zambia and Zimbabwe. Isolates of pathotype VIa from both beans and wild legume species were predominant in central, eastern and southern Africa. Isolates of pathotypes I, III, IVa, IVb and Va were also found. There was considerable variation for pathogenicity among isolates and several novel pathotypes were identified based on the reactions of a set of differential bean cultivars.

INTRODUCTION

The aim of this study was to investigate the pathogenicity of BCMV isolates obtained from samples of infected beans and wild legume species in central, southern and eastern Africa. Several sources of resistance to BCMV are available but it is essential for plant breeders to have knowledge of the distribution of and variation among BCMV isolates in the region where new cultivars are to be deployed to achieve a sustainable improvement in crop production. Pathotypes of BCMV are identified by their pathogenicity on a set of differential bean cultivars. Hitherto, ten pathotypes of BCMV (referred to previously as pathogenicity groups) have been identified on the basis of differential host responses (Drijfhout, 1978). Monoclonal antibodies differentiate BCMV isolates into two serotypes and there is a correlation between pathotype and serotype where isolates belonging to pathotype III, VIa and VIb also belong to the "A" serotype, while isolates of all other pathotypes belong to the "B" serotype (Wang et al., 1984).

MATERIALS AND METHODS

Surveys

Field surveys were carried out in collaboration with personnel of Centro Internacional de Agricultura Tropical (CIAT) and National Bean Programmes in Lesotho, Malawi, Swaziland, Uganda and Zimbabwe (January 1990), Ethiopia (September 1990), Morocco (November 1990), Rwanda, Tanzania, Uganda and Zaire (May 1991), Burundi, Kenya and Rwanda (November 1991). In addition, samples were received at Wellesbourne directly from Africa.

Host plants, virus isolates, inoculations and maintenance of cultures

Seeds of test plants were germinated in plastic boxes on damp cellulose wadding by incubating them for four days at 25°C. The germinated seed was planted in M2 Levington

compost (Fisons, UK) in 7 cm plastic pots. Plants were sown in an insect-proof glasshouse at 26°C; from October to March supplementary lighting was provided to give a daylength of 16 hours. Seeds of the genera *Chenopodium* and *Nicotiana* were sown directly in sifted peat compost and germinated seedlings pricked out into individual pots.

Infected plant material was collected as leaves. If samples other than bean were collected, additional herbarium material was preserved in a flower press and photographs taken to aid identification. For each sample, records were taken of location, altitude, host species, cultivar (if known) and disease severity. Leaf samples were placed between two sheets of filter paper (Whatman No.1), lightly moistened with water and placed in a polythene bag. The samples were stored in cool bags in the field and later refrigerated at 4-5°C, until they reached the laboratory (usually within 2-3 weeks). Additional similar leaf samples were immediately dried in plastic scintillation vials containing approximately 10 g of coarse $CaCl_3$. Samples (fresh or dried) were homogenised in 1% K_2HPO_4 solution containing 0.1% Na_2SO_3 (1 g/ml) and used for sap transmission to appropriate test seedlings including: *P. vulgaris* cv. Double White (Dubbele Witte), *Chenopodium quinoa*, *Nicotiana benthamiana*, *N. clevelandii*, *Vigna unguiculata* and *V. radiata*. Sap inoculum for sub-culturing was prepared in the same way. Some virus isolates were obtained from infected seedlings grown from seed samples collected in Africa.

BCMV strains representing pathotypes: I (NL1), III (NL8), IVa (NVRS), IVb (NL6), Va (NY15), VIa (NL3), VIb (NL5) and VII (NL4) (Drijfhout, 1978) were maintained in bean cv. Double White and are referred to as "standard" strains to be used for comparison with unidentified isolates. Cultures of blackeye cowpea mosaic potyvirus (BlCMV), cowpea aphid-borne mosaic potyvirus (CAMV), peanut mottle potyvirus (PMV) and alfalfa mosaic virus (AMV) were also maintained for comparison.

Differential bean cultivars

Original seed was obtained from E. Drijfhout (Agricultural Research University, Wageningen, The Netherlands) and multiplied at Wellesbourne in insect-proof polythene tunnels. Up to 22 cultivars were used to characterise BCMV isolates on the basis of systemic infection (Drijfhout, 1978). A minimum of two cultivars per host resistance group were used in each test and four seedlings of each cultivar were inoculated as soon as the primary leaves unfolded. In addition, two further plants of cultivars in resistance group 8, 9a, 9b and 10 (which possess the dominant *I* gene) were inoculated with each isolate and maintained in a controlled environment cabinet at 32°C to identify those isolates which induced systemic necrosis only at this higher temperature.

Each plant was scored for symptoms at intervals up to 4 weeks after inoculation. Differential cultivars that developed questionable symptoms were back-inoculated to plants of cv. Double White to check for virus infection, or the suspect plants were tested for infection by ELISA. Potentially mixed infections were back-inoculated from individual cultivars to a new set of differential cultivars. Isolates were assigned to pathotypes according to the reaction patterns of the differential host cultivars. The reaction pattern of the differential host cultivars following inoculation with the standard BCMV strains was investigated to confirm the original descriptions by Drijfhout (1978).

Enzyme-linked immunosorbent assay (ELISA)

A number of monoclonal antibodies and polyclonal antisera were used in ELISA to aid the identification of the BCMV isolates and other viruses (Clark & Adams, 1977). Monoclonal antibodies bc-1-3 (BC3) and 197 were broad-spectrum, reacting in plate-trapped antigen ELISA (PTA-ELISA) with all BCMV strains and other potyviruses including BlCMV, CAMV and PMV. In contrast, monoclonal antibodies bc-1-1A4 (BC1) and I2 were highly specific to BCMV strains of the "A" serotype only. Each survey sample was tested with these monoclonal antibodies by PTA-ELISA in duplicate wells. To aid identification, samples were also tested in direct double-antibody sandwich ELISA (DAS-ELISA) with a number of polyclonal antisera raised to specific BCMV strains and other potyviruses.

RESULTS

BCMV isolates from *P. vulgaris*

In general, the reaction patterns observed following inoculation of the differential bean cultivars with the standard strains of BCMV were consistent with those described by Drijfhout (1978). Small differences that were observed can probably be attributed to environmental variation (particularly temperature). BCMV was isolated from 149 of the 647 samples of *P. vulgaris* collected in Africa (Table 1). From the remaining 498 samples either no virus was detected, or BCMV was detected by ELISA but not isolated. Viruses other than BCMV were isolated from some samples or detected by ELISA but not isolated (Spence & Walkey, 1994). Variation in the reaction patterns observed on differential cultivars following inoculation with the BCMV isolates from Africa suggested that each could be considered as distinct. However, the isolates have been assigned, wherever possible, to the pathotype to which their reaction patterns most closely conformed. In some cases, assignment to a pathotype was clear-cut as reaction patterns were identical to those of the standard strain representative of the pathotype. In other cases there were small but distinct and consistent differences between the reaction phenotype observed and that of standard strains. This variation included the failure of an isolate to infect one particular cultivar from a particular host resistance group even though other cultivars of the same group were infected. Isolates whose reaction phenotype deviated from that of the standard strain by variations such as this were still assigned to the pathotype they most closely resembled. Isolates whose reaction patterns did not conform to any previously described pathotype were considered to represent novel pathotypes.

Approximately 53% of BCMV isolates from *P. vulgaris* were assigned to pathotype VIa, the reaction phenotypes on differential bean cultivars being similar to NL3. Isolates of pathotype VIa occurred widely in all the areas surveyed except Ethiopia. Isolates assigned to pathotype III, being similar to NL8, had a more limited distribution, being found only in Burundi, Rwanda and Tanzania. Isolates assigned to pathotype I were found occasionally in Burundi, Ethiopia, Tanzania and Uganda and an isolate assigned to pathotype IVa was found in only one sample from Zimbabwe. The distribution of isolates assigned to pathotype IVb was more widespread and they were frequently isolated from infected seed. The distribution of isolates assigned to pathotype Va was restricted to western Kenya, which was in marked contrast to the rest of the country where all other isolates obtained were assigned to pathotype VIa. Isolates of apparently novel pathotypes were found infecting *P. vulgaris* in

all of the countries surveyed except Uganda.

In Ethiopia there was a much lower incidence of BCMV than in the other areas surveyed and the absence of isolates of the "A" serotype is significant. When infection by BCMV was found, the symptoms were generally mild. Only two isolates of BCMV were obtained which were both of the "B" serotype and were assigned to pathotypes I and IVb. In addition, three isolates of apparently novel pathotype were found at different locations in the Rift Valley, these were confirmed to be of the "B" serotype of BCMV.

In most countries there was little difference between the strains found at or near research stations and those found in farmers' fields. In Malawi, however, isolates assigned to pathotype VIa were only found near the Bunda College and Makapwa Research Stations while isolates assigned to pathotype IVb were only found in farmers' fields. The recovery of a single isolate conforming to pathotype IVa from the Harare Research Station, Zimbabwe, may have originated from imported virus-infected seed because no other isolates of this type were found in Zimbabwe.

The "A" serotype of BCMV was detected by ELISA in several bean plants collected in a very limited survey in Morocco. However, BCMV was always present in a mixed infection with alfalfa mosaic virus (AMV) and as a consequence the reaction of differential bean cultivars to these isolates was not determined.

Full details of all BCMV isolations made during the survey have been documented by Spence & Walkey (1994).

<u>BCMV isolates from wild legume species</u>

BCMV was isolated from several wild legume species in Kenya, Malawi, Rwanda and Uganda (Table 2). Isolates 28 and 30 from *Crotalaria incana* and isolate 38 from *Glycine max* obtained from Nakabango, Uganda were all of the "A" serotype and produced a similar set of reaction patterns on differential host cultivars. Systemic veinal necrosis was observed in cultivars of host resistance groups 1 and 4, but this reaction was not the typical hypersensitive reaction associated with the action of the *I* gene. The phenotypic reaction pattern was not similar to any previously described BCMV pathotype and the isolates were therefore considered to comprise a novel pathotype. Isolate 465 from *Cassia hirsuta* (synonym *Senna hirsuta*) obtained at Bukalasa, Uganda was of the "B" serotype and following inoculation of differential host cultivars the reaction pattern conformed to pathotype I. An isolate of the "A" serotype (499) from *Macroptilium atropurpureum* obtained at Mubuku, Uganda conformed to pathotype VIa following inoculation of differential bean cultivars.

An isolate of the "A" serotype (531) from *Vigna unguiculata* growing as a weed in a bean plot in Rwanda conformed to pathotype VIa while three other isolates of the "A" serotype (820, 830 and 836) from *Cassia sophera* (synonym *Senna sophera*) obtained from different sites in Rwanda displayed a novel set of reactions patterns on differential cultivars and were considered to be previously undescribed pathotypes.

Isolate 145 from a *Rhynchosia* spp. (not in flower) growing at Champhira, Malawi was of the "B" serotype. This isolate conformed to pathotype IVa following inoculation of differential bean cultivars.

At Nyangusu, near Kisii, Kenya an isolate (956) of the "B" serotype was obtained from *Vigna vexillata* and the reaction pattern on differential host cultivars assigned it to pathotype Va. At Nyangena, c. 30 km from the Nyangusu site, an isolate (963) of the "B" serotype obtained from *Crotalaria comanestiana* produced a novel reaction pattern on differential bean cultivars and was therefore considered to represent a novel pathotype. The survey indicated that herbaceous wild legume species were numerous in Burundi, Malawi, Rwanda, Uganda and the wetter parts of Ethiopia and Kenya. Fewer herbaceous legumes were observed in other areas and no samples were collected from wild legumes in Lesotho, Swaziland, Zaire or Zimbabwe as none were observed to have virus symptoms.

Full details of all BCMV isolations made from wild legumes in the different countries have been documented by Spence & Walkey (1994).

DISCUSSION

BCMV isolates were assigned to pathotypes according to their pathogenicity phenotype on differential host cultivars and could readily be classified as belonging to either the "A" or "B" serotype by ELISA. Pathotype and serotype were strongly correlated. The results clearly demonstrated the widespread occurrence and predominance of the "A" serotype in central, eastern and southern Africa; this serotype was completely correlated with the occurrence of a temperature-independent necrotic response by cultivars carrying the *I* gene. The study also demonstrated the variation in pathogenicity which existed between isolates from wild populations of BCMV. The widespread occurrence of isolates similar to NL3 and NL8 and of other isolates of the "A" serotype with novel pathogenicity phenotypes, emphasizes the importance of incorporating resistance to these so-called necrotic strains into new cultivars. Many cultivars that have been introduced into bean improvement programmes in Africa carry only the dominant *I* resistance gene and are therefore susceptible to "black root" when infected by any isolate which provokes necrosis in the presence of this gene. In areas where necrosis-inducing isolates exist it is essential to incorporate additional resistance genes such as $bc2^2$ or *bc*3 into breeding programmes. Exceptionally, necrosis-inducing isolates of the "A" serotype were not located in Ethiopia. However, three isolates of a novel pathotype were found that did induce necrosis in host group 8 cultivars even though they were of the "B" serotype. In Ethiopia, cultivars carrying the *I* gene alone may be sufficient to confer resistance against BCMV in most areas, but plant breeders need to be aware of the existence of this novel pathotype which could influence the durability of any newly introduced resistant bean cultivars. Because of the considerable variation that between isolates, the use of local isolates is considered more appropriate for screening local germplasm for resistance. The occurrence of BCMV in several wild legume species has significance as if such species have a role in the epidemiology and evolution of BCMV this could also affect the durability of resistant cultivars.

ACKNOWLEDGEMENTS

We thank personnel from National Bean Programmes and CIAT Regional Bean Programmes in Africa for their assistance in the survey work and Drs G.I. Mink and H.J. Vetten for antibodies. The work was funded by the Overseas Development Administration through the Natural Resources Institute. BCMV isolates were imported into the UK under MAFF Licence No. PHF 1227A/39(60)

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Table 1. Number of isolates of BCMV derived from countries in Africa and assigned to particular pathotypes

Country[1]	BCMV Pathotypes											
	I	II	III	IVa	IVb	Va	Vb	VIa	VIb	VII	Novel	T
BU	1	0	8	0	2	0	0	10	0	0	5	26
ET	1	0	0	0	1	0	0	0	0	0	3	5
KE	0	0	0	0	0	1	0	0	0	7	7	15
LO	0	0	0	0	0	0	0	2	0	0	1	3
MW	0	0	0	0	1	0	0	7	0	0	6	14
RW	0	0	4	0	2	0	0	22	0	0	3	31
WD	0	0	0	0	1	0	0	1	0	0	1	3
TZ	1	0	2	0	0	0	0	6	0	0	6	15
UG	1	0	0	0	2	0	0	11	0	0	0	14
ZR	0	0	0	0	0	0	0	1	0	0	3	4
ZA	0	0	0	0	1	0	0	4	0	0	1	6
ZW	0	0	0	1	2	0	0	8	0	0	2	13
Total	4	0	14	1	12	1	0	79	0	0	38	149
%	3	0	9	1	8	1	0	53	0	0	25	100

[1] BU, Burundi; ET, Ethiopia; KE, Kenya; LO, Lesotho; MW, Malawi RW, Rwanda; WD, Swaziland; TZ, Tanzania; UG, Uganda; ZR, Zaire; ZA, Zambia; ZW, Zimbabwe.

Table 2. BCMV isolates obtained from wild legume species in Africa

Isolate number	Host species	Country of origin	BCMV serotype	BCMV Pathotype
28	*Crotalaria incana*	Uganda	A	Novel
30	*Crotalaria incana*	Uganda	A	Novel
38	*Glycine max*	Uganda	A	Novel
145	*Rhynchosia* sp.	Malawi	B	IVb
465	*Cassia hirsuta*	Uganda	B	I
499	*Macroptilium atropurpureum*	Uganda	A	VIa
531	*Vigna unguiculata*	Rwanda	A	VIa
820	*Cassia sophera*	Rwanda	A	Novel
830	*Cassia sophera*	Rwanda	A	Novel
836	*Cassia sophera*	Rwanda	A	Novel
956	*Vigna vexillata*	Kenya	B	Va
963	*Crotalaria comanestiana*	Kenya	B	Novel

[1] Serotype determined using monoclonal antibodies 197 and I2

THE OCCURRENCE OF BEAN COMMON MOSAIC VIRUS IN WILD AND FORAGE LEGUME SPECIES IN UGANDA*

T.N. Sengooba[1], D.G.A. Walkey[2], N.J. Spence[2], D.J. Allen[3] and A. Femi Lana[4]

[1] Namulonge Research Institute, Uganda; [2] Horticulture Research International, Wellesbourne, UK; [3] CIAT Regional Bean Programme, Arusha, Tanzania; and [4] Sokoine University of Agriculture, Morogoro, Tanzania.

ABSTRACT

Necrosis inducing BCMV isolates were obtained from a range of wild and forage legume species growing in Uganda. Thirteen distinct isolates were identified on the bases of symptoms, pathogenicity in bean differential cultivars, serology, immunosorbent electron microscopy, seed-and aphid-transmission. Some isolates conformed with the characteristics of previously described strains but others showed novel properties. Two isolates (429 and 472) caused necrosis in I-gene carrying bean cultivars but gave negative tests against 1-2 monoclonal antibodies specific for "A" serotypes of BCMV. All isolates reinfected the natural host from which they were obtained. The origin and ecological significance of these isolates is discussed.

INTRODUCTION

Bean common mosaic virus (BCMV) is a disease of global importance occurring wherever common beans (*Phaseolus vulgaris)* are grown and can cause severe crop losses in eastern, central and southern Africa. The virus is recognised as comprising two serotypes based on their pathogenicity reactions on a range of differential bean genotypes (Drijfhout, 1978; Morales & Bos, 1988). Serotype "A", also known as the necrotic strains, induce a hypersensitive and often lethal necrosis in bean genotypes possessing the dominant I resistance gene, while "B" serotypes (non-necrotic strains) cause only mosaic symptoms in susceptible bean cultivars (Drijfhout, 1978; Wang, 1983; Vetten et al., 1992).

Strains of the "A" serotype have been reported to predominate in the major bean growing regions of eastern, central and southern Africa with the exception of Ethiopia, where only "B" serotypes have been found (Goothales, 1986; Vetten et al., 1991; Spence & Walkey, 1994). The predominance of "A" serotype strains in Africa, which is not the centre of origin of *Phaseolus vulgaris* suggests that these necrotic strains may have originated in some other, indigenous legume species in Africa.

* The substance of this paper has now appeared in revised form in Plant Pathology 46, 95-103 (ed.)

Recent serological and protein sequencing studies have indicated that the "A" and "B" serotypes may be distinct viruses and it has been suggested that the "A" serotypes should be called bean common mosaic necrosis virus (BCMNV) (Vetten et al., 1992, Khan, 1993). Until the debate on the systematics of BCMV is resolved, both the "A" and "B" serotypes are referred to as BCMV in this paper.

Previous studies have reported the occurrence of necrotic "A" serotypes similar to the standard NL3 strain (pathogenicity group VIa; Drijfhout, 1978) infecting bean crops in Uganda and the isolation of novel isolates from *Crotalaria incana* and *Glycine max* which were similar but distinct from the "A" serotype standard strain NL8 (pathogenicity group III; Spence & Walkey, 1994). This paper reports the results of a more extensive survey of legume weed and forage species throughout Uganda in which numerous "A" serotypes isolates have been found and characterised. New hosts of the virus and novel strains are reported.

For the purposes of this paper the definition of novel isolate, novel strain, strain type, standard strain, pathogenicity group and pathogenicity phenotype are as defined by Spence & Walkey (1994).

MATERIALS AND METHODS

Collection, isolation and maintenance of virus isolates

Samples were collected from wild and forage legume species showing suspected virus symptoms in various regions of Uganda during 1992 and 1993. Leaf samples were refrigerated at 4° C prior to laboratory testing, or were dried in vials containing calcium chloride (Spence & Walkey, 1994). Virus inoculum was prepared by grinding leaf material in 1% phosphate solution (K_2HPO_4 containing o.1% Na_2SO_2), in the proportion of 1ml solution to 1g leaf material, and inoculated to bean seedlings of cv. Double White. ELISA serology tests were also made on the collected leaf samples (Spence & Walkey, 1994).

Isolates which gave positive tests in ELISA for BCMV were further propagated in bean plants cv. Double White to increase virus concentration before they were stored in liquid nitrogen. Additional cultures of each isolate were maintained in cv. Double White by sub-culturing and by harvesting infected seed from these plants. Virus-free seed stocks of cv. Double White were used in all experiments and the isolates were maintained in an aphid-proof screenhouse (Clovis Lande) at Namulonge and in aphid-proof glasshouses at Wellesbourne.

The BCMV standard strains, NL3 and NL8 (Drijfhout, 1978), isolate 30 (a novel NL8 strain-type isolated from *Crotalaria incana* in Uganda, Spence & Walkey, 1994) and isolate 458 (a NL3 strain-type identified in Uganda, Spence & Walkey, 1994) which were used as controls in various experiments, were supplied by HRI, Wellesbourne and maintained in bean cv. Double White as described above.

Differential bean cultivars

The virus isolates were identified and characterised according to the reactions they induced (pathogenicity phenotype) in a selected range of differential bean cultivars (Drijfhout, 1978). The cultivars used are shown in Table 1. Four seedling of each were raised in an aphid-proof screenhouse and sap-inoculated with the test isolate at the primary leaf stage using standard procedures (Morales, 1989). The standard strain of NL3 and Ugandan isolate 458 were also inoculated to four seedlings as controls. Plants were observed for 4 weeks, after which symptomless plants were tested by ELISA and back-tested on cv. Double White to detect any latent infection.

Host range studies

Each test isolate was return inoculated to the legume species from which it was isolated. The test seedlings were grown in Levington (M2) compost in a glasshouse maintained at approximately 21° (day) and 17°C (night). Inoculated seedlings were observed for symptoms for 6 weeks and symptomless seedlings were tested for latent infection by ELISA. A range of legume species (Table 5) known to occur naturally in Uganda were also tested in a screenhouse at Namulonge for their susceptibility to isolate 458 (a NL3 strain type isolated from *P. vulgaris* in Uganda; Spence & Walkey, 1994) and isolates 197 and 308 found in this study. Seeds were supplied by the International Livestock Centre for Africa (ILCA) in Ethiopia and by CIAT from Colombia. All seeds were scarified prior to germination on damp filter paper in petri dishes at 22°C for 4 days. When the radicals had emerged the seeds were planted in sterilised soil in clay pots. Samples from all seed batches were tested before virus inoculation to ensure that they were free from seed-borne virus. Four seedlings were inoculated with each test isolate. Virus inoculum was prepared as described above. All inoculated seedlings were tested by ELISA using Mab I-2 and back-tested on cv. Double White 4 to 6 weeks after inoculation to confirm virus infection.

Enzyme-linked immunosorbent assay (ELISA) and electron microscopy

Direct ELISA using polyclonal antibodies (Pab) and indirect ELISA using Pab's and monoclonal antibodies (Mab's) were carried out using standard procedures (Clark & Adams, 1977; Kemeny, 1991). A reaction was considered positive if the absorbency value at 405nm was twice that of the healthy control. The following antibodies were used in ELISA and electron microscopy tests : Pab's to standard BCMV strains NL3, NL8, NL6, isolate 30 (a novel "A" serotype BCMV strain isolated from *Crotalaria incana* in Uganda, Spence & Walkey, 1994), blackeye cowpea mosaic (BlCMV), peanut mottle (PnMoV), and cassia severe mosaic (CSMV) viruses. All Pab's were supplied by N.J. Spence. Mab's I-2, 197 and 463 were supplied by G.I. Mink, University of Washington, USA.

Leaf samples showing virus symptoms were examined in an electron microscope using the 'quick-dip' method (Brandes, 1957) to detect the presence and size of any virus particles. Immunosorbent electron microscopy (ISEM) (Derrick, 1973, Walkey & Webb, 1984) was used as an aid to identify each isolate and to investigate their relationship with the standard BCMV strains and other potyviruses.

Seed-and aphid-transmission

The seed-transmission of the thirteen test isolates was assessed in *P. vulgaris* cv. Double White in three experiments. Firstly, seeds were collected from the plants used for maintenance of stock isolate cultures. Seedlings germinated from this seed were visually assessed for virus symptoms. Secondly, seed was harvested from 45 plants grown in fifteen 20cm clay pots each containing three seedlings. Each seedling was inoculated at the trifoliolate leaf stage (considered to be early enough to insure infection but not too soon to prevent seed set; Morales & Castano, 1987). The experiment was carried out in a screenhouse (Rossel-type) which allowed daytime temperatures of 30 to 36°C. Harvested seeds were germinated and seedlings evaluated for visual virus symptoms. Thirdly, the above experiment was repeated in a Clovis-Lande screenhouse which provided daytime temperatures 4°C cooler than the Rossel house.

Aphid-transmission tests were carried out using a virus-free colony of *Aphis fabae*. The aphids were cultured on bean genotype MCM 5001 (a cultivar protected by the I gene and known to be resistant to BCMV infection; H. Gridley, personal communication. Test virus transmissions were made using the "leaf-piece" method (Walkey, 1991). Apterous aphids were starved for 1 h and placed on infected leaves for acquisition feeds 10min.long. One centimetre squares, each with several feeding aphids, were cut from the leaves and placed on the test bean seedling cv. Double White. Five or ten seedlings were used for each of the ten isolates tested. Inoculated seedlings were kept in an isolation chamber for 24 h before the aphids were killed by watering with Ambush (50g/l Cypermethrin). Test plants were evaluated for visual symptoms 3 to 4 wk after inoculation.

RESULTS

Isolation of virus isolates

598 samples were collected from 43 different legume species from 20 genera showing virus-like symptoms at different locations in Uganda. Eight per cent (51/598) of these samples induced virus symptoms when inoculated to bean cv. Double White. 38 of the 51 samples (74 per cent) reacted positively in ELISA tests against Mab 197 which indicated the presence of BCMV or a related potyvirus. In further ELISA tests, 11 of the 38 isolates (from nine different legume species, Table 2) reacted positively with Mab I-2 (Table 2) which is specific for necrotic, "A" serotypes of BCMV (G. Mink, personal communication; Spence & Walkey, 1994). In addition, isolate 472 gave a negative reaction against Mab I-2 but a positive reaction against Mab 463 and Pab 30, which are specific for BCMV and BCMV "A" serotypes, respectively (Spence, 1992). Isolate 429 also reacted negatively against Mab I-2 but positively against Pab 30.

Electron microscope examination of leaf samples infected with these 13 isolates showed that all contained elongate, rod-shaped particles of approximately 750nm in length, consistent with the known length of BCMV (Morales & Bos, 1988).

BCMV "A" serotypes were also detected by Mab ELISA tests in *Albizia coriaria* Welw. ex Oliv., *Desmodium intortum* (Mill.) Urb, *D. uncinatum* (Jacq.) DC, *Rhynchosia resinosa* (A.

Rich.) Bak, *Tephrosia barbigera* Welw. ex Bak. and *T. paniculata* Bak., but virus was not isolated in bean cv. Double White from these species.

Characterization of isolates

The 13 "A" serotype isolates were further characterized in pathogenicity, serological and transmission tests.

Reactions on differential bean cultivars

The pathogenicity phenotype of the 13 isolates was determined by inoculation of the differential bean cultivars as shown in Table 3. Isolates 741, 197, 218, 463, 473 and 496 caused reactions that were typical of the standard strain of NL3 (pathotype group VIa) and isolates 472 and 534 reactions that were typical of the standard NL8 strain (pathotype III). Isolate 472 was serologically atypical, however, in that it did not react with Mab I-2 (Table 3). The responses of the other five isolates were distinct from those of known strains of the virus and must be considered novel isolates. Isolates 308 and 574 differed from the standard NL3 strain in not infecting host resistance group 2 and would correspond with the theoretical pathogenity genotype V9 (Drijfhout 1978; Spence & Walkey, 1994). Isolate 598 differed from the standard NL3 strain in not infecting host resistance group 3 cultivars and corresponds with theoretical genotype V7. Isolate 429 differed from the standard NL8 strain by latently infecting host resistance group 6 cultivars, so corresponds with theoretical genotype V11. Isolate 524 also differed from the standard strain NL8 in infecting cv. Pinto 114 of host resistance group 5.

All 13 isolates caused systemic necrosis at both 26 and 32 C in susceptible bean cultivars carrying the I gene, indicating that they are temperature-independent isolates.

Serological relationships

In ELISA tests the 13 isolates did not react with Pab's to PnMoV, BlCMV or CSMV, except for isolates 429 and 472 which gave positive reactions against PnMoV antiserum (Table 3). Considerable variation was observed in their reactions against Pab's to BCMV strains NL3 and NL8, and isolates 741 and 534 did not react against Pab's to isolate 30 from Uganda. As mentioned earlier, all 13 isolates were detected by Mab 197 which is specific for BCMV and related potyviruses, but isolates 429 and 472 did not react with Mab I-2 which is specific for "A" serotypes. Similarly, isolates 197 and 308 did not react with Mab 463 which is normally specific for all BCMV strains.

In ISEM tests, all isolates were decorated by Pab's to both NL3 and NL8 standard strains except 429 and 472 which were decorated by NL8 but not by NL3 antibodies.

Host range in legume species and virus transmission

In screenhouse tests, all 13 isolates were found to reinfect the original host from which they were isolated. In a further experiment isolate 197 (a standard NL3 strain-type) isolate 308 (a novel "A" serotype strain) and isolate 458 (NL3 strain-type originally isolated from *P. vulgaris* in Uganda), were inoculated to a range of indigenous and introduced legume species

that occur in the wild in Uganda. The symptoms induced by the viruses and the results of the ELISA to test for latent infection in these hosts are shown in Table 5. The results showed that the three isolates varied in their virulence in the 14 legume species that became infected and that the majority of the species infected developed no visual symptoms. The following additional species were inoculated but did not become infected : *Calopogonium mucunoides, Crotalaria brevidens, C. laburnifolia, Indigofera hirsuta, I. erecta, Macroptilium atropurpureum* and *Neonotonia wightii*.

Eight of the test isolates and isolate 458 were found to be seed-transmitted in bean cv. Double White with percentage transmission ranging from 31 to 64 percent (Table 6). *Aphis fabae* transmitted ten of the test isolates as well as isolate 456 and the standard strain NL3 in a non-persistent manner from infected to healthy seedlings of cv. Double White.

DISCUSSION

This study has confirmed that necrosis-inducing "A" serotypes of BCMV are prevalent in Uganda in many wild legume species. Some of the isolations made were in areas of scrub vegetation distant from cultivated *P. vulgaris*. These results provide further evidence in support of the theory that the wetter, humid areas of Uganda and central Africa, where the number of wild endemic legume species is high, could be the geographical centre of origin of the "A" serotype strains of BCMV (Spence & Walkey, 1994). In contrast, the non-necrotic "B" serotype strains that occur in Africa are likely to have been introduced as seed-borne virus, together with *P. vulgaris* from Latin America (F. Morales, personal communication). This study, together with other reports (Spence & Walkey, 1994), has clearly demonstrated that the "A" serotype strains are readily transmitted to beans from wild legume species by local aphid species and that they may be seed-transmitted in *P. vulgaris*.

The results of the experimental sap transmission of isolates 197, 308 and 458 to a range of locally occurring legumes species are interesting (Table 5). Firstly, they show *Crotalaria spinosa* and *Indigofera spicata* to be hosts of BCMV for the first time and secondly, they indicate that the infection induced in many species is latent. Such symptomless infection suggests that many of these species are tolerant to these isolates, which may indicate co-evolution of the "A" serotype strains with these species in this region, so providing further evidence that central Africa is close to the centre of origin of these strains.

The independent origin of the "A" serotype strains in this region of Africa would strongly support the proposal that the "A" and "B" serotype strains are two distinct viruses and that the "A" serotypes should be called bean common mosaic necrosis virus (McKern *et al*, 1992; Vetten *et al.*, 1992).

The prevalence both of susceptible wild legume host species and of aphid vectors of the virus in this region of Africa would also provide the ecological opportunity for the evolution of novel strains of BCMV. Such novel strains could result from the recombination of virus RNA's when two virus strains jointly infect the same host plant. This process is known to occur between closely related potyviruses (Lecoq & Purcifull, 1992; Goldbach, 1992). Previous studies have identified novel "A" serotype strains of BCMV infecting *Crotalaria incana* and *Glycine max* in Uganda and *Senna sophera* in neighbouring Rwanda (Spence &

Walkey, 1994). In the present study, novel isolates were found infecting *Lablab purpureus* (isolate 598), *P. lunatus* (574), *Senna hirsuta* (472), *S. sophera* (429, 524) and *Vigna vexillata* (308) (Tables 1 & 3).

The variation shown by these isolates was characterized by their pathogenicity genotype in differential cultivars of common bean (Table 3). Some of the isolates had phenotype responses that clearly identified them as being related to the standard NL3 or NL8 strains of BCMV. The phenotype responses of these isolates were not necessarily identical to those of NL3 and NL8 in every cultivar of each host resistance group. Some isolates failed to infect an individual cultivar of a particular host group, even though they infected all other cultivars of that host group. Where such small variation occurred the isolate was considered to be of the NL3 or NL8 strain-type (Spence & Walkey, 1994). Isolates 197, 218, 463, 473, 496, 741 (NL3 strain-types) and 472 and 534 (NL8 strain-types) were in this category (Table 4), although it should be pointed out that isolate 472 showed additional atypical serological reactions (Table 3).

Other isolates, however, showed phenotypic expression that differed considerably from the standard strains in that they failed to infect any cultivar of a specific host resistance group. Such isolates were considered to be novel. The response of isolate 598 from *Lablab purpureus*, for instance, indicated that it possessed pathogenicity genes P1 and P2 (Table 4), which suggests that it belongs to the theoretical virus pathogenicity group V7 proposed by Drijfhout (1978); in addition, it carries the temperature-independent Px gene for necrosis proposed by Spence & Walkey (1994). This is the first report of the isolation of a BCMV strain from this pathogenicity group. Similarly, isolate 429 (from *Senna sophera*) is novel; it carries pathogenicity genes P2 and $P2^2$ (as well as Px), so placing it in theoretical pathogenicity group V11. Isolates 308 (from *Vigna vexillata)* and 574 (from *P. lunatus*) have similar phenotype responses that suggest that they carry pathogenicity genes $P1^2$ and P2 and belong to pathogenicity group V9. These would then belong to the same strain group as isolate 836 (from *S. sophera* in Rwanda; Spence & Walkey, 1994).

The reactions of the isolates in ELISA to BCMV Mab's I-2 and 463 and to BCMV Pab's 301 NL6 and NL8, also indicated significant variation in their viral proteins (Table 3).

The horticultural importance of the widespread occurrence of these temperature-independent, necrosis inducing "A" serotype strains of BCMV in Uganda and their aphid vectors is considerable. The basis of most national bean improvement programmes in Uganda and elsewhere in east, central and southern Africa has been the introduction of new, high yielding cultivars carrying the I resistance gene, effective against "B" serotype strains of BCMV. The ability of the necrotic "A" serotype strains to overcome the I gene resistance emphasizes the importance of protecting new bean cultivars in this region against the potentially devastating necrotic strains. The Ugandan national bean breeding improvement programme is now incorporating germplasm into its new I gene carrying cultivars that possess the recessive resistance genes bc3 or bc2, which afford total resistance against all known pathogenicity genes of BCMV (H. Gridley, personal communication).

ACKNOWLEDGEMENTS

We wish to thank the Rockefeller Foundation, the CIAT East African Regional Programme and the Natural Resources Institute for financially supporting these studies. We also thank Sam Sequya, Moses Luyiira, Yakubu Sekamwa and Lawrence Kamya for their technical assistance.

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Table 1. Differential cultivars of common bean used in these studies.

Host groups[1]	Resistance genes	Cultivars
1	bc-u	Double White, The Prince, Sutter Pink, Stringless Green Refugee, Common Red Mexican.
2	bc-u, bc-1	Pure Gold Wax, Redlands Greenleaf C
3	bc-u, bc-1^2	Redlands Greenleaf B, Great Northern 59, Great Northern 123
4	bc-u, bc-2	Michelite, Sanilac
5	bc-u, bc-1, bc-2	Pinto 114
6	bc-u,bc-1^2, bc-2^2	Monroe, Red Mexican 35, Great Northern 31
(7)	-	-
8	bc-u, I	Widusa, Black Turtle Soup
9a	bc-u, bc-1, I	Jubila
9b	bc-u, bc-1, I	Top Crop, Improved Tendergreen
10	bc-u, bc-1^2, I	Amanda

[1] Drijfhout (1978).

Table 2. Legume species from which BCMV necrotic "A" serotype isolates were obtained in Uganda.

Species	Isolate	Sympoms in natural host	Survey site	Location (village /district)
Centrosema pubescens Benth.	741	mild mosaic	fallow field	Nakabango, Jinja
Crotalaria sp.	218	mild mosaic	roadside scrub	Nabbingo, Mpigi
C. incana L.	463[1]	mild mosaic	fallow field	Namulonge, Mpigi
Lablab purpureus (L.) Sweet	598	mosaic	banana plantation	Kirinyabigo, Mpigi
Phaseolus lunatus L.	574	mosaic	roadside scrub	Kawanda, Mpigi
Senna bicapsularis (L.) Roxb.	496	chlorotic spots	scrubland	Bajja, Masaka
Senna hirsuta (L.) Irw. & Barn	197	mosaic	edge of bean field	Bukalasa, Luwero
S. hirsuta	472	vivid mosaic	edge of bean field	Bukalasa, Luwero
S. hirsuta	473	vivid mosaic	edge of bean field	Bukalasa, Luwero
S. sophera (L.) Roxb.	429	mosaic	roadside scrub	Kimanya, Masaka
S. sophera	524	chlorotic streaks	roadside scrub	Ruhanga, Kabale
S. sophera	534	mild mosaic	roadside scrub	Kabale, Kabale
Vigna vexillata (L.) A. Rich.	308	mild mottle	banana plantation	Namalere, Mpigi

[1] An additional "A" serotype was isolated from a different *C. incana* plant at this site.

Table 3. Reactions in ELISA tests of the "A" serotype legume isolates of BCMV.

Isolate	Mab[1]			Pab[2]					
	I-2	197	463	30	NL6	NL8	PnMoV	BlCMV	CSMV
741	+	+	+	-	-	+	-	-	-
197	+	+	-	+	+	+	-	-	-
218	+	+	+	+	-	-	-	-	-
308	+	+	-	+	+	+	-	-	-
429	-	+	nt	+	-	+	+	-	-
463	+	+	+	+	-	-	-	-	-
472	-	+	+	+	+	+	+	-	-
473	+	+	+	+	+	-	-	-	-
469	+	+	nt	nt	nt	nt	nt	nt	nt
524	+	+	nt	nt	nt	-	nt	nt	nt
534	+	+	+	-	-	+	-	-	-
574	+	+	+	+	+	-	-	-	-
598	+	+	+	+	+	+	-	-	-
464	+	+	nt	nt	nt	nt	nt	nt	nt

[1] Mab I-2 is specific for "A" serotype strains of BCMV; 197 is specific for all BCMV strains and selected potyviruses; 463 is reported to be specific for all BCMV strains.

[2] Pab 30 was prepared against a novel "A" serotype strain found in Uganda (Spence & Walkey, 1994).

nt = not tested.
PnMoV = Peanut mottle virus.
BlCMV = Blackeye cowpea mosaic virus.
CSMV = Cassia severe mosaic virus.

Table 4. Pathogenicity phenotypes of the 13 isolates, their probable pathogenicity genes and their strain grouping.

Bean host group	Standard strain		Isolate							
	NL3	NL8	197 473	218 496	463 741	472 543	598	308 574	524	429
1	+	+		+		+	+	+	+	+
2	+	-		+		-	+	-	-	-
3	+	-		+		-	-	+	-	-
4	+	+		+		+	+	+	+	+
5	+	-		+		-	+	+	+	-
6	-	-		-		-	-	-	-	+
8	N	N		N		N	N	N	N	N
9[a]	N	-		-		-	nt	N	nt	-
9[b]	N	-		+		-	N	N	N	-
10	-	-		-		-	-	-	-	-
Path. genes	*P*O	*P*O		*P*O		*P*O	*P*O	*P*O	*P*O	*P*O
	*P*1	*P*2		*P*1		*P*2	*P*1	*P*1[2]	*P*2	*P*2
	*P*1[2]			*P*1[2]			*P*2	*P*2	*P*1?	*P*2[2]
	*P*2			*P*2						
	*P*x	*P*x		*P*x		*P*x	*P*x	*P*x	*P*x	*P*x
Strain -type	NL3	NL8		NL3		NL8	Novel	Novel	Novel	Novel
Virus Genotype[1]	V12	V4		V12		V4	V7	V9	V7?	V11

+ = Positive reaction; - = negative reaction; N = temperature - independent necrotic reaction; nt = not tested; ? = questionable presence of P1 pathogenicity gene and possible relationship to theoretical virus genotype group V7.

[1] Theoretical virus genotype group, Drijfhout (1978).

Table 5. Experimental infection of wild legume species by three "A" serotype isolates.

	Isolate					
	197		308		458	
Host Species	Sympts[1]	ELISA	Sympts	ELISA	Sympts	ELISA
Calopogonium caeruleum	-	-	-	+	-	-
Canavalia ensiformis	mo,ss	+	mo,s	+	mm	+
Centrosema pubescens	-	+	lc	+	-	+
Crotalaria incana	-	+	-	+	mm	+
C. spinosa	-	+	ss	+	-	+
Desmodium intortum	-	+	-	+	-	+
D. uncinatum	-	+	-	+	-	+
Indigofera spicata	-	-	-	+	-	+
Macroptilium lathyroides	mo	+	nt	nt	mo	+
Rhynchosia minima	-	+	-	+	-	+
Senna hirsuta	mm	+	mo	+	mm	+
Vigna radiata	-	+	-	+	-	+
V. unguic-ulata	-	-	mm	+	-	-
V. vexillata	-	+	mo	+	-	+

[1] mo = distinct mosaic; mm = mild mosaic; lc = leaf curl; s = moderate stunting; ss = slight stunting; - = negative reaction; + = positive reaction in ELISA test.

Table 6. The seed transmission of various "A" serotype isolates in bean cv. Double White.

Isolate	Seedlings infected/seedlings tested			Total	Percentage infection
	Exp.1	Exp. 2	Exp. 3		
197	4/6	6/14	nt	10/20	50
308	nt	nt	7/11	7/11	64
429	nt	nt	5/16	5/16	31
473	2/3	24/56	8/13	32/72	44
496	1/4	nt	5/8	6/12	50
534	0/6	nt	10/21	10/27	37
574	nt	nt	10/19	10/19	53
741	5/8	9/26	12/14	26/48	54
458	1/6	23/44	10/14	34/64	53

1 Isolate 458 was a NL3 strain-type originally isolated from *P. vulgaris* in Uganda, and included as a control; all other isolates were isolated in the present study from other legume species.

nt = Not tested.

Table 7. Aphid transmission of various "A" serotype isolates by *Aphis fabae* in bean cv. Double White.

Isolate [1]	No. seedlings infected /No. inoculated
197	3/6
218	3/9
308	4/6
463	4/7
472	2/5
473	4/10
496	2/8
534	1/5
574	2/6
598	5/5
458	3/10
NL3	5/10

[1] The standard strain NL3 and isolate 458 (a NL3 strain-type originally isolated from *P. vulgaris*) were included as controls. All other isolates were isolated from other legume species in this present study.

DETECTION OF NON-PROTECTED I-GENE IN ADVANCED BREEDING LINES

D. Cishahayo[1], R. Buruchara[2] and T. Ntawurutwa[1].

[1] Institute des Sciences Agronomique du Rwanda (ISAR), B. P. 138, Rubona, Rwanda, [2] Centro Internacional de Agricultura Tropical (CIAT),B. P. 259, Butare, Rwanda.

INTRODUCTION

For a number of years now, the national bean programmes of Rwanda, Burundi and Zaire in the Great Lakes Region of central Africa have been handling large numbers of segregating populations, generated either from crosses made in their breeding programmes or received directly from CIAT, Colombia. Many of the crosses have one or more of the parents with the I-gene which offers protection against certain strains of BCMV. However, the I-gene materials give a hypersensitive reaction to necrotic strains of BCMV (particularly the NL3 and NL8) resulting in systemic necrosis and sometimes death of plants. Considering that these strains have been shown to occur in the region (Spence and Walkey, 1993) and in some cases their effect on-station has been considerable, national bean programmes have shown interest in detecting the presence of the unprotected I-gene in their advanced lines. This allows decision-making on how and where best to use advanced lines, including protecting the I-gene by incorporating the recessive genes bc_{2-2} or bc_3. The objective of this study was to develop effective methodologies for field and greenhouse detection of the I-gene in advanced lines. This paper presents results obtained from field trials.

MATERIALS AND METHODS

Field trials were conducted at ISAR, Karama station in south-east Rwanda, situated at an altitude of 1300 masl and receiving a bimodal rainfall of about 900 mm per year. The site is a hotspot for BCMV and the NL3 strain has been isolated from the station (Spence and Walkey, 1993). The common bean line AFR 13 infected with NL3 was used as the source of the virus.

Time of planting infector and test plants.

To determine the appropriate time of planting, an infector line was planted early relative to test lines so as to maximize infection. AFR 13 and A 321 were used as infector and test lines, respectively. AFR 13 gives a mosaic reaction whereas A 321 (an I-gene line) develops black root if infected with necrotic strains of BCMV. Test lines were planted alternately, between two infector rows. During the 1993A season, two planting methods were compared.

Method 1. At the initial planting date (12/11/93), all infector rows were sown with AFR 13. At the same time the first treatment of test lines was planted. On the three subsequent planting dates done at weekly intervals, only the test line was planted.

Method 2. Both the infector and test lines were sown on the initial planting date as well as on each of the three subsequent planting dates, done at weekly intervals. A split plot design was used, with planting of infector plants as main plots and test lines as subplots.

During the 1993B season, a trial was conducted to determine the effect of establishing screening trials for the I-gene at the beginning of the season or establishing the trial about three weeks later. Late planting necessitated the use of irrigation to supplement the 234mm of rainfall received throughout the trial period. In both cases, the test line was planted as described in Method 1 above, except for the fact that 5 planting dates were used at 2 weekly intervals.

Screening for the presence of I-gene.

A total of 175 lines from different breeding programmes in the Great Lakes Region were screened at Karama in 1993 B season. Infector rows of AFR 13 were sown late in the season (1/4/93) and the test lines were sown 26 days later. An augmented design was used, with A 321 as the black root susceptible check and AND 811 as the mosaic susceptible check. Evaluation was done at weekly intervals until harvest. The trial received 1237 mm of water; 75 mm from rain and 1162 mm from irrigation.

RESULTS AND DISCUSSION

Time of planting of infector rows in relation to test plants

Planting of infector rows of AFR 13 before test lines resulted in a significantly higher incidence of black root on A 321 than when both were planted at the same time (Table 1). There was a significant method x date interaction. Late planting of the test lines resulted in early appearance of black root symptoms soon after emergence, but it took 32 days from emergence for black root symptoms to appear, when both infector and test lines were planted at the same time. However, when test plants were sown three weeks later than infector plants, black root symptoms did not develop until 18 days after test plant emergence. Sowing (by Method 1) in which A 321 was sown three weeks after AFR 13, also led to increased plant mortality from black root.

During the 1993B season, sowing the trial three weeks later resulted in early appearance of black root (16.4 days compared to 20.5 days) (Table 2), increased the percentage of plant mortality due to black root (4.7% and 11.9% from early and late planting, respectively), and reduced the number of escapes (12.9% versus 2.9%)(Table 3).

Our results indicate that it is advisable to sow an infector crop before the test lines. This allows detection of black root earlier and reduces disease escape. Under field conditions, transmission of the virus from infector to test lines relies on aphids. Thus, it is important that dates of planting are timed to ensure that both virus and aphids are present to facilate transmission. The optimum method at Karama station appears to be by sowing 3 weeks into the season combined with planting of test lines 2 to 4 weeks after planting of infector rows. However, under such conditions irrigation is necessary during dry periods.

AFR 13 was used as the infector line because it is susceptible to the virus but, at the same time, it maintains a good amount of vegetative growth; being a climbing cultivar, it serves as a source of virus for a longer period than bush types. Since most advanced lines are likely

to be genetically uniform, it is not necessary for all plants of test lines to be affected to confirm presence of the I-gene. Sowing of test lines 2 to 4 weeks after the infector rows enables early detection of black root. This gives the option of using the same infector plants to evaluate several entries serially, as long as the infector crop remains viable and irrigation is possible. Dry conditions also favour aphid infestation.

Screening for the presence of I-gene

A total of 39 cultivars (22.2 %) gave the black root reaction. Four entries (2.3 %) remained free of mosaic and black root and the rest gave a mosaic reaction. These four together with those showing dubious reactions are to be tested further using artificial inoculation in the screenhouse. Entries found black root susceptible are shown in Table 4.

This study demonstrates conditions and methods under which screening of advanced lines for the presence or lack of I gene can be done under field conditions with a good degree of success. In the absence of laboratory or greenhouse facilities to conduct artificial inoculation this offers an alternative or even a complementary approach.

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Table 1. The effect of two methods of sowing an infector (AFR 13) and test line (A 321) on the time of appearance and incidence of black root.

	Days from emergence to first infection		% dead plants at first evaluation	
Sowing dates[u]	Method 1[v]	Method 2[x]	Method 1	Method 2
DO	32.0 a[y]	32.0 a	8.0 b	9.5 a
D1	26.4 b	25.0 b	6.8 b	5.4 ab
D2	18.8 c	22.2 c	4.8 b	0.6 b
D3	18.0 c	19.4 d	21.5 a	9.7 a

[u] = Sowing at weekly intervals.
[v] = All infector plants were sown at initial planting date (DO). Only test plants were sown at weekly intervals.
[x] = Both infector and test lines were planted at each sowing date.
[y] = Values followed by the same letter in each column are not significantly different at P = 0.05.

Table 2. Comparison of planting early and late in the season, and planting test lines at different times after the infector plants on the time of appearance of black root.

Sowing date[u]	Number of days to first black root	
	Early planting[v]	Late planting[w]
DO	27.2 a	24.4 a
D2	23.0 bc	14.0 b
D4	20.2 c	14.0 b
D6	16.0 d	14.0 b
D8	16.0 d	15.4 b
Mean	20.5	16.4

[u] = Sowing at two weekly intervals
[v] = Initial sowing at start of season
[w] = Initial sowing 3 weeks later
[y] = Values followed by the same letter in each column are not significantly different at P = 0.05

Table 3. Effects of time of planting on escape, the incidence of black root, and on plant mortality attributed to other causes.

Time of planting	No. escapes (%)	Black root incidence (%)	Mortality from other causes (%)
Early (start of season)	12.9 b	13.0 a	4.7 a
Late (3 wks later)	1.9 a	14.0 a	11.9 b

Values followed by the same letter within each column are not statistically different at P = 0.05.

Table 4. Origin of advanced bean lines found susceptible to black root.

Origin	Advanced lines
CIAT	AND 682, AND 875, CNF 4708, AND 886 AND 688, BAT 308, CNF 5547, CNF 5506, XAN 97, A 796, FEB 162, VDN 186, VDN 172, RAB 494, CNF 5538, PF 16, CNF 5513.
RWANDA	RWR 719, RWK 8, RWR 432, RWR 306, RWR 867, RWK 7, RWK 1, RWK 10, RWK 5, RWK 11.
BURUNDI	MORE 90039, MORE 90030, MORE 90035, MORE 90062, MORE 90040, MORE 90003, MORE 91002, SCAM-80-CM/2, SCAM-80-CM/15.
ZAIRE	MLB-42-89A, MLB-12-88B, MLB-13-88B.

BEAN PRODUCTION CONSTRAINTS IN AFRICA WITH SPECIAL REFERENCE TO BREEDING FOR RESISTANCE TO BEAN COMMON MOSAIC VIRUS IN UGANDA

H.E.Gridley

CIAT, Kawanda Research Institute, P O Box 6247, Kampala, Uganda.

BEAN PRODUCTION CONSTRAINTS

Bean production constraints comprise fungal, bacterial, viral and insect pests and a range of soil fertility problems. Wortmann and Allen (1994) have recently compiled information on the constraints in bean producing countries in Africa to assist CIAT (Centro International de Agricultura Tropical) and national bean research programmes in defining priorities.
Based on the estimated bean production area affected, the five principal constraints of high (yield loss > 300 kg/ha) and moderate (100-300 kg/ha lost) importance across all countries of eastern and southern Africa are given in Table 1. Similar data for the highland (>1500m) and mid-altitude (1000m to 1500m) areas of eastern Africa are also included in Table 1 as these areas account for 70% of bean production in sub-Saharan Africa.

Across sub-Saharan Africa, angular leaf spot (*Phaeoisariopsis griseola*) is the most serious constraint, estimated to be of high importance in 67% of the bean producing area, followed by low nitrogen (53%), anthracnose (*Colletotrichum lindemuthianum*)(47%), bean stem maggot (46%) and low phosphorus (44%) (Table 1). The two soil fertility constraints and bean stem maggot are the three least widespread amongst the constraints of moderate importance, with bruchids the most widespread at 95%, followed by phosphorus fixation by iron oxides, at 69%.

Of the five most widespread biotic constraints of high importance, three have already been noted, with common bacterial blight (*Xanthomonas campestris pv. phaseoli*) at 29% and bean common mosaic virus at 22%, making up the top five. Three of these five occur amongst the top five of moderate importance which also includes rust (*Uromyces appendiculatus*) at 44%, and phoma blight (*Phoma exigua var. diversispora*), at 26%.

The constraints of high importance in eastern Africa are very similar to those across sub-Saharan Africa although the root/stem rot complex and charcoal rot (*Macrophomina phaesolina*) are more serious in this region. Also the prominence of the constraints changes with altitude (Table 1), notably with anthracnose switching from a major problem at the higher to a minor one at the lower altitudes, whilst common bacterial blight shows the opposite trend. Overall, four of the five most important biotic constraints are common to both regions, namely, angular leaf spot, anthracnose , common bacterial blight and bean common mosaic virus.

Biotic factors have long been recognized as important constraints to bean productivity but it is clear that the abiotic constraints of low nitrogen

DISCUSSION

Introductions are a fast and cost effective means of increasing genetic variation in a breeding programme. Selection in well adapted segregating populations potentially should generate a higher frequency of lines with improved yield potential than occurs amongst introduced homozygous breeding lines, which inevitably have been selected in a 'foreign' environment. However, retention of only resistant progenies and lines inevitably drastically reduces the amount of genetic variation within populations for yield and other traits. To counteract this, and in view of the well documented low heritability for yield, a relatively large number of populations were utilized to create a diverse pool of genetic variation; the number of progenies screened and lines tested were maximized within available resources (Table 1). Multisite replicated yield testing was imposed from the preliminary yield trial onwards to reduce the genotype x environmental variance.

No reselection was consciously practised within the lines, following selection of resistant (usually F2) single plants, to allow exploitation of inherent genetic variation for yield and locally important biotic and abiotic constraints in different countries which received the African BCMV nursery. Preliminary results from the evaluation of progenies (from selection in F2 derived lines) indicate that such variation can be exploited practically but there is need to confirm this in a further season of testing.

To date, breeders have mainly utilized the dominant I gene and more recently the recessive genes bc3 or $bc2^2$ in breeding for resistance. The former has been used for over 20 years in Latin America and, although producing a hypersensitive reaction with the necrotic strains, has yet to be overcome. However, breeders and pathologist cannot feel complacent, noting the regular breakdown of disease resistance in many crops, and the recent detection of novel strains of BCMV in Africa (Spence and Walkey, 1994), so posing a future problem for the resistant sources currently being utilized.

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Table 1. The five principal constraints to bean production of high and moderate importance in sub-Saharan Africa and eastern Africa.

ABIOTIC/BIOTIC

sub-Saharan

high importance	%ha[1]	moderate importance	%ha
Angular leaf spot	67	Bruchids	95
Low nitrogen	53	FeP[2]	69
Anthracnose	47	Bean stem maggot	51
Bean stem maggot	46	Low phosphorous	47
Low phosphorous	44	Low nitrogen	46

eastern Africa - high importance

>1500m	%ha	1000-1500m	%ha
Anthracnose	92	Angular leaf spot	70
Angular leaf spot	89	Common bacterial blight	68
Low nitrogen	70	Low nitrogen	40
Low phosphorous	70	Bean common mosaic virus	32
Bean stem maggot	62	Bean stem maggot	30

BIOTIC

sub-Saharan Africa

high importance	%ha	moderate	%ha
Angular leaf spot	67	Rust	44
Anthracnose	47	Bean common mosaic virus	37
Bean stem maggot	46	Anthracnose	30
Common bacterial blight	29	Common bacterial blight	29
Bean common mosaic virus	22	Phoma blight	26

eastern Africa - high importance

>1500m	%ha	1000-1500m	%ha
Anthracnose	92	Angular leaf spot	70
Angular leaf spot	89	Common bacterial blight	68
Root stem rot complex	35	Bean common mosaic virus	32
Halo blight	31	Anthracnose	21
Bean common mosaic virus	25	Charcoal rot	19

[1]. Percent of production area (in hectares) where the constraint is considered of high/moderate importance.

[2]. Phosphorus fixation by iron oxides.

Table 2. Number of segregating populations, progenies screened and lines tested in the breeding programme for BCMV resistance in Uganda initiated in three seasons.

Number of:		Phase number/starting season			Total
		1/90a	2/91a	3/92a	
-Segregating populations	F2	19	43	38	100
	F3	3			3
	F4	1			1
- single plant progenies screened:		1881 (F3/F4/F5)	2835 (F3)	2325 (F3)	7041
- lines tested:	PBR(1)	182	182	405	769
	IBR	109	40	103	252
	ABR	45	21	(none)	66

1. Yield trials: PBR-preliminary, IBR-intermediate, ABR-advanced.

Table 3. Percent yield increase over released cultivars of superior BCMV resistant lines[1], derived from segregating populations.

Seed size			
Large/		Small	
% CAL 96[2]		% MCM 5001[2]	
9 sites/3 seasons		9 sites/2 seasons	
UBR(93) 16	18	UBR(92) 12	28
UBR(93) 14	16	UBR(92) 13	26
UBR(93) 4	14	UBR(92) 27	20
UBR(93) 12	9	UBR(92) 26	13
UBR(93) 1	6	UBR(92) 9	12

1. UBR-code for Uganda black root resistant line.
2. Released cultivar.

DISCUSSION OF SESSION 1

Lana : Are there specific antisera for 'novel' isolates, like those assigned to a novel pathotype in Ethiopia ?

Spence : There are no antisera yet that are specific to these novel isolates. We now have many isolates that do not conform to patterns of previously described pathotypes and there remain areas in Africa (like Mozambique, Sudan and the whole of West Africa) where collections have not been made.

Lana : CIAT and its collaborators should produce a distribution map of BCMV pathotypes and non-conforming strains.

Spence : There are such maps, published in NRI Bulletin 63 !

Lana : How long will the bc3 gene remain effective ? Why are you worried ?

Pastor-Corrales : Genes for resistance to BCMV seem fairly stable, but the pyramiding of several genes is preferable to using one in isolation.

Lana : Can bc2 and bc-3 be combined ?

Gridley : Yes, but a lot of work would be involved !

Spence : Nothing from our survey suggests there is a problem with bc3.

Pastor-Corrales : With fungal pathogens, one can identify a site with lots of variation in the pathogen. Can this be done with BCMV ?

Spence : The dot-blot test for identification of BCMV can be used in the field without grinding.

Lana : It would be helpful to have a simple diagnostic test.

Spence : We need good, healthy controls with this test. We are trying to develop the simplest and most convenient test but polysaccharides in wild species can give misleading results. We will continue work on this at Wellesbourne over the next year.

Lana : Can Ethiopia be used for screening for NL3 ?

Gridley : Because of the risk of accidental introduction of NL3 into Ethiopia, all seed imports from elsewhere in Africa have been burned. Aid projects remain a problem, though.

Opio : How is work progressing with large seeded types ?

Gridley : Between half and three-quarters of our materials are medium to large seeded.

Buruchara : Entries in the black-root nursery from Karama are not well-adapted and are rust susceptible. I like the idea of diversity of materials maintained in breeding programmes.

Lana : Can we have transparency in breeding ?

Gridley : Yes, certainly !

Sengooba : What advice can we get for multiple disease resistance ?

Gridley : Multilocational screening is helpful in picking up susceptibility to other diseases. In Uganda, Bukalasa is a good site for screening against both CBB and ALS as well as BCMV. Our strategy is to maintain levels of resistance against other diseases rather than to make crosses specifically for multiple resistance.

Buruchara : Results from sub-projects on CBB and rust should help to identify resistant parents.

Gridley : We have more information on the material now. We have got the crosses but remain unsure about the level of screening.

Pastor-Corrales : CIAT headquarters does have a number of lines with multiple disease resistance.

Gridley : We should get these lines and use them as parental material.

Lana : I propose that CIAT collates everyone's results in producing distribution maps.

Pastor-Corrales : It might be more appropriate that NRI does so.

Spence : Certainly, we must get together to collect information and produce something !

Pastor-Corrales : How will you do this ? An expanded series of differential cultivars is needed.

Spence : We are working with a breeder to produce engineered differentials. Bean mixtures have also been screened against BCMV.

Sengooba : Perhaps certain wild legumes might be useful differentials ?

Lana : There remains the problem of the role of wild legumes in BCMV epidemiology. What rates of transmission occur ?

Sengooba : I would like to show you some data on transmission between different virus hosts.

Spence : We still know little about aphid transmission in the "wild" : I didn't see any evidence of it during my recent trip. Some isolates seem adapted to wild hosts, providing evidence of co-evolution. Cultivated plants may be more susceptible, from a narrower gene-pool.

Pastor-Corrales : There is lots of evidence for co-evolution between fungal pathogens and common beans, in both small and large-seeded types.

Spence : We are using PCR analysis to reveal differences.

Pastor-Corrales : The centre of diversity of the virus may be Africa not Latin America, which is the centre of origin of beans. Studies of diversity and co-evolution can help us to understand the system.

Spence : Yes, I agree.

Lana : Are sequences between strains of the black root pathogen identical ? Are they the same between strains from bean and wild legumes ?

Spence : Beans and wild legumes next to each other may have different infections, perhaps from different sources. Such information could help in predicting the evolution of strains.

Pastor-Corrales : Do different strains have different DNA finger-prints ?

Spence : We are working on this with other viruses and hope to investigate this in BCMV in the future.

Lana : Have the isolates of Spence and Sengooba been compared ?

Spence : Yes ! Selection appears to be for variation in BCMV.

Pastor-Corrales : Sometimes there is diversity in characters other than pathogenicity !

Buruchara : On current evidence, necrotic strains of BCMV are originally African, indicating that *P. vulgaris* may not be the original host.

Spence : Genetic diversity in the host permits us to identify diversity in the pathogen.

Sengooba : This kind of research is appropriate to Universities.

Spence : Much depends on funding, and collaboration between groups may be essential.

Buruchara : Breeders and pathologists in the region have discussed the issue of whether or not to condone release of material possessing the unprotected I gene. We recognise that farmers are likely to think they have been given a new disease rather than a new susceptibility. How much will black-root be a problem if we release an I gene variety ? Farmers will react sharply !

Opio : We had this problem here, in Uganda. Farmers reported to the Ministry that a new disease had been introduced !

Schmit : Black-root is a problem only on research stations !

Sengooba : The use of White Haricot as a spreader seems likely to undermine our credibility with farmers.

Spence : There are reservoirs of BCMV on-station.

RACE CHARACTERISATION AND IDENTIFICATION OF RESISTANCE TO HALO BLIGHT *Pseudomonas syringae* pv. *phaseolicola*) IN AFRICA

Dawn M. Teverson* and J.D. Taylor+

* Natural Resources Institute, Chatham Maritime, Kent ME4 4TB, UK and +Horticulture Research International, Wellesbourne, Warwick CV35 9 EF, UK.

ABSTRACT

Isolates from Africa and other bean growing areas were categorised into nine races on the basis of the responses of eight differential cultivars after artificial inoculation. Nearly nine hundred isolates were identified as Pseudomanas syringae pv. phaseolicola on the basis of their pathogenicity to common bean. 175 were selected for detailed race determination. A 'race map' was produced indicating the frequency and occurrence of the nine races. Alternative hosts were also investigated.

One thousand and forty eight Phaseolus bean accessions were evaluated for resistance to six races of the pathogen. Race specific resistance genes (R-genes) were detected in 49.4% of accessions at frequencies ranging from 35% for R4 down to only 0.2% for R5. A list of useful sources of race non-specific resistant cultivars is given.

INTRODUCTION

The following paper was presented at the Pan African Working Group Meeting on Bacterial and Viral Bean Diseases. It is the summary of seven years work and therefore only a proportion of the information could be presented. The following is a summary of the parts of the study most pertinent to the practical control of halo-blight in Africa.

Halo-blight of common bean (*Phaseolus vulgaris*) caused by the bacterium *Pseudomonas syringae* pv. *phaseolicola*, is seed-borne and spread through the crop by rain-splash. It is favoured by cool temperatures and is found at medium to high altitude (1500-2500m) in the tropics.

Resistant cultivars are the only practical means of controlling halo-blight in countries where farmers keep their own seed and alternative hosts make the carry-over of disease from one season to the next a significant problem. However, the deployment of resistance without an awareness of pathogenic variation within the pathogen population could result in costly failure. To avoid this, it is necessary to identify variants (strains/races) of *P.s.* pv. *phaseolicola* before the deployment of resistant cultivars, ensuring that only appropriate resistance sources are deployed.

Two races of the pathogen (races 1 and 2) have long been recognised on the basis of the differential reaction of the *P.vulgaris* cultivar Red Mexican U13 (Walker and Patel, 1964).

These races are widely distributed throughout the world, including Africa. More recently a third race was described from Africa which was recognised by the differential response of the cultivar Tendergreen (Taylor & Teverson, 1985; Mabagala & Saettler, 1992). The differential reactions of cultivars to these races is shown in Table 1.

MATERIALS AND METHODS

Halo-blight infected material was collected by the authors and collaborators worldwide but with special empasis on Africa. Isolates were selected on the basis of colony characteristics on King's Medium B (King, et al., 1954) and were usually the predominant organism. Although *P. vulgaris* was the main host, collections were made of tropical legumes representative of seven different genera.

A total of 1106 isolates were examined, representing 394 disease occurrences and including 54 reference strains. Detailed race determinations were made on 175 selected isolates. More technical information can be obtained from publications by the authors and collaborators (Taylor et al., 1996a & b; Teverson et al., in prep.).

Pathogenicity tests were carried out as detailed in (Taylor et al., 1996a). Bean seedlings were spray inoculated at primary leaf stage and kept under humid conditions for two days. After this period they were returned to normal glasshouse conditions and symptoms were evaluated 10 days after inoculation. Symptoms were graded on a 1 - 5 scale where one is completely resistant and five completely susceptible. Using this system 1048 accessions were evaluated for resistance to six or more races.

RESULTS

Eight hundred and ninety three isolates of *P.s.* pv *phaseolicola* were grouped into three provisional race categories corresponding to the original races 1 and 2 and the new race 3 on the basis of the responses of the three differential cultivars shown in Table 1. A more extensive series of eight differential cultivars was developed as a result of screening nearly 1000 host accessions with a group of isolates representative of different geographical areas and the three previously categorised races. As a result of these tests, which suggested a complex race structure, the original three races were each initially sub-divided, resulting in the identification of 6 races (races 1-6). Subsequent tests were then done on a selection of 175 isolates using the revised differential series and the isolates were categorised into nine races. The chronological order of identification of races is shown in Fig. 1 and the developed race structure based on the interaction of nine races and eight differential cultivars is shown in Table 2.

The geographical distribution of the 172 isolates is shown in Table 3. Race 6 occurred most frequently and comprised 3% of the isolates categorised. Nineteen per cent of isolates were race 4 in contrast to only 4 % of isolates categorised as race 3. However, both of these isolates produced the characteristic hypersensitive resistance on cv. Tendergreen. Race 1 accounted for 12% of the isolates. In total, the three most frequent races 6,4, and 1, accounted for over 60% of all *P.s.* pv. *phaseolicola* isolates characterised.

The geographical distribution of race 6 was widespread. It was detected in seven African countries, as far north as Ethiopia and as far south as Lesotho. Race 1 occurred throughout the world, with 21 isolates originating from nine different countries; race 7 was also distributed worldwide.

Races 3 and 4 were, with the exception of one isolate, confined to east and central Africa. Six race 5 isolates were identified, all except one of which originated from Africa. Race 8 was also confined to Africa, mainly Lesotho, with a single isolate from Tanzania. Race 9 was rare and represented by only two isolates, one each from Malawi and Colombia.

The host range distribution of 172 selected isolates is shown in Table 4. Of the 11 different host species of *P.s.* pv. *phaseolicola* represented, *Phaseolus vulgaris* was the most frequent (78% of isolates). *Neonotonia wightii*, a pasture legume and common weed around bean fields, was host to 4% of isolates whilst *Phaseolus coccineus* and *Lablab purpureus* were host to 6% and 3% of isolates, respectively. The other seven species were each host to fewer than 2% of isolates. The widest host range was shown by race 7 with 18 isolates derived from seven different hosts.

Two types of resistance were detected in the 1048 *Phaseolus* bean accessions which were evaluated for resistance. Race specific resistance was characterised by highly incompatible interaction phenotypes (resistant, grade 1) between certain combinations of races and accessions, but complete compatibility (susceptible, grades 4-5) with others. Race non-specific resistance was characterised by generalised resistance to all races. It could vary from a relatively high level resistance (grades 1-2) to intermediate levels of resistance (grades 2-4).

Approximately half of the 1048 accessions tested showed specific resistance to one or more races of the pathogen. From genetic studies accession groups could be classified according to the likely resistance (R) genes they carried. By inference the frequency of these genes in the seven accession groups could then be estimated. Thus R1 was estimated to be present in 10.3% of accessions, R2 in 0.3%, R3 in 25.0%, R4 in 35% and R5 in 0.2%. Details of this work are given in Taylor et al., (1996b). Similarly, details of the gene-for-gene relationship postulated to explain in the interaction between races and cultivars, and the interpretation of the relationship is detailed in the third paper of the series, (Teverson et al., in prep.).

Race non-specific resistance was shown by only 1% of accessions tested. Accessions showing useful levels of this type of resistance are shown in Table 5. These accessions originate from diverse sources. HBr72 is a North American green bean, whilst 2702/2 was found as a low frequency component of a high altitude Rwandan bean mixture.

DISCUSSION

The objective of this study of pathogenic variation was to probe alternately for variation in pathogen isolates and host cultivars (Taylor et al., 1996a & b), as pathogenic races can only be identified in relation to specific host resistance and vice versa.

The practical significance of this work is illustrated by the often uneven geographic distribution of races (Table 3). For example, races 3 and 4 were almost entirely confined to east and central Africa and, similarly, race 8 was found mainly in Lesotho. In constrast, some races had worldwide distribution, in particular races 1, 2, 6 and 7, which probably reflects their distribution via the international seed trade.

The wide natural host range of the halo-blight pathogen (Table 4), represents 11 species of tropical legumes from seven different genera, including grain and fodder legumes and leguminous weeds. Inoculation studies (Teverson, 1991) showed that the potential range was even more extensive. In particular, the occurrence of *P.s.* pv. *phaseolicola* in certain weed species (*Neonotonia wightii* and *Desmodium* sp.) was thought to provide an important reservoir of infection for the carry-over of disease between seasons (Teverson et al., 1993).

In conclusion, it is suggested that use of race non-specific resistance together with race specific resistance appropriate to particular regions (eg. R3 in east and central Africa) could provide an effective strategy for establishing enhanced and durable resistance to halo-blight.

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Table 1. Differentiation of *Pseudomonas syringae* pv. *phaseolicola* races.

	Races		
Differential cultivars	1	2	3
Canadian Wonder	+	+	+
Red Mexican U13	-	+	+
Tendergreen	+	+	- (HYP)

+, susceptible; -, resistant (hypersensitive); HYP, severe hypersensitive reaction.

Table 2. Race differentiation in *Pseudomonas syringae* pv. *phaseolicola* with an extended range of differential cultivars.

Cultivars	Race 1	2	3	4	5	6	7	8	9
Canadian Wonder	+	+	+	+	+	+	+	+	+
ZAA54 (A52)	+	+	+	+	-	+	+	+	+
Tendergreen	+	+	-	-	+	+	+	+	+
Red Mexican U13	-	+	+	+	-	+	-	+	-
1072	+	-	+	-	-	+	-	+	+
ZAA55 (A53)	+	+	-	-	-	+	+	+	+
ZAA12 (A43)	+	-	-	-	-	+	-	-	-
Guatemala 196-B	-	+	-	-	-	+	-	+	-

\+ = compatible (susceptible)
\- = incompatible (resistant)
1072 = *Phaseolus acutifolius*

Table 3. Frequency of occurrence and geographic distribution of races of *Pseudomonas syringae* pv. *phaseolicola*.

Origin	Race .... (Number of isolates)									
	1	2	3	4	5	6	7	8	9	T
Africa										
Burundi			3	4						7
Ethiopia		2		1		2	1			5
Kenya				1	2	5	3			11
Lesotho	4	1				5		12		22
Madagascar	1									1
Malawi		1			1				1	3
Mauritius		1								1
Rwanda	2	2*		20						24
Swaziland						1				1
Tanzania		1	2	2	2	9	4	1		21
Uganda				4						4
Zaire		1	1	1						3
Zambia						5				5
Zimbabwe		3				5	1			9
America										
Canada				1						1
Colombia	1		1			3	1		1	7
Mexico						4				4
Peru	1					2				3
U.S.A.	1	1					1	1		4
Europe										
Bulgaria						1				1
France		1			1	1				3
Germany						5				5
Italy	2									2

Spain							2	2		4
Sweden		1								1
U.K.	7					4	1			12
Other Areas										
Australia		1					1			2
New Zealand	2						2			4
N. Yemen		2								2
	21	17	7	33	6	55	18	13	2	172

* One isolate of this total was reisolated from 1302A, the race 4 type strain, from Rwanda.

Table 4. Host range distribution and number of isolates of *Pseudomonas syringae* pv. *phaseolicola* races.

Origin	Race									
	1	2	3	4	5	6	7	8	9	T
C. cajan						3				3
Desmodium sp.				2			1			3
Lablab purpureus					1	2	1	1		5
Macroptilium atropurpureum		1								1
Neonotonia wightii					2	1	4			7
Phaseolus acutifolius							2			2
P. coccineus	8			2		1				11
P. lunatus	1	1								2
P. vulgaris	11	15*	7	29	3	48	7	12	2	134
Vigna angularis							2			2
V. radiata	1						1			2
	21	17	7	33	6	55	18	13	2	172

* One isolate of this total was reisolated from isolate 1302A, the race 4 type strain, from Rwanda.

Table 5. Bean assessions with potentially useful levels of race non-specific resistance.

Accession No.	Designation	Origin	Disease grade (average)
136	Wis HBR 72	USA	1.0
1049	AFJ 29	France	1.0
1055	Valliant	France	1.2
1054	NAC 6S	France	1.3
1051B	NDM 14	France	1.5
138	GN *1 Sel 27	USA	1.6
2702/2	Component of farmer's mixture	Rwanda	1.6
208	Pajuro	Peru	1.7
29	Mezcla de 6 lineas (Urobonobono)	Burundi	1.9
938	PI 150414	El Salvador	2.0
1004	GLP-X92	Kenya	2.0
173	Gloriabamba	Mexico	2.1
137	Jules	USA	2.5
263	Poroto	Peru	2.6

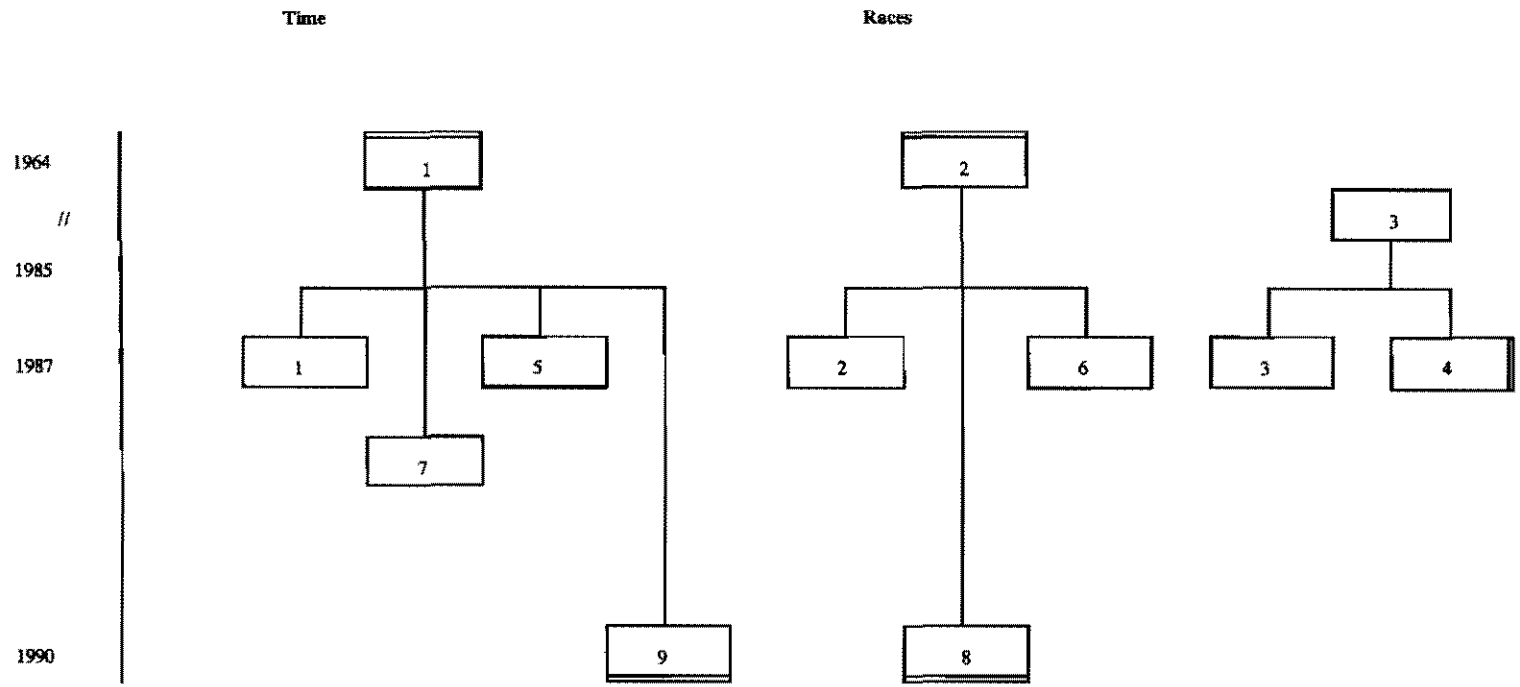

Fig. 1. Chronological order of identification of races of *Pseudomonas syringae* pv. *phaseolicola*.

HALO BLIGHT RESISTANT VARIETIES

Veronique Schmit

Programme Haricot, ISABU, BP 795, Bujumbura, Burundi

ABSTRACT

In order to introduce resistance to halo blight mainly into the cultivar Doré de Kirundo, a total of 3096 crosses have been made at the ISABU station of Moso during the years 1990-1991, 1991-1992 and 1992-1993, and 475 hybrid pods have been harvested. The mean success rate was 15,3%, best in the third year (28,2%); success in the two years before was respectively, 5% and 7,1%. Two backcrosses were made with the recurrent parent Doré de Kirundo in order to restore the interesting characteristics of that variety which is very much appreciated by farmers. The F1 hybrids are multiplied in Moso.

Segregating populations, either created in Burundi or introduced from CIAT, have been evaluated mainly in Gisozi, although some evaluation with inoculation has taken place in Moso in the second rainy season of 1993. As the selection pressure is often too low, the material has to be harvested in bulk. Therefore, during the second season of 1992-1993, artificial inoculation was done with a local strain of halo blight, on F2 populations in Moso, and on F2, F3, F4, F5, F7, BC2F2 and BC1F4 in Gisozi.

At Moso, that technique allowed the selection of 40 symptomless plants as well as a resistant population of Doré de Kirundo x Aroana in F2.

At Gisozi, the individuals which were selected for their high resistance to halo blight included 26 F2, 12 F3, 14 F4, 34 F5, 39 BC2F2, 66 BC1F3 and 6 BC1F4. Out of 33 F7 populations, 16 have been selected. The evaluations made in Gisozi also confirmed the high degree of resistance to halo blight which was detected in the Doré de Kirundo x Aroana F2 population.

In 1993-1994, the material could only be multiplied and bulk harvested in Gisozi during the two consecutive rainy seasons, and in Moso during the second rainy season.

INTRODUCTION

Since 1990, the Bean Programme of ISABU has been in charge of the sub-project "Halo blight resistant varieties". Halo blight, caused by *Pseudomonas syringae* pv. *phaseolicola*, is important in Rwanda and Zaïre but is not the most devastating disease in Burundi. Nevertheless, the risks must not be neglected in medium and high altitude regions where low temperatures during the night create favourable conditions for the development of the disease. It can also be a problem in seed multiplication fields. In Burundi, the cultivar Doré de Kirundo, which was released in 1984 and became very popular among farmers, was withdrawn in 1988 because of its high susceptibility to the disease. Genes for resistance to halo blight have been identified in other local cultivars and can be introduced through hybridization to susceptible traditional or released cultivars.

The main objective of the sub-project is to introduce resistance to halo blight in commercial varieties of the region.

MATERIALS AND METHODS

Materials

Doré de Kirundo has been used as recurrent parent. Initially the varieties Calima, Urubonobono, A 410, A 321, PVA 779, HM 21-7, H 75, Aroana, and SM 1197 were used as donor parents. HM 21-7 is not highly resistant to halo blight and was dropped as donor since the second rainy season of 1993. On the other hand, A 321, A 410 and Aroana being susceptible to black root, have been eliminated from the crossing block during that same season. Doré de Kirundo is used as the susceptible check in the segregating nurseries and A 321 is the resistant check.

Methodology

The main activities of the sub-project are:

- hybridization at the ISABU station of Moso. In the crossing block, varieties are planted every 15 days in order to have them flower simultaneously. The row and plants are separated by distances of 1 m and 20 cm, respectively. An insecticide (dimethoate) is sprayed at 2-3 weekly intervals during the vegetative and reproductive stages. Crosses are made on floral buds without emasculation just before opening.

- F1 hybrid multiplication and checking whether the F1s are hybrids at Moso. Seeds from the same pod are planted in one row, with distances of 1 m between the rows and 10 cm between the plants.

- Carrying backcrosses on Doré de Kirundo (recurrent parent), in order to recover the interesting characteristics of that variety as well as to keep resistance to halo blight. However, only two backcrosses were made in 1990-1991 and in 1991-1992.

- Evaluation and individual selection at Moso and Gisozi. Until the first rainy season of 1993, evaluation was carried out under natural selection pressure. Because of low disease incidence, even at Gisozi where the heavy rains and low temperatures would normally favour disease development, it was decided to inoculate the different progenies. The inoculation is carried out at the primary leaves stage, some 10 to 15 days after planting, by cutting the tips of the primary leaves with scissors which have been immersed in a bacterial suspension containing about 10^8 bacteria/ml. The first evaluation is made about 10 to 14 days after inoculation. A second evaluation is made at the pod filling stage. The technique has been used with success at Moso and Gisozi in 1993B. The evaluation is based on the scale of severity and intensity established at CIAT by van Schoonhoven and Pastor-Corrales (1992). As for the progenies evaluated under natural selection pressure, either the populations were bulk harvested when the pressure was too low, or the single pod descent method was used.

The activities are carried out during both rainy seasons: the first rainy season, season A,

from October to January (e.g. 1993A: from October 1992 to January 1993), and the second rainy season, season B, from February to May (e.g. 1993B: from February 1993 to May 1993).

RESULTS

Crossing programme

From 1991A until 1993B, a total of 3096 crosses were made and 475 hybrid pods were obtained, with a mean success rate of 15,3 %. The results are presented in Tables 1 and 2, and show that the success rate was better in 1993, because the hybridization technique was mastered better.

The first backcrosses were carried out in 1991B on the F1s obtained in 1991A, and also in 1992A on the F1 obtained in 1991B. A total of 76 F1 families were involved and 722 backcrosses were made, with a success rate of 12,6%.

The second backcrosses were carried out in 1992A on the BC1F1 hybrids obtained in 1991B, and in 1992B on the BC1F1 hybrids obtained in 1992A. Seven hundred and twenty backcrosses were carried out on 74 BC1F1 families, with a success rate of 21,1%.

Evaluation and selection

The evaluation of resistance to halo blight began in 1992A with 18 F4 populations which came as F3s from CIAT and were multiplied in Moso in 1991B. Since the selection pressure was not severe, the single pod descent method was used at harvest and the F5 progeny was planted in 1992B at Gisozi. Within each population, the four best resistant plants were selected and the rest of each population was harvested as a bulk. The parent combinations of the selected individuals were the following: AND 303 x PVA 1320, PVA 15 x PVA 1320, PVA 1438 x PVA 1320, PVA 773 x PVA 1320, Doré de Kirundo x A 182, Nain de Kyondo x A 410, Doré de Kirundo x A 410, G 20523 x A 410, G 20523 x G 4450.

Twelve F2 populations from CIAT were also planted in Gisozi in 1992A. After being multiplied, an individual selection of 50 plants was carried out in the F3 in 1992B. In 1991-1992, no hybrid population from the crosses made in Moso was evaluated in Gisozi because those populations were used in backcrosses in Moso. The selection of the materials created at ISABU began in 1993A.

At the Moso station, the following materials were multiplied in 1993A: 40 F1 populations, 39 F2 populations, 43 BC1F2 populations and 82 BC2F1 populations.

At Gisozi, the materials planted in 1993A included 29 F3 populations, 26 BC1F3 populations, 65 F4 populations and 34 F6 populations of bush and semi-climbing beans, and 6 F4 populations of climbing beans. Because of the low selection pressure of halo blight, those materials were bulk harvested and planted again in 1993B.

During 1993B, 19 F2 populations were inoculated tentatively in Moso. The symptoms

appeared 8 days after inoculation and an individual selection was carried out. Within a total of 1860 plants evaluated, 40 symptomless plants were selected: 25 plants among the crosses Doré de Kirundo x H 75 and 15 plants among the crosses Doré de Kirundo x HM 21-7, as well as one population from the cross Doré de Kirundo x Aroana. On the other hand, the following materials were multiplied in Moso: 269 F1 and 46 BC1F1, 37 BC2F2, 23 BC1F3 and 38 F3 populations. Those materials were bulk harvested.

During 1993B at Gisozi, the families included F2, BC2F2, BC1F3, F3, BC1F4, F4, F5 and F7 populations. The F7 came from crosses made at CIAT (Colombia). The results of the individual selections after inoculation are presented in Tables 3 and 4. In F7, 16 populations were selected out of 33 populations.

The results (Table 3) show that HM 21-7 is not highly resistant to halo blight and therefore very few highly resistant hybrids were obtained: between 0 and 0,9 % depending on the progeny, with a mean of 0,5% for all progenies.

The evaluation of the F2 populations carried out at Gisozi confirmed the high degree of halo blight resistance in the Doré de Kirundo x Aroana population.

The young plants were highly affected by the inoculation and frequently died. The number of dead plants were counted at flowering time. Other factors (including bean fly, root rots) may have contributed to the death of the plants as was the case in the BC1F4 progeny, where the death rate is 46,6% for the resistant check A 321. These data, as well as the halo blight scoring at R8, are presented in Table 4.

The susceptible check, Doré de Kirundo, was heavily affected by the inoculation: most plants were already dead at flowering time, while the resistant check A 321 was symptomless or expressed only very light symptoms. These data show the low level of resistance of the BC2F2, with scores of 6,3 to 7,6, with a mean of 7,2.

As for the crosses with A 321, the generally low resistance can be explained by the susceptibility of the halo blight resistant plants to black root, which were eliminated during the multiplication of the early progenies in Moso. As shown by Teverson (1991), the resistance gene R3 to halo blight is identical with the resistant gene I to BCMV. This would explain why A 321, A 410 and Aroana which have the I gene, are also resistant to the race 3 of halo blight.

Because of the political problems during the year 1993-1994, the materials were only multiplied in Gisozi in 1994A, and in Moso in 1994B. In Gisozi, in 1994B the materials are being evaluated under natural selection pressure. The populations which were planted in Gisozi and Moso during 1993-1994 are:

a. in Gisozi, in 1994A: F2, F3, F4, F5, BC1F2, BC1F4, BC1F5, and advanced lines (F6 and F8); CIAT's International Bean Nursery for Halo Blight in 1994B: F3, F4, F5, BC1F3, BC1F5, and advanced lines (F6, F7, F9, BC1F6); CIAT's IBNHB.

b. in Moso, in 1994B: F2, F3, BC1F2, BC2F3, BC1F4.

CONCLUSIONS AND PERSPECTIVES

Individuals or populations with a high level of resistance to halo blight have been selected within the materials created in Burundi and introduced from CIAT. The best resistant lines among the advanced lines will be introduced for varietal selection in the near future.

In order to avoid damage due to bean fly infestation, the treatment of seeds with endosulfan (2g/per kg seeds) before planting is recommended.

Inoculation in the field in Gisozi is recommended in order to obtain severe and uniform selection pressure. Inoculation with a mixture of local races and evaluation of advanced lines under controlled conditions are planned in collaboration with the Phytopathology Programme of ISABU. That method would allow the Bean Programme to confirm the resistance of the materials which were selected in the field.

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van Schoonhoven, A. and Pastor-Corrales, M.A. (1992). Systeme Standard pour l'Evaluation du Germoplasme du Haricot. CIAT, Cali, Colombia, 49 pp.

Table 1. Number of crosses made at the ISABU station of Moso between 1991A and 1993B, number of hybrid pods harvested and success rate.

Season	Number of crosses	Number of hybrid pods	Success rate (%)
91A	553	16	2,9
91B	180	21	11,7
92A	550	39	7,1
92B	540	40	7,4
93A	778	227	29,2
93B	495	132	27
TOTAL	3096	475	15,3

Table 2. Parent combinations, number of crosses made, number of hybrid pods harvested and success rate during 1990-91, 1991-92 and 1992-93.

Parents		Number of crosses	Number of hybrid pods	Success rate (%)
Recurrent	Donor			
Doré de Kirundo	A410	322	28	8,7
	A321	317	45	14,2
	Urubonobono	403	51	12,7
	Calima	578	122	21,1
	PVA779	361	75	20,8
	H75	225	30	12,8
	Aroana	285	18	6,3
	HM21-7	333	39	11,7
	SM1197	98	31	31,6
	77/2	8	5	62,5
HM21-7	Calima	52	17	32,7
	PVA779	41	2	4,9
	SM1197	73	12	16,4
TOTAL		3096	475	15,3

Table 3. Number of plants evaluated (eval.) and number of individual plants selected (select.) for halo blight resistance at Gisozi, Burundi, 1993B.

Parents	No. plants	F2	F3	F4	F5	BC2 F2	BC1 F3	BC1 F4	Total
D x C	Eval.	164	172	1108	146	1988	1301	477	5356
	Select	3	3	8	6	22	31	2	75
	(%)	1.8	1.7	0.7	4.1	1.1	2.4	0.4	1.4
D x A	Eval.	268	-	-	-	-	-	-	268
	Select	17	-	-	-	-	-	-	17
	(%)	6.3	-	-	-	-	-	-	6.3
D x H 75	Eval.	251	28	116	-	395	333	-	1123
	Select	5	0	2	-	4	3	-	14
	(%)	2.0	0	1.7	-	1.0	0.9	-	1.2
D x HM 21-7	Eval.	487	48	541	108	1800	2467	269	5720
	Select	1	0	3	1	11	13	2	31
	(%)	0.2	0	0.6	0.9	0.6	0.5	0.7	0.5
D x PV A779	Eval.	51	93	141	27	458	272	59	1101
	Select	0	6	0	0	2	15	0	23
	(%)	0	6.5	0	0	0.4	5.5	0	2.1
D x U	Eval.	-	25	86	56	98	329	164	758
	Select	-	3	1	2	0	4	0	10
	(%)	-	12	1.2	3.6	0	1.2	0	1.3
D x A410	Eval.	-	16	-	56	59	-	161	292
	Select	-	0	-	2	0	-	1	3
	(%)	-	0	-	3.6	0	-	0.6	1.0

D x A321	Eval.	-	-	-	29	-	-	44	73
	Select	-	-	-	1	-	-	1	2
	(%)	-	-	-	3.4	-	-	2.3	2.7
Various from CIAT	Eval.	-	-	-	1230	-	-	-	1230
	Select	-	-	-	22	-	-	-	22
	(%)	-	-	-	1.8	-	-	-	1.8
Total	Eval.	1221	382	1992	1652	4798	4702	1174	15921
	Select	26	12	14	34	39	66	6	197
	(%)	2.1	3.1	0.7	2.1	0.8	1.5	0.5	1.2

Table 4. Percentage of dead plants in the halo blight nurseries in Gisozi and mean score for halo blight.

Combination	Value*	F2	F3	F4	F5	BC2F2	BC1F3	BC1F4
A 321 (check)	% dp	9,6	4,9	10,6	28,1 2	10,8	10,5	46,6
	Score	2,5	2,3	2,5		2,2	2,5	2
Doré de Kirundo (check)	% dp	90,3	82,0	83,5	92,3	76,3	68,1	91,0
	Score	7,5	6,3	8	7	8,1	8	6
Doré[1] x Calima	% dp	68,2	65,2	78,2	84,2	44,8	37,6	91,8
	Score	4	3,7	5,4	3,4	6,7	4,5	4,6
Doré x Aroana	% dp	22, 4						
	Score	3,5						
Doré x H 75	% dp	55,6	50,0	80,2		31,2	54,0	
	Score	4	5	3		6,3	5	
Doré x HM 21-7	% dp	77,3	68,7	87,7	86,4	55,9	44,6	95,7
	Score	5	4	5,9	5,7	7	5,2	6
Doré x PVA 779	% dp	39,2	70,3	76,4	81,5	63,2	30,9	89,8
	Score	5	5	6	4	7,6	3	6
Doré x A 410	% dp		75,0		73,2	41,7		90,2
	Score		4		6	7,5		4
Doré x Urubonobon	% dp		56,0	54,9	57,0	53,3	32,6	92,0
	Score		6	5	3,5	7,6	4,5	4,7
Doré x A 321	% dp				79,3			93,2
	Score				8			6
Mean	% dp	52,5	64,2	75,5	76,9	48,4	39,9	92,1
	Score	4,3	4,6	5,1	5,1	7,2	4,4	5,2

1 Doré = Doré de Kirundo; * % dp = percentage of dead plants; Score = score for halo blight

STUDIES OF COMMON BACTERIAL BLIGHT OF BEANS IN UGANDA

Fina Opio

Namulonge Research Institute, P.O. Box 7084, Kampala, Uganda

INTRODUCTION

Common bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *phaseoli* (XCP), is the second most prevalent disease of beans *(Phaseolus vulgaris)* in Uganda; the first is angular leaf spot (Sengooba, 1985). The disease is most important in the lowland, high rainfall areas. It is also in these areas where common bean is most important in the diet as a protein supplement to the starchy staple, Matoke.

Research work on CBB in Uganda, which was initiated in 1987, has been carried out cooperatively by a breeder and a pathologist. Work of the pathologist (1990-1992) is presented here. Objectives were the following :

1. To study the nature and extent of variation in pathogenicity of *Xanthomonas campestris* pv. *phaseoli*.

2. To determine the ability of XCP to survive on weeds, non-host crops and in soil.

3. To determine whether there are differences among genotypes in their ability to transmit CBB with a view to selecting for low seed transmission efficiency.

4. To assess crop loss associated with CBB.

5. To form an Eastern African common bacterial blight nursery.

Reviews of the work carried out toward each objective are given separately in the text that follows. Fuller details have been published elsewhere (Opio et al., 1992, 1993, 1994, 1996).

VARIATION IN PATHOGENICITY

Naturally infected leaves, stems and pods of *P. vulgaris* that bore characteristic symptoms of CBB were collected from most bean growing areas of Uganda, supplemented by collections made in Ethiopia, Rwanda and Tanzania. Isolations were made on yeast dextrose carbonate agar (YDCA) and MXP (Claflin et al., 1987) a semi selective medium for XCP. Colonies of MXP that were yellow and hydrolysed starch were selected and considered XCP. Single colonies were selected for purification and subculturing and were confirmed to be XCP by pathogenicity tests. Pathogenicity tests were made by inoculating the first trifoliolate leaves of cultivar Kanyebwa with a bacterial suspension prepared from 48 hr old cultures grown on YDCA. The concentration of the suspension was adjusted to optical density 0.5 at 640hm to contain 5 x 10^8 colony forming units (cfu) per millilitre. This was diluted to 10^7 cfu/ml before inoculating the bean leaves by the razor blade method (Pastor-Corrales et al.,

1981).

In studying the nature and extent of the variation in XCP isolates, reactions of different hosts (*P. vulgaris* and *P. acutifolius*), phage-typing and growth characteristics on different media were used. Twenty genotypes of *P. vulgaris* and eight of *P. acutifolius* were used. Out of 98 isolates collected, 30 were selected for these studies. The selection was made on the bases of their contrasting origins.

The results revealed the existence of two types of pathogenicity. There were differences in aggressiveness of the isolates on the 20 *P. vulgaris* genotypes (Table 1) indicating a quantitative relationship, and no host specificity. On *P. acutifolius*, the 30 isolates tested interacted differentially with tepary bean genotypes and eight distinct physiologic races of XCP were defined (Table 2), suggesting an underlying gene-for-gene relationship. Although variation was found in parameters including phage-type, the production of brown pigment and in vitro growth rate, there was little consistent relationship between these characterictics and pathogenic variation.

SURVIVAL

The role of infested soil, infested bean debris, weeds and non-host crops in the survival of XCP was investigated between April,1990 and October,1992, at two sites (Kawanda and Bukalasa) in Uganda.

For soil, fields were first sown with beans. After harvest, the field was then subdivided into different plots. The treatments applied to the different plots was varying the time when beans was sown again in that field. A range of 1 - 4 seasons was used (Table 3).

Infested bean debris was sampled from beans during the cropping season. Then the debris were left standing in the field. Sampling continued weekly. Soil samples were taken at the pod filling stage when CBB was severe in the field, at harvest time, and thereafter at monthly intervals throughout the experimental period. The soil samples were randomly taken from different parts of the field. The bacteria were recovered from soil samples by serial dilutions in 0.01m phosphate buffer (pH 7.2) and plating on YDCA and MXP.

The infected debris left standing in the field were sampled weekly for XCP, from immediately after harvest up to the next planting. Weeds and non-host crops within and around severely infected debris were also sampled. XCP was extracted by grinding of the material in 0.01m phosphate buffer (pH 7.2), serial dilution and plating on MXP and YDCA.

XCP survived on infected debris, in soil and on weeds for several months in the absence of a bean crop. Crop rotation resulted in a decrease in the XCP population in the absence of beans. The population dynamics of XCP appeared to depend on the movement of this bacterium from infected leaves (or/and infested debris) to the soil. This is because the XCP population was highest at physiological maturity of the host when CBB was most severe and immediately after harvest when most of the infected crop residue had dropped to the ground. The XCP population declined thereafter. This decline may have been due to decomposition of infected debris, suggesting that XCP cannot survive as a free bacterium in the soil in the absence of debris, confirming previous findings (Weller and Saettler, 1980.)

XCP was isolated from the following weeds: *Senna hirsuta Aeschynomene sensitiva, Ageratum conyzoides, Siegesbeckia orientalis, Bidens pilosa, Sonchus oleraceus, Amaranthus hybridus, Amaranthus thunbergii, Commelina benghalensis, Oxalis latifolia, Digitaria scalarum, Galinsoga parviflora, Sorghum verticilliflorum, Erucastrum arabicum,* and *Portulaca oleracea*. The non-host crops include *Zea mays* and *Glycine max*. All the weeds and non hosts were symptomless carriers of XCP. XCP was recovered from four weeds 3-4 months after harvest of the bean crop; and at six months from *Senna hirsuta*. This indicates that, in absence of the bean crop, *Senna hirsuta* would harbour epiphytic XCP even in long dry seasons which in most of Uganda is less than 5 months. Hence it bridges the gap between bean crops and perpetuates XCP inoculum. XCP could not be recovered from soil nor from any weed species after seven months.

This means that, under Uganda conditions, effective rotation to control CBB would involve a minimum of one year (i.e. two cropping seasons).

VARIATION IN SEED TRANSMISSION

Ten elite bean lines from breeders in different countries in East Africa were evaluated for their ability to transmit XCP. The lines were : K.20, CAL 96, RWR 136 and MCM 5001 from Uganda; 2395-B-b-1 and 2439-B-b-1 from Tanzania; Awash 1, Roba and ICA Linea 64 from Ethiopia; and GLP 585 from Kenya. Kanyebwa and Kasuka Nywele were susceptible checks and XAN 112 was a resistant check. Seed of each line was increased from a single seed in the screen house. The seeds were all inoculated by soaking in a bacterial suspension for four hours before planting.

The inoculum concentration used was 5 x 107 cfu/millilitre. The isolates used were 1068, 1073, 1010, and 1029 (see Table 1). The design was a split plot with isolates in main plots and genotypes in subplots.

Records were taken on incidence of CBB as percentage of infected seedlings at 10, 18, 35 and 56 days after sowing. Disease transmission efficiency (TE) was calculated as

$$TE = \frac{NDS \times 100}{TGS}$$

where NDS is the number of diseased seedlings at 10 days after sowing and TGS is the total number of seedlings that germinated.

The results showed significant differences ($P \leq 0.05$) between genotypes in their ability to transmit XCP from seed to seedling. K.20 had the highest infected seedlings at 10 and 18 days after sowing. These were followed by RWR 136, 2395-B-b-1, GLP 585, 2439-B-b-1, Awash 1, CAL 96, Roba and ICA Linea 64, respectively. All seedlings from seeds of MCM 5001 and XAN 112 were healthy for all treatments. These results indicate that these two genotypes had very low seed transmission of the disease and could be used by breeders for developing bean varieties that are resistant to CBB and with low transmission efficiency of the disease. A significant interaction between isolates and genotypes was obtained in this study, indicating that the rate of seed transmission in beans depends both on XCP isolate and host genotype.

ASSESSMENT OF YIELD LOSS

The effect of common bacterial blight on seed yield of common bean was determined in field experiments during three seasons at Bukalasa, Uganda. Different levels of disease were generated using chemical treatments, three bean genotypes differing in susceptibility, and three seed symptom categories. The chemicals were cupric carbonate, cupric sulphate and water, and the bean genotypes were Kanyebwa (susceptible), K.20 (intermediate) and XAN 112 (resistant). The seed categories were "severely diseased", "slightly diseased" and "symptomless".

The experimental design was a split plot, with chemicals as main plots and genotypes x categories in subplots. Severity and incidence were assessed from pre-flowering (R5) to physiological maturity, at weekly intervals. A 1-9 scale was used for assessing severity.

Total and clean seed yields were taken at harvest. Regression analysis to evaluate the relationship between disease severity and yield employed the SPSS/PC V.3 programme. Percentage reduction in yield for each season was calculated using the intercepts of yield loss models developed. The data were expressed as percentage of the minimum attainable yield (Bowen et al., 1991) using the following formula.

$$\text{Percentage YL} = \frac{\text{YR x D}}{\text{MAY}} \times 100$$

where YL is the yield loss, YR is the reduction in yield induced by one unit increase in disease severity, D is the disease severity, and MAY is the maximum attainable yield (the intercept) for each season.

Yield losses in beans associated with CBB varied with susceptibility of the genotype and stage of growth at the time of infection. Losses in seed yield ranged from 26.6 to 61.7% in the susceptible genotype Kanyebwa, and from 6.2 to 7.8% in the tolerant genotype K 20. XAN 112 suffered no significant yield loss at any stage of growth.

COMMON BACTERIAL BLIGHT REGIONAL NURSERY

A regional common bacterial blight nursery was put together in Uganda. This nursery is made up of materials resistant to CBB in Eastern Africa. This nursery now has 50 entries. There are a few susceptible and intermediate lines for comparative purposes.

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Table 1. Leaf lesion size (mm) in 20 genotypes of *Phaseolus vulgaris* 14 days after inoculation with 30 isolates of *Xanthomonas campestris* pv. *phaseoli*.

ISO-LATE	HOST GENOTYPES*																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	Mean
1068	4.8	6.3	5.5	5.4	3.8	3.6	2.5	2.8	5.8	5.9	3.0	3.5	1.9	3.0	5.8	4.2	0.8	3.1	0.2	0.7	3.6
1010	6.4	5.6	5.3	3.2	3.4	3.2	2.3	2.3	2.6	2.4	1.0	0.7	2.8	1.2	0.2	0.5	0.7	0.6	0.9	0.1	2.7
1073	6.1	4.8	3.5	4.7	2.5	3.2	1.3	3.7	3.8	2.0	1.8	2.8	2.3	1.8	0.8	2.7	2.3	0.8	1.3	0.5	2.6
1029	6.3	5.8	6.3	6.0	2.8	5.3	4.7	2.7	0.5	0.5	0.9	0.7	2.3	0.6	2.6	0.7	0.6	0.4	0.4	0.4	2.5
1067	4.9	3.0	6.1	3.2	5.1	2.2	2.1	3.0	3.0	1.0	3.5	0.6	0.2	4.2	0.4	3.0	2.1	0.3	0.3	0.3	2.4
1011	5.1	5.4	6.8	4.0	4.2	3.3	2.7	2.7	1.3	2.3	0.6	2.8	0.8	1.1	0.5	0.7	1.0	1.0	0.5	0.5	2.4
1038	4.3	5.3	5.8	1.3	3.9	2.6	2.6	2.6	0.4	2.5	0.7	0.5	0.9	0.5	1.9	0.5	1.1	0.9	0.7	0.3	2.0
1069	5.3	4.0	4.0	6.3	2.6	3.2	2.4	2.3	0.3	0.7	1.0	1.3	0.8	0.5	1.5	0.4	0.8	0.8	0.6	0.4	2.0
1032	6.3	4.4	3.7	5.1	2.3	1.0	1.3	2.4	3.0	1.0	0.6	0.5	2.3	0.5	0.5	1.0	1.0	0.6	0.6	0.4	1.9
1099	5.8	5.6	6.6	5.8	2.3	0.4	2.7	1.1	0.7	0.5	2.7	0.5	0.4	0.7	0.6	0.3	0.5	0.5	0.2	0.5	1.9
1046	5.4	5.4	6.2	5.2	1.3	0.7	0.9	0.7	0.9	0.9	2.1	2.6	0.4	0.6	0.9	0.5	1.0	1.1	0.5	0.2	1.9
1005	5.2	3.3	3.4	2.4	2.6	3.4	0.8	2.3	3.0	2.7	1.9	0.6	0.8	0.5	0.5	0.5	0.6	0.4	0.5	0.1	1.8
1030	5.3	6.4	4.3	5.7	3.4	0.9	0.5	0.5	0.9	0.7	2.0	0.5	0.5	0.5	0.3	0.3	1.0	0.5	0.5	0.3	1.8
1112	5.3	5.2	5.4	3.7	2.4	1.4	1.2	1.4	1.1	1.1	1.1	1.1	0.5	1.0	1.1	0.5	1.3	0.8	0.5	0.5	1.8
1048	4.8	3.7	4.3	2.6	2.3	0.5	2.3	1.8	2.2	0.5	1.7	0.5	0.7	1.1	1.9	1.3	1.0	1.0	0.2	0.4	1.7
1066	5.2	4.8	6.2	6.0	1.3	0.6	1.0	2.0	1.0	0.3	0.3	0.5	1.1	0.4	0.3	0.4	0.5	0.6	0.4	0.3	1.7
1096	3.9	3.8	3.9	3.9	2.7	2.7	0.5	0.5	0.4	0.7	0.6	0.5	0.3	0.6	0.5	0.4	0.6	0.5	1.0	0.3	1.4

1007	4.8	5.8	5.2	3.5	2.2	0.7	0.6	0.9	0.1	0.6	0.2	0.2	0.1	0.2	0.2	0.3	0.2	0.1	0.1	0.3	1.3
1024	5.9	3.2	3.8	4.9	2.3	0.5	0.5	0.5	0.3	0.5	0.5	0.5	0.5	0.7	0.5	0.3	0.5	0.4	0.3	0.3	1.3
1002	2.8	3.8	3.3	1.9	3.6	2.3	1.3	1.2	0.2	2.0	1.2	0.5	0.4	0.5	0.1	0.4	0.4	0.6	0.6	0.4	1.3
1034	5.9	3.5	3.8	4.6	2.3	0.5	0.5	0.5	0.3	0.5	0.5	0.5	0.4	0.4	0.5	0.3	0.5	0.4	0.3	0.3	1.3
1090	3.2	2.9	4.0	4.0	2.6	1.3	0.5	0.1	0.3	0.6	0.7	0.6	0.2	0.5	0.5	0.3	0.5	0.5	0.5	0.2	1.2
1083	3.3	2.9	3.4	4.3	3.0	0.8	0.5	0.5	0.2	0.7	0.5	0.4	0.5	0.5	0.3	0.3	0.6	0.2	0.2	0.2	1.2
1065	4.0	2.3	2.0	2.0	1.3	1.0	0.3	0.3	1.0	0.5	0.5	1.3	2.3	1.0	0.7	0.6	0.6	0.3	0.2	0.3	1.1
1042	2.0	6.2	1.3	5.0	0.6	0.4	0.7	0.5	0.2	1.2	0.7	0.2	0.2	0.7	0.5	0.4	0.6	0.5	0.5	0.1	1.1
1043	2.3	2.3	2.4	0.8	1.4	0.6	1.1	0.8	0.4	0.6	0.6	0.7	0.5	0.5	0.1	0.4	0.7	0.5	0.5	0.4	0.9
1040	0.9	5.5	2.3	1.2	1.3	0.6	1.1	0.2	1.3	0.2	0.2	0.5	0.2	0.2	0.2	0.7	0.4	0.2	0.1	0.1	0.9
1026	0.6	0.5	2.0	0.5	0.5	2.4	0.7	3.1	1.8	0.5	0.0	0.0	0.8	0.8	0.0	0.0	0.4	0.0	0.0	0.0	0.7
1033	2.5	3.0	3.6	0.9	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
1074	1.8	2.7	2.8	1.8	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5

SE +/- 0.04
CV % 23.9

*1= Red Wolaita, 2=ZPv 292, 3= Kanyebwa, 4= Kasuka nywele, 5= K20, 6= Tara, 7= PI 207262, 8= G 9857, 9= G6772, 10= Jules, 11= BAC 6, 12= BAC 5, 13= XAN 112, 14=IAPAR 14, 15= ICA Linea 64, 16= MX 259-8, 17= IAPAR 16, 18= XAN 169, 19= Amanda 75-20, 20= XAN 159.

Table 2. Interaction between 30 isolates of *Xanthomonas campestris* pv. *phaseoli* and eight genotypes of tepary bean, *P. acutifolius* and definition of XCP races.

Isolate No	T1[a]	T5	T8b	T19	T21	T22	PI 321638
Race 1 1010	-	-	-	-	-	-	+
Race 2 1005 1032 1069	-	-	-	+	-	-	-
Race 3 1034	+	-	-	-	-	+	-
Race 4 1038 1067	-	-	+	+	-	-	+
Race 5 1024 1048	-	-	-	+	-	-	+
Race 6 1065	-	-	-	+	+	-	+
Race 7 1068 1083	-	-	+	+	-	-	-

Race 8	-	-	-	-	-	-	-
1011							
1046[b]							

+, compatible (susceptible) reaction.
- , incompatible (resistant) reaction.
[a] Responses of L 242-45 are similar to T1 so are omitted
[b] Remainder of isolates

Table 3: The sequence of crops used in determining the survival period of *Xanthomonas campestris* pv. *phaseoli* in the field.

Treatment		1990[a]A	1990[b]B	1991A[a]	1991B[b]	1992A	1992B
Experiment 1	1	Bean	Bean	Bean	Bean	Bean	Bean
	2	Bean	Maize	Bean	Maize	Bean	Maize
	3	Bean	Maize	Soybean	Bean	Maize	Soybean
	4	Bean	Maize	Soybean	Maize	Bean	Maize
	5	Bean	Fallow	Fallow	Bean	Bean	Maize
Experiment 2							
	1	Fallow	Bean	Bean	Bean	Bean	Bean
	2	Fallow	Bean	Maize	Bean	Maize	Bean
	3	Fallow	Bean	Maize	Soybean	Bean	Maize
	4	Fallow	Bean	Maize	Soybean	Maize	Bean
	5	Fallow	Bean	Fallow	Fallow	Fallow	Bean

[a] A refers to the first rainy season which is from March to June.

[b] B refers to the second rainy season which is from August to December.

RECENT RESEARCH ON COMMON BACTERIAL BLIGHT OF BEANS AT CIAT HEADQUARTERS

M. A. Pastor-Corrales,
Centro Internacional de Agricultura Tropical,
A. A. 6713, Cali, Colombia.

ABSTRACT

Common bacterial blight (CBB), an important bacterial disease of common beans in both Latin America and Africa, is caused by Xanthomonas campestris pv. phaseoli (XCP) and Xanthomonas campestris pv. phaseoli var. fuscans (XCPF). The two causal agents produce indistinguishable symptoms although XCPF is regarded as more virulent than XCP. XCPF produces a dark brown melanin pigment on tyrosine containing media but it is time consuming to distinguish the two by conventional methods. Non-pathogenic xanthomonads associated with bean plants and debris can also be identified mistakenly as XCP and XCPF on semi selective media. Recent work on CBB at CIAT, Colombia has covered the following areas: use of two medium repetitive DNA probes in the quick identification of XCP and XCPF for diagnostic purposes. The same probes have also been used to study the genetic diversity of Latin American isolates. Evaluation of Phaseolus acutifolius germplasm available in CIATs' Genetic Resources Unit has been made with the objective of identifying new sources of resistance and to verify resistance of the species.

It was shown that the two medium repetitive probes (P2 and P7) can be used to identify and unequivocally distinguish between XCP and XCPF, making pathogenicity tests unnecessary. The RFLPs patterns generated by the two probes to the restricted DNA of XCP and XCPF were very different for the two CBB pathogens. The probes can also be used to rapidly separate the two causal agents of CBB from non-pathogenic xanthomonads usually associated with bean plants or seed.

The main results obtained in genetic diversity studies are that isolates of the two CBB pathogens can be separated into families. Isolates from the same geographical origin (eg country) tend to have very similar RFLP patterns. It was also interesting to note that there were no XCPF isolates and/or families from Costa Rica or the Caribbean countries.
This implies that isolates of XCP and XCPF of different geographical origin are not clonal as previously thought. Pathogenicity tests conducted with selected isolates of both pathogens confirm that they also have differences in pathogenicity.

Evaluation of 303 accession of P. acutifolius has been made with artificial inoculation under field conditions. Out of 147 cultivated accessions, 90% were resistant while 85 - 89 % of the wild accessions were susceptible. However, some of the cultivated accessions were more susceptible than the common bean susceptible check. The inference of these results is that during the process of domestication, CBB-susceptible genotypes were either not selected or may have not survived. On the other hand, wild accession have not been subjected to the same selection pressures and their survival did not relay on CBB resistance. CBB resistance in wild accessions might well be a random feature.

DISCUSSION OF SESSION 2

Following paper by Opio

Pastor-Corrales : How do you rate the pathogen in Uganda ?

Opio : *P. vulgaris* cannot detect races in XCP, unlike *P. acutifolius* in which differential interactions between host genotypes and isolates are demonstrable.

Sengooba : I note you have found symptomless weed hosts of XCP. What is their role in transmission ?

Opio : More studies are needed.

Lana : Rain, and perhaps insects, are means of CBB spread. Is that correct ?

Pastor-Corrales : Yes, studies in Latin America show that populations of XCP/XCPF survive permanently in weeds. It is the weather that triggers disease development and spread.

Following paper by Male-Kayiwa

Lana : You said seedlings are more resistant than older plants. Shouldn't it be the other way around ?

Gridley : There is considerable additive gene action.

Opio : Expression of symptoms does depend on the stage of plant development as well as on time of infection.

Pastor-Corrales : Beans are more susceptible to CBB as seedlings as well as at flowering. Primary leaves show strong symptoms but there is no correlation with reactions of first or second trifoliolates.

Buruchara : Evaluation of both leaves and pods is important.

Teverson : Do you inoculate pods as well, at CIAT ?

Pastor-Corrales : Yes, we do. We use about 20 pods, and that's a lot of work, inoculating between the seeds. Pod age is critical.

Following paper by Pastor-Corrales

Gridley : As regards levels of resistance to CBB, Opio has tested some of the putative resistant materials here in Uganda and found that, in some cases, resistance held and, in others, it did not. How do you explain that ?

Opio : We have already been doing this ! Materials have been received from many parts of Africa including Sudan and Ethiopia. We are ready to distribute nurseries on request. CIAT materials have been exchanged too. Now CIAT headquarters should be asked to send back lines for our nurseries.

Pastor-Corrales : The best source of resistance is derived from *P. vulgaris* x *P. acutifolius*.

Lana : Should BCMV and CBB be considered together in breeding, since they appear together in the field ?

Pastor-Corrales : Certainly ! You can get the next CBB nurseries from Cali in Dec'94 in time for next season.

Buruchara : Do we need to consider bacterial diseases other than CBB and halo blight ?

Opio : We have found brown spot (*Pseudomonas syringae* pv. *syringae*) which we believe is a minor pathogen. We have isolates.

PART 2:

Planning of Future Research Activities on Bacterial and Viral Bean Diseases in Africa.

Reports from the Working Group Sessions.

R. Buruchara,
CIAT,
P. O. Box 6247, Kampala, Uganda.

Introduction:

The primary objective of this meeting was to review past and current research on bacterial and viral bean diseases, identify future strategies, and prioritize research and non-research activities or themes that could be the basis for developing regional sub-projects. The framework developed was meant to serve as a guide for Steering Committees of the bean networks for orienting, approving or rejecting sub-projects.

Planning Methodology: PPO.

A modification of the Project Planning by Objective (PPO) method applied in the Pathology Working Group Meeting on fungal bean diseases in 1992 and described by Scheidegger and Buruchara (1992) was used. The planning period was 3 years and the areas of intervention were restricted to bacterial and viral diseases in Africa.

Application of PPO in the pan-African meeting on bacterial and viral bean diseases.

Only common bacterial blight (CBB), halo blight (HB) and bean common mosaic virus (BCMV) were considered for detailed discussions. Other bacterial and viral diseases were regarded to be of minor importance to warrant consideration or allocation of research resources at this stage. The same principle and procedure as applied in the 1992 bean fungal meeting, to identify problems, solutions and activities was used. Neither verifiable indicators nor resources necessary for suggested activities were determined. This was because such details were regarded justifiable at sub-project level. But allocation of responsibilities among participating and non-participating institutions, and prioritization at pan-Africa level was done.

Results: Problem Analysis

Results on problem analysis are found in the following 4 pages. The logic and steps used are similar to those described by Scheidegger and Buruchara (1992). The BCMV tree go over two pages.

LIST OF PARTICIPANTS

Dr. Robin Buruchara
CIAT Bean Programme
Kawanda Research Station
P.O. Box 6247
Kampala, Uganda

Dr. Howard Gridley
CIAT Bean Programme
Kawanda Research Station
P.O. Box 6247
Kampala, Uganda

Prof. Allan Femi Lana
Sokoine University of Agriculture
P.O. Box 3062
Morogoro, Tanzania

Ms Beatrice S. Male-Kayiwa
Namulonge Agricultural and Animal Production Research
Institute
P.O. Box 7084
Kampala, Uganda

Mr. Theogene Ntawurutwa
Pathologiste au Programme Haricot
de l' ISAR - Rubona
BP 138
Butare, Rwanda

Dr. Fina Opio
Namulonge Agricultural and Animal Production Research
Institute
P.O. Box 7084
Kampala, Uganda

Dr. M.A. Pastor-Corrales
Bean Programme, CIAT
A.A. 6713, Cali, Colombia

Dr. Veronique Schmit
Programme Haricot
ISABU BP 795
Bujumbura, Burundi

Dr. Theresa Sengooba
Namulonge Agricultural and Animal Production Research Institute
P.O. Box 7084
Kampala, Uganda

Dr. Nicola Spence
Horticulture Research International
Wellesbourne, Warwick
CV35 9EF, U.K.

Dr. Dawn Teverson
Horticulture Research International
Wellesbourne, Warwick
CV35 9EF, U.K.