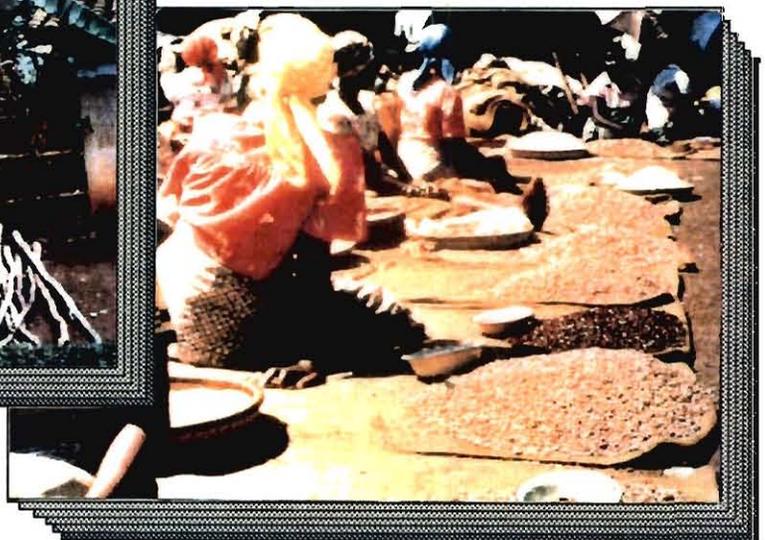


Interspecific Solutions to Intractable Problems of Common Bean



A Proposal for:

Administration Générale de la Coopération au
Développement (AGCD, Belgium)

Submitted by:

CIAT
Centro Internacional de Agricultura Tropical
Cali, Colombia

**Collaborating
Partner:**

Faculté des Sciences Agronomiques de l'Université de
Gembloux (FSAGx)

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INTERSPECIFIC SOLUTIONS TO INTRACTABLE PROBLEMS OF COMMON BEAN

BROADENING THE GENETIC BASE OF COMMON BEAN, *Phaseolus vulgaris*, AND EXPLOITATION OF THE BIODIVERSITY FOUND IN SECONDARY GENE POOLS OF *P. Coccineus* AND *P. polyanthus*, THROUGH GERMPLASM CORE COLLECTIONS AND INTERSPECIFIC HYBRIDIZATIONS

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Acronyms

AGCD	Administration Générale de la Coopération au Développement
BARN	Bean Advanced Research Network
BGMV	Bean golden mosaic virus
BNF	Biological Nitrogen Fixation
BRU	Biotechnology Research Unit (CIAT)
CEC	Commission of European Communities
CIAT	Centro Internacional de Agricultura Tropical
DNA	Deoxyribonucleic acid
FSAGx	Faculté des Sciences Agronomiques de l'Université de Gembloux
GRU	Genetic Resources Unit (CIAT)
PCR	Polychain Reaction
PROFRIJOL	Programa Cooperativo Regional de Frijol para Centro América, México y el Caribe
RAPD	Random amplified polymorphic DNA
RFLP	Restriction fragment length Polymorphism
RIL	Recombinant inbred lines

1.0 Summary

Title:

Broadening the genetic base of common bean, *Phaseolus vulgaris*, and exploitation of the biodiversity found in secondary gene pools of *P. coccineus* and *P. polyanthus*, through germplasm core collections and interspecific hybridizations.

Short title:

Interspecific solutions to intractable problems of common bean.

Objectives:

Improve the productivity and adaptation of common bean in Latin America and Africa through the incorporation of resistance genes to ascochyta blight, bean golden mosaic virus (BGMV), and bean fly, which are highly expressed in some *P. coccineus* and *P. polyanthus* accessions and not in *P. vulgaris*. To preserve and rationally manage the germplasm collections of these secondary gene pools.

Key words:

Biodiversity - genetic resources - *Phaseolus vulgaris*, *P. coccineus*, *P. polyanthus* - common bean- productivity - core collection - interspecific hybridization - breeding - ascochyta blight - BGMV - bean fly.

Abstract:

In the past, a collaborative project between CIAT (Colombia, South America) and the University of Gembloux (Belgium) in the general areas of *Phaseolus* germplasm management, characterization, and breeding was sponsored by the Administration Générale de la Coopération au Développement (AGCD, Belgium). The project was very successful in achieving several objectives in relation to:

- ◆ the management of a worldwide collection of three species: *P. coccineus*, *P. polyanthus*, and *P. lunatus*, including the development of maintenance and seed increase methodologies, and base line evaluations of morphoagronomic and biochemical characters;
- ◆ the creation of interspecific hybrids and the restoration of their fertility;
- ◆ the implementation of a breeding program based on the selection of parental genotypes for disease resistance and good adaptation.

To benefit from the germplasm characterization and pre-breeding work already accomplished, we propose a new research project, which is based on the genetic improvement of common bean by broadening its genetic base through interspecific hybridization with the secondary gene pools of *P. polyanthus* and *P. coccineus*. The major objective of the interspecific hybridizations is to incorporate genes found in the secondary gene pools for high levels of resistance to three major common bean constraints: ascochyta blight, BGMV, and the African Bean fly, for which, in common bean, only low levels of resistance have been found.

Cooperating partners:

The proposed project will be carried out jointly by:

- ◆ The Centro Internacional de Agricultura Tropical (CIAT)
- ◆ The Faculté des Sciences Agronomiques de l'Université de Gembloux (FSAGx)

Names of principal scientists:

Senior staff from the Bean Program at CIAT:

- ◆ Dr. Julia L. Kornegay, Leader, Bean Program.
- ◆ Prof. Dr. J. P. Baudoin, Head of the Department of Tropical Phytotechnology, University of Gembloux.

Total Budget:

The total budget is US\$376,905, broken down as follows:

<i>Budget</i>	<i>Year 1</i>	<i>Year 2</i>	<i>Year 3</i>	<i>Year 4</i>	<i>Total</i>
<i>AGCD</i>	<i>97,295</i>	<i>95,357</i>	<i>91,241</i>	<i>93,012,</i>	<i>376,905</i>
<i>CIAT</i>	<i>in kind</i>	<i>in kind</i>	<i>in kind</i>	<i>in kind</i>	<i>in kind</i>
<i>Total</i>	<i>97,295</i>	<i>95,357</i>	<i>91,241</i>	<i>93,012,</i>	<i>376,905</i>

2.0 Background and Justification

Common bean is an important source of protein and a key element in the sustainability of farming systems in Latin America and sub-Saharan Africa



Abiotic and biotic constraints have severe effects on bean yields

The food legume, *Phaseolus vulgaris*, represents an inexpensive and important nutritional source of plant protein for poor consumers and small-scale farmers in the tropics. In eastern and southern Africa, common beans are second only to maize as a source of dietary protein. Because neither cereal nor legume proteins contain a balanced complement of essential amino acids, both types of protein are necessary in the diet. Additionally, beans are the third most important source of calories in this part of Africa. Beans continue to be an important source of protein and calories in Latin America as well, especially among the lower income strata.

Because of the high levels of agronomic diversity within the crop, common beans are grown in a wide variety of cropping systems in Latin American and sub-Saharan Africa. Beans play an important role not only in the diet of the people of these regions, but also *in the sustainability of farming systems*. For example, beans can contribute significantly through biological nitrogen fixation to the N nutrition of the entire system. In Cajamarca, Peru, BNF improved yields of both beans and maize in on-farm trials in farmers' fields (Pineda et al., n.d.).

Nevertheless, bean yields remain low in farmers' fields because of the severe incidence of abiotic and biotic constraints. Bean yields for the period 1988-1990 averaged only 495 kg ha⁻¹ in Latin America and 678 kg ha⁻¹ in Africa (CIAT, 1992) even though yield potential in these regions is well over 2,000 kg ha⁻¹. Production must increase 42% in Latin America and 72% in Africa over 1988 levels to meet demand in year 2000 (Janssen, 1988). However, most farmers



Bean fly causes severe yield losses in East Africa



Ascochyta blight occurs in Africa and Latin America



BGMV is the primary bean production constraint in some Latin American areas

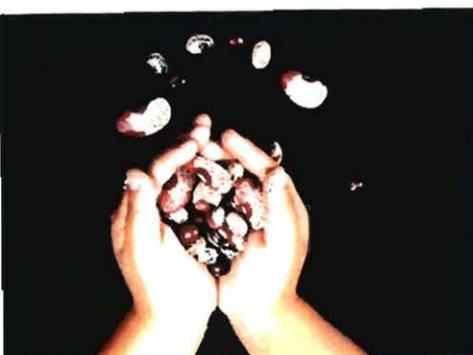
in the tropics do not have the means for correcting production constraints through added inputs. For these farmers, solutions to any constraint must come from within the seed.

Although biotic constraints are numerous, three of the most destructive are bean fly (*Ophiomyia* sp.), ascochyta blight (caused by the fungus *Phoma exigua* var. *diversispora*) and BGMV (bean golden mosaic virus).

In East Africa, the bean fly is the most important entomological pest of beans, causing severe yield losses from Ethiopia in the north to Malawi in the south. Although seed-dressing is effective, chemicals applied to seed can be dangerous to farmers who plant seed by hand. Only small genetic differences have been observed in *P. vulgaris* for resistance to bean fly.

Ascochyta blight occurs in the highlands of both Africa and the Americas, and is especially destructive in Rwanda and Burundi above 1600 m.a.s.l. (CIAT, 1985; CIAT, 1986).

BGMV as of yet occurs only in the Americas but is the number-one production constraint in the lowlands of Brazil, Central America, Mexico, and the Caribbean. Bean production over a million hectares has been abandoned because of BGMV in Brazil alone. Although some genetic resistance to BGMV exists within *P. vulgaris*, BGMV is known to be extremely variable, and the possibility of new races developing cannot be ignored. Also, new, more aggressive races of the viral vector, the *Bemisia* whitefly could contribute to a breakdown of resistance. All possible avenues of genetic control should be explored to combat BGMV.



CIAT maintains in trust for humanity the world's largest common bean germplasm collection



The secondary gene pools of common bean represent part of the biodiversity from South and Central America

To develop more productive and stable bean varieties, breeders are constantly drawing on the resources and genetic diversity of germplasm collections. CIAT maintains in trust for humankind the world's *Phaseolus* bean germplasm collection which consists of more than 26,000 accessions available for distribution, including both wild and cultivated forms of *Phaseolus vulgaris* and related taxa. For the first 15 years of its existence, the Bean Program at CIAT concentrated its efforts on the characterization and utilization of common bean landraces and wild *P. vulgaris* in genetic improvement programs. Nevertheless, within the *P. vulgaris* gene pool, insufficient genetic variation has been found to overcome several major production constraints. Better sources of resistance to these constraints have been identified in alien germplasm, mainly the secondary gene pools.

The secondary gene pools of *Phaseolus vulgaris* are composed of three species, *P. acutifolius*, *P. coccineus*, and *P. polyanthus*, and other related taxa such as *P. glabellus*, *P. purpurescens*, and new wild taxa recently collected. The secondary gene pool represents an additional source of genetic variability, being part of the *wide biodiversity from South and Central America*, and providing an important reservoir of traits poorly or not expressed in the primary gene pool such as in the case of ascochyta blight, BGMV, and bean fly (CIAT, 1987). Resistances to other pathogens such as *Xanthomonas campestris*, *Uromyces appendiculatus*, and *Sclerotinia sclerotiorum* have also been recovered in interspecific crosses with *P. coccineus* (Miklas et al., 1993). Resistance to *Sclerotinia* is especially rare within *P. vulgaris*. This experience and others confirm the potential of *P. coccineus* to improve the common bean. Interspecific

*The exploitation of alien
germplasm depends on
certain conditions*

hybridization with the secondary gene pools may therefore be utilized to resolve several major bean production problems. To exploit fully this alien germplasm, a number of conditions must prevail:

- ◆ a better understanding of the genetic organization, the phyletic relationships, and the genetic components of the botanic group;
- ◆ a refinement of the in-vitro embryo culture techniques needed to create new interspecific hybrids with interesting donor genotypes;
- ◆ an intensive breeding program and a molecular-marker assisted selection process to hasten the breeding process and increase its efficiency.

3.0 Overall Project Objectives

The project has clearly defined objectives



The project is focused on the genetic improvement of common bean through laboratory and field experiments

This collaborative project is essentially aimed at the genetic improvement of common bean, integrating laboratory and field experiments. The major objective of the breeding program will be to incorporate genetic resistance to ascochyta blight, a very destructive fungal disease of common bean prevailing in the highlands of both Latin America and Africa; BGMV, a major viral disease transmitted by whiteflies in Central America, the Caribbean basin and Brazil; and bean fly, the most important, destructive, and widespread insect pest in Africa attacking common bean. For these three constraints, no strong host resistance has been found so far in common bean germplasm. In comparison, very high levels of resistance have been observed in many accessions of the secondary gene pool, particularly in *P. polyanthus*, *P. coccineus*, and related taxa (Obando et al., 1990a; Schmit and Baudoïn, 1992).

The reasons outlined above highly justify the emphasis that this research proposal places on the genetic characterization and transfer of alien resistance genes from the secondary gene pools into the primary common bean gene pool. It is also planned to combine the resistance genes with other useful characteristics of the donor species, in the lines derived from interspecific hybrids.

A secondary long-term purpose of the project is to better understand the evolution of the genus *Phaseolus* through intensive studies of a few of its species. *Phaseolus* is a widespread genus of the Americas, many species of which are native to endangered ecological zones such as the subhumid premontane forest. An in-depth understanding of the genetic diversity of the genus *Phaseolus* could



A deep understanding of the genetic diversity of common bean will improve the understanding of biodiversity



contribute to a general understanding of biodiversity in these ecological zones, and eventually, to efforts in conservation of biodiversity. Studies of diversity in wild and cultivated *P. vulgaris* are already under way at CIAT, and the studies proposed for *P. coccineus* and *P. polyanthus* would complement them.

This research proposal will be a case model responding to three main objectives:

- 3.1** To broaden the genetic base of a major crop, *P. vulgaris*, by the rational exploitation of the *wealth of biodiversity* available in the genus, particularly in cross-compatible species.
- 3.2** To facilitate identification of genotypes with high levels of desirable traits and the transfer of these traits from the secondary gene pools, through the *development of core collections*, and with the help of molecular markers.
- 3.3** To increase the productivity of a major food legume, which plays a *key role in sustainable cropping systems prevailing in both Latin America and Africa*, through interspecific hybrids.

4.0 General Impact of the Research

The project will have a positive impact on the conservation of genetic diversity of common bean



Research capacities of national institutions will be strengthened through this project

The research project will have a positive contribution in increasing and maintaining genetic diversity in *P. vulgaris*, and in the preservation of the environment through the following:

- ◆ developing core collections for alien gene pools closely related to common bean and harboring useful traits that are poorly or not expressed in the primary gene pool;
- ◆ introgressing into common bean durable resistance to pests and diseases from *P. coccineus* and *P. polyanthus*, and new architectural traits;
- ◆ reducing use of pesticides in bean-based cropping systems; and
- ◆ promoting sustainable agriculture with the creation of improved climbing and bush bean varieties that play a key role in the intercropping systems of Latin American and African highlands.

The objectives and activities of the research proposal are consistent with the priorities defined in the Bean Advanced Research Network (BARN), which was organized by CIAT in 1990. Research capacities of national institutions will be strengthened, not only by the methods and technologies developed in this project, but also by favoring the participation of young research fellows, from both developing and developed countries.

5.0 Components of Research

The proposed work plan covers a series of simultaneous activities

The project outputs and activities are outlined in Figure 1 (Work Breakdown Structure). Figure 2 shows the organization of the project, including financial and evaluation reporting. To achieve the project objectives a sequential methodology, covering a series of activities being tackled simultaneously, must be developed. These outputs and their related activities are as follows:

5.1 The genetic organization of *P. coccineus* and *P. polyanthus* gene pools is determined

5.1.1 Core collections of *P. coccineus* and *P. polyanthus* are developed, based on available passport data, morphoagronomic traits, and molecular characterizations

The establishment of core collections is a priority of the Bean Advanced Research Network (BARN)

Core collections serve to promote the use of alien germplasm collections for genetic improvement; to facilitate their conservation and the study of their genetic organization; and to identify useful traits within these collections (Brown, 1989). Therefore, the establishment of core collections is considered a priority by BARN. The use of a broad-based sample of germplasm accessions, similar to a core collection, has already proved useful in identifying *P. vulgaris* landraces tolerant of low soil phosphorus availability (Beebe et al., 1992), thus demonstrating the value of such collections in germplasm utilization. A core collection would be especially useful in the case of *P. coccineus* and *P. polyanthus*, since their long growth cycle, aggressive climbing habit, and difficult agronomic management have discouraged many bean

CIAT has valuable experience in the formation of a core collection of common bean



researchers from studying these species extensively. A core collection would permit concentration on a relatively small number of accessions, thus minimizing problems of agronomic management.

CIAT already has experience in the formation of a core collection of *P. vulgaris*, using passport data to derive an agroecological characterization of the collection site which, combined with morphoagronomic traits, served to identify a sample of the broadest genetic variability (Tohmè et al., n.d.). A similar procedure will be followed in the case of *P. coccineus* and *P. polyanthus*, for which many morphoagronomic, passport, and molecular data are available (Schmit, 1988) or will be obtained.

In this work, priority will be given to an in-depth molecular characterization of selected populations, using DNA molecular markers and combining RFLP and RAPD methods. In Gembloux and CIAT, preliminary work on seed proteins and cpDNA have contributed to clarifying the phyletic position of several taxa in relation to the *P. polyanthus/P. coccineus* complex (Schmit and Debouck, 1991; Schmit et al., 1991 and 1992; Baudoin et al., 1992; Schmit et al., n.d.). In particular, investigations have shown the uniqueness of the *P. polyanthus* gene pool with the center of origin located in Guatemala; the very close relationship between *P. polyanthus* and *P. costaricensis*; the separation between *P. polyanthus* and *P. coccineus*; and the isolation of *P. glabellus* among the sampled populations.

More research is needed, however, to better understand the genetic organization of *P. coccineus* germplasm, and to more precisely define the boundary between this species and other taxa. The divergence between Andean and Mesoamerican *P. polyanthus* populations also needs to be examined. Data obtained from the present study will be



The core collection will be evaluated for resistance to Ascochyta blight, BGMV and bean fly

subjected to multivariate analysis to identify subgroups within *P. coccineus* and *P. polyanthus* similar to the definition of races by agromorphologic traits in *P. vulgaris* (Singh et al., 1991). The definition of within-species subgroups may contribute to the identification of useful economic traits, if such traits are better expressed in a particular subgroup. Such work will help identify allelic frequencies, detect duplicate groups, and develop prediction models for combining ability among and within taxa. This information will help guide breeders select those parental genotypes which combine both discriminatory useful traits and good cross-compatibility—two factors essential for undertaking a wide crossing program. The building of a core collection is also intended to correlate the useful traits in the secondary gene pools with agroecological data and molecular markers.

5.1.2 Core collections are evaluated for resistance to important constraint factors such as ascochyta blight, BGMV, and bean fly

Once defined, the core collection will be evaluated under controlled greenhouse and field conditions for response to inoculations and infestations by ascochyta blight, BGMV, and bean fly. These evaluations will involve the expertise of several scientists at CIAT, Colombia and in Africa, whose time inputs in conducting these evaluations will be given at no charge to the project.

Ascochyta blight will be evaluated in CIAT's experimental farm in Popayan, Colombia (1,700 m.a.s.l., 17°C mean annual temperature), where the disease is endemic but where field inoculations have been utilized to ensure uniformity of disease pressure.



The project involves the collaboration of national programs through the PROFRIJOL regional bean project

BGMV will be evaluated initially in controlled greenhouse conditions that have proven useful in revealing genetic differences in the past (Morales and Niessen, 1988). Promising accessions will be tested in Central America in collaboration with national programs, through the PROFRIJOL regional bean project. Research programs exist in Guatemala, Mexico, El Salvador, and Honduras with ample experience in evaluation of BGMV.

Bean fly can only be evaluated in Africa or Asia, because the pest does not exist in the New World. CIAT's entomologist stationed in Tanzania dedicates most of his research effort to bean fly. The entomologist has identified reliable field evaluation sites for the different *Ophiomyia* species, and is perfecting a mass-rearing technique for the insect. Therefore, the development of screening methodology is well under way, and is awaiting the potential genetic diversity.

Once the core collection has been evaluated, further accessions from the reserve collection will also be evaluated selectively, based on the geographic origin or genetic subgroup of promising accessions in the core.

5.1.3 Inheritance studies are conducted to identify the genetic control of individual traits



After the core collections are evaluated and genotypes with contrasting responses to a given constraint are identified, crosses will be made among these materials to determine the genetic control of the traits in segregating populations. The information concerning the inheritance of genes controlling a trait, whether it is simple or complex, dominant or recessive, will allow the breeders to define breeding strategies needed to introgress resistance traits into *P. vulgaris*.

These populations will be intraspecific, or will combine *P. coccineus* and *P. polyanthus* to avoid genetic and chromosomal abnormalities, which can occur in interspecific crosses with *P. vulgaris* and which can distort normal segregation ratios. Because individual plant evaluation is not reliable, evaluation must be done on families or populations. Therefore, an F_1 population will be generated to reveal tendencies to recessiveness, additivity, or dominance of resistance. Recombinant inbred lines (RILs) will be developed for evaluation in F_6 generation with several repetitions, to reveal segregation patterns and estimate the number of genes involved.

5.1.4 Molecular probes combining RFLP and RAPD methods are used to tag resistance genes

The populations developed in the inheritance studies will subsequently be used in molecular analyses to identify DNA markers which are linked to genes of interest. This part of the project will focus on BGMV and bean fly, because gene tagging of ascochyta resistance is being realized elsewhere. However, if multiple resistance is identified in the evaluation of the core, in accessions combining ascochyta resistance with BGMV and/or bean fly resistance, then it may be feasible to utilize these accessions in populations and to obtain information on molecular markers of ascochyta resistance with only small additional cost. This would only be undertaken if the sources of ascochyta resistance were different than those used in studies elsewhere. These markers will also be mapped on the *Phaseolus* linkage map. Once this work is under way, it will facilitate completion of the second output of the research proposal, which is as follows:

Phenotypic data for marking of resistance genes will be derived from RILs. If populations between *P. polyanthus* and *P. coccineus* are free of abnormalities, these populations will be utilized for marking as they may present greater DNA polymorphism than intraspecific crosses. But if the crosses of *P. coccineus* and *P. polyanthus* are suspected of physiological abnormalities or distortions of segregation patterns, it will be necessary to mark the genes in intraspecific crosses.

Tagging will be performed with random primers to generate random amplified polymorphic DNA (RAPD) markers. Linked RAPD segments will be isolated from gels, reamplified, and used as probes on previously mapped populations, to locate resistance genes on the *P. vulgaris* gene map (Nodari et al., 1993).

*New interspecific hybrids
will be developed*



The investigation will consider several factors influencing genotypes

5.2. Interspecific hybridization and selection techniques are developed which permit the introgression of useful genes into common bean

5.2.1 Tissue culture techniques are developed to rescue proembryos of interspecific crosses

New interspecific hybrids will be developed in the framework of the genetic improvement program. The parental genotypes from the donor species will be selected on the basis of their high levels of expression of useful characters poorly represented in *Phaseolus vulgaris*. The most interesting crosses—new combinations between, for example, *P. vulgaris* and *P. glabellus*; and hybrids involving the cytoplasm of the donor parents (to avoid quick reversal to the recurrent common bean)—will no doubt require the development of an efficient tissue culture technique that uses proembryos (Baudoin and Marechal, 1991). Proembryos refer to those developmental stages preceding cotyledon initiation, such as the globular and the early heart-shaped stages. It is precisely at these stages that incompatibility barriers provoke embryo abortion in *Phaseolus* interspecific crosses. Few studies have been conducted on in vitro culture of early immature embryos in this genus, and it is an area that begs attention. Indeed, a breakthrough in this field would open the way to obtaining hybrids between specific genotypes by avoiding cytoplasm-genome-maternal tissue interactions. From previous work conducted in Gembloux on *Phaseolus* species, an efficient technique of extraction and transfer of proembryos to the culture medium was developed, and a performing media inducing germination defined (Mergeai and Baudoin, 1990; Schmit et al., 1991; Chavez et al., 1992).

There is now a need to improve the efficiency of the media used for embryo rooting and ex-plant hardening. Nutritional and hormonal requirements are bound to be more exacting than those usually observed in classical embryo rescue techniques (with embryos at the cotyledonary stage). The investigations will consider several factors: effect of amino acids, nitrogen sources, natural plant extracts, requirements for high osmoticum, role of hormones and other stimuli, and influence of genotypes. Efforts will also be directed to understanding the mechanisms of abortion at the cell biochemistry level and to identify possible correlations between hybrid embryogenesis and molecular characterization of the parental genotypes. These techniques will be directly applied to the new interspecific hybrids requested by the breeders.

5.2.2 Interspecific hybridization and selection methodologies, combining classical and molecular techniques, are developed to successfully transfer resistance traits into common bean

Past experience has shown that successful introgression of traits through interspecific hybridization requires a very strict and intensive methodology of selection that favors high recombination rates and breakup of undesirable linkage blocks (Baudoin, 1991; Baudoin and Marechal, 1991; Baudoin et al., 1993). Recurrent selection schemes and congruity backcrossing are two procedures usually adopted in breeding interspecific hybrids. Methodologies employed in the past will be strengthened through knowledge obtained in the present project about the inheritance of the useful characters from the donor parents and



the discovery of molecular markers linked to the targeted genes. These two major factors will greatly facilitate genetic gains in the breeding process. Also, stabilizing useful genes expressed in the advanced progenies of interspecific lines is also an essential task requiring careful monitoring and selection by the breeders.

Choice of genotypes entering interspecific crosses also influences success of the crosses. In the present case, *P. vulgaris* of different genetic origins will be tested for their ability to recombine with *P. coccineus* and *P. polyanthus*. In addition to cultivated common bean, some wild *P. vulgaris* originating in regions such as Guatemala where they have been sympatric with the other species, will be included in crosses. Recent studies have identified regions in which variability of wild *P. vulgaris* is far greater. It is possible that, in these regions, *P. vulgaris* has experienced some introgression from other species, and in this case, chromosome pairing and genetic recombination may occur more readily when these are included in the interspecific crosses. Although wild accessions have been utilized in interspecific crosses before, data had not been available to select the wild *P. vulgaris* parents systematically.

As previously mentioned, a special focus will be placed on introgressing genes for resistance to ascochyta blight, BGMV, and bean fly into common bean. Preliminary screenings of various *Phaseolus* materials under laboratory and field conditions have revealed very high levels of resistance to ascochyta blight, particularly in *P. polyanthus*, but also in several populations of *P. coccineus*, *P. purpurescens*, *P. salicifolius*, *P. oligospermus*, *P. pluriflorus*, and *P. pauciflorus*; some being cross-compatible with common bean (Schmit and Baudoin 1987 and 1992; Obando et al., 1990b).



Although hybrids have been developed between ascochyta susceptible *P. vulgaris* genotypes and resistant *P. polyanthus* populations, they have not been submitted to a rigorous breeding process involving intermating and backcrossing cycles, which is contemplated in this project. The use of molecular-marker-assisted selection will enable the breeders to simultaneously select for resistance to several traits within the same segregating populations. For example, the development of markers linked to bean fly resistance will allow the breeders to select for resistance to this pest and ascochyta blight at CIAT, Colombia, without having to send early generation segregation populations to Africa. Only later in the breeding process, would it be necessary to confirm bean fly resistance levels in controlled infestation studies in Africa.

The time frame of this project does not permit that advanced introgressed lines, with stable trait expression in common bean phenotypes, be completed. The time frame does permit that interspecific populations be developed, using recurrent selection and backcrossing schemes, and that selection for the presence of resistance genes be made within segregating populations. However, as the results of this project are of great importance to CIAT, the breeding work begun in the project will later be completed by CIAT.

Investigations to be conducted directly in the course of the project will be as follows:

- ◆ inheritance studies of ascochyta blight, BGMV, and bean fly in segregating populations;
- ◆ tagging of genes for BGMV and bean fly resistances;



- ◆ interspecific crosses involving the most interesting donor genotypes will be made for three years; and
- ◆ in the last cycle of crosses, segregating populations will be monitored with the support of marker-assisted selection techniques (pending of course the identification of molecular markers tightly linked to the genes of interest).

Investigations to be conducted only partially in the course of the project or totally outside the present project will be as follows:

- ◆ a study of *Phoma exigua* var. *diversispora* variability and aggressiveness in relation to the ecogeographical distribution of the pathogen, based on DNA fingerprinting, isozyme electrophoresis, or other physical characterizations, is being carried out at the University of Bonn, Germany in the framework of a STD-3 project financed by the CEC.
- ◆ identification of *Phaseolus* molecular markers linked with the genes of resistance to ascochyta blight, using RFLP with random genomic and cpDNA probes, PCR and RAPD techniques, applied to inbred or isogenic populations, is also currently being conducted by the University of Bonn under the same STD-3 project with backing from the University of Gembloux.
- ◆ construction of linkage maps for *P. vulgaris*, *P. polyanthus*, and *P. coccineus* taxa, including genes responsible for agronomic and morphological factors, and biochemical markers, is being started at CIAT in the Biotechnology (BRU) and Genetic Resources (GRU) Units, and at the University of Gembloux.

Throughout the project, attention will be paid to avoid duplicating work; and, moreover, the results from this project will complement investigations already conducted to provide solutions to major constraints in bean improvement and production in the tropics.

6.0 Reporting and Evaluation



Annual progress reports will be submitted to the donor agency. No formal evaluation has been planned, but if the donor agency so desires this can be programmed. However, no funds have been budgeted for this purpose and additional funds would be required.

7.0 Calendar of Activities

The project will cover a 4-year period (Appendix A). Although it is not expected to complete the overall project objectives during this time, we do expect to make significant advances in launching a very promising breeding program based on the wealth of biodiversity displayed in the *Phaseolus* gene pools. Progress in each of the various activities will also often depend upon success achieved in other activities. Consequently, a strict schedule of activities is not proposed, but rather a tentative calendar of operations starting as of 1994.

8.0 General Project Structure and Budget Estimates

The research will be conducted with the collaboration of scientists in Latin America, Europe and Africa

The project will be coordinated by Dr. J. L. Kornegay, leader of the Bean Program at CIAT. She will receive collaboration from CIAT scientists, Dr. S.P. Singh, genetics/plant breeder; Dr. M. A. Pastor Corrales, pathologist; Dr. F. Morales, virologist; and Dr. K. Ampofo, entomologist based in Arusha, Tanzania; and from Dr. J. P. Baudoin, Faculté des Sciences Agronomiques de l'Université de Gembloux (FSAGx).

The University of Gembloux will provide consultancy to ensure close collaboration between the two institutes and to transfer technologies from Gembloux to CIAT.

To complete the molecular and tissue culture studies at the University of Gembloux, one Belgian Junior Research Fellow will be employed full-time during the course of the project (Table 1). At CIAT, two Colombian Research Assistants will be employed full-time during the course of the project: one assistant will be responsible for the management and daily evaluation of the core collections and supervise the interspecific hybridizations. The second assistant will conduct the molecular evaluations for gene tagging, and will receive training from Gembloux on proembryo rescue techniques. A full-time Field Laborer is also needed to carry out the interspecific pollinizations, and provide agronomic care for field and greenhouse plantings and harvests.

Financial resources will be allocated to CIAT and the University of Gembloux in order to execute the different components of the project. The annual contribution to the University of Gembloux will be paid directly by AGCD to avoid transfer of funds from CIAT to Gembloux. The estimates of the research costs at Gembloux have been calculated at the exchange rate of US\$1.00 = BF33 (Belgian Francs).

Table 1: Budget CIAT + Gembloux

LINE ITEM	1994	1995	1996	1997	TOTAL
CIAT (In US Dollars)					
PERSONNEL					
Research assistants (2)	22,000	23,100	24,255	26,680	96,035
Field laborer for crossing (1)	6,200	6,510	6,525	6,851	26,086
Temporary laborers	1,500	1,600	1,700	1,750	6,550
Total personnel	29,700	31,210	32,480	35,281	128,671
TRAVEL					
To Gembloux and within Colombia	1,000	3,400	700	3,900	9,000
Total travel	1,000	3,400	700	3,900	9,000
OPERATIONS					
Laboratory supplies	5,000	5,000	5,000	3,000	18,000
Field supplies	1,500	1,500	1,500	1,500	6,000
Screenhouse	6,000	3,000	1,000	1,000	11,000
Total operations	12,500	9,500	7,500	5,500	35,000
INDIRECT COSTS	6,480	6,617	6,102	6,702	25,901
CONTINGENCIES	2,160	2,206	2,034	2,234	8,634
TOTAL CIAT	51,840	52,932	48,816	53,617	207,205
GEMBOUX (In Belgian Francs)					
PERSONNEL					
Research fellow	700,000	800,000	800,000	800,000	3,100,000
Total personnel	700,000	800,000	800,000	800,000	3,100,000
TRAVEL					
To Colombia and within Colombia	150,000	150,000	150,000	150,000	600,000
Total travel	150,000	150,000	150,000	150,000	600,000
OPERATIONS					
Growth chamber facilities	200,000	100,000	100,000	50,000	450,000
Consumables	380,000	300,000	300,000	250,000	1,230,000
Total operations	580,000	400,000	400,000	300,000	1,680,000
INDIRECT COSTS	70,000	50,000	50,000	50,000	220,000
Subtotal in Belgian Francs	1,500,000	1,400,000	1,400,000	1,300,000	5,600,000
Conversion to US\$	45,455	42,425	42,425	39,395	169,700
GRAND TOTAL	97,295	95,357	91,241	93,012	376,905


ABRAHAM E. ESPINO
 FINANCIAL CONTROLLER

EGR1 - PSE
 02-AUG-03
 PROPOSED

9.0 Representatives of the Collaborative Project

All the important questions on the program will be dealt with between the responsible parties of the entities involved in the project or by their representatives:

CIAT

- ◆ Dr. Gustavo A. Nores, *Director General*
- ◆ Dr. William Scowcroft, *Deputy Director General-Germplasm Division*
- ◆ Dr. Julia L. Kornegay, *Leader of the Bean Program*
- ◆ Dr. Abraham Espino, *Controller*

University of Gembloux

- ◆ Prof. C. Deroanne, *Rector of the Faculty*
- ◆ Prof. J. P. Baudoin, *Head of the Department of Tropical Phytotechnology*

10.0 References

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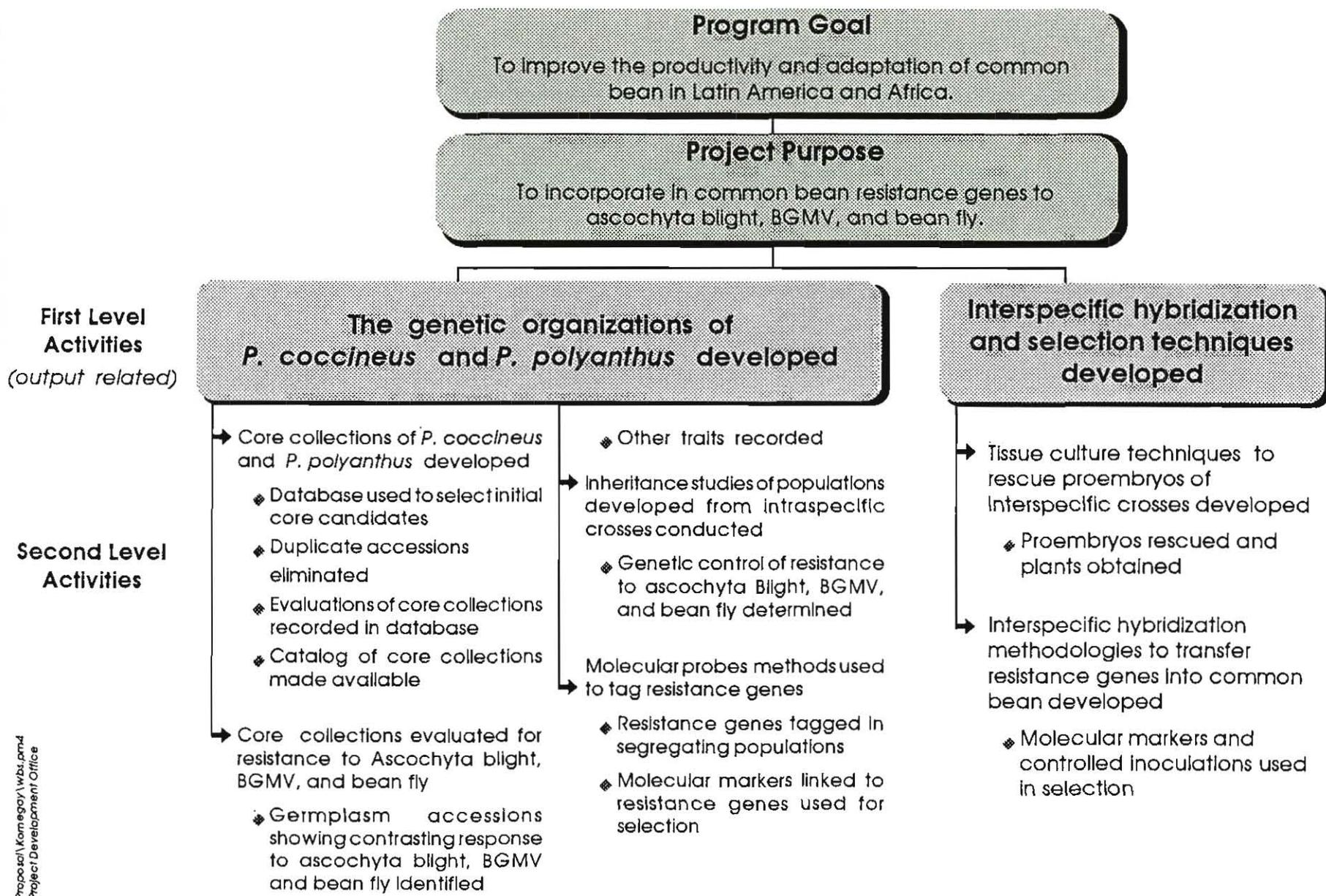
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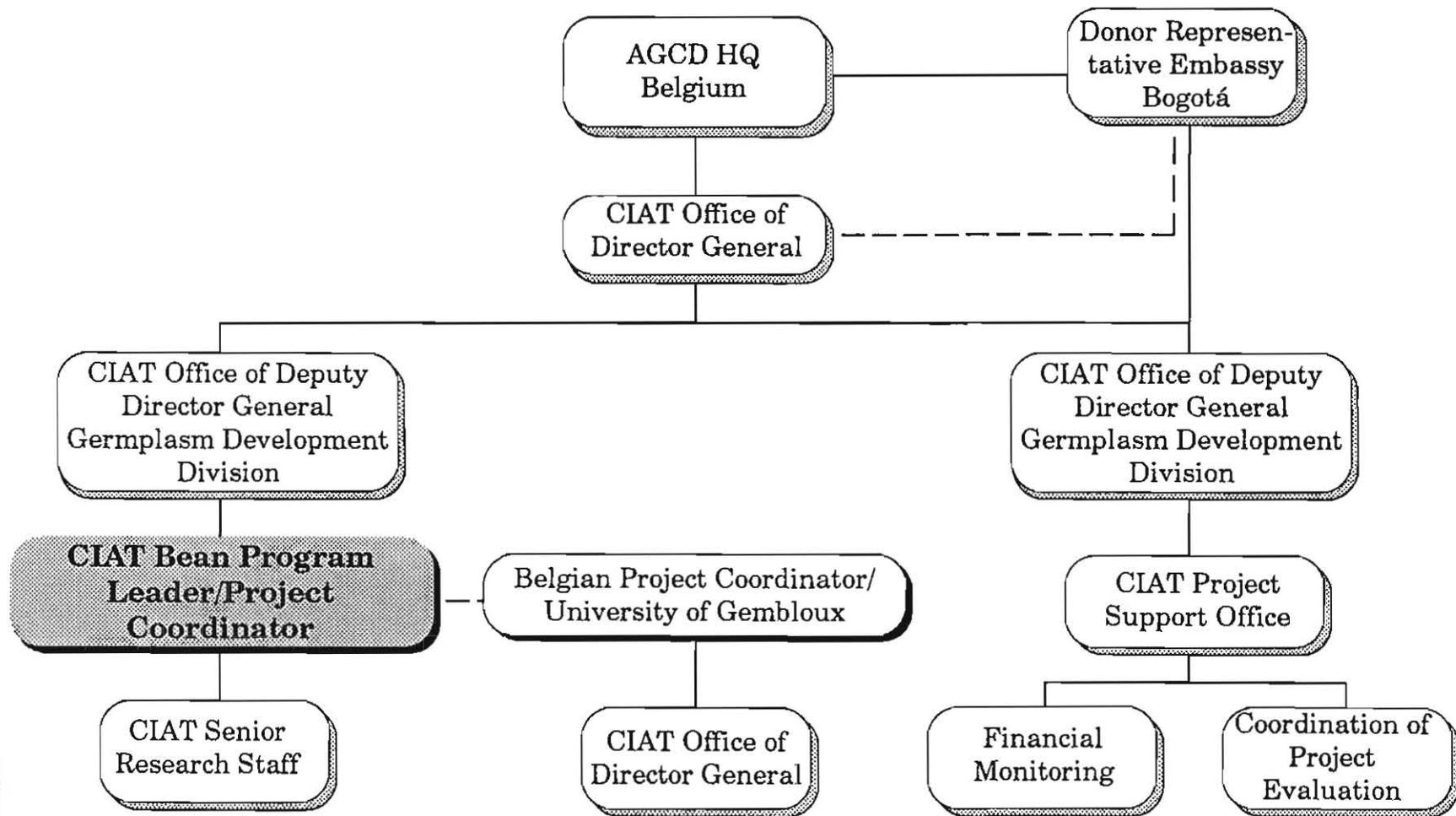
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Figure 1: Work Breakdown Structure, Linking Project Activities to Project Outputs



Proposal\Komegov\wbs_pm4
 Project Development Office

Figure 2
Project Organization Chart



Communication lines - - - - -
 Management and financial reporting lines _____

Appendix B-1



CIAT C.V.

Centro Internacional de Agricultura Tropical

Name:

Julia L. Kornegay

Position in Project:

Project Coordinator

Citizenship:

American

Country of Residency:

Colombia

Education:

Ph.D., Plant Breeding
Cornell University, 1985.

M.Sc., Plant Pathology
North Carolina State University, 1979

B.Sc., Horticultural Science
North Carolina State University, 1976

Languages:

English -Native
Spanish -Conversational

Management Experience:

Centro Internacional de Agricultura Tropical (CIAT)
Cali, Colombia
Leader, Bean Program
1993 to present

International Research Experience:

Centro Internacional de Agricultura Tropical (CIAT)
Cali, Colombia
Senior Scientist, Research Geneticist. Bean Program
1987 to 1993

Centro Internacional de Agricultura Tropical (CIAT)
Cali, Colombia.
Postdoctoral Fellow. Plant Breeder. Bean Program
1985 to 1987

**Awards and
Memberships:**

Centro Internacional de Agricultura Tropical (CIAT)

Cali, Colombia.

Visiting Research Associate. Entomologist/Plant Breeder.
Bean Program
1983 to 1984.

Outstanding Publication and Research Award (OPRA)
CIAT, 1990.

Vice-president, Board of Trustees.
Centro Cultural Colombo-Americano
Cali, Colombia.

Member of the American Society of Agronomy.

Member of the Bean Improvement Cooperative.

Publications:

- Kornegay, J. L., C. Cardona, and C.E. Posso. 1993 Inheritance of Resistance to Mexican bean weevil, determined by bioassay and biochemical tests. *Crop Sci.* 30: (n.d.).
- Kornegay, J., J. White, O. and Ortiz de la Cruz. Growth habit and gene pool effects on inheritance of yield in common bean. *Euphytica* 62:171-180
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Kornegay, J. L., C. Cardona, A. and van Schoohoven. 1986. The mechanisms of resistance in common beans to the leafhopper *Empoasca kraemeri*. *Entom. Exp. Appl.* 40:273-279.

Kornegay, J. L., M. K. Beute and J. C. Wynne. 1980. Inheritance of resistance to *Cercospora arachidicola* and *Cercosporidium personatum* in six Virginia-type peanuts (*Arachis hypogae*) lines. *Peanut Sci.* 7:4-9.

Appendix B-2



CIAT C.V.

Centro Internacional de Agricultura Tropical

Name:	Stephen Beebe
Position In Project:	Germplasm Specialist
Citizenship:	American
Country of Residency:	Colombia
Education:	Ph.D., Plant Breeding and Genetics University of Wisconsin, 1978 M.Sc. Plant breeding and Genetics University of Wisconsin, 1976 B.Sc. Horticulture Iowa State University, 1974
Languages:	English -Native Spanish -Conversational
International Research Experience:	Centro Internacional de Agricultura Tropical (CIAT) Cali, Colombia Head, Bean Germplasm Characterization Section 1992 to present Centro Internacional de Agricultura Tropical (CIAT) Cali, Colombia Head of Bean Breeding Program 1985 - 1992 CIAT - ICTA Guatemala Bean Breeder In Regional Project 1981 - 1985

-2-

S. Beebe

**Teaching and Thesis
Supervisory:**

Organizing Course for National Program Bean Breeders
Lecturing in short courses
Supervision of several M.Sc. and one Ph.D. theses

Major Publication:

"Breeding for Disease Resistance" in "Common Beans:
Research for Crop Improvement. Schoonhoven and
Voyses, Editors.

Appendix C

Collaborative Project on *Phaseolus* Germplasm CIAT-University Gembloux

Donor: Administration Générale de la Coopération au Développement (AGCD, Belgium)

Place: Centro Internacional de Agricultura Tropical

Duration: 7 years

Status: Completed

Amount: US\$459,630

This project was a continuation of a previous one which started in 1977. The persons involved since 1977, are the following:

CIAT-University of Gembloux (Belgium) Collaborative Program on *Phaseolus* Germplasm

CIAT is responsible for conserving not only common beans (*P. vulgaris*), but also other *Phaseolus* cultivated beans, namely, lima bean (*P. lunatus*), tepary (*P. acutifolius*), and runner bean (*P. coccineus/P. polyanthus*). Because CIAT's Bean Program works almost exclusively on the common beans, the germplasm of other cultivated beans did not receive sufficient attention. The University of Gembloux has a group of scientists specialized in *Phaseolus* germplasm. Since the late 1970s, the Belgium Government provided financial support to link the university experts with CIAT's needs on these species. Two projects were successfully executed during 1986 to 1991.

Lima bean (*P. lunatus*) germplasm research by Research Associate Alain Maquet

This project started in May 1986 and terminated in April 1991.

Runner bean (*P. coccineus/P. polyanthus*) germplasm research by Research Associate Veronique Schmit

This project focused on the efficient use of *P. coccineus/P. polyanthus* in the improvement of common bean. It started in 1984 and terminated in 1989.

Research Participants in Previous Cooperation

University of Gembloux

Daniel Debouck (Junior Research Fellow)
Paul Gepts (Junior Research Fellow)
Elizabeth Lewinson (Junior Research Fellow)
Thierry Vanderborcht (Junior Research Fellow)
Veronique Schmitt (Junior Research Fellow)
Alain Maquet (Junior Research Fellow)
Robert Maréchal (Project Coordinator)

CIAT

Douglas Laing (Proj.Coordinator)
Robert Luse (Head GRU)
Leonard Song (Head GRU)
William Roca (Head GRU)
David Wood (Head GRU)
Jeremy Davis (Bean Breeder)
Masaru Iwanaga (Head GRU)

CIAT has in-house facilities for the production of high-quality training materials and video programs for scientific and extension agricultural activities.

This proposal was produced and published, using CIAT's computer layout and graphic composition facilities and outside low-cost copying services