SCIENTIFIC REPORT

Collaborative project between CIAT (Cali, Colombia) and the University of Gembloux (Belgium)

SHORT TITLE OF THE PROJECT

Interspecific Solutions to Intractable problems of Common Bean

Third annual report : October 1998 - September 1999

Annual Report October 1998 - September 1999

Partners :

Centro Internacional de Agricultura Tropical (CIAT, Cali, Colombia).
Faculté Universitaire des Sciences Agronomiques de Gembloux (FUSAGX, Gembloux, Belgium).

Title :

Broadening the genetic base of common bean, *Phaseolus vulgaris*, and exploitation of the biodiversity found in the 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.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. General Objectives of the project

The general objective of the project is to 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*. Another objective is to preserve and rationally manage the germplasm collections of these secondary gene pools.

Therefore, investigations of the project are aimed :

- to understand the genetic organization, the phyletic relationships and the genetic components of the botanic group;
- to refine the *in vitro* embryoculture techniques needed to create new interspecific hybrids with interesting donor genotypes;
- to conduct an intensive breeding program and a molecular-marker assisted selection process among the interspecific hybrids.

II. Activity Report

1. Introduction

The project started in October 1996, with the first disbursement of funds from AGCD.

In CIAT, an agronomist with ample experience in the Bean Team, Ing. Agr. César Cajiao, was contracted under this project. Ing. Cajiao has been working for many years in the Popayán environment where most of the field trials will be carried out, and previously served in CIAT's bean breeding program selecting for disease resistance. He is therefore very well qualified to carry out the field work planned under this project.

In Gembloux, a young research fellow, Ir. Pascal Geerts, was contracted for laboratory experiments. The main objectives of the FUSAGX team are :

- the development of new interspecific hybrids with *P. vulgaris*, using mainly the most promising donor genotypes (*P. coccineus* and *P. polyanthus*) as female parents. Such crosses require the implementation of an efficient tissue culture technique with proembryos, (e.g. at the globular and early heart-shaped development stages). The Belgian research fellow, appointed in the framework of this project will concentrate his activities on the improvement of the embryoculture technique.
- provide guidelines to develop an intensive breeding program, using the most appropriate parental genotypes, interspecific combinations and selection schemes.
- complement investigations conducted at CIAT, particularly providing data on the genetic organization of the *Phaseolus* gene pool, the molecular characterization of *P. coccineus* and *P. polyanthus* genomes, the genetic boundary between taxa of primary and alien gene pools of *P. vulgaris* and the combining ability among and within taxa.

Activity report will be presented in a sequential methodology, covering a series of activities being tackled simultaneously.

2. The genetic organization of P. coccineus and P. polyanthus gene pools (CIAT)

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

Previously we reported;

- Seed of about 80% of accessions has been obtained in sufficient quantities to initiate phenotypic evaluations.
- A pattern has emerged that indicates that accessions from Chiapas in southern Mexico and other sites further south have lower seed production than accessions to the north of Mexico.
- A nursery for vegetative propagation of accessions has been established to make most efficient use of scarce seed.

In 1998-99 we can report the following highlights:

- The process of seed production is finally nearing completion. Intransigent accessions were delivered to the Genetic Resources Unit for planting in Tenerife.
- Seed was shared with national programs in Kenya and in Brazil.
- Vegetatively propagated cuttings served to economize on seed use for disease nurseries.

While seed production was much more laborious than originally anticipated, it is finally nearing completion, with the result that quite sizable seed stocks are available for these accessions, from 400-1000 or more seed in almost all cases (**Table I**). This is more seed than is commonly stored for these species. We have set ourselves the goal of having 1,000 seed of each core accession, in the expectation that the core collection could become a tool for scientists with interest in these species. For example, a seed request was received from Kenya for accessions of *P. coccineus*, and it was possible to respond using the core collection. Another set was dispatched to the Brazilian national bean program for evaluation for resistance to white mold (**Table II**). Thus, the core is becoming a useful tool for supplying a rational subset of germplasm of these species to bean researchers. This is especially pertinent to the sustained research effort on these species, since they are of very difficult management agronomically, and a core collection permits studying broad genetic diversity with a modest number of accessions.

However, about 19 accessions remained essentially intransigent and have produced less than 400 seed in almost three years. Indeed, seven accessions have produced less than 100 seed in that period. These are obviously very ill-adapted to the Popayán environment, therefore cuttings of these accessions were delivered to the GRU for planting in the Tenerife site, to seek better seed production there.

Thirty accessions that had been tentatively eliminated from the original selection of the core due to lack of seed were finally planted in July, 1999. These include 18 accessions of *P. coccineus* (PC) and 12 of *P. polyanthus* (PP).

The system that was developed for vegetative propagation was used successfully to economize on seed use during the evaluations of the core, but now that seed is more available, this activity has been scaled down.

Table I. Range in seed production of Core accessions and selections for Ascochyta, Bean Fly and BGMV. Popayán 1998 B – 1999 A.

No of seeds	Entries	%	Cumulative %
> 2000	14	10.9	10.9
1501 - 2000	26	20.1	31.0
1001 - 1500	30	23.2	54.2
801 - 1000	10	7.8	62.0
601 - 800	16	12.4	74.4
401 - 600	14	10.9	85.3
201 - 400	9	7.0	92.3
101 - 200	3	2.3	94.6
51 - 100	3	2.3	96.9
11 - 50	3	2.3	99.2
1 - 10	0	0.0	99.2
0	1	0.8	100.0
TOTAL	**129**		

Table II. Seed shipments of core accessions of *P. coccineus /P. polyanthus* and of populations of F_2 with *P. vulgaris*. 1999 A.

Country	Type of Material	Observations
KENYA	Accessions	12 Entries (*P. coccineus*)
PUERTO RICO	Accessions	112 Entries (64 *P. coccineus* , 48 *P. polyanthus*)
BRAZIL	Accessions	92 Entries (50 *P. coccineus* , 42 *P. polyanthus*)
GUATEMALA	Populations F_2	249 x ICTA HUNAPU (*) - 385 seeds
		282 x ICTA HUNAPU (*) - 1000 seeds
		283 x ICTA HUNAPU (*) - 1990 seeds

(*)
249-(8)A-F_{11} = BAT 338 x G35252
282-(2)A-F_{12} = G15428 x G35174
283-(6)A-F_{12} = G15428 x G35174

2.2. Core collection are evaluated for resistance to important constraint factors such as Ascochyta blight, Bean Golden Mosaic Virus (BGMV), and Bean Fly

In 1998-9 we can report the following highlights:

- The core has been evaluated for anthracnose-ANT (Mesoamerican and Andean races); angular leaf spot-ALS (Mesoamerican and Andean races); and Ascochyta blight-ASC.
- Accessions of both wild and cultivated *P. polyanthus* were universally and highly resistant to ASC, thus the resistance is an ancestral trait.
- Both wild and cultivated *P. coccineus* present a range of reactions to ASC, with similar values. Thus, the reaction of PC to ASC has not changed substantially with domestication.
- However, within *P. coccineus* there is certain geographical stratification, such that Mexican and European accessions often present intermediate to susceptible reactions to ASC, while accessions to the south of Mexico tend to be more resistant.
- Reaction to common bean ALS is variable but generally low, and does not appear to follow gene pool lines of the pathogen closely, although susceptibility to Mesoamerican isolates is slightly greater.
- Reaction to common bean ANT is almost universally resistant in both species.

The core has been evaluated for reaction to common bean anthracnose-ANT (Mesoamerican and Andean races); angular leaf spot-ALS (Mesoamerican and Andean races); and Ascochyta blight-ASC (**Table III**).

Table III. Data on reaction to pathogens that cause anthracnose (Mesoamerican and Andean races), angular leaf spot (Mesoamerican and Andean races) and Ascochyta, of accessions in the core collection.

				ANT - A			ANT - M			ANT	ALS - A			ALS - M			ALS	ASCOCHYTA		
Ident.	ORIG	SP.	BS	Range	Mean-A		Range	Mean-M		Mean	Range	Mean-A		Range	Mean-M		Mean	Range	Mean	
G35006	GTM	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 2,0	1.3		1.2	3.0 - 5.0	3.2	*
G35007	GTM	COCC	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0		1.0	0.0		*
G35008	GTM	PLAN	C	1,0 - 3,0	1.3		1.0	1.0		1.1	1,0 - 4,0	1.6		1.0	1.0		1.3	2.0 - 3.0	2.5	
G35014	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 4,0	1.9		1.4	3.0 - 4.0	3.8	
G35021	MEX	COCC	C	1,0 - 3,0	1.3		1,0 - 6,0	2.1		1.7	1.0	1.0		1.0	1.0		1.0	6.0 - 7.0	6.8	
G35023	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	4.0 - 5.0	4.5	
G35025	TUR	COCC	C	1,0 - 2,0	1.2	*	1.0	1.0	*	1.1	1,0 - 3,0	1.3		1,0 - 6,0	2.2		1.8	4.0 - 7.0	5.8	
G35034	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 5,0	3.0		1,0 - 6,0	2.5		2.8	1.0	1.0	
G35036	GTM	PLAN	C	1,0 - 2,0	1.2		1,0 - 2,0	1.2		1.2	1.0	1.0	*	1.0	1.0		1.0	1.0 - 2.0	1.8	
G35040	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.3		1,0 - 5,0	2.1		1.7	1.0 - 2.0	1.3	
G35056	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.1		1,0 - 6,0	3.0		2.1	1.0 - 2.0	1.8	
G35060	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 6,0	2.3		1,0 - 3,0	1.5		1.9	1.0 - 2.0	1.5	
G35066	MEX	COCC	C	1,0 - 4,0	1.6		1.0	1.0		1.3	1.0	1.0		1,0 - 4,0	1.7		1.4	5.0 - 6.0	5.3	
G35077	DEU	COCC	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0		1.0	1.0		1.0	6.0 - 7.0	6.8	
G35084	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0	1.0	
G35086	GTM	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 2,0	1.1		1.1	5.0 - 7.0	6.0	
G35090	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 5,0	2.7		4,0 - 6,0	5.0		3.9	2.0	2.0	
G35093	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.4		1,0 - 7,0	3.3		2.4	2.0	2.0	
G35099	GTM	PLAN	C	1,0 - 4,0	1.8		1.0	1.0		1.4	1,0 - 3,0	1.6		1,0 - 6,0	2.8		2.2	1.0 - 3.0	2.0	*
G35100	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1,0 - 6,0	3.8		2.4	1.0	1.0	*
G35103	MEX	COCC	C	1,0 - 5,0	1.6		1.0	1.0		1.3	1,0 - 3,0	1.3		1,0 - 6,0	2.0		1.6	4.0 - 6.0	5.0	

Ident.	ORIG	SP.	BS	ANT - A Range	ANT - A Mean-A		ANT - M Range	ANT - M Mean-M		ANT Mean	ALS - A Range	ALS - A Mean-A		ALS - M Range	ALS - M Mean-M		ALS Mean	ASCOCHYTA Range	ASCOCHYTA Mean	
G35105	MEX	COCC	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0	*	1.0	6.0 - 8.0	7.0	
G35113	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 2,0	1.2	*	1.1	2.0 - 4.0	3.3	
G35116	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	3.0 - 4.0	3.5	
G35121	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 4,0	2.0		1.5	4.0 - 6.0	4.8	
G35123	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 3,0	1.3		1,0 - 3,0	1.2		1.3	2.0	2.0	*
G35143	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1,0 - 4,0	1.6		1,0 - 5,0	2.6		2.1	1.0 - 6.0	3.6	
G35148	MEX	COCC	C	1.0	1.0		1,0 - 2,0	1 1		1.1	1.0	1.0		1,0 - 5,0	2.5		1.8	3.0 - 6.0	5.0	
G35155	GBR	COCC	C	1,0 - 3,0	1.3		1.0	1.0	*	1.1	1.0	1.0		1,0 - 5,0	1.9		1.4	6.0 - 7.0	6.8	*
G35157	MEX	COCC	C	1,0 - 2,0	1.1		1.0	1.0		1.1	1,0 - 4,0	1.8		1,0 - 3,0	1.9		1.8	6.0 - 7.0	6.8	
G35169	GTM	COCC	C	1,0 - 2,0	1.3		1.0	1.0		1.2	1.0	1.0		1,0 - 5,0	3.0		2.0	3.0 - 5.0	4.3	
G35171	RWA	COCC	C	2,0 - 5,0	3.3		1,0 - 2,0	1.1		2.2	1,0 - 3,0	1.7		1,0 - 6,0	3.3		2.5	6.0 - 8.0	6.8	
G35172	RWA	COCC	C	1,0 - 2,0	1.5		1.0	1.0		1.3	1,0 - 4,0	2.3		2,0 - 6,0	3.7		3.0	6.0 - 7.0	6.8	
G35177	GTM	COCC	C	1,0 - 2,0	1.1		1.0	1.0		1.1	1.0	1.0		1.0	1.0		1.0	3.0 - 4.0	3.3	
G35182	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 5,0	3.0		1.0	1.0		2.0	2.0	2.0	
G35194	GTM	COCC	C	1,0 - 3,0	1.6		1,0 - 2,0	1.2		1.4	1,0 - 3,0	1.2		1,0 - 3,0	1.5		1.4	4.0 - 5.0	4.3	
G35220	GTM	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 4,0	1.6		1.3	3.0 - 4.0	3.8	
G35243	PRT	COCC	C	1.0	1.0	*	1.0	1.0		1.0	1.0	1.0		1,0 - 3,0	1.4		1.2	4.0	4.0	
G35252	PRI	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 5,0	2.0		1.5	3.0 - 4.0	3.8	
G35258	GTM	PLAN	C	1.0	1.0		1,0 - 2,0	1 1		1.1	1.0	1.0		1.0	1.0		1.0	2.0	2.0	
G35263	CRI	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0	1.0	
G35266	HND	COCC	C	1,0 - 2,0	1.1		1.0	1.0		1.1	1.0	1.0		1,0 - 2,0	1.2		1.1	3.0	3.0	
G35267	COL	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 4.0	3.0	
G35268	TUR	COCC	C	0.0		*	1.0	1.0	*		1.0	1.0	*	1.0	1.0		1.0	5.0 - 8.0	6.5	

Ident.	ORIG	SP.	BS	ANT - A Range	ANT - A Mean-A		ANT - M Range	ANT - M Mean-M		ANT Mean	ALS - A Range	ALS - A Mean-A		ALS - M Range	ALS - M Mean-M		ALS Mean	ASCOCHYTA Range	ASCOCHYTA Mean	
G35306	COL	PLAN	C	1.0	1.0		1,0 - 2,0	1.1		1.0	1.0	1.0	*	1.0	1.0		1.0	1.0	1.0	
G35311	MEX	COCC	C	1,0 - 2,0	1.2		1.0	1 0		1.1	1.0	1.0		1,0 - 2,0	1.1		1.1	3.0 - 6.0	4.5	
G35313	MEX	COCC	S	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0		1.0	1.0 - 3.0	1.8	*
G35327	NLD	COCC	C	1.0	1.0		1.0	1.0	*	1.0	1.0	1.0		1.0	1.0		1.0	3.0 - 5.0	4.3	
G35336	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 5,0	2.9		3,0 - 7,0	4.9		3.9	1.0 - 2.0	1.5	
G35337	MEX	PLAN	C	1,0 - 2,0	1.1		1.0	1.0		1.0	1,0 - 2,0	1.5		1,0 - 4,0	1.3		1.4	1.0 - 2.0	1.5	
G35341	MEX	COCC	C	1,0 - 2,0	1.1		1.0	1.0		1.1	1.0	1.0		1.0	1.0		1.0	2.0 - 4.0	3.5	
G35345	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 5,0	1.7		1.3	1.0 - 2.0	1.4	
G35346	MEX	COCC	C	1,0 - 5,0	3.0		1,0 - 6,0	4.1		3.6	1.0	1.0		1,0 - 7,0	3.8		2.4	4.0 - 6.0	5.0	
G35347	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0 - 2.0	1.8	
G35348	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.6		1.0	1.0		1.3	1.0 - 2.0	1.7	*
G35349	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 3,0	1.3		1,0 - 4,0	1.9		1.6	2.0 - 3.0	2.3	
G35350	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 4,0	2.5	*	1,0 - 3,0	1.3		1.9	2.0 - 4.0	2.2	
G35353	MEX	COCC	C	1,0 - 2,0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 3,0	1.1		1.1	3.0 - 7.0	4.6	
G35358	COL	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0		1.0	3.0 - 4.0	3.3	
G35359	COL	PLAN	C	1,0 - 2,0	1.1		1.0	1.0	*	1.1	1.0	1.0		1,0 - 6,0	2.8		1.9	0.0		*
G35369	CRI	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0	2.0	
G35372	COL	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0 - 2.0	1.5	*
G35373	COL	PLAN	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0		1.0	1.0	1.0	*
G35375	COL	COCC	C	1.0	1.0		1.0	1 0		1.0	1.0	1.0	*	1.0	1.0		1.0	3.0	3.0	
G35377	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 3,0	1.8		1,0 - 5,0	3.6		2.7	2.0 - 3.0	2.5	*
G35381	MEX	COCC	E	1,0 - 7,0	3.3		1,0 - 2,0	1.1		2.2	1.0	1.0		1,0 - 4,0	1.4		1.2	4.0 - 7.0	5.5	
G35382	MEX	COCC	S	1,0 - 2,0	1.3		1.0	1.0		1.2	1.0	1.0		1.0	1.0	*	1.0	3.0 - 5.0	3.8	

				ANT - A			ANT - M			ANT	ALS - A			ALS - M			ALS	ASCOCHYTA	
Ident.	ORIG	SP.	BS	Range	Mean-A		Range	Mean-M		Mean	Range	Mean-A		Range	Mean-M		Mean	Range	Mean
G35391	MEX	C/P	C	1.0	1.0		1,0 - 2,0	1.2		1.1	1.0	1.0		1.0	1.0		1.0	1.0 - 5.0	2.5
G35394	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	4.0 - 5.0	4.5
G35401	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	4.0 - 5.0	4.5
G35404	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0	2.0
G35405	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0		1.0	3.0 - 4.0	3.6
G35406	MEX	COCC	C	1,0 - 2,0	1.1		1,0 - 2,0	1.1		1.1	1.0	1.0		2,0 - 4,0	3.2		2.1	3.0 - 7.0	4.4
G35411	MEX	COCC	C	1,0 - 3,0	1.2		1.0	1.0		1.1	1.0	1.0		1,0 - 2,0	1.1		1.1	3.0 - 5.0	4.0
G35412	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	3.0 - 6.0	3.5
G35414	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 3,0	1.7		1,0 - 5,0	3.6		2.6	1.0	1.0
G35425	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 4.0	2.7
G35432	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0	*	1.0	1.0 - 2.0	1.5
G35441	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.1		1,0 - 5,0	1.5		1.3	1.0	1.0
G35448	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	6.0 - 7.0	6.8
G35453	MEX	PLAN	C	1.0	1.0		1,0 - 3,0	2.0		1.5	1.0	1.0		1.0	1.0		1.0	2.0	2.0
G35454	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1,0 - 3,0	1.3		1.0	1.0		1.1	3.0 - 5.0	4.5
G35458	MEX	PLAN	C	1.0	1.0		1,0 - 2,0	1.2		1.1	1,0 - 3,0	1.3		1.0	1.0		1.2	1.0 - 3.0	1.8
G35464	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	0.0	*
G35471	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0 - 2.0	1.3
G35476	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	3.0 - 5.0	4.0
G35485	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1,0 - 5,0	1.6		1,0 - 5,0	2.7		2.1	6.0 - 7.0	6.5
G35493	MEX	COCC	S	0.0		*	0.0		*		0.0		*	0.0		*		0.0	*
G35498	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 5,0	2.7		1.9	1.0	1.0
G35500	MEX	COCC	C	1.0	1.0		1,0 - 2,0	1.2		1.1	1.0	1.0		1,0 - 2,0	1.1		1.1	3.0	3.0

				ANT - A			ANT - M			ANT	ALS - A			ALS - M			ALS	ASCOCHYTA		
Ident.	ORIG	SP.	BS	Range	Mean-A		Range	Mean-M		Mean	Range	Mean-A		Range	Mean-M		Mean	Range	Mean	
G35503	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.1		1,0 - 7,0	3.1		2.1	1.0 - 2.0	1.5	
G35505	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*
G35506	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 4.0	3.3	
G35509	MEX	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 4.0	2.4	
G35513	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0 - 2.0	1.5	
G35526	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 2,0	1.1		1.0	1.0		1.1	1.0	1.0	
G35531	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 3,0	1.4		1,0 - 5,0	2.9		2.1	1.0	1.0	
G35547	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 6,0	2.7		1,0 - 7,0	3.1		2.9	1.0 - 4.0	1.6	*
G35549	MEX	PLAN	C	1.0	1.0		1.0	1 0		1.0	1.0	1.0		1,0 - 2,0	1.1		1.1	1.0 - 3.0	1.2	
G35552	MEX	PLAN	C	1.0	1.0		1.0	1 0		1.0	1,0 - 4,0	1.9		1,0 - 4,0	2.3		2.1	1.0	1.0	
G35569	GTM	PLAN	C	1.0	1.0		1.0	1.0	*	1.0	1.0	1.0	*	1,0 - 5,0	1.7		1.3	0.0		*
G35570	GTM	PLAN	C	1.0	1.0		1,0 - 3,0	1.2		1.1	1,0 - 2,0	1.3		1,0 - 3,0	1.3		1.3	1.0	1.0	
G35571	GTM	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	4.0	4.0	
G35573	CRI	PLAN	C	1,0 - 2,0	1.2	*	1.0	1.0		1.1	1,0 - 3,0	1.3		1.0	1.0		1.2	0.0		*
G35575	MEX	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0		1.0	0.0		*
G35582	GTM	PLAN	C	1.0	1.0		1.0	1 0		1.0	1,0 - 3,0	1.3		1,0 - 5,0	1.6		1.5	2.0	2.0	
G35588	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1,0 - 5,0	2.2		1.6	1.0 - 2.0	1.5	
G35593	GTM	PLAN	C	1.0	1.0		1.0	1.0		1.0	1,0 - 5,0	1.4		1.0	1.0		1.2	1.0	1.0	
G35593-B	GTM	PLAN	H	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	1.0 - 3.0	1.1	
G35595	GTM	PLAN	C	1.0	1.0		1,0 - 2,0	1.2		1.1	1,0 - 2,0	1.2		1,0 - 3,0	1.4		1.3	1.0 - 4.0	1.6	*
G35597	GTM	COCC	C	0.0		*	1.0	1.0	*		1.0	1.0	*	1.0	1.0		1.0	0.0		*
G35599	GTM	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	3.0	3.0	
G35601	GTM	COCC	C	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 4.0	3.3	

Ident.	ORIG	SP.	BS	ANT - A Range	ANT - A Mean-A	ANT - M Range	ANT - M Mean-M		ANT Mean	ALS - A Range	ALS - A Mean-A		ALS - M Range	ALS - M Mean-M		ALS Mean	ASCOCHYTA Range	ASCOCHYTA Mean	
G35609	MEX	COCC	C	1.0	1.0	1,0 - 2,0	1.1		1.1	1,0 - 4,0	1.3		1,0 - 5,0	1.9		1.6	4.0 - 6.0	5.2	
G35616	GTM	PLAN	C	1.0	1.0	1.0	1.0		1.0	1,0 - 2,0	1.1		1,0 - 4,0	1.5		1.3	1.0 - 2.0	1.5	
G35621	MEX	COCC	C	1,0 - 3,0	1.4	1.0	1.0		1.2	1.0	1.0		1.0	1.0		1.0	2.0 - 3.0	2.3	*
G35622	VEN	PLAN	C	1.0	1.0	1.0	1.0		1.0	1,0 - 6,0	2.8	*	3,0 - 6,0	4.1		3.5	1.0 - 2.0	1.3	
G35623	YUG	COCC	C	1.0	1.0	1.0	1.0	*	1.0	1.0	1.0		1.0	1.0		1.0	3.0 - 5.0	3.5	
G35625	COL	PLAN	C	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1,0 - 6,0	2.5		1.8	1.0 - 2.0	1.3	
G35642	PER	PLAN	C	1,0 - 2,0	1.1	1.0	1.0	*	1.1	1.0	1.0	*	0.0		*		2.0	2.0	
G35644	PER	PLAN	C	1.0	1.0	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0	*	1.0	2.0	2.0	
G35658-A	MEX	COCC	C	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	5.0	5.0	
G35708	MEX	COCC	C	1,0 - 2,0	1.1	1.0	1.0		1.1	1.0	1.0		1.0	1.0		1.0	1.0 - 2.0	1.5	
G35755	GTM	PLAN	E	1.0	1.0	1.0	1.0		1.0	1,0 - 2,0	1.2		1,0 - 6,0	2.7		1.9	1.0	1.0	
G35757	GTM	COCC	S	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	4.0 - 5.0	4.8	
G35758	GTM	PLAN	S	1.0	1.2	1.0	1.0		1.1	1.0	1.0		1.0	1.0		1.0	1.0	1.0	
G35759	GTM	COCC	S	1.0	1.0	1.0	1.0		1.0	1.0	1.0	*	1.0	1.0	*	1.0	1.0 - 2.0	1.3	*
G35765	GTM	COCC	C	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	3.0 - 5.0	4.0	
G35769	GTM	COCC	C	1.0	1.0	1.0	1.0		1.0	1,0 - 2,0	1.1		1.0	1.0		1.1	2.0 - 3.0	2.8	
G35771	GTM	PLAN	C	1.0	1.0	1.0	1.0		1.0	1,0 - 2,0	1.3		4,0 - 6,0	4.5		2.9	1.0	1.0	*
G35777	GTM	COCC	E	1,0 - 3,0	1.3	1.0	1.0		1.1	1.0	1.0		1.0	1.0		1.0	4.0 - 6.0	4.8	
G35780	GTM	PLAN	C	1.0	1.0	1.0	1.0		1.0	1,0 - 5,0	2.0		1,0 - 6,0	3.9		2.9	1.0	1.0	*
G35837	GTM	COCC	S	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	3.0	3.0	
G35837-A	GTM	COCC	S	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1,0 - 6,0	2.6		1.8	2.0 - 3.0	2.8	*
G35838	MEX	COCC	S	1.0	1.0	1.0	1.0		1.0	1.0	1.0	*	1.0	1.0	*	1.0	2.0 - 4.0	2.8	*
G35845	MEX	COCC	S	1.0	1.0	1.0	1.0		1.0	1.0	1.0		1.0	1.0	*	1.0	1.0	1.0	

				ANT - A			ANT - M			ANT	ALS - A			ALS - M			ALS	ASCOCHYTA		
Ident.	ORIG	SP.	BS	Range	Mean-A		Range	Mean-M		Mean	Range	Mean-A		Range	Mean-M		Mean	Range	Mean	
G35846	MEX	COCC	S	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0	*	1.0	2.0 - 4.0	2.8	*
G35851	GTM	COCC	S	1.0	1.0	*	1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	4.0 - 5.0	4.3	
G35856	GTM	COCC	S	1.0	1.0		1.0	1.0		1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 3.0	2.8	*
G35869	COL	COCC	H	1,0 - 3,0	1.4		1.0	1.0		1.2	1.0	1.0		1.0	1.0		1.0	4.0 - 6.0	4.5	
G35871	MEX	COCC	S	1.0	1.0	*	1.0	1 0		1.0	1.0	1.0	*	1.0	1.0		1.0	1.0 - 2.0	1.3	*
G35872	MEX	COCC	S	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0	*	1.0	3.0 - 4.0	3.3	*
G35875	GTM	COCC	S	1.0	1.0		1.0	1.0		1.0	1.0	1.0	*	1.0	1.0		1.0	2.0	2.0	*
G35877	GTM	PLAN	S	1.0	1.0		1.0	1 0		1.0	1.0	1.0		1.0	1.0		1.0	1.0	1.0	
G35884	YUG	COCC	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0		1.0	6.0 - 8.0	7.3	
G35907	ROM	COCC	C	1,0 - 2,0	1.2	*	1.0	1.0	*	1.1	1.0	1.0		1.0	1.0		1.0	4.0 - 6.0	4.8	
G35980	COL	PLAN	H	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0		1.0	4.0	4.0	*
G35991	COL	PLAN	H	1.0	1.0	*	1.0	1 0	*	1.0	1.0	1.0	*	1 0	1.0	*	1.0	2.0	2.0	
G35995	COL	PLAN	C	1,0 - 2,0	1.5	*	1.0	1.0	*	1.3	1.0	1.0	*	2,0 - 4,0	3.0		2.0	2.0 - 3.0	2.8	
G35997	COL	PLAN	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	*	1.0	1.0	*	1.0	2.0 - 4.0	2.8	
G35999	COL	COCC	C	1.0	1.0		1.0	1.0	*	1.0	1.0	1.0		1.0	1.0		1.0	2.0 - 3.0	2.5	
G36011	YUG	COCC	C	1,0 - 3,0	1.7	*	1.0	1.0	*	1.3	1.0	1.0	*	1.0	1.0		1.0	6.0 - 7.0	6.8	
G36022	ROM	COCC	C	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0		1.0	1.0		1.0	5.0 - 8.0	5.1	
S29704	CRI	COST	S	1.0	1.0		1.0	1.0		1.0	0.0		*	0.0		*		1.0 - 2.0	1.5	
S32391	ECU	PLAN	C	1.0	1.0		1.0	1.0	*	1.0	1.0	1.0		1.0	1.0	*	1.0	2.0	2.0	*
S33174	COL	COCC	C	0.0		*	1,0 - 2,0	1.2			0.0		*	1.0	1.0	*		2.0	2.0	
S33526	COL	PLAN	C	1,0 - 3,0	1.5	*	1.0	1.0	*	1.3	1.0	1.0	*	1.0	1.0	*	1.0	1.0	1.0	
S33624	COL	PLAN	C	1,0 - 2,0	1.3	*	1.0	1.0	*	1.1	1.0	1.0		1.0	1.0		1.0	1.0	1.0	
S33626	COL	COCC	H	1,0 - 2,0	1.1		1,0 - 2,0	1.3	*	1.2	1.0	1.0		1.0	1.0	*	1.0	3.0 - 4.0	3.3	

Ident.	ORIG	SP.	BS	ANT - A Range	ANT - A Mean-A	ANT - M Range	ANT - M Mean-M	ANT Mean	ALS - A Range	ALS - A Mean-A	ALS - M Range	ALS - M Mean-M	ALS Mean	ASCOCHYTA Range	ASCOCHYTA Mean	
S33669	COL	PLAN	C	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0 - 2.0	1.3	*
ENT 356-1A-F6		COCC		0.0		0.0			1.0	1.0	1.0	1.0	1.0	0.0		
G2333		VULG		1.0	1.0	1.0	1.0	1.0								
KABOON		VULG		1.0	1.0											
BAT841		VULG		2,0 - 3,0	2.3	3,0 - 6,0	4.1	3.2								
BAT76		VULG		2,0 - 4,0	3.1	5,0 - 9,0	7.2	5.2								
ICA PIJAO		VULG		3.0 - 8.0	5.4	7.0 - 9.0	8.7	7.1								
CALIMA		VULG		7,0 - 9,0	8.6	1,0 - 2,0	1.5	5.1								
BAT332		VULG							1.0	1.0	1.0	1.0	1.0			
G2858		VULG							3,0 - 5,0	4.1	8.0	8.0	6.1			
DON TIMOTEO		VULG							5,0 - 9,0	6.3	7,0 - 8,0	7.9	7.1			
CALIMA		VULG							7,0 - 9,0	8.0	1,0 - 4,0	3.2	5.6			
CARGAM ANTO		VULG							7,0 - 9,0	7.1	6,0 - 7,0	6.1	7.1			
G35182		PLAN												1.0 - 3.0	2.0	
G10859		VULG												4.0 - 7.0	5.9	
G02641		VULG												7.0 - 8.0	7.8	
Entries to re-evaluate				23		28			35		19			35		

Inoculations with mixtures of isolates of the ANT and ALS pathogens were carried out in screenhouses in the Popayán station of CIAT. Ten plants were established of each accession. Inoculations with ASC were carried out in the field in Popayán on five meter rows. Isolates of all three pathogens were obtained locally. ANT and ALS pathogens are known to display adaptation through co-evolution with the bean host, and the isolates used had been classified as Mesoamerican or Andean in reaction with common bean. All reactions were registered on a scale of 1 (immune) to 9 (totally susceptible).

* Evaluations to be repeated

ANT - A : Anthracnose –Inoculation with a mixture of local isolates of Andean reaction Nos. 2 and 56 from Popayán, in screenhouse.

ANT - M : Anthracnose –Inoculation with a mixture of local isolates of Mesoamerican reaction Nos. 17, 20 and 43 from Popayán, in screenhouse.

ALS - A : Angular leaf spot - Inoculation with a mixture of local isolates of Andean reaction Nos. 243 and 265 from Popayán, in screenhouse.

ALS - M : Angular leaf spot - Inoculation with a mixture of local isolates of Mesoamerican reaction Nos. 242 and 244 from Popayán, in screenhouse.

ASCOCHYTA : Inoculation with local isolates of the ascochyta pathogen in the field in Popayán.

Both wild and cultivated *P. polyanthus* were universally and highly resistant to ASC. Although only two wild accessions of PP were evaluated, results with wild were nonetheless consistent with results with the cultivated. Thus it appears that the resistance may be an ancestral trait derived from the wild *polyanthus* and has not been substantially altered during domestication. On the other hand, both wild and cultivated *P. coccineus* present a range of reactions to ASC. The wild presented values from 1.0 to 5.5, and the cultivated from 1.3 to 7.0. Thus, the reaction of *coccineus* to ASC has not changed substantially with domestication either. However, within *P. coccineus* there is certain geographical stratification, such that Mexican and European accessions often present intermediate to susceptible reactions to ASC, while accessions to the south of Mexico tend to be more resistant. This could feasibly reflect the lateness of these latter accessions, as noted in last year's report.

The contrast between *polyanthus* and *coccineus* presents an interesting evolutionary question. It has been shown that the nuclear DNA of these two species presents greater similarity than either do with *vulgaris*, while the non-nuclear DNA of *polyanthus* is more closely related to *vulgaris*. This suggests that the nucleus of *polyanthus* was heavily introgressed by *coccineus* at some point in its evolution. Wild *P. polyanthus* is known to exist only in Guatemala to date, and two of these accessions presented ASC readings of 1.0. Wild Guatemalan *coccineus* presents ASC reactions from 1.3 to 4.8. If *polyanthus* has been heavily introgressed from *coccineus*, why does it present such a narrow range of ASC values? Does the uniformly high reaction of both the wild and cultivated *polyanthus* suggests that this introgression was limited to certain populations of wild *coccineus*, even among the several populations in Guatemala? Again, this should be taken within the context of the limited sample size of wild PP, and should be interpreted as a topic of future study and not as a conclusion.

Reaction of PP and PC to common bean ALS is variable but generally low. One might have speculated that Mesoamerican isolates of the pathogen could have attained greater adaptation to the PP/PC hosts than Andean isolates, since PP and PC are believed to have evolved essentially in Middle America. In fact, the Mesoamerican isolates were slightly more virulent on about 25% of the accessions, although disease reaction did not follow gene pool lines of the pathogen closely. The reaction of a given accession with the two types of isolates was similar in more cases than it was distinct. Curiously, in the few cases in which the Andean isolates gave a more virulent reaction, this was observed only with *polyanthus*. This suggests some subtle differences in the evolution of the two species in relation to the ALS pathogen. Could PP have experienced some stage of its evolution in contact with

Both wild and cultivated *P. polyanthus* were universally and highly resistant to ASC. Although only two wild accessions of PP were evaluated, results with wild were nonetheless consistent with results with the cultivated. Thus it appears that the resistance may be an ancestral trait derived from the wild *polyanthus* and has not been substantially altered during domestication. On the other hand, both wild and cultivated *P. coccineus* present a range of reactions to ASC. The wild presented values from 1.0 to 5.5, and the cultivated from 1.3 to 7.0. Thus, the reaction of *coccineus* to ASC has not changed substantially with domestication either. However, within *P. coccineus* there is certain geographical stratification, such that Mexican and European accessions often present intermediate to susceptible reactions to ASC, while accessions to the south of Mexico tend to be more resistant. This could feasibly reflect the lateness of these latter accessions, as noted in last year's report.

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Andean isolates of ALS? This could be studied further with selected, virulent Andean isolates of the pathogen.

Reaction to ANT gave the least discrimination among accessions of the three pathogens studied. Both species are almost universally resistant to either Mesoamerican or Andean isolates of the pathogen. While a few individual plants were registered with reactions as high as 7, the highest average value for an accession was 4.1 in G35346, a Mexican accession of PC inoculated with Mesoamerican isolates. The same accession presented a reaction of 3 with Andean isolates.

One accession of *P. costaricensis* was also included in the evaluations, and this accession was resistant to all three pathogens.

The core was dispatched to Puerto Rico in an attempt to evaluate resistance to BGMV, but disease pressure was unusually light and did not permit discrimination of differences among accessions.

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

Previously we reported;

- The F_1 generation of PC x PP resistant and susceptible to BGMV was planted and F_2 seed has been harvested.
- The F_1 generation between putative susceptible and resistant PC and PP for Bean Fly has been planted and harvest of F_2 seed has begun.
- Interspecific selections that are susceptible to Ascochyta and display PC or PP morphology are being crossed with PC and/or PP to create the F_1 for subsequent genetic studies.

In 1997-98 we can report the following:

- In the cross of PC x PP resistant and susceptible to BGMV, F_3 families have been obtained for BGMV testing.
- F_2 population of the BGMV cross has transplanted to the field for inoculation with ASC.
- F_2 plants of four crosses for ASC resistance are now planted and will be transferred to the field for inoculation. One of the four populations is being increased in the screenhouse to obtain F_3 families.

The study of the inheritance of resistance requires the development of populations between susceptible and resistant parental genotypes that permit drawing conclusions about

the number of genes present and the type of gene expression. For this purpose we have made the necessary crosses for BGMV, for BSM and for Ascochyta. Populations are in different stages of development (**Tables IV-VI**).

Table IV. Hybrid seeds for inheritance study of BGMV resistance.

Obs	Identification	Type of cross	F_2 seed	F_3 Families
1	G35171 x G35337	Pc x Pp	682	
2	G35171 x G35347	Pc x Pp	718	
3	G35172 x G35337 (*)	Pc x Pp	537	153 **
4	G35172 x G35347	Pc x Pp	575	

(*) : Advanced to F_3 during 1999 A .
(**) : Total number of harvested families. Families with : 15 – 25 seeds: 50 Entries
: ≥ 25 seeds: 103 Entries

Table V. Hybrid seeds for inheritance study of BSM resistance.

Obs	Identification	Type of cross	F_2 seed
1	G35353 x G35337	Pc x Pp	712
2	G35353 x G35347	Pc x Pp	482
3	G35353 x G35023	Pc x Pc	346
4	G35353 x G35350	Pc x Pp	541
5	G35341 x G35337	Pc x Pp	531
6	G35341 x G35347	Pc x Pp	422
7	G35341 x G35023	Pc x Pc	354
8	G35341 x G35350	Pc x Pp	398

Table VI. Hybrid seeds for inheritance study of Ascochyta resistance.

Obs	Identification (*)	Type of cross	F_1 seed	F_2 seed
1	356-1 A-F_6 x G35358 (**)	Pc x Pc	58	618
2	356-1 A-F_6 x G35369 (**) §	Pc x Pc	38	835
3	356-1 A-F_6 x G35509 (**)	Pc x Pc	47	521
4	356-1 A-F_6 x G35498	Pc x Pp	1 (***)	
5	G35884 x G35336	Pc x Pp	13 (***)	
6	G35884 x G35441	Pc x Pp	9 (***)	
7	G35884 x G35498	Pc x Pp	4 (***)	

(*) : 356-1A- F_6, an interspecific line with tendency toward P. coccineus = ((G35876 x G3807) x G35023) x BAT 1297.
(**) : Planted in the field July 22 1999.
(***) : Partial data. 1999 B.
(§) : Planted in the screenhouse July 22 1999 to advance to F_3.

In one of the crosses created to study the inheritance of BGMV resistance, G35172 x G35337, representing PC resistant x PP susceptible, harvest of F_2 seed was completed and F_3 families have been obtained for BGMV testing. Some 103 F_3 families have 25 seed or more, and another 50 families have 15-25 seed. Plans are underway to inoculate this population in the next year.

To study the inheritance of resistance to ASC, a highly susceptible interspecific progeny with PC phenotype, 356-1A, was identified in the field in 1997. Experience has borne out the initial observation regarding the extreme susceptibility of this line. This line was crossed to resistant PC accessions including G35369. The F_2 populations has been planted in plastic bags and will soon be transplanted to the field for inoculation. One of the populations will be also be planted in the screenhouse for F_3 seed production.

In the course of the evaluations of the core collection, we realized that the accession G35172, which figures as a resistant parent for the study of BGMV resistance, is also highly susceptible to ASC (rating of 6.8). In the study of BGMV inheritance, G35172 is crossed to a *P. polyanthus* accession (G35337) that is resistant to ASC (rating of 1.5). Therefore, we have the opportunity to study the inheritance of ASC resistance in PP, using the plants that have remained of the BGMV population. In the course of our evaluations we have gained confidence that individual plants can be evaluated effectively, therefore we are preparing to evaluate the F_2 plants. We have transplanted more than 120 F_2 plants of this population to the field for inoculation with ASC. We also have the opportunity to multiply these plants by cuttings, if this be necessary to obtain a larger plot size.

A few F_1 plants of the BGMV inheritance crosses were transplanted to the field after obtaining F_2 seed for the inheritance studies. Casual observations indicated that these F_1 plants expressed very high levels of susceptibility, suggesting that resistance is definitely not dominant in nature, and is more likely recessive than additive.

Thus we have expectations that in the course of the next year we will have fulfilled the objectives of studying the inheritance of resistance to these two diseases. Unfortunately, we will probably not have similar data on the inheritance of resistance to Bean Fly (or Bean Stem Maggot – BSM), due to problems in obtaining data on this pest in Tanzania. An unusually dry year reduced the incidence of the pest in the first months of this year, and no data are yet forthcoming to confirm the reaction of parental sources of resistance.

2.4. Molecular probes combining RFLP and RAPD methods are used to tag resistance genes. This objective is pending the obtention of data on segregating populations.

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

3.1. Tissue culture techniques are developed to rescue proembryos of interspecific crosses

3.1.1. Introduction

Interspecific hybridization within *Phaseolus* gene pool is aimed at solving some major problems of the cultivation of *P. vulgaris* in the tropics. Regarding Ascochyta leaf blight, field screening in CIAT demonstrated that the most resistant genotypes to this disease belong to *P. polyanthus*.

Growing *Phaseolus* interspecific hybrids often requires using embryoculture. This allows the rescue of aborting immature embryos and the recovery of mature plants suitable for breeding programmes. Incompatibility barriers between *Phaseolus* species usually causes abortion of the globular or early heart-shaped stage embryos. This is the case for crosses between *P. polyanthus* (used as female parent) and *P. vulgaris*. Some interspecific crosses have been attempted by Lecomte (1997) between these two species but, although fertilized ovules were obtained, up to 60 % of globular embryos failed to develop due to undefined incompatibility barriers between embryo and mother plant. Only 7 % of these globular embryos could reach the cotyledonar stage when cultivated *in vitro* before dying (Lecomte 1997). Success in *Phaseolus* embryo rescue actually has been limited to embryos more than 8 days old that had reached at least the late heart-shaped stage (**Table VII**).

Table VII. Regeneration of interspecific hybrids within *Phaseolus* using embryoculture.

Crosses (female x male)	Age (days)	Stage	Reference
P. vulgaris L. x *P. ritensis* Jones	16-23	LC	**(1), (14)**
P. vulgaris L. x *P. lunatus* L.	12-24	C-LC	**(2), (16), (18)**
P. vulgaris L. x *P. acutifolius* A. Gray	14-28	C-LC	**(3), (6) ,(7), (8), (9), (12), (15), (16), (19), (20)**
P. coccineus L. x *P. acutifolius* A. Gray	15-20	C-LC	**(4), (17)**
P. coccineus L. x *P. vulgaris* L.	15-20	C-LC	**(5)**
P. vulgaris L. x *P. filiformis* Bentham.	7-24	LC	**(11), (14)**
P. vulgaris L. x *P. angustissimus* A. Gray	16-23	LC	**(10), (14)**
P. polyanthus Greenman. x *P. vulgaris* L.	19-33	LC	**(13)**

(1) Braak & Kooistra (1975); **(2)** Mok *et al.* (1978); **(3)** Rabakoarihanta *et al.* (1980); **(4)** Alvarez *et al.* (1981); **(5)** Shii *et al.* (1982); **(6)** Prendota *et al.* (1982); **(7)** Pratt & Bressan (1983); **(8)** Thomas & Waines (1984); **(9)** Pratt *et al.* (1985); **(10)** Belivanis & Doré (1986); **(11)** Weilenmann de Tau *et al.* (1986); **(12)** Parker & Michaels (1986); **(13)** Camarena & Baudoin (1987); **(14)** Petzoldt & Dickson (1987); **(15)** Andrade & Jackson (1988); **(16)** Cabral & Crocomo (1989); **(17)** Ben Rejeb & Benbadis (1989); **(18)** Kuboyama *et al.* (1991); **(19)** Jung *et al.* (1992); **(20)** Mejia-Jimenez *et al.* (1994); LC = Late Cotyledonar; C = Cotyledonar.

In vitro rescue of very immature embryos requires a culture medium that can support their growth and development. The use of inadequate media results in embryo necrosis, callus formation or premature germination, which leads in turn to weak and unbalanced seedlings.

For early heart-shaped embryos (6 days old), Mergeai *et al.* (1997) obtained the best embryo development on a modified Gamborg *et al.* (1968) maturation medium containing 400 $mg.l^{-1}$ NH_4NO_3, 1 $mg.l^{-1}$ thiamin HCl, 5 $mg.l^{-1}$ nicotinic acid, 0.5 $mg.l^{-1}$ pyridoxine, 1,000 $mg.l^{-1}$ L-glutamine, 1,000 $mg.l^{-1}$ casein hydrolysate, 100 $mg.l^{-1}$ myoinositol, 0.028 $mg.l^{-1}$ N_6-Benzyladenine (N_6B), 30 $g.l^{-1}$ sucrose, and 8 $g.l^{-1}$ DIFCO agar. With this last medium, 60 % of the embryos germinated and 30 % developed into plantlets. Some modifications were proposed by Lecomte (1997) to improve the embryo regeneration rate through *in vitro* culture : suppression of N_6B and incorporation of 25 $\mu g.l^{-1}$ abscisic acid (ABA), of 200 $mg.l^{-1}$ calcium ($CaCl_2x2H_2O$) and of 0.06 $mg.l^{-1}$ gibberellic acid (GA_3). Improvements were brought later by Geerts *et al.* (1999a). They proposed to replace minerals of Gamborg *et al.* (1968) by those of Phillips *et al.* (1982), and to use 25 $\mu g.l^{-1}$ ABA as growth regulator (described as the P4 medium). They also reported the interest to cultivate the embryos in a dehydration medium for 2 weeks after germination and before cultivation on the rooting medium. The dehydration medium is similar to the germination medium but lacks the hormone and amino acid and contains 100 $g.l^{-1}$ sucrose and 5 $g.l^{-1}$ activated charcoal. Dehydration also stimulated subsequent development of the germinated embryos in rooting medium. With the modifications proposed by Geerts *et al.* (1999a), the average germination rate of early heart-shaped embryos was about 50 % and the plantlet survival rate reached 60 %.

Results obtained with globular embryos are relatively poor (Smith 1971). Lecomte (1997) underlined the benefits of an osmotic gradient in the culture medium. In particular, the use of a double-layer medium as described by Liu *et al.* (1993) enabled 60 % of cultivated globular embryos to reach the late cotyledon stage and 13 % to germinate but no plantlet could be rescued. Another possibility to regenerate plants from globular embryos could be the *in ovulo* or pod culture, during the first days, before extracting the embryos. For example, Dherte (1995) obtained interesting results with an *in ovulo* culture technique adapted for 4 days old *Phaseolus* embryos : 38 to 55 % of the embryos reached at least the early heart-shaped stage after 10 days of *in ovulo* culture. However, only one plantlet on about 300 germinated embryos could be rescued. Lazaridou *et al.* (1993) described a pod culture technique that permits to regenerate hybrid plants from *Vicia faba* x *V. narbonensis* embryos. Ridolfo (1998) adapted the technique described by Lazaridou *et al.* (1993) to 4 days old *Phaseolus* pods. He observed that in *P. vulgaris* pods (containing about 6 to 8 seeds with

globular embryos), one or two seeds inflated and gave rise to early heart-shaped embryos. This result suggested that it could be possible to regenerate at least 12 % of globular embryos. Pod culture is therefore a mean to avoid problems occurring during embryo extraction.

In the work reported here, rather than using embryo rescue or *in ovulo* culture, we assessed the interest of a pod culture technique adapted from Lazaridou *et al.* (1993) taking into account the *in vivo* evolution of osmolality within pods during the early development (2-40 days after pollination).

During the first two years of our research, we optimized a protocol for histological studies. In the present report, we have reviewed embryogenesis in *Phaseolus* from ovule to seed and we have made some preliminary observations related to the abortion of embryos in *P. vulgaris* (♀) x *P. polyanthus* crosses.

3.1.2. Main results obtained in the first two years of the project

Several experiments were carried out during the first two years of the project. The main results were as follow :

(a) The maturation of immature embryos depends on the type of salts and hormones in the medium. A 20 % increase of the maturation rate of early heart-shaped embryos is obtained by replacing the mineral of Gamborg *et al.* (1968), NH4NO3 (400 $mg.l^{-1}$) and N_6-benzylaminopurine (0.028 $mg.l^{-1}$) by the salts of Phillips *et al.* (1982) and 25 $\mu g.l^{-1}$ ABA. Gibberellin (GA_3) has an inhibitory action on ABA, the latter preventing precocious germination. The addition of 200 mg/l calcium to a Phillips medium has a negative effect on the development of *Phaseolus* embryos : callus formation appeared at the suspensor level.

(b) The plantlet survival rate of germinated embryos depends on the cultivation conditions during embryoculture. For early-heart shaped embryos, the transfer of the embryos on a dehydration medium as described by Hu & Zanettini (1995) for one week before cultivating them on a rooting medium permitted to increase the acclimatization rate by more than 10 %.

(c) For globular embryos (4 days old), a pod culture in a sequence of liquid Phillips media, each completed with different polyethylene glycol concentrations to adjust osmotic pressure to the one observed *in vivo*, permitted to obtain, easily, late cotyledonar embryos. So far we do not know whether the increased rate of embryo development is more related to the osmotic pressure adjustment or to the increased number of globular embryos

cultivated without damage.

(d) The selection of *P. vulgaris* and *P. polyanthus* genotypes adapted to embryoculture and having a high crossing compatibility is an important step to reach the objectives of our research. It allows to identify the hybrid combinations that have the highest probability to produce interesting adult plants through embryo rescue. We selected two *P. polyanthus* genotypes (NI 1015 and G 35348) and one wild *P. vulgaris* genotype (G 21245) whose hybrid pods reached a good size and were late to abort.

(e) A protocol for histological studies was devised from the one described by Lecomte (1998). It will allow us to understand better the process that leads to embryo abortion and the role of osmotic pressure during early embryo development.

3.1.3. Current objectives in the framework of the project

Considering our results obtained during the first two years of the project, we concentrated the activities conducted in the third year on the following objectives :

A. Evaluation of an *in vitro* pod culture technique for the cultivation of early globular embryos (2 days old) with evaluation of the impact of the osmotic treatments on the embryo development, according to the results obtained by Geerts *et al.* (1999b).

B. Assessment of the crossing compatibility between selected *P. polyanthus* and *P. vulgaris* genotypes;

C. Comparison between embryo development in selfpollinated and hybrid seeds by histological studies. In a first step we concentrated our efforts on *Phaseolus vulgaris* (used as female) x *P. polyanthus.*

3.1.4. Material and method

Plant Material

One cultivated (NI 637) and one wild (G 21245) *P. vulgaris* genotypes, and two *P. polyanthus* genotypes (NI 1015, G35348) were grown in a growth chamber under controlled conditions (day/night temperature of 24/20°C, light intensity of 580 µE/m²sec. and a day length of 11h30). Genotypes were chosen according to their adaptation to interspecific crosses with *P. vulgaris* (Second Annual Report 1998).

Embryoculture : Technique adapted for globular embryos

A single *P. vulgaris* genotype (NI 637) was used to assess the interest of *in vitro* pod culture on the development of early globular embryos (2 days old). The pod culture device was adapted from Lazaridou *et al.* (1993). Young pods, collected 2 days after pollination, were sterilized and directly cultivated under light for maturation-germination processes in Petri dishes containing a Phillips gelified medium during one week. Two different maturation-germination media were used : the modified Phillips *et al.* (1982) medium as described by Geerts *et al.* (1999a, P4, see chapter 3.1.1.) or a sequence of two new modified Phillips *et al.* (1982) media, described as Po0 and Po1. These media are adapted from the results obtained by Monnier (1976) and Liu *et al.* (1993) for very immature embryos. New modified Phillips *et al.* (1982) media (Po0 and Po1) are described in **Table VIII**. After one week, embryos were extracted from ovule reaching at least 2 mm and transferred to P4 or Po1 media for two weeks in order to induce germination. Embryos were then transferred to a dehydration medium (G6) during one week before cultivating them on a rooting medium (G7). Media G6 and G7 are described in **Table VIII**.

Two pod sterilizing methods were applied : (i) Lazaridou *et al.* (1993) method : pods are sterilized in 15 % sodium hypochlorite (10 min.) and rinsed two times in autoclaved water (St1 method), or (ii) Randall (1998) method : pods are also sterilized in sodium hypochlorite solution but containing ten droplets per liter of polyoxyethylenesorbitan monolaurate (Tween 20) and cultivated on media containing 0,1 to 2 ml/l of Plant Preservative Mixture (PPM) (St2 method). PPM contains a mixture of two isothiazolones, which are a class of broad-spectrum, widely used industrial biocides, and reported as non-phytotoxic at concentrations suitable for the prophylactic control of microbial contaminants in plant tissue culture.

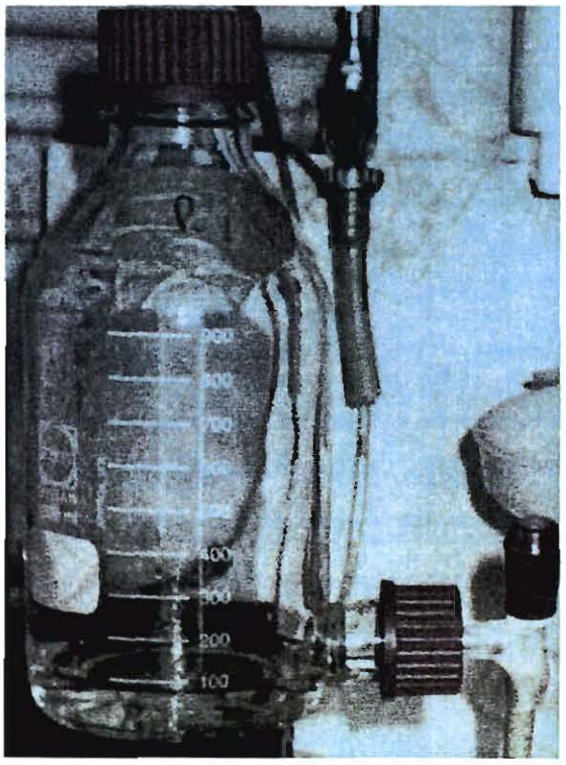
Three pod culture conditions were compared. They are summarized in **Table IX**. The difference between the three culture conditions was principally based on the evolution of osmolality in media. Values of osmolalities were adapted considering the results obtained by Geerts *et al.* (1999b) on the *in vivo* evolution of osmolality of pods. The three pod culture conditions were as follows :

(a) culture conditions described by Geerts *et al.* (1999a), (IG6 conditions) : pods are cultivated in P4 medium characterized by a constant osmolality of 350 mosm. This medium will permit us to compare the results with those of previous experiments, in particular those of Ridolfo (1998);

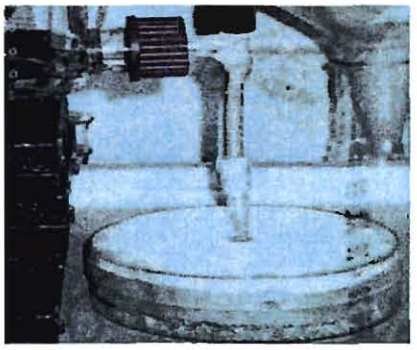
(b) culture conditions described by Geerts *et al.* (1999a) but adapted from the results obtained

by Monnier (1976) and Liu *et al.* (1993), described as IP0-1 conditions : pods are cultivated on a gelified Po0 medium essentially characterized by a higher concentration in sucrose (osmolality of 580 mosm) and a lower content of minerals;

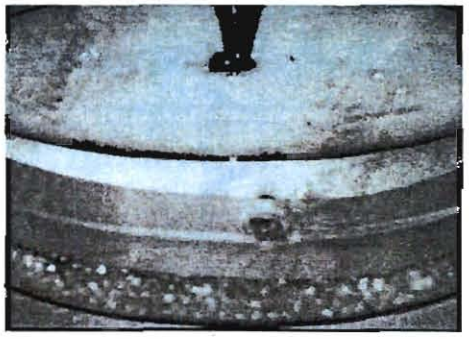
(c) same culture conditions as IP0-1 conditions, but adapted from the results obtained by Geerts *et al.* (1999b) on the *in vivo* evolution of pod osmolality, described as IP0-2 conditions : pods are laid down on sterilized glass balls (3 mm) in a Petri dish (15 cm x 1 cm) containing 100 ml of a liquid Po0 medium (osmolality of 580 mosm). During the first five days of culture, 100 ml/day of a liquid Po1 medium (350 mosm) was added in the Petri dish by dripping, permitting a constant evolution of the medium. To avoid losses by evaporation during the dripping of Po1 medium, a special design was investigated (**Figure 1**). A one liter bottle decanter with an inferior tap directly connected to the top of a Petri dish was set up. Petri dish was completely sealed but two small apertures were made : one on the top-center permitting the entry of liquid Po1 medium and a lateral one permitting to maintain a 100 ml volume of medium in the Petri dish by the discharge of the excess of liquid (**Figure 1**). Everyday during pod culture, 1 ml of the medium in the Petri dish was sampled to control the evolution of osmolality by osmolality measurements.



(1)



(2)



(3)

Figure 1. The design used for liquid media experiments. A one liter decanter (photo 1) with an inferior tap, is connected to a Petri dish (Φ = 15 cm, photo 2) completely sealed but with two small apertures : one on the top-center permitting the entry of liquid medium from the bottle decanter (photo 2) and a lateral one permitting to discharge the excess of liquid (photo 3).

Table VIII. Mineral composition of the basal media : Po0, Po1, G6 and G7.

ELEMENTS	Po0	Po1	G6	G7
		Major elements (in mg.l^{-1})		
CaCL2x2H2O	300	600	150	150
MgSO4x7H2O	220	435	134	134
KNO3	1050	2100	250	250
NaH2PO4xH2O	42	85	2500	2500
KH2PO4	162	325	150	150
NH4NO3	500	1000	400	
		Minor-elements		
MnSO4x7H2O	7,50	15	10	10
ZnSO4x7H2O	2,50	5	2	2
CuSO4x5H2O	0,050	0,1	0,025	0,025
CoCl2x6H2O	0,050	0,1	0,025	0,025
KI	0,50	1	0.75	0.75
H3BO3	3,70	7,4	3	3
NiCl2x6H2O	0,018	0,4		
Na2MoO4x2H2O	0,2	0,024	0.25	0.25
FeSO4x7H2O	12,50	25	27.85	27.85
Na2.EDTA	18,62	37,25	37.25	37.25
		Vitamins		
Thiamin	0,25	1	1	1
Nicotinic acid	1,25	5	5	5
Pyridoxine	0,125	0,5	0,5	0,5
Myoinositol	25	100	100	100
		Sugars		
Sucrose	100000	80000	100000	5000
		Amino acids		
L glutamine	250	1000	1000	100
casein hydrolisate	250	1000	1000	100
		Hormones		
Abscisic acid	0,025			
Tryptophan	1,128			
NAA	0,019	0.019		
Adenin		1.35		
N6 benzylaminopurine		0.225		0.028
		Gel and other		
DIFCO agar	5000	5000	8000	5000
Activated charcoal			5000	

(*) Po0, Po1, G6 and G7 represent respectively two new modified Phillips *et al.* (1982) media, the dehydration medium described by Hu & Zanettini (1995) and the Mergeai *et al.* rooting medium (1997).

Table IX. *In vitro* culture conditions (IG6 and IP0) used during the experiments.

	Maturation	Germination	Dehydration	Rooting	Acclimatization
IG6	P4	P4	G6	G7	Jiffy pots
IP0	Po0	Po1	G6	G7	Jiffy pots
light intensity (in µE / m²sec)	60	Obscurity	60		
Photoperiod	12.30/11.30 hours				
Temperature (Day/Night)	26/26°C				24/20°C
Relative Humidity	100%		70%		70%-50%
Days of culture	7 (in pods)	14	7	14	28

Three experiments were conducted (**Table X**). For each experiment, two culture conditions

were compared using a different sterilizing method.

Table X. Culture conditions tested and sterilizing methods used during the three experiments.

Experiment	*Culture conditions tested*	*Sterilizing methods used*
Experiment 1	IG6 or IP0-1	St1
Experiment 2	IG6 or IP0-1	St2 with 1 ml/l of PPM in the maturation and germination medium.
Experiment 3	IP0-1 or IP0-2	St2 with 0,4 ml/l of PPM in the maturation medium and 0,1 ml/l of PPM in the germination medium

The pod growth (PG) was assessed using the following parameter : PG = (Pf - Pi) / Pi x 100 where Pf is the final length (measured after one week of culture) and Pi, the initial length of pod. At extraction, the number and the length (in mm) of evolved ovules, the stage of embryo (globular, heart-shaped or cotyledonar) and the presence of a suspensor were noted. After 14 days of *in vitro* culture, embryo growth and development on the maturation medium was assessed using the following parameters : (i) the germination rate or rate of embryo showing normal development of root axis, shoot primordia and at least one cotyledon; (ii) the growth rate (LE) calculated as LE = (Lf - Li) / Li x 100 where Lf is the final length (measured between the two apices of the embryo) and Li, the initial length of embryo. After 50 days of *in vitro* cultivation, plantlet morphology was examined in order to evaluate success in acclimatization. Plantlet survival was calculated as the number of plantlets still surviving after 50 days of culture (plantlets with a good rooting ramification and one or more trifoliate leaves).

Evolution of osmolality in media

Osmolality of media was measured with an osmometer using a cryoscopic method (Fiske ONE-TEN osmometer, Massachusetts, USA). All values of osmotic pressure are given in osmolality (mosm). The translation in bar is given by the following expression : Osmotic pressure (in bar) = - Osmolality * 2,453 / 1000.

Interspecific crosses and histological studies

The two *P. polyanthus* genotypes (NI 1015 and G 35348) used as paternal parent were evaluated for their crossing compatibility with the wild *P. vulgaris* genotype (G 21245). More than 150 crosses were made for each combination between *P. vulgaris* (used as female) and *P. polyanthus*.

For histological studies, ovary tissues and pods were harvested at different stages of

maturity (from 1 day before the opening of the flower, D-1, to 15 days after the opening of the flower, D+15) from plants growing in a growth chamber. At least one seed per maturity stage was collected. Protocol can be summarized as follows : plant material is freshly harvested and eventually nicked with a scalpel to facilitate penetration by fixing and embedding solutions. Objects were fixed in 1.2 % glutaraldehyde in 0.3 M phosphate buffer (pH 6.8) for 24 h at 4°C, rinsed in phosphate buffer (pH 6.6), dehydrated in a graded ethanol series and pre-embedded for 1 h in a mixed solution (50/50) of absolute ethanol and ™Technovit 7100 resin. Objects were then embedded in pure ™Technovit 7100 resin for four days at 4°C. Seeds were coated in special mould (™Histoform S, Kulzer).

Sections were cut, 2 µm thick, with a Zeiss microtome (microm HM 360) fitted with a tungsten knife. They were stained with an adapted Toluidine blue O procedure from Gutmann (1995 – general structure) or with fluorescent auramine-O (for cuticle identification) and viewed with a Nicon Eclipse E800 light and fluorescent microscope. Pictures were taken with a JVC 3-CCD color video camera and images were treated with image Archive Plus program of Sony.

3.1.5. Results and discussion

3.1.5.1. Embryoculture : technique adapted for globular embryos

During the experiment 1, more than 50 % Petri dishes were lost due to the media contamination. These dishes were not considered in the data analysis but led us to try to improve the sterilizing method used in the experiments 2 and 3.

The statistical analysis shows no interactions between the culture conditions and the sterilizing methods (data not shown). Therefore, the experiments 1 and 2 are studied in a single analysis, considering two factors : the culture conditions and the sterilizing method. Indeed, culture conditions were the same in these experiments.

Experiment 3 is studied separately considering one factor : the culture conditions. Finally, we also discuss the interest to diminish drastically the PPM concentrations in media regarding the results obtained with IP0-1 conditions, used in all experiments.

3.1.5.1.1. Experiment 1 and 2

Pod growth

Table XI shows the results obtained for the pod growth between sterilizing methods (St1or St2) and culture conditions (IG6 or IP0-1).

Table XI. Evaluation of the influence of sterilizing methods (St1or St2) and culture conditions (IG6 or IP0-1) on pod growth, with N = number of replicates.

Factor		*N*	*Pod growth (in %)*	*Standard error*
Sterilizing methods	St1	18	105,2 a	3,0
	St2	38	93,0 b	4,0
Culture conditions	IG6	25	96,4 a	5,3
	IP0-1	32	97,6 a	3,2

Different small letters indicate a significant difference for one given parameter (P<0,05).

An average pod growth of 97 % was obtained. No statistical difference was observed between culture conditions (P = 0,874). However, the pod growth under IP0-1 conditions was more regular. **Table XI** shows that the standard error was lower under IP0-1 conditions. A Statistical difference in pod growth was observed between sterilizing methods (P = 0,047). The pod growth decreased when using St2 method (**Table XI**). However, using PPM during the pod culture, permitted to reduce the contamination rate by 100 %, so that the number of replicates considered during experiment 2 could be increased markedly, from 18 in experiment 1 to 38 in experiment 2 (**Table XI**).

Ovule growth

Table XII shows the results obtained for ovule mean lengths between sterilizing methods (St1 or St2) and culture conditions (IG6 or IP0-1). **Figure 2** shows the frequencies of extracted ovules by length classes between culture conditions while **Figure 3** shows the frequencies of pod in relation with the number of ovules reaching at least 2 mm long within the pod after one week culture. Finally, **Figures 4 and 5** illustrate respectively the growth of ovules within the pod under IG6 or IP0-1 conditions.

Table XII. Evaluation of ovule mean lengths between sterilizing methods (St1 or St2) and culture conditions (IG6 or IP0-1), with N = number of replicates.

Factor		*N*	*Ovule mean length (in mm)*	*Standard error*
Sterilizing methods	St1	19	2,34 a	0,11
	St2	38	2,28 a	0,10
Culture conditions	IG6	25	2,05 a	0,08
	IP0-1	32	2,50 b	0,08

Different small letters indicate a significant difference for one given parameter (P<0,05).

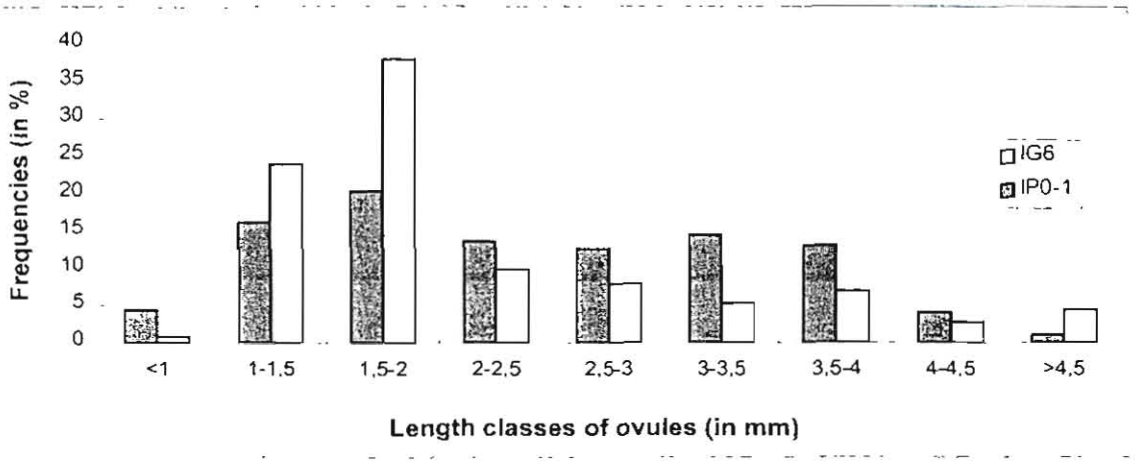


Figure 2. Frequencies (in percent) of extracted ovules by length classes in function of culture conditions (IG6 or IP0-1). Frequencies are calculated on 832 extracted ovules under IG6 conditions and on 1085 under IP0-1 conditions.

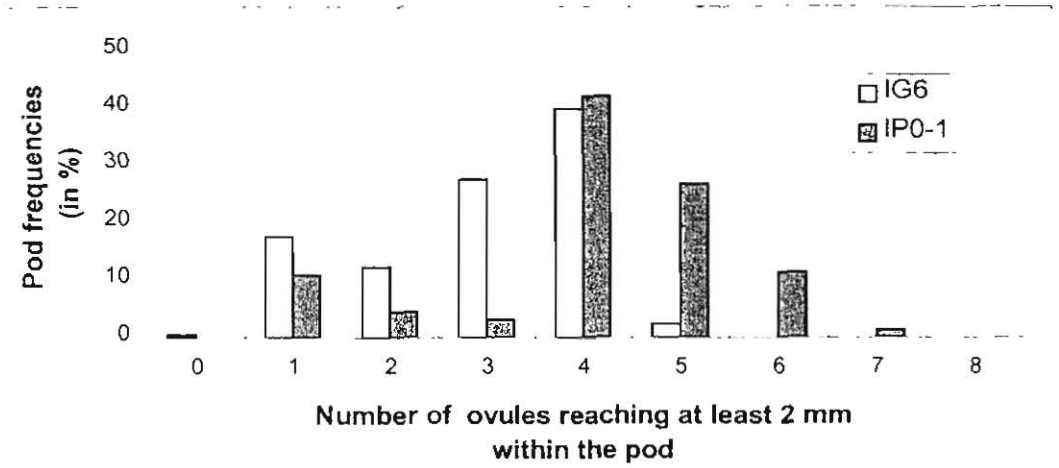
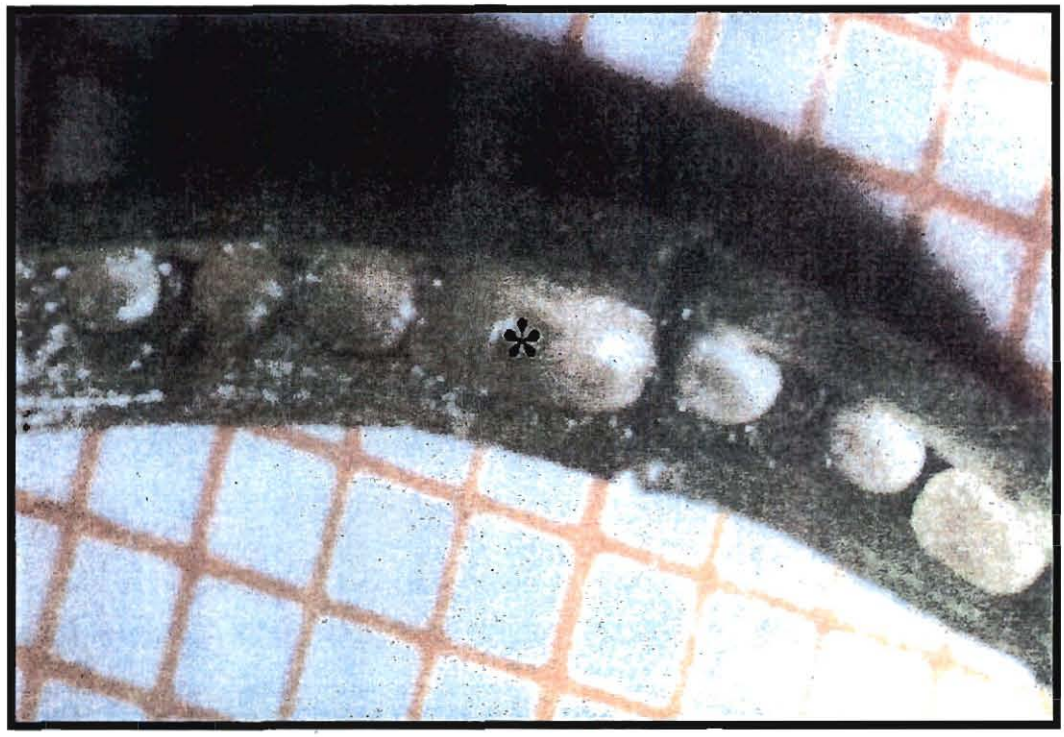


Figure 3. Pod frequencies (in %) in relation with the number of ovules reaching at least 2 mm long within a pod after one week culture. Two culture conditions are compared (IG6 or IP0-1). Frequencies are calculated on 181 pods for IG6 conditions and on 219 in IP0-1 conditions.

The ovule mean length was 2,31 ± 0,10 mm at the time of extraction of the embryo. **Table XII** shows no statistical difference in ovule mean length between sterilizing methods (P = 0,785) but a highly significant statistical difference between culture conditions (P = 0,000). The growth of the ovules increased markedly under IP0-1 conditions. More precisely, **Figure 2** shows that the percentage of ovule reaching 2 mm after one week of culture was higher under IP0-1 than under IG6 conditions. In fact, **Figure 3** shows that a higher number of ovules per pod evolved under IP0-1 conditions : 1 to 4 ovules evolved under IG6 conditions while 4 to 6 under IP0-1 conditions. It is worth noting that under IP0-1 conditions, 6 ovules evolved within the pod for about 15 % cultivated pods while such a number was not observed

under IG6 conditions (**Figure 3**). This difference is illustrated by the **Figures 4** and **5** showing also that the stage of embryo development after one week culture depends on culture conditions.



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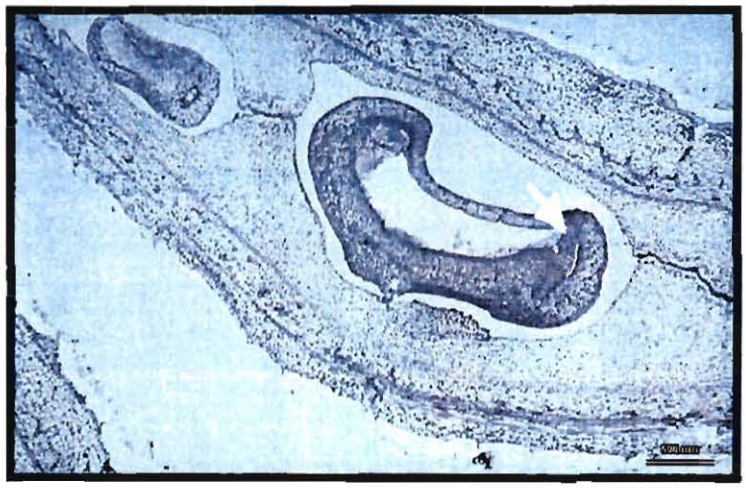

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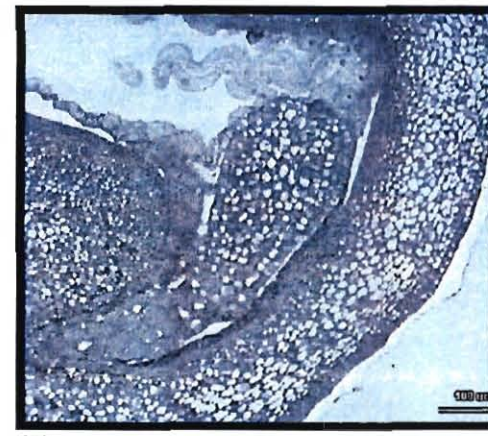
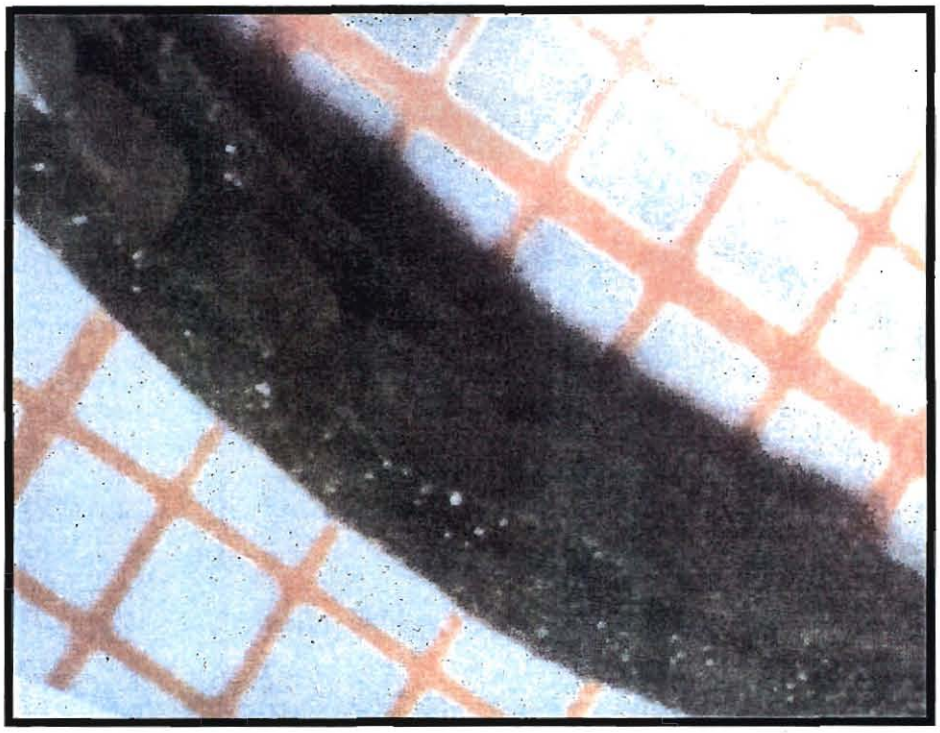


(3)

Figure 4. (1) Growth of ovules within a pod cultivated during 5 days under IG6 conditions : only one ovule evolved (asterisk). (2) Median longitudinal section of a pod cultivated under IG6 conditions during 5 days (magnification 10 x). Only one seed with a globular embryo (arrowhead) evolved. (3) globular embryo at higher magnification (60x).



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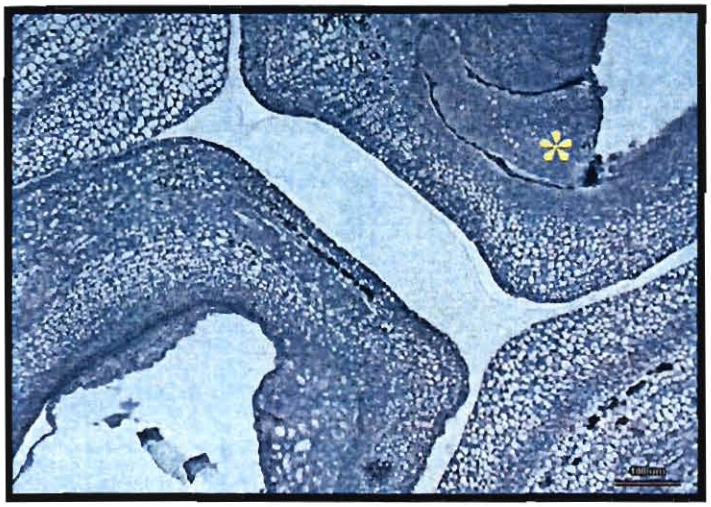

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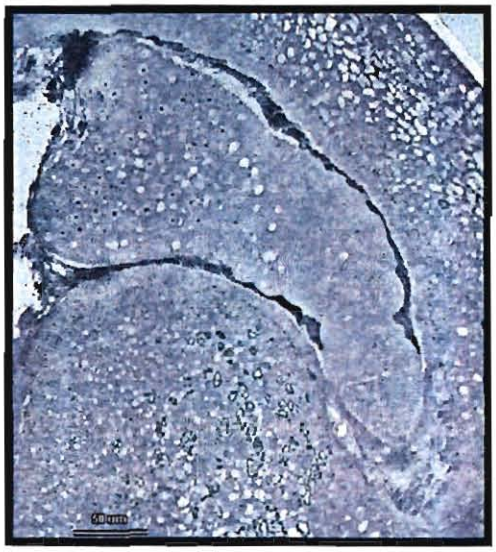


(3)

Figure 5. (1) Growth of ovules within a pod cultivated during 5 days under IP0-1 conditions : 5 ovules evolved. (2) Median longitudinal section of a pod cultivated under IP0-1 conditions during 5 days (magnification 20 x). showing 2 evolved ovules and a early heart-shaped embryo (asterisk). (3) early heart-shaped embryo at higher magnification (100x).

Embryo development

Table XIII and **XIV** summarize the data obtained during experiment 1. **Table XIII** shows the number of extracted embryo with their stage of development and their germination rate while **Table XIV** gives the growth of embryos, the germination rate and the plantlet survival rate under IP0-1 conditions.

Table XIII. Number (n) of extracted embryo during experiment 1 with their stage of development and their rate of germination (mean in %).

Culture conditions	Stage of embryo	n	Germination rate (in %)	Standard error
IG6	*Globular*	9	0	
	Early heart-shaped	17	0	
	Heart-shaped	20	0	
	Cotyledonar	1	0	
IP0-1	*Globular*	12	0	
	Early heart-shaped	67	4,5	2,6
	Heart-shaped	181	18,5	2,9
	Cotyledonar	26	50,0	10,0
Total number of extracted embryos		333		

Table XIV. Evaluation of the growth of embryos (in %), the germination rate (in %) and the plantlet survival rate (in %) between culture conditions (IG6 or IP0-1) during experiment 1. With N = number of replicates.

Culture conditions	*Factor*	*N*	*Percentage*	*Standard error*
IG6	*Growth of embryos*	9	0	
	Germination rate	9	0	
	Plantlet survival rate	9	0	
IP0-1	*Growth of embryos*	9	70,6	3,8
	Germination rate	9	45,5	7,8
	Plantlet survival rate	9	2,8	2,2

Most of the extracted embryos reached late heart-shaped stage and germination rate was up to 50 % when embryos reached cotyledon stage at extraction (**Table XIII**). On the 333 extracted embryos (**Table XIII**), only 47 embryos from 270 ovules (17,41 %) were extracted under IG6 conditions while 286 embryos from 517 ovules (55,32 %) were extracted under IP0-1 conditions. Moreover, only embryos growing under IP0-1 conditions evolved (**Table XIV**). Finally, on the 45,5 % germinated embryos cultivated under IP0-1 conditions, only 3 % give rise to plantlets that survived the first 50 days of culture (**Table XIV**).

During the experiment 2, no embryo development was observed when adding 1 ml/l PPM in the germination medium Po1. Only one embryo, grown under IG6 conditions, could evolve but no plantlet survival was observed.

Discussion and conclusions

During the experiment 1, a high level of pods were infected by different fungi and bacterial agents. More than 50 % of replicates (Petri dishes) were lost. The normal sterilizing method was not efficient enough for pods. Therefore, we proposed, in experiment 2, to use a novel product, the PPM, that acts as a general decontaminating agent. However, the level of PPM added to media is very critical. In our experiment 2, we used the highest level proposed in the literature as well for pod as for embryo development. Using this PPM level in the media allowed to prevent media completely against contamination.

Our results showed that pod growth was slightly affected by the presence of PPM in the medium but also that the PPM level was low enough, permitting a normal ovule growth without any significant differences between the two sterilizing methods (P = 0,785). At pod level, the use of a high PPM content was thus very interesting when contamination could not be controlled by the use of sodium hypochlorite as the single sterilizing method.

On the contrary, when cultivating embryos, the use of 1 ml/l of PPM in the maturation medium was totally inefficient. Indeed, no germination could be observed during experiment 2. It was supposed that the level of PPM was too high and affected the first steps of embryo development. Therefore, in experiment 3, we diminished drastically PPM from the media on which pods and extracted embryos were cultivated.

The results obtained in comparing culture conditions showed the importance to adapt media to the *in vivo* conditions. In particular, our results demonstrated the importance to adjust the osmolality level of the maturation media (in our experiment with sucrose) to the one observed *in vivo*. The ovule development within a pod was more regular, suggesting a better distribution of nutrient supply from the medium. Moreover, the embryo development was much better : more than 60 % embryos were extracted with an osmotically adjusted medium. However, it remains to clarify whether this result is due to osmotic adjustment or is also related to the higher sucrose level.

Finally, the number of plantlets obtained during our two first experiments was relatively low. Only, an adjusted medium without PPM enabled to obtain a plantlet survival rate of 3 %, i.e. plantlets surviving the first 50 days of culture. Due to contamination problems, we could not rescue a normal plantlet : only one plantlet reached more than 3 cm long (**Figure 6**). However, this result is promising. Indeed, the plantlet survival is higher than in any other techniques used before. For example, with 4 days old ovules, Derthe (1996) obtained only 1 plant from 300 cultivated embryos while Ridolfo (1998) with similar ovules obtained good results on germination rates but poor results in plantlet survival rates.

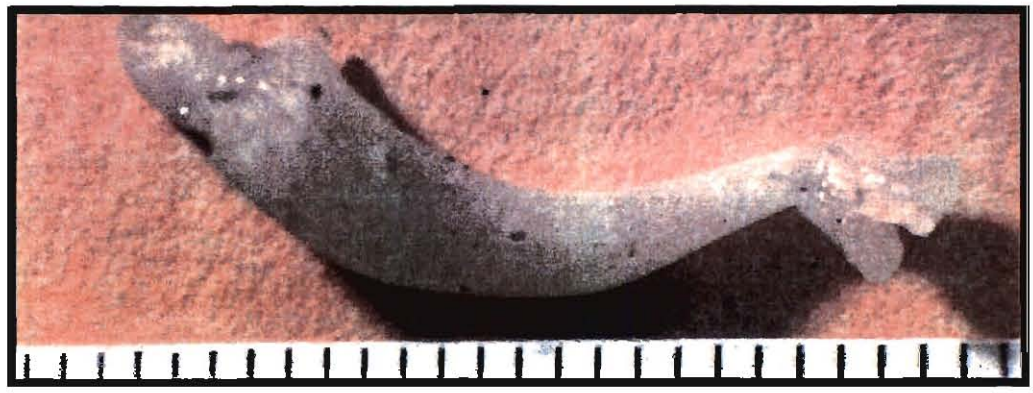


Figure 6. Plantlet rescued from a two days old pod using IP0-1 conditions. Initiation of roots are visible (black points) and leaf apex is present (scale : 0,5 mm).

3.1.5.1.2. Experiment 3.

Evolution of in vitro osmolality in media

During experiment 3, it was important to follow the osmolality of the medium in order to evaluate the good function of our design. Results are given in **Figure 7**. During the first days of culture, medium osmolality decreased as regularly as in *in vivo* conditions (P = 0,974). However, after 4 days, the osmolality of the medium increased significantly. This osmolality increase was due to the difficulties encountered to protect pods from external contamination and results could therefore only be partially exploited.

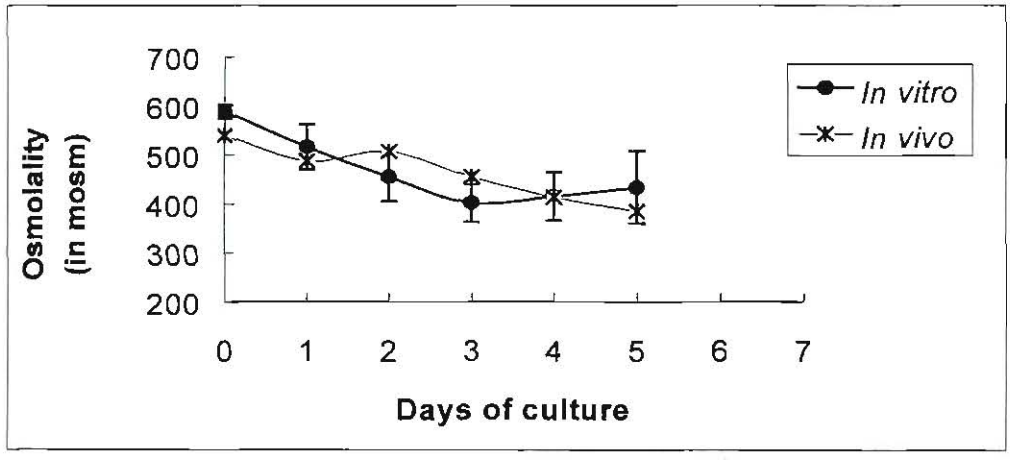


Figure 7. Evolution of osmolality in liquid medium during *in vitro* pod culture (•) compared to the *in vivo* conditions observed for pods (*) by Geerts *et al.* (1999).

Pod and ovule growths

Table XV shows the means of pod growth and ovule length between IP0 culture conditions. **Figure 8** shows the pod frequencies in relation with the number of ovules reaching at least 2 mm within the pod after one week culture between IP0 (1 and 2) culture conditions.

Table XV. Mean pod growths (in %) and mean ovule lengths (in mm) between IP0 culture conditions. N = number of replicates.

Factors	*Culture conditions*	*N*	*Mean*	*Standard error*
Pod growth (in %)	IP0-1	19	108,5 (a)	3,5
	IP0-2	19	106,8 (a)	3,8
Ovule length (in mm)	IP0-1	19	2,58 (a)	0,09
	IP0-2	19	2,60 (a)	0,11

Different small letters indicate a significant difference for one given parameter (P<0,05).

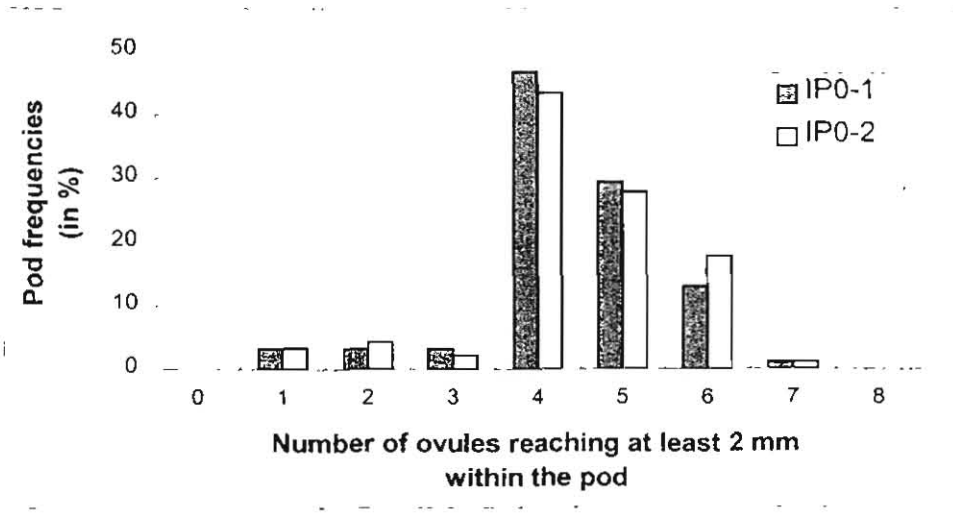


Figure 8. Pod frequencies (in %) in relation with the number of ovules reaching at least 2 mm long within a pod after one week culture. Two culture conditions are compared (IP0-1 or IP0-2). Frequencies are calculated on 94 pods for IP0-1 conditions and on 100 in IP0-2.

A mean pod growth of 107,65 % was obtained while a mean length of 2,59 mm was attained for ovules. These results were slightly better than those obtained in experiments 1 and 2. Moreover, **Table XV** shows that no statistical differences were observed for pod growths and ovule lengths between IP0 conditions. However, the variability in the results was higher under IP0-2 conditions. This variability could be attributed to the effect of contamination occurring after 4 days in most of the Petri dishes. Despite this high variability, **Figure 8** shows the interest to use IP0-2 conditions. Indeed, in 18 % of the pods cultivated under IP0-2 conditions, 6 ovules reached more than 2 mm while such evolution was observed in only 13 % of the pods when cultivated under IP0-1 conditions. This difference suggests a better distribution of the nutrient supply under IP0-2 conditions than under IP0-1 conditions.

Embryo development

Table XVI compares the number of extracted embryos per cultivated ovules, the embryo growth, and the germination rate between IP0 techniques. **Figure 9** illustrates a germinated embryo cultivated under IP0-2 conditions.

Table XVI. Comparison of the number of extracted embryos from cultivated ovules, the embryo growth (in %), and the germination rate (in %) between IP0 techniques.

Parameters	*IP0-1*	*IP0-2*
Extracted embryos/ovules	263/543 (48,43 %)	218/541 (40,29 %)
Embryo growth (in %)	74,8 ± 9,0	71,8 ± 3,6
Germination rate (in %)	5,8 ± 14,0	1,2 ± 6,8

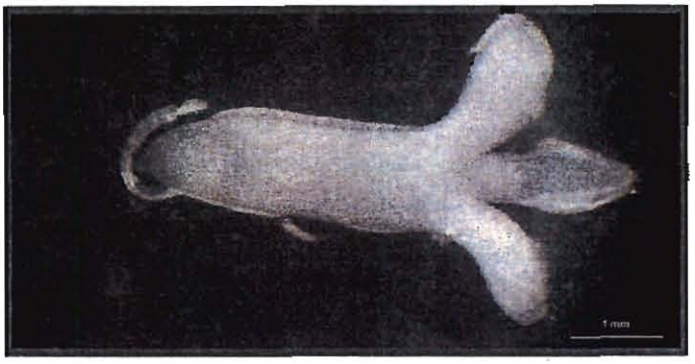


Figure 9. Germinated embryo after five days of culture under IP0-2 conditions and transferred on Po1 with low PPM concentration during two weeks.

In comparison to the experiment 2 where no germination was observed, the germination rate under IP0-1 conditions was 5,8 % with a high variability. This result suggests that PPM remained toxic for the extracted embryo at a level of 0,1 ml/l.

The success of IP0-2 conditions on embryo growth was very limited. Embryo growth was probably affected by the pod contamination. Only in three replicates, no contamination occurred. In these replicates, 7 embryos (on 24 cultivated pods) germinated well (**Figure 9**).

3.1.5.1.3. Conclusion and perspectives

Results obtained for the three experiments show the interest to adapt media to the *in vivo* conditions (Geerts *et al.* 1999b). Especially, during experiment 1 using a medium (Po0) adapted for very young pods (2 days old), the germination rate reached 50 %. Moreover, preliminary results show an interesting potential to use a design in which a constant evolution of osmolality in the medium is obtained. Indeed, the ovule development within a pod was more regular suggesting a better distribution of nutrient supply from the medium. Also, when no contamination is observed, cotyledonar embryos can easily be obtained and germinated well. However, the plantlet survival remains very low (3 %) and, therefore, the technique of plantlet regeneration obtained from two days old embryos has to be improved.

It is also important, in a latter study, to compare adjusted media using different osmoticum : i.e. polyethylen glycol (PEG) as proposed by Ridolfo (1998) and mannitol

(Emons *et al.* 1993). Results will show the importance of high sucrose levels and the interest to use media osmotically adjusted to the *in vivo* conditions.

During the experiments, a high level of contamination was observed. The use of a new technique of decontamination was thus necessary. We investigated the potential of the combination of Tween 20 and PPM at different concentrations as proposed by Randall (1998). It appears that during pod culture, 1 ml/l of PPM gave the best results while a drastic decrease of concentration was necessary when cultivating extracted embryos. More investigations are needed to precise the best levels to use.

3.1.5.2. Histological studies : Interspecific crosses using *P. polyanthus* as paternal parent

The main objective of histological studies is to understand better the process that leads to embryo abortion by studying the nutrient transport of occurring at this time within immature seeds. In particular, we compared embryo development in selfpollinated and hybrid seeds.

In this report we present in a first part (Part I) a review of histological studies made on *Phaseolus* species from pollination to cotyledonar stage of embryo development and illustrate them with our own observations. In a second part (Part II), we present preliminary observations made on interspecific hybrids obtained between *P. vulgaris* (G 21245, used as female) x *P. polyanthus* (NI 1015).

3.1.5.2.1. PART I : REVIEW of PHASEOLUS EMBRYOGENESIS

3.1.5.2.1.1. Megasporogenesis and pollination

Phaseolus vulgaris ovules (megagametophyte) are borne alternately as slight protuberances by periclinal divisions of cells lying below the epidermal layer on the two margins of the placenta, which is located on the inner dorsal surface of the laterally flattened ovary (Weinstein 1926). Later, nucellus is formed by anticlinal and periclinal divisions, as an undistinguished mass of cells. At the same time, two integument primordia are formed on each side of the nucellus (Sterling 1954), the outer and the inner integument, leaving a small opening at the free end, termed micropyle (**Figure 10**). At the other end, or chalazal end, ovule is raised on a stalk known as the funiculus (**Figure 11**). The outer integument grows much faster than the inner one and envelops it progressively. Therefore, ovule is turned at right angles to the funiculus and the micropyle reaches a position parallel to the funiculus while nucellus is then at right angles to the latter (**Figure 12**). The outer integument is initially five cells thick (**Figure 10**) but reaches at maturity a massive structure. Cells are rich in fatty acids and starch corpuscles with dense cytoplasm and small vacuoles. The inner integument is only two cells in thickness, except near its upper margin where it reaches four or five cells thick (**Figure 10**). At anthesis, these cells have also dense cytoplasm with numerous small vacuoles and scattered starch grains. This starch is gradually depleted when cells divide. At the micropylar end of the ovule, a large hypodermal mother cell (megasporocyte or archesporial cell) of the nucellus become differentiated before the integument primordia. Ovule is crassinucellated with a zigzag micropyle : the mother cell is separated from the inner integument by a range of cells : the nucellar cap (**Figure 10**).

Megasporogenesis is monosporic. The megasporocyte, differentiated by the prior division of a nucellar cell, undergoes meiosis by transverse division to give a linear triade of haploid megaspores. Megaspores are characterized by their callose deposition, allowing to assert their genetic individuality (Rodiewicz 1970). Only the chalazal megaspore is functional and grows considerably along the micropylar-chalazal axis to give the embryo sac. At the same time the nucleus of the megaspore is partitioned into two nuclei, one of which is pushed to the micropylar pole and the other to the chalazal pole of the cell. Later, both nuclei divide twice into eight haploid nuclei by mitosis. The megaspore that contains then eight nuclei in a common cytoplasm, grows considerably to form the embryo sac (**Figure 13**). This multinucleate state of the embryo sac is very transient as the nuclei are organized as cells in a characteristic pattern : the three micropylar nuclei give the oosphere and two synergids (**Figure 14**); the three chalazal nuclei organize as antipodal cells; and the two polar haploid nuclei, in the middle of the embryo sac, define the central cell (**Figure 15**). A large vacuole separating the antipodal cells from the micropylar cells is then constituted in the embryo sac. This vacuole push the two polar nuclei close to the egg apparatus (**Figure 15**). Antipodals are ephemera and degenerate before fertilisation (**Figure 15**). Synergids disappear soon after fertilisation, one synergid being destroyed by the penetration of pollen tube (**Figure 14** and **14**).

At the time of fertilisation, cells of the inner integument are in direct contact with the embryo sac and elongate to give a characteristic layer of tissue called the integumentary tapetum. The great majority of the pollen tubes, with their two male nuclei, do not penetrate the stigma (Weinstein 1926). The pollination takes place usually within 8 or 9 hours after pollination (Tan & Jackson 1988), a single pollen tube enters through each micropyle to reach directly the each embryo sac. Just after anthesis, ovules fill the ovarian cavity and curves toward the stylar end to a campylotrope position (Bocquet 1959).

3.1.5.2.1.2. Fertilization

Fertilization of the oosphere is preceded by the transfer of pollen grains of one flower to the stigmatic surface of the same (selfpollination) or another flower (cross-pollination) on the same or a different plant and by the subsequent germination of pollen grains. A tube is developed from the grains reaching the vicinity of the embryo sac by the way of style and ovary tissues (**Figure 17**). From the style, pollen tube grows downward over the outer integument, then between the inner ovary wall and the inner integument of the ovule, toward the placenta. When the placental region is reached, the pollen tube executes a sharp turn and

continues its journey between the placenta and the outer integument to the micropyle. In *Phaseolus* sp., porogamy is observed : pollen tube enters the ovule through the micropyle. Pollen tube reaches the embryo sac at the micropylar end, penetrates one of the synergids, accelerating its destruction in the process (**Figure 18**). Growth of pollen tube could be stopped by osmotic pressure conditions, causing rupture of the tube at its weakest point (Russell 1982), or by changes in oxygen tension (Stanley & Linskens 1967). The role of synergids in fertilization (processes that leads to prior degeneration of one of the two synergid; predisposition of one of the synergid to prior degeneration; role of the post-degenerating synergid) remains points of discussion. Also, the remaining filiform apparatus, observed between the synergids (**Figure 14** and **16**), could point out a role in the nutrition of the zygote. Indeed, in these transfer cells, nutrient could transient preferentially from the inner integument to the zygote. However it is not clear if this apparatus is from embryo sac or synergid origin or is controlled by the embryo himself. After the passage through the cytoplasm of the degenerating synergid, the cytoplasm and the two male nucleus of the pollen grain are discharged and fusion between the egg and a male nucleus occurs just after the fusion between polar cells (**Figure 19**) and the second male nucleus. The triple fusion between one male nucleus and the two priorly fused polar nuclei of the embryo sac gives rise to the primary endosperm nucleus. At that time, two cells are thus formed : the diploid zygote and the primary endosperm nucleus (**Figure 14**). The zygote is sharply pointed at the micropylar end and its nucleus is located at the chalazal end (**Figure 19**).

Zygote displays a mitotic activity to develop into the embryo, whereas the primary endosperm nucleus differentiates into a nutritive tissue know as the endosperm (**Figure 20**). Embryo and endosperm are enclosed in an ovule transformed into a seed within the ovary.

3.1.5.2.1.3. Zygote development

After fertilization, the egg is transformed into the zygote, product of the fusion of two gametes (**Figure 19**), the sperm contributing the paternal genome and the egg providing the maternal counterpart. The zygotic phase of embryo formation is known as zygotic embryogenesis.

3.1.5.2.1.3.1. Diploïd embryo or zygote

The egg enlarges after fertilization as it is transformed into a zygote. At that time a polarity is already established in the egg by the presence of a large micropylar vacuole (**Figure 19** and **18**). The position of this vacuole remains unchanged as the egg is transformed

into the zygote. Nuclei and cytoplasm are pushed at chalazal end while standard changes are described in many different species (van Lammeren in *Z. mays* 1986, Mansfield & Briarty in *Arabidopsis* 1991, Schulz & Jensen in *C. bursa-pastoris* 1992, Sumner in *Brassica campestris* 1992,...) such as alterations in the form of the endoplasmic reticulum, increase of the starch grains in the plastids, elaboration of a new generation of ribosomes and their transformation into polysomes and increased dictyosome activity. These last changes are consistent with a higher level of mRNAs content in the zygote. Raghavan (1990) shows not only an increase of RNA content but also a gradient in its accumulation, with a high concentration in the chalazal part. All these changes during the formation of the zygote and his polarity reflect the capture of information necessary for his subsequent division to form the embryo.

Suspensor and embryo-proper

One of the important concepts to emerge from light microscopic analysis of embryo development in plants is that early division sequences of the zygote are carried forward according to a blueprint characteristic of each species. In *Phaseolus vulgaris*, as in most of angiosperm species, the first division of the zygote is asymmetric and periclinal, cutting off a large vacuolate basal cell attached to the embryo sac wall (micropylar end) and a small, densely cytoplasmic organelle-rich terminal cell toward the embryo sac cavity (chalazal end). The egg has a limited amount of cytoplasm, which is spread in a thin layer surrounding the vacuole except near the nucleus. Fifteen or sixteen hours after the opening of the flower, 3 or 4 celled embryos are observed. The first two divisions of the zygote are transverse resulting in the formation of a filament of three cells (**Figure 21**) while the third division is vertical. The two basal cells of the three celled pro-embryo give the suspensor. The terminal cell gives rise to the embryo-proper. The two suspensor cells are easily detected until late in the history of the embryo. Indeed, they evolve into swollen cells with a globoid shape. Xuhan (1995) observe that during heart-shaped and early cotyledon stages, both the cell length and the nucleus diameter of the basal cell increase about 10 times compared to those of the embryo-proper cells. The development of the suspensor is thus very rapid both in terms of size and fresh weight (**Figure 22**). Histological studies show that the basal cells extend into the maternal tissue and thus play certainly a role in the attachment of the embryo to the maternal seed tissue, fixing his position. During proembryo stage, other works (Brady & Combs 1988, Xuhan 1995) demonstrate that these basal cells play also an active role in the transition of nutrients. Indeed, besides their size, nuclei and organelles are abundant in these cells. Moreover, Sussex *et al.* (1973) and Brady & Walthall (1985) show their important activity in

both RNA and protein synthesis of the embryo while Alpi *et al.* (1975) underline the important levels of gibberellins in the suspensor. Brady & Combs (1988) conclude that the terminal cell can only develop into an embryo if the suspensor is present or, at least, is replaced by physiological concentrations of gibberellins until the heart stage of embryo development. This conclusion is supported by the works of Yeung & Sussex (1979) and Yeung (1980) who study the importance of suspensor during *in vitro* development of *P. coccineus* L. embryo and its replacement by specific hormones, as gibberellin. In particular, they note that the suspensor also exerts its influence when placed next the embryo-proper, suggesting that it acts either by synthesizing and/or secreting substances or is more efficient in digesting the surrounding medium so that the embryo can readily absorb substances necessary for its growth. In particular, Clutter *et al.* (1977) note a high amylase activity in the suspensor of *P. vulgaris* whereas it is not detected in the embryo-proper. In conclusion, these early observations are coherent with the hypothesis that suspensor may synthesize substances not produced by the embryo-proper and may also be the site of hormone synthesis. Later, Xuhan (1995) shows cell wall ingrowths formation initiated in the outer walls of suspensor cells at the globular stage. The wall invaginations develop independently either in the suspensor cells connected to the endosperm or in the integument suggesting a double way of nutrient transfer : integument-suspensor and integument-endosperm-suspensor. The same author also observes transfer-cell-type wall ingrowths in the neck cells bordering the basal suspensor (**Figure 23**) suggesting a direction of the nutrient flux from surrounding tissue via the basal part to the upper embryo-proper without symplasmic barriers. Moreover, in soybean, Chamberlin *et al.* (1993) show that no wall ingrowths are seen between the cell of the embryo head and the endosperm still at the globular stage (**Figure 24**). Therefore, suspensor may have not only a mechanical and physiological function but is also probably an organ for apoplasmic and symplasmic transport of materials to the developing embryo-proper. Recent works of Szczuka & Rodkiewicz (1996) support this hypothesis. Indeed, in several species, they note the presence of a thin cuticle layer over the globular embryo-proper but not on the suspensor (**Figure 23**). The cuticle layer formed over the surface of the embryo may be impervious to solute exchange, as suggested by Chamberlin *et al.* (1993) or, at least, decreases this exchange between the embryo and the endosperm. Therefore, it supports that, early in the embryogenesis, only suspensor acts as an uptake region. Later cuticle is formed around the suspensor while it disappears at the cotyledon surface (Szczuka & Rodkiewicz 1996). At torpedo stage, embryo should absorb materials directly from the endosperm and cotyledons appear then to play an active role in the transport of substances into early embryo (Johansson

& Walles 1994) while the role of suspensor is clearly reduced. Finally, after torpedo stage, cuticle is formed over all the embryo and may act as a factor for induction of some maturation processes in the embryo. At the time of germination, the communication between embryo and endosperm is partially restored by the degradation of the cuticle layer on the radicular part (Szczuka & Rodkiewicz 1996). In conclusion, all these observations are in concordance with the hypothesis of an important physiological role played by the suspensor early in the embryogenesis in *Phaseolus vulgaris* and corroborate with the results obtained by Yeung (1980) and Lecomte (1997) in *P. vulgaris*, Chamberlin *et al.* (1993) in soybean or Szczuka & Rodkiewicz (1996) in several other species.

Cotyledons

In describing embryogenesis, the term "proembryo" is used to designate the early stages of development of the embryo; initiation of cotyledons is considered as a good cut-off point for the end of the proembryo stage. Multiplication of the suspensor cells ceases at about the time that the dermatogen begins to differentiate. The embryo is then at globular stage (Brown 1917). In *Phaseolus vulgaris*, cotyledons are initiated 4-5 days after anthesis at the chalazal side of the embryo (**Figure 24**). At that time, the embryo is at heart shaped stage. Eleven days after anthesis, at cotyledonar stage, the cotyledons grow rapidly, and fill only in four days the entire space of the integuments (**Figure 25**). By this time, endosperm has been almost entirely digested, and the little that remains of it is to be found near the chalazal end of the seed cavity (**Figure 26**). The increase of weight and nitrogen content of the cotyledons is maximum between 22 and 34 days after anthesis (Öpik 1966). The cotyledon parenchyma cells first become very highly vacuolate, but soon the large vacuoles are divided up and converted to reserve protein bodies, while expansion continues until senescence after germination. Starch grains grow within plastids to reach diameters of 50 microm.

At the end of embryogenesis, the mature embryo has attained a basic organization consisting of a bipolar axis with two cotyledons and, at each pole, an apical meristem. The point of attachment of the cotyledons separates the embryonic axis into a hypocotyl-root region and an epicotyl-plumule region. The primordial root (radicle) is situated at the lower end of hypocotyl, whereas the primordial shoot (plumule) is attached to the stem part called epicotyl.

3.1.5.2.1.3.2. Triploïd embryo or endosperm

After fertilization, divisions of the primary endosperm nucleus begin much earlier than those of the zygote nucleus (Brown 1917). **Figure 19** shows that the fusion between female

and male nucleoli occurs after complete fusion in endosperm nuclei. In *Triticum aestivum*, this prior division of endosperm is particularly revealed. Bennett *et al.* (1973) show that after 24 h after pollination, 16 free nuclei are observed while only a two-celled pro-embryo is developed. The cell divisions of primary endosperm conforms to nuclear-type in which division is not accompanied by cytokinesis resulting in the formation of free nuclei in the central cell. Cellularization occurs later (**Figure 27**), to form a partially cellularized endosperm with a multinucleate mass of protoplasm and populated by numerous small vacuoles called the liquid endosperm. The development of these structures before the division of the zygote can be considered as an adaptive value in ensuring the availability of an adequate food supply to the developing embryo. In contrast to embryo, where only cotyledons are accumulating reserve, all endosperm cells dedicate their synthetic machinery to the production and accumulation of food reserves.

Coenocytic endosperm

Divisions of the coenocytic endosperm are more irregular than those of the zygote and not simultaneous (**Figure 21**). Moreover, these divisions occur only in the portion directly in contact with the embryo and gradually progress toward the chalazal as the embryo increases in size (**Figure 28**). At the heart stage of embryo development, cell division is gradually confined to the region adjacent to the tips of the enlarging cotyledons, near the seed coat (Yeung & Cavey 1988) and persists until the early cotyledon stage of embryo development to give cellular endosperm. Cellularization of the endosperm begins with free nuclei at the micropylar end of the embryo sac and extends to the chalazal end (Xuhan & van Lammeren 1994 – **Figure 27** and **26**).

Up to the early cotyledon stage of embryo development, the cellular endosperm completely encloses the embryo and thus may play a role in regulating nutrient flow to the developing embryo (**Figure 24**). Indeed, any material entering the developing embryo must cross this layer of cells. Also, following the initial phase of cellularization, invaginations of the plasma membrane similar to transfer cells are seen on the wall of the endosperm cells surrounding the embryo in several legume species such as in *Vigna sinensis* (Hu *et al.* 1983) and *Vicia faba* (Johansson & Walles 1994). Later, these cells separate readily from one another, indicating that the bond between endosperm and seed coat is stronger than between individual endosperm cells.

At the same time, cuboidal cellular endosperm cells are developed from free cell wall formations (Yeung & Cavey 1988). Later, these endosperm cells, formed by freely growing

walls, stretch to form a membranous sheet over the embryo (**Figure 27**). This outermost layer of the cellular endosperm incorporated into the seed as a single layer of cells around the embryo is also described in soybean (Yaklich & Herman 1995– **Figure 26**). Two methods of wall formation are described (Yeung & Cavey 1988) : freely growing walls are initiated directly from the central cell wall or later also from the walls of the newly formed endosperm cells. Moreover, the cellular endosperm is initially loosely attached to the developing embryo while it firmly adheres to the inner surface of the seed coat at maturity when sandwiched between embryo and seed coat. Cellular endosperm never appears in the region of degenerating nucellus (chalazal end) which is covered only by the wall of the liquid endosperm (**Figure 27**).

Cellularization of the endosperm is associated with a particular cycle of DNA synthesis and mitosis. Endoreduplication and polyploidy are two conventional routes followed by endosperm cells (Raghavan 1997). This DNA amplification during the early stages of endosperm development may be important for the synthesis and accumulation of storage products.

Liquid endosperm

Liquid endosperm, formed by numerous small vacuoles of the embryo sac, surrounds the embryo during early embryogenesis (**Figures 11, 14, 17 and 18**). Moreover, the embryo is enclosed in the seed coat and the seed is in turn enclosed within the pod. Therefore, a point of discussions from these observations concerns the importance of anaerobic conditions for the development of globular embryo. Indeed, Boyle & Yeung (1983) show the presence of a high specific activity of alcohol dehydrogenase involved in anaerobiosis during early embryo development followed by a continuous and gradual decline.

After 5 days after anthesis, the liquid endosperm is reabsorbed (**Figure 28**). Yeung & Cavey (1988) suggest that this reabsorption occurs at the chalazal end of the seed, especially in the region of the degenerating nucellus, where cellular endosperm is not present. Indeed, wall ingrowths are present in this region (Sage & Webster 1990). Autoradiographic evidence suggests that these wall ingrowths are involved directly with the transport of assimilates into the embryo sac (Chamberlin *et al.* 1993). At the cotyledon stage, all traces of liquid endosperm disappear while the membranous sheet becomes firmly attached to the inner surface forming a single wall (**Figure 26**). This wall is characterized by a meshwork of fibrils and folds. This organization is an adaptation that enables the endosperm to accommodate rapid expansion of the seed without damage to either the endosperm cells or the

integumentary tapetum of the seed coat. As the seed matures and dries, this sheet also dries down, with no apparent degradation of its cells (Yeung & Cavey 1988).

3.1.5.2.1.4. Nucellus

At anthesis, a single row of nucellar cells, the epistase, is present in the micropyle region (**Figure 10**) and nucellar tissue forms a U-shaped cap over the embryo sac in the chalazal region (**Figure 11** and **27**). Cells of the chalazal end divide to give the hypostase. Its function is not clear. It contains large, prominent starch grains and could serve as an nutritive tissue for the developing endosperm (Brink & Cooper 1947). Nucellar tissue degenerates during the 3 days after anthesis as the embryo and embryo sac enlarge. By 4-5 days, it is detectable only in the chalazal region while it totally disappears at the end of embryogenesis (**Figure 25**).

3.1.5.2.1.5. Funiculus and vascular tissues

The developing seed is attached to the maternal tissue of the fruit by an organ called funiculus (**Figure 11**). Pod nutrients must traverse the funiculus to reach the seed coat. A characteristic solid core of vascular tissue originates from the differentiation of the procambium and is present near the main vasculature of the pod (**Figure 30**). The amount of vascular tissue diminishes progressively to become a small strand prior to enter into the seed, phloem elements being located at chalazal end while xylem elements being located towards the micropylar end of the seed (**Figure 11** and **28**). Thus, the vascular tissue within the funiculus appears as a conical structure with an intertwining network of vascular tissue : sieve and vessel mature elements can be found to lie side by side. It is thus a single primary vascular trace comprising xylem and phloem that enters the placenta into the funicular region and this toward the chalazal end of the embryo sac (Brady & Combs 1989 – **Figure 30**). At anthesis, xylem and phloem are made up of continuous files of mature and immature vessel and sieve elements, companion cells, elongated procambial cells, and associated parenchyma. One day after anthesis, two recurrent branches develop from the procambial strands of the central region and run on either side of the embryo sac of the seed from the primary chalazal trace parallel to the placenta toward the area of the micropyle where the embryo is developing. There is also some limited tertiary procambial activity perpendicular to the lateral traces. At cotyledon stage, funiculus increases in size by mitotic activity within the procambium and the surrounding parenchyma. After cotyledon stage, the enlargement of funiculus is due to cell expansion, intercellular spaces, and maturation of vascular elements.

3.1.5.2.1.6. Seed coat

Seed coat is primarily constituted by an outer and an inner integument and encloses the embryo. Later, five tissues can be detected from inside to outside as described by Yeung (1983 & Blackman- **Figure 26**). (i) : the innermost layer differentiated into the integumentary tapetum which encloses the embryo cavity, (ii) : a tissue of 6-8 layers of branched parenchyma cells, (iii) : a tissue of compactly arranged parenchyma cells with small intercellular air spaces and surrounding the vascular tissue, (iv) : a tissue of large vacuolated parenchyma cells containing osmiophilic substances within their vacuoles and with prominent intercellular air spaces appearing at heart stage of embryo development as the seeds start to expand rapidly, and (v) : the outermost layer differentiated into macrosclereids. Since strengthening tissues can often be found in the mature seed coat, it is generally believed that this organ functions solely as a protective covering for the embryo. However, recent works show that the seed coat may have a nutritive role and may be important in controlling the development of the embryo (Yeung & Blackman 1983). Among all the parenchyma cells within the seed coat, only the branched parenchyma cells show dramatic changes in their ultrastructure through the maturation of the seeds (Yeung & Blackman 1983) with, as most noticeable change, the reduction of endoplasmic reticulum cisternae.

3.1.5.2.1.7. Flower and seed abortion studies within *Phaseolus vulgaris*

In this chapter, we review the different incompatibility barriers, appearing as well in self fertilization as in wide crosses.

Incompatibility barriers

To preserve their identity and stability, species have developed genetic mechanisms involved in the control of pre- and post-pollination and fertilization, known as incompatibility barriers. This incompatibility defines the inability of the functional gametes to affect sexual recombination and eventual seed set in a particular combination. Self-incompatibility describes intraspecific reproductive barriers, between gametes from the same individual or from individuals of the same species. Cross-incompatibility is used in the other cases. While self-incompatibility has been largely studied, much less is known about cross-incompatibility (Raghavan 1997).

Self-incompatibility

Briefly, two type of self-incompatibility are recognized : heterotrophic and homomorphic types. These types are distinguished by floral morphology. In the heterotrophic

type, cross-compatible plants have visible differences in floral morphology. Many genetical studies have been made to characterize the self-incompatibility barriers. They are summarized by Lewis (1960) and Lewis & Jones (1992). For each type, one or two genes involved in the seed abortion have been identified :

1. Heterotrophic type (distyly or tristyly) : role of alleles *S* and *s*, the S-gene in distyly; role of S-and M-genes in tristyly.
2. Homomorphic type (gametophytic or sporophytic) : role of S-gene or pollen grain.

Several studies (Adams 1967 and Tamas *et al.* 1979; cited by Sage & Webster 1987) on spatial and temporal abortion within a morphological unit (a raceme) of *P. vulgaris* indicate that the probability of floral buds giving rise to mature fruit varies with position of a bud within a unit : axillary and basal buds, the first to flower, have the lowest probability of aborting, while distal buds, the last to flower, have the highest probability. This abortion occurs within 3 to 4 days after anthesis for axillary and basal fruits while this abortion occurs 0 to 1 day after anthesis for distal fruit (Sage & Webster 1987). The factors inducing this homomorphic type of abortion have not been identified by Sage & Webster (1990). These authors are the first to make an anatomical comparison between aborting and non-aborting seeds within *Phaseolus*. The following observations on aborting seeds are made :

1. there is enlargement of some basal suspensor cell and penetration into the outer integument but no further embryo development;
2. nucellus cells are devoid of starch and are vacuolated;
3. starch depletion, vacuolation and cell wall distortion are observed in the integuments;
4. vascular differentiation is observed (tertiary and secondary vascular development).

The first anatomical abortion -related changes are in integumentary and nucellar cells. These include starch depletion, increased cell vacuolation, and distortion of wall cells of the middle cell layers of the outer integument. These rapid changes indicate that the nutritional balance of aborting seed is disrupted early in the abortion process. Studies by Nakamura (1988) indicate that abortion induced processes are not initiated in embryonic tissue in *Phaseolus*. The most important factor could be the disruption of vascular system at the chalazal end by degeneration of nucellar tissue. Subsequently embryo seed/fruit development ceases due to a failure of conduction. However, Sage & Webster (1990) note that the first changes related to abortion in maternal seed are not vascular disruption in the chalaza. For the latter, abortion could be due to diversion of assimilates from aborting axillary or basal buds to non-aborting seeds 4 days after anthesis.

Pre-fertilization cross-incompatibility: male sterility and pollen abortion

Chemical and environmental manipulations of the flower can be responsible for pollen abortion and male sterility. However, most of the time, male function can be abolished only by gene action, and this at any stage of microsporogenesis or microgametogenesis. Thus, anther primordium is a prime target of action of male-sterility genes. Phenotypic variations, such as the absence of stamens with anthers or the presence of anthers devoid of sporogenous tissues, are due to defective gene action at the earliest stage of differentiation of the primordium. When perfectly normal anthers are produced, pollen development can be impaired by irregularities in the division of the sporogenous cells. This event interacts with the development of somatic tissues, such as the tapetum, and with the dynamics of microsporogenesis. Later, viable pollen can be incapable of affecting fertilization due to the failure of anther dehiscence, the presence of an abnormal exine configuration on the pollen, or the failure of pollen tube growth.

Genic, cytoplasmic, and gene-cytoplasmic are the three types of genetic male steriles so far identified in which genic male sterility occurs predominantly and is controlled by nuclear genes under homozygous recessive conditions independent of the type of cytoplasm. At a molecular level, it is established that the male sterility is related to the rearrangement of the mitochondrial genome. A low rate of replication in recombined mitochondria, particularly in the tapetum cells, could limit ATP production in the anther primordium, leading to sterility, such as the cytoplasmic male steriles of *P. vulgaris* (Johns *et al.* 1992). Also, as demonstrated in bean (Chase & Ortega 1992), the produced toxic protein acts like any toxic compound, causing the death of the cells in its immediate vicinity.

Post-fertilization cross-incompatibility

Most of the works carried out on comparative histology of the ovule in wide crosses underline a delay in endosperm development. Importance of the delay is function of the cross. The genetic balance between the different ovule tissues is also one of the most important factors. In particular, the lost of balance between nucellus and endosperm leads to an abnormal distribution of nutrients to the embryo.

Although a wide range of irregularities is displayed by the hybrid embryos, in the case of post-fertilization abortion processes, embryos generally begin to grow in a relatively healthy way. Therefore, embryo abortion is most of the time related with other factors than genetical; In other words, embryo does not abort by himself. In fact, a 2:1 ratio between

maternal and paternal genomes in the endosperm is generally required for seed set. In crosses between diploid and tetraploid varieties of *Citrus*, Esen & Soost (1973) postulate that a deviant ratio in the chromosome number between embryo and endosperm causes abnormalities in the endosperm and, as a consequence, embryo abortion. Thus, deleterious genes may exert their primary effect on the endosperm rather than on the embryo. This is pointed out by success obtained with some embryo culture methods : these provide an artificial medium supplied with nutrient substances that are normally identified in the endosperm and used to obtain transplantable seedlings from aborted seeds of interspecific crosses. Therefore, the early disintegration of the endosperm, beginning soon after fertilization, is commonly cited as the most important factor in embryo abortion. Indeed, endosperm is an immediate source of food for the embryo that is deprived by this disintegration. In general, the initial rate of development of the endosperm in nonviable crosses does not differ from that observed in successful crosses, but afterward, it decreases slightly. The structural changes are more dramatic than the decrease in cell number. In particular, cells of the endosperm become vacuolated at the chalazal end and less dense than those at the micropylar end. Moreover, cell walls are dissolved leading to the fusion of the protoplasm and the nuclei as observed in aborting selfpollinated *Phaseolus* seeds (**Figure 31**). In *Lycopersicum pimpinellifolium* the endosperm is then reduced to a single or a few giant cells surrounding the embryo (Cooper & Brink 1945).

Poor endosperm development and embryo abortion have also been associated in *Oenothera lamarckiana* with the proliferative growth of a tissue from the nucellus (Renner 1914). This hyperplastic growth of the nucellus, causing somatoplastic sterility, is also associated with a deficiency in the development of conducting elements within the seed, foreshadowing a suppression of nutrient transport to the endosperm. In other cases, infertile crosses show a proliferation of the integument (endothelium). This tissue proliferation begins to grow without restriction and extends into the embryo sac as a burgeoning mass of cells. This endothelium burgeoning is observed in aborting selfpollinated *Phaseolus* seeds (**Figure 32**). In any case, the hyperplastic growth of the nucellus or the proliferated tissue of the endothelium cause under-nutrition of the embryo.

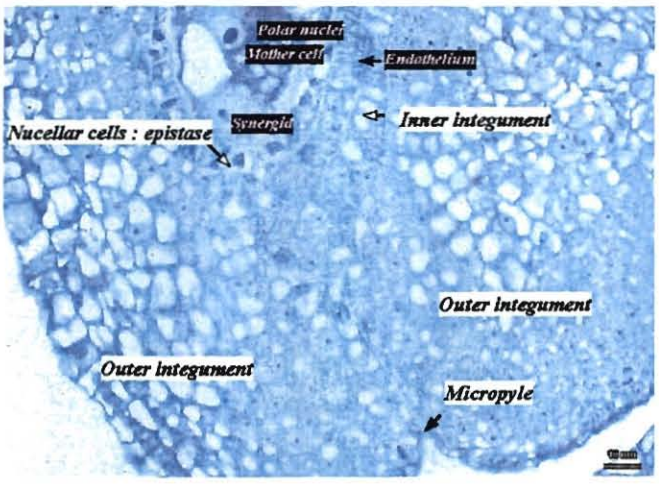


Figure 10. A median longitudinal section showing a mother cell (archesporial cell) and its environment at D-1 (*P. vulgaris* G21245). Ovule is crassinucellated : the mother cell is separated from the inner integument by a range of cell : the endothelium.

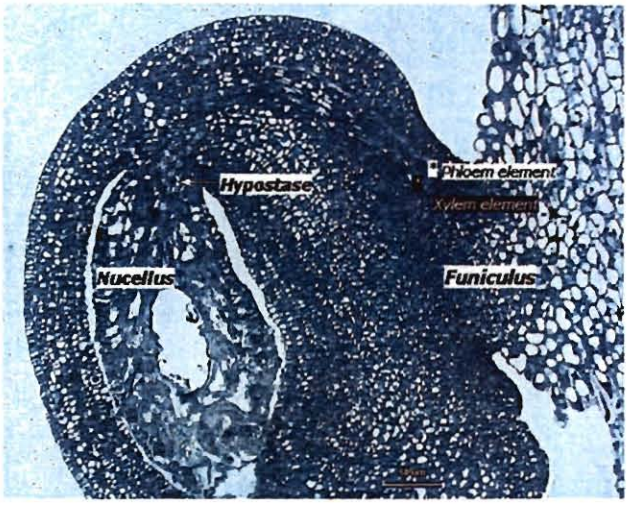


Figure 11. A median longitudinal section of *P. vulgaris* (G21245) ovule at D0 showing the funiculus to nucellus organisation.

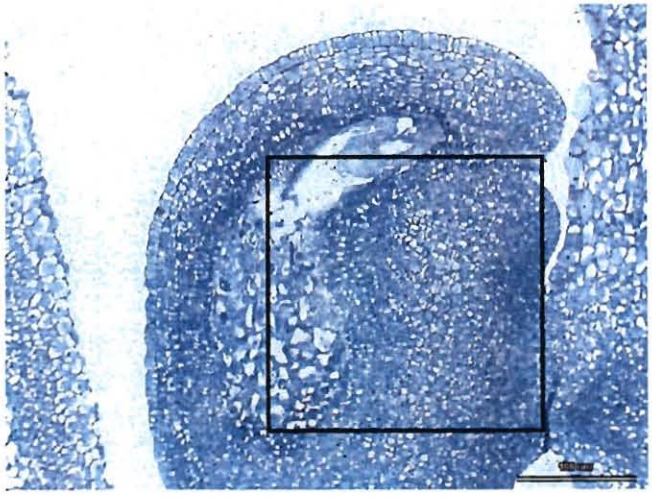


Figure 12. A median longitudinal section showing an ovule of *P. vulgaris* (G21245) at D-1. Ovule is turned at right angles to the funiculus.

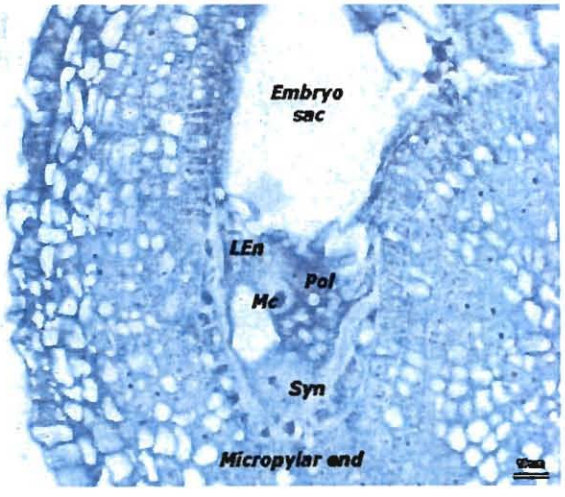


Figure 13. A median longitudinal section of *P. vulgaris* (G21245) ovule at D-1 showing the monosporic type of megasporogenesis : eight nuclei are formed in the embryo sac : three ephemera antipodals at the chalazal end, two synergids (Syn) at the micropylar end, two polar nuclei (Pol) and the mother cell (Mc) forming the central cell in the middle of the embryo sac. Liquid endosperm surrounds nuclei.

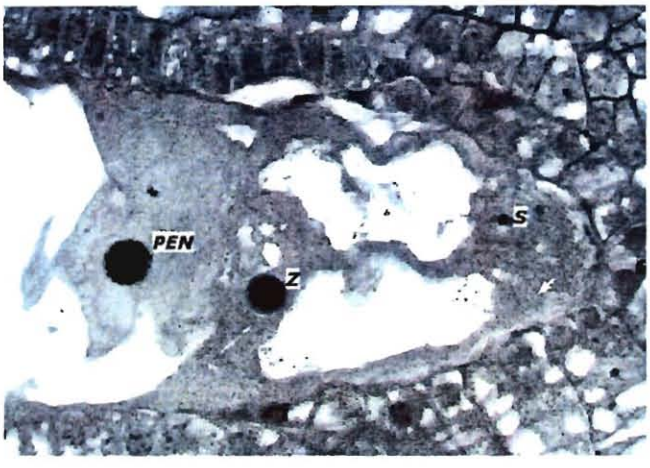


Figure 14. A median longitudinal section of *P. vulgaris* (G21245) seed at D0 showing the formation of the primary endosperm nucleus (PEN), the zygote (Z) and the degeneration of synergids. Arrowhead shows the filiform apparatus between synergids.

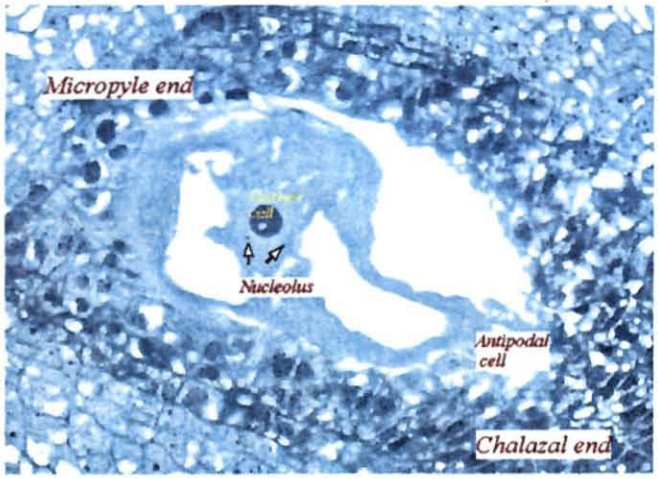


Figure 15. A median longitudinal section of *P. polyanthus* (NI1015) ovule at D-1 showing a degenerating antipodal cell at the chalazal end. Polar nuclei and mother cell, in the middle of the embryo sac, define the central cell.

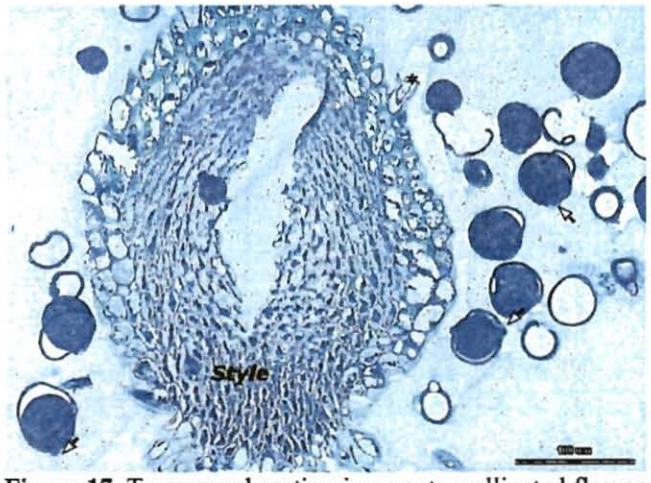


Figure 17. Transversal section in an auto-pollinated flower of *P. polyanthus* (NI1015) at D+1 showing pollen germination (arrowheads) and pollen tube penetration into style (asterisk).

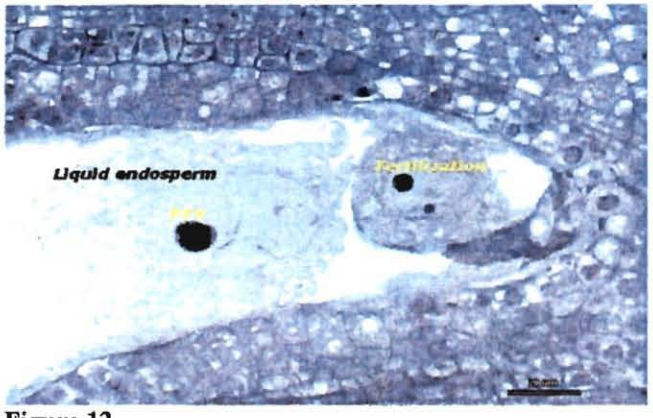


Figure 12

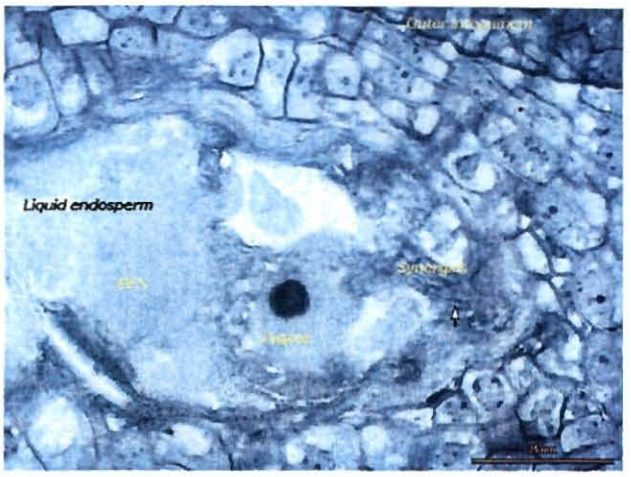


Figure 16. A median longitudinal section of *P. polyanthus* (NI1015) seed at D+1 showing the zygote surrounded by liquid endosperm next the primary endosperm nucleus (PEN). The two synergids disappear and only a filiform apparatus remains intact between them (arrowhead).

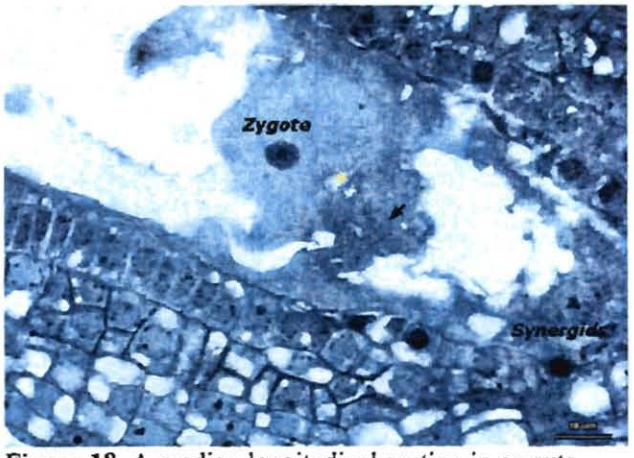


Figure 18. A median longitudinal section in an auto-pollinated flower of *P. vulgaris* (G21245) at D0 showing pollen tube penetration (arrowhead) through a synergid and the point of release of the two male nuclei (asterisk).

Figure 19. A median longitudinal section in an auto-pollinated flower of *P. polyanthus* (NI1015) at D+1 showing fertilisation process. The figure shows that primary endosperm nucleus (PEN) is formed before the fusion of female and male nucleolus of the zygote nucleus. Liquid endosperm and cytoplasm surround PEN and zygote. Divisions of the primary endosperm begin thus earlier and is conformed to nuclear-type.

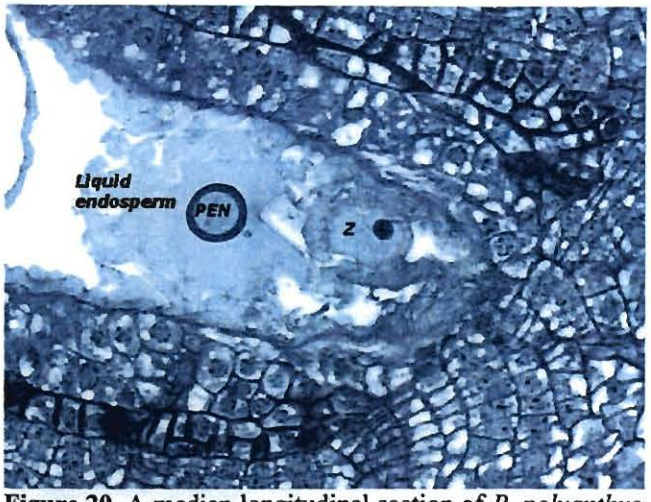


Figure 20. A median longitudinal section of *P. polyanthus* (NI1015) seed at D+2 showing the result of double fertilisation. PEN = primary endosperm nucleus; Z = zygote. Zygote is surrounded by liquid endosperm.

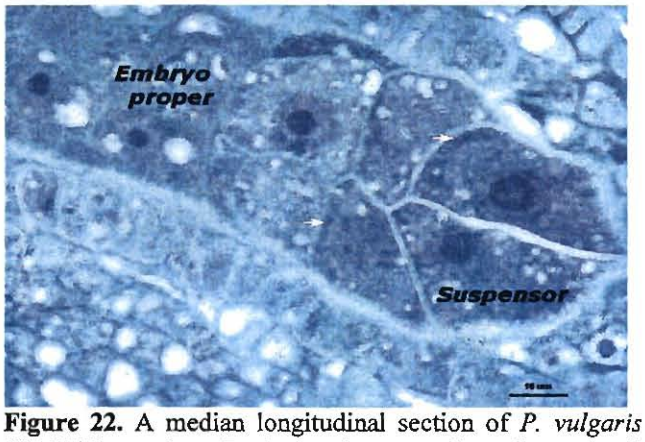


Figure 22. A median longitudinal section of *P. vulgaris* (G21245) seed at D+4 showing transfer-cell-type wall ingrowths in the neck cells bordering the basal suspensor (arrowheads). Cell wall ingrowths are also present in the outer walls of the suspensor cell. No wall ingrowths are seen in the embryo-proper.

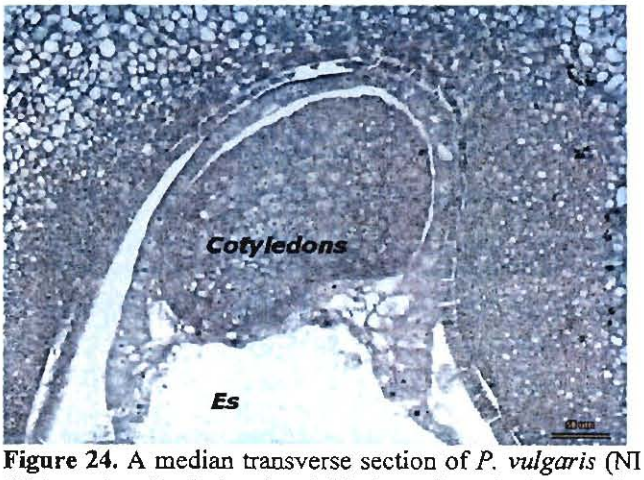


Figure 24. A median transverse section of *P. vulgaris* (NI 637) seed at D+6 showing initiation of cotyledons at the chalazal end of the embryo. Cellular endosperm encloses the embryo. Es = embryo sac.

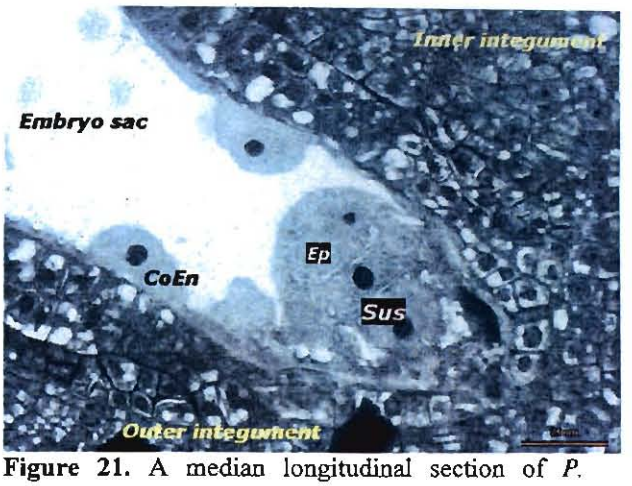


Figure 21. A median longitudinal section of *P. polyanthus* (NI1015) seed at D+2 showing the two first transverse divisions of the zygote resulting in the formation of the three-celled pro-embryo. The two basal cell give the suspensor (Sus); the terminal cell give the embryo-proper (Ep). Coenocytic endosperm (CoEn) is formed by divisions of the PEN. Liquid endosperm is largely reduced.

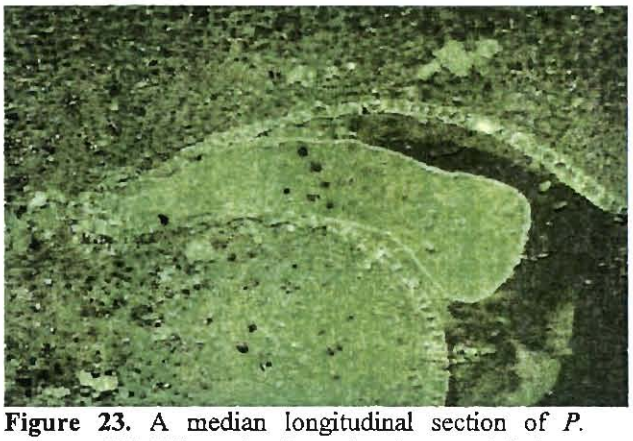


Figure 23. A median longitudinal section of *P. vulgaris* (NI 637) seed at D+5 showing a cuticle layer formed over the surface of the globular embryo-proper but not on the suspensor.

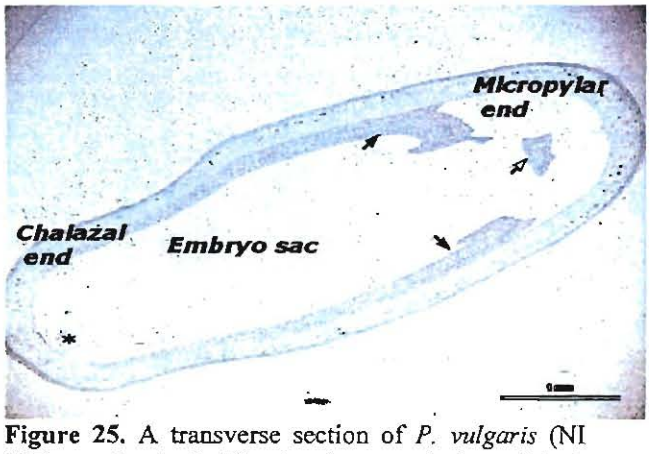


Figure 25. A transverse section of *P. vulgaris* (NI 637) seed at D+13 showing cotyledons (black arrowheads), embryo axis (white arrowhead) and remaining endosperm at the chalazal end (asterisk).

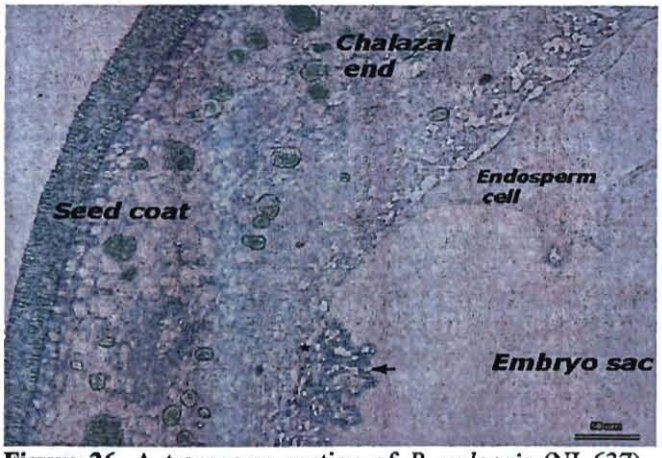


Figure 26. A transverse section of *P. vulgaris* (NI 637) seed at D+13 showing cotyledons (black arrowheads) and the remaining endosperm at the chalazal end of the seed. A single layer of stretch endosperm cells between cotyledon and seed coat is incorporate into the mature seed (asterisk).

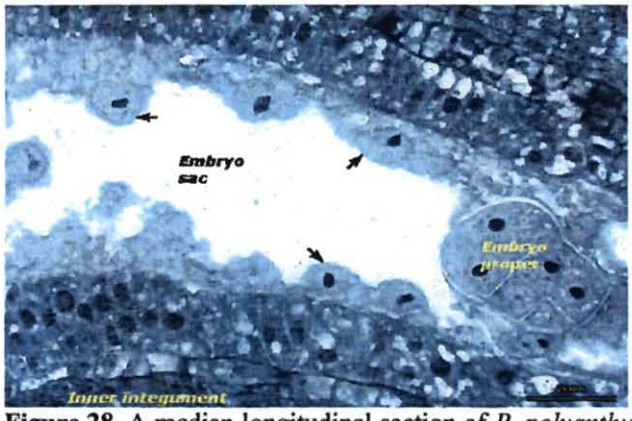


Figure 28. A median longitudinal section of *P. polyanthus* (NI1015) seed at D+4 showing cellular endosperm formation (arrowhead) and its division in the portion in contact with the embryo. Liquid endosperm is partly reabsorbed.

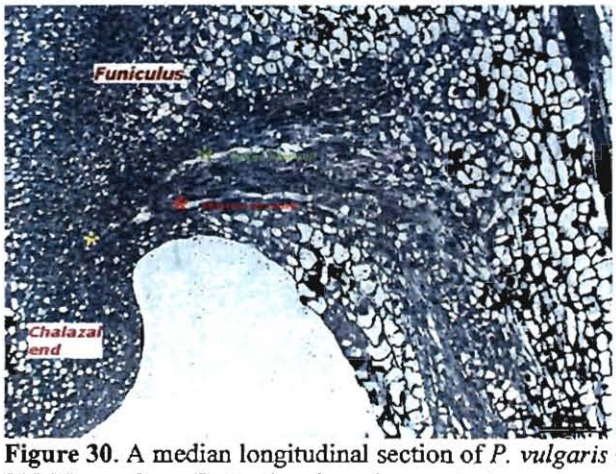


Figure 30. A median longitudinal section of *P. vulgaris* (G 21245) seed at D+3 showing the conical structure of vascular elements with a single point at the entry of the seed (yellow *). Xylem elements are located at the micropylar side of the funiculus (green *) and phloem elements at the chalazal end (red *).

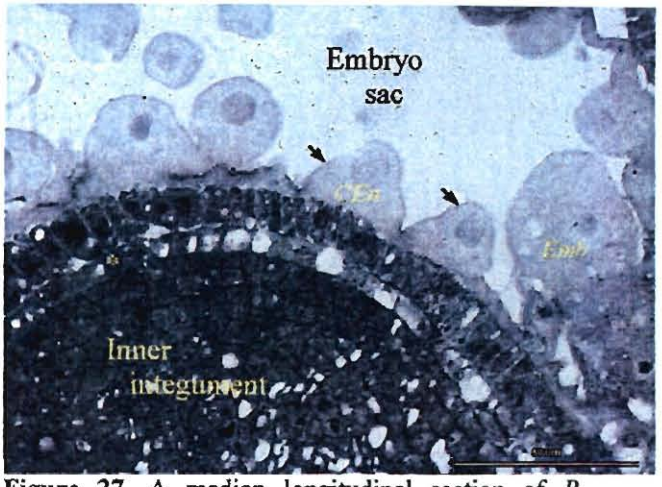


Figure 27. A median longitudinal section of *P. vulgaris* (G 21245) seed at D+3 showing embryo (Emb), embryo sac (Es), and cellular endosperm formation (CEn) by freely growing walls (arrowhead). These cells stretch to form, later, a membranous sheet over the embryo. CEn never appears in the region of degenerating nucellus. Inner integument is showed by an asterisk.

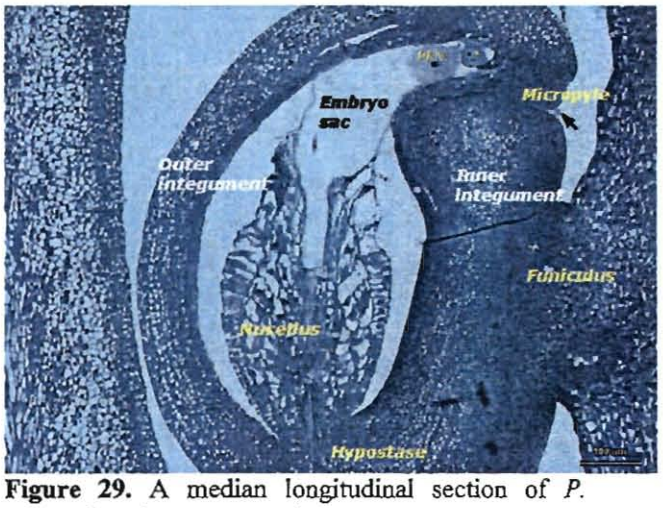


Figure 29. A median longitudinal section of *P. polyanthus* (NI1015) seed at D+1 showing the U-shaped cap of the nucellus over the embryo sac. Arrowhead shows a remain part of pollen tube passing through the micropyle.

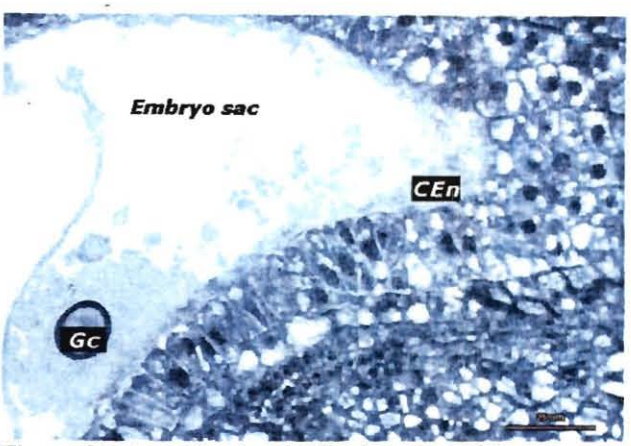


Figure 31. A median longitudinal section of *P. polyanthus* (NI1015) aborting seed at D+3 showing the fusion of the protoplasm of the cellular endosperm (CEn) and the generation of a giant cell at the chalazal end (Gc).

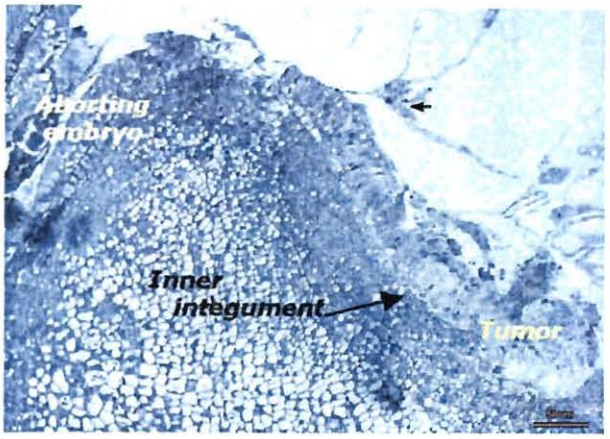


Figure 32. A median longitudinal section of *P. polyanthus* (NI1015) aborting seed at D+5 showing an aborting embryo with the formation of a proliferating tissue from the inner-epidermis of the integument.

3.1.5.2.2. PART II : EMBRYOGENESIS of PHASEOLUS HYBRIDS

3.1.5.2.2.1. Introduction

In the interspecific hybridizations *P. polyanthus* (♀) x *P. vulgaris*, embryo abortion appears in 60 % of studied cases at globular stages, 4 to 8 days after pollination according to the crosses. To understand this early abortion, a comparative histological study was carried out by Lecomte (1998) on ovule development in two *Phaseolus* species, selecting one genotype for each species. She showed that *P. polyanthus* embryos of develop more slowly than *P. vulgaris* embryos. In addition, suspensor basal cells in *P. polyanthus* are bigger than *P. vulgaris* ones. For a same development stage, nucellar cap and transfusion tissue remain in *P. vulgaris* but disappear in *P. polyanthus*. These observations suggest a nutritive requirement higher in *P. polyanthus* than in *P. vulgaris*. Lecomte (1998) concluded that a discordance in the absorption rate of nutriments by parental embryos can be responsible of disturbances in the secretion and degradation processes prevaling during the development of hybrid embryos *P. polyanthus* (♀) x *P. vulgaris*. Other histological examinations on *Phaseolus* ovule development demonstrated also the major role of integument and nucellus in embryo and endosperm nutrition. Particularly, the first histological studies made by Lecomte (1997) on *P. polyanthus* (♀) x *P. vulgaris* showed a general degradation of endothelium, integuments and nucellar cells leading to a disruption of vascular vessels and consequently to nutrition problems of embryo via the funical-chalazal-nucellus-endosperm way.

To complete these observations we studied first the problems of abortion in the reciprocal cross using *P. vulgaris* as female, considered as a control. Indeed, such hybrid

embryos are easier to obtain and can develop into mature seed. The differences in the expression of incompatibility barriers between the two reciprocal crosses (*P. polyanthus* used as female or male), will enable us to identify more precisely the most important changes related to abortion in *P. polyanthus* (♀) x *P. vulgaris* crosses.

3.1.5.2.2.2. Interspecific crosses

On 104 crosses made between the wild genotype of *P. vulgaris* (G 21245) and *P. polyanthus* (NI 1015), 47 pods were collected for histological studies at different stages of maturity. Pods were taken before any sign of abortion. On the 57 remaining crosses, 14 pods evolved to maturity and provide hybrid seeds (**Figure 33** and **32**). Germination of one seed gave an hybrid plant. The other hybrid seeds were dried and preserved for further studies. The hybrid plant was evaluated for morphological traits (**Figure 35**) and isozyme markers (using starch gel electrophoresis – **Figure 36**).

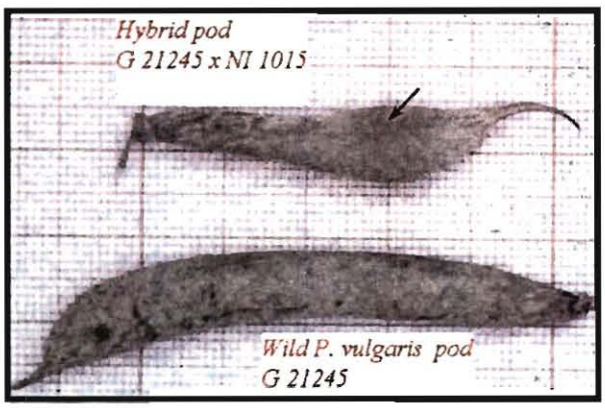


Figure 33. Hybrid pod with one seed at maturity (arrowhead).

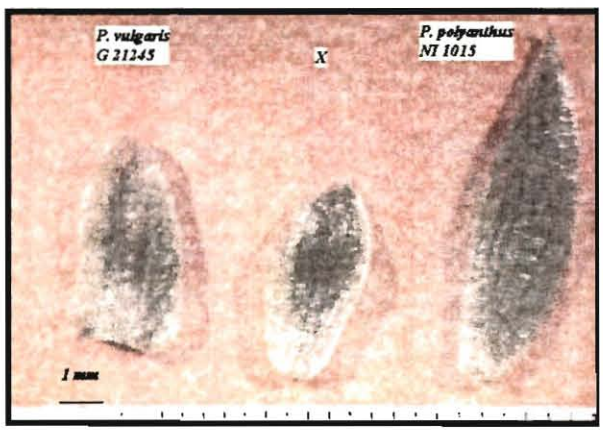


Figure 35. Flower bract of hybrid having an intermediate form between *P. vulgaris* (small bracts) and *P. polyanthus* (bracts with sharp basal part).

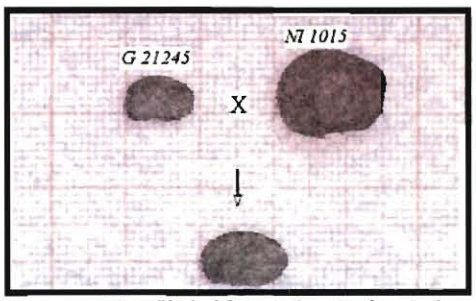


Figure 34. Hybrid seed obtained between *P. vulgaris* (♀) and *P. polyanthus* has an intermediate size.

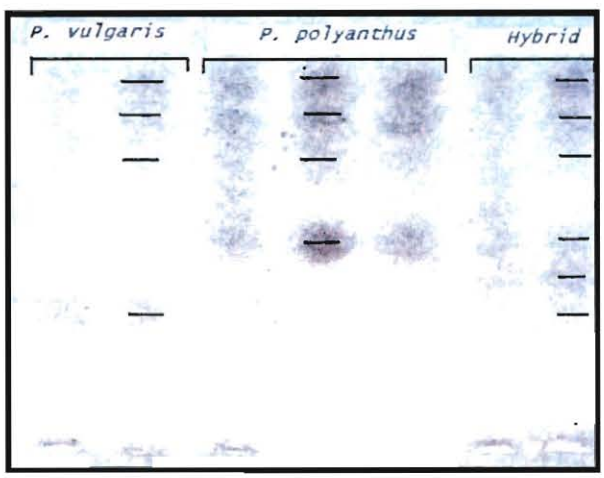


Figure 36. Isozyme on starch gel electrophoresis characterising the hybrid obtained between *P. vulgaris* (♀) x *P. polyanthus*. Six bands are observed for the hybrid as a result of the interaction of the two different bands from each species.

Results pointed out the relative easiness to abotain hybrids between G 21245 (as female parent) and NI 1015 : success rate was more than 10 %.

The hybrid plant we obtained is currently evaluated for its potential to be crossed with *P. polyanthus* (used as female parent).

3.1.5.2.2.3. Histological studies

Observations made on hybrids between *P. vulgaris* (♀) x *P. polyanthus* are summarized below. Figures are compared to what is observed in a normal developing *P. vulgaris* seed.

One day after pollination, the development of the different structures in the hybrid appears normal. Only a vacuole separating the first two endosperm cells is observed (**Figure 37**). Some hypertrophy of the vascular elements is also observed (**Figure 38**). However, the nucellus shows normal cells.

Two days after pollination, we can point out the weak development of the coenocytic endosperm (**Figure 39**). Indeed, only four nuclei are observed in comparison to 20 to 30 in normal developing seeds. Besides this difference, another discriminating factor is the low development of the zygote which is still one or two-celled while zygotes in selfpollinated seeds are 16 to 24-celled at this stage (**Figure 39**). Its division has not yet started.

Three days after pollination, four observations are made in relation with abortion of G 21245 x NI 1015 pods :

1. A 16-celled hybrid embryo can be observed. Suspensor is loosely attached to the embryo-proper (**Figure 40**).
2. Coenocytic endosperm is vacuolated and slight cellularization could be observed (**Figure 40**). We also observe a delay in endosperm development. The nutritive function of the endosperm could therefore be greatly affected.
3. Xylem elements and their parenchyma are hypertrophied at the chalazal side while phloem elements are well developed (**Figure 41**). This hypertrophy limits nutrient exchange between nucellus, outer integument and placental region. Procambial branches into the nucellus are also hypertrophied.
4. A proliferation of endothelium tissue of the inner integument is observed (**Figure 42**). This tissue grows into the embryo sac at the expense of nutritive elements.

After three days of development, most of the embryos aborted. However, in some cases, one or two seeds continue to develop within a pod. In these cases, the development of the embryo was three to four days slower than in *P. vulgaris* seeds (**Figure 42** and **41**). We observed also that the proliferating tissue from the endothelium was limited in size (**Figure**

43). Finally, it was noted that the cell divisions of the embryo-proper were more irregular than in selfpollinated seeds of *P. vulgaris*. More precisely, vertical divisions were more abundant, leading to the growth of the embryo into the inner integument, cutting off the cellular endosperm which usually completely surrounds the non hybrid embryos (**Figure 44**).

In conclusion, in hybrid seeds obtained between *P. vulgaris* (♀) x *P. polyanthus*, embryos generally begin to grow in a relatively healthy way. Only the presence of a vacuole between developing coenocytic cells and some hypertrophy of the xylem elements are noted. This early disintegration of the endosperm, beginning soon after fertilization, is commonly cited as the most important factor in embryo abortion. In general, the initial rate of development of the endosperm in hybrid crosses did not differ from that observed in selfpollinated seeds, but afterward, decreases in cell number. Structural changes are also observed. In particular, endosperm cells become vacuolated and cellularization is delayed.

Embryo abortion is also associated with the proliferative growth of a tissue from the endothelium. This tissue proliferation begins to grow without restriction and extends into the embryo sac as a burgeoning mass of cells. It is supposed that the proliferated tissue causes under-nutrition of the embryo.

In parallel to the development of a weak endosperm, we note an hypertrophy of xylem elements and their parenchyma at the chalazal end of the seed while sieve elements develop normally. Later, hyperplastic growth of the nucellus is also associated with a deficiency in the development of conductor elements within the seed.

We suppose that the hypertrophy of xylem elements could be due to the export via the nucellus of substances (that could be hormone growth substances) produced in the embryo sac by the hybrid endosperm cells and/or by the proliferating tissue of the endothelium. In other word, deleterious genes of the hybrid endosperm could lead to the production of substances that induces the division of the endothelium and the hyperplastic growth of xylem elements and nucellus. The proliferating tissue, as the hyperplastic growth of vascular elements, foreshadow a suppression of nutrient transport to the endosperm and as a consequence leads to a weak development of the embryo. We note that the development of the hybrid embryo was three to four days slower than in selfpollinated seeds.

Median longitudinal sections in hybrid seeds obtained between :
P. vulgaris **(G 21245) x** ***P. polyanthus*** **(NI1015)**

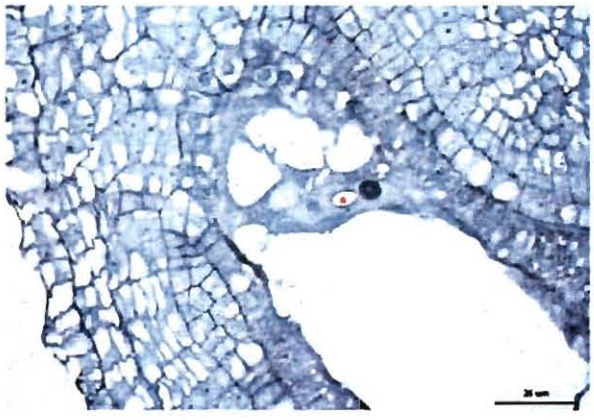


Figure 37. Section at D+1 showing the first divisions of the primary endosperm nucleus : a vacuole separates the two first cells formed (red asterisk).

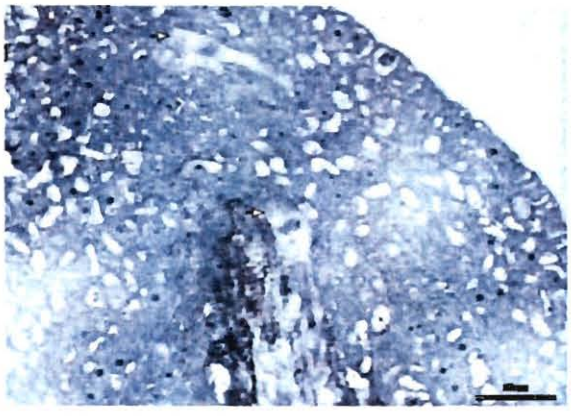


Figure 38. Section at D+1 showing some hypertrophied cells of xylem elements (arrowheads).

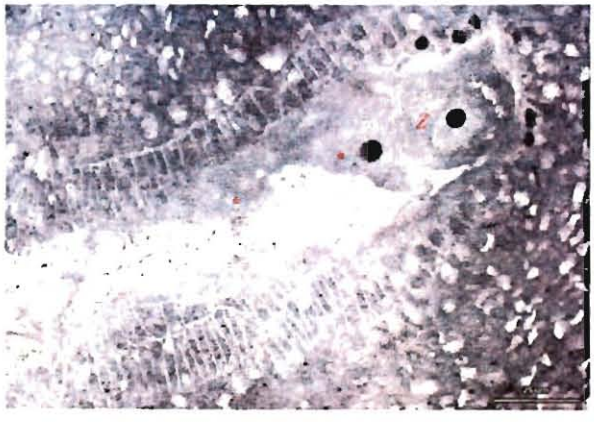
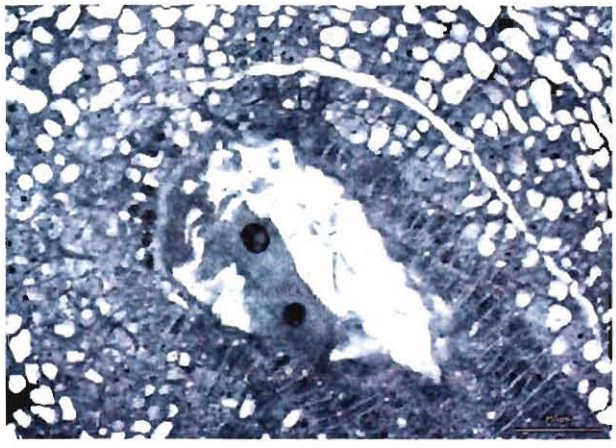
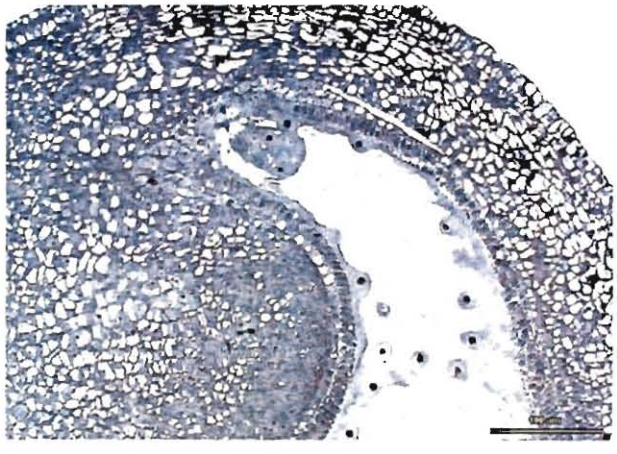


Figure 39. Section at D+2 showing a 4 celled coenocytic endosperm (asterisk) and the zygote that has still not divided.

Median longitudinal sections in normal seeds of ***P. vulgaris*** **(G 21245).**



At D1, no vacuole appears between cells of coenocytic endosperm.



At D2, embryo is 16 to 24 celled and coenocytic endosperm nuclei are abundant. Cellularization is already in process.

Median longitudinal sections in hybrid seeds obtained between :
P. vulgaris **(G 21245) x** ***P. polyanthus*** **(NI1015)**

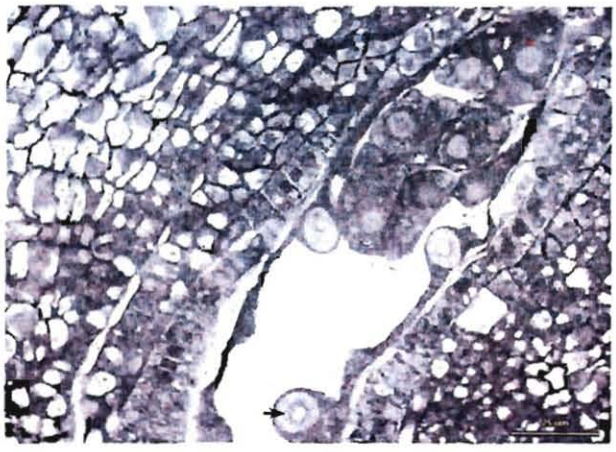


Figure 40. Section showing a 3 days old hybrid with a loosely attached suspensor (asterisk). Coenocytic endosperm is not cellularized and nuclei contains a prominent vacuole (arrowhead).

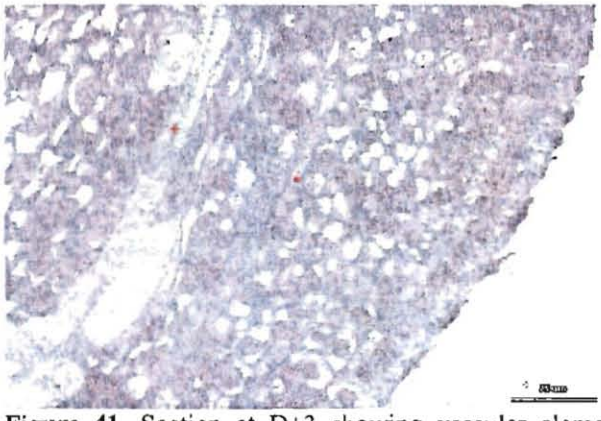


Figure 41. Section at D+3 showing vascular elements. Xylem elements and their parenchyma are hypertrophied (+) while sieve elements appears well developed (*).

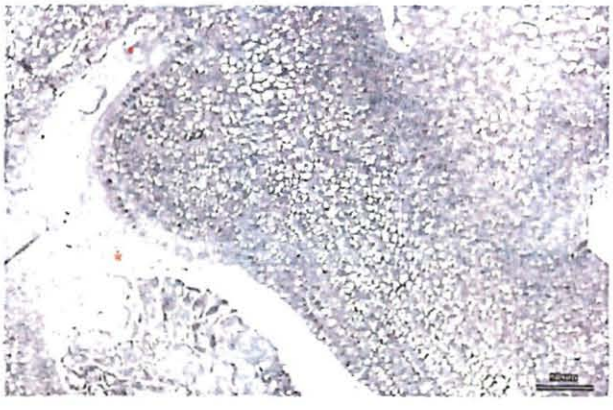
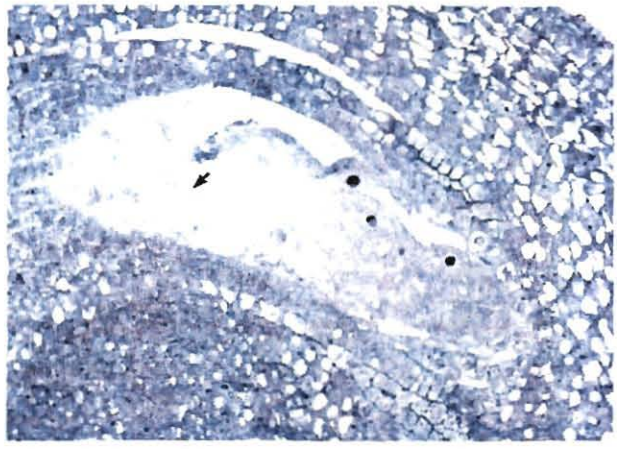
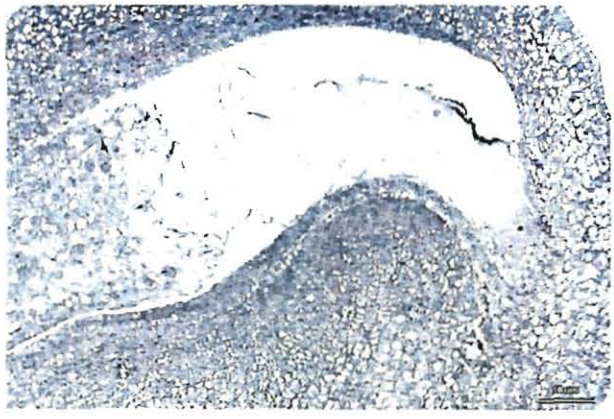


Figure 42. Section at D+3 showing the proliferation of a tissue from the endothelium (*). Hybrid embryo degenerates (red point).

Median longitudinal sections in normal seeds of ***P. vulgaris*** **(G 21245).**



Suspensor is firmly attached to the 3 days old embryo-proper and endosperm is partially cellularized (arrowhead).



Section at D+3 showing that endothelium is one cell thick, no proliferation is observed. Embryo is

well constituted.

Median longitudinal sections in hybrid seeds obtained between :
P. vulgaris **(G 21245) x** ***P. polyanthus*** **(NI1015)**

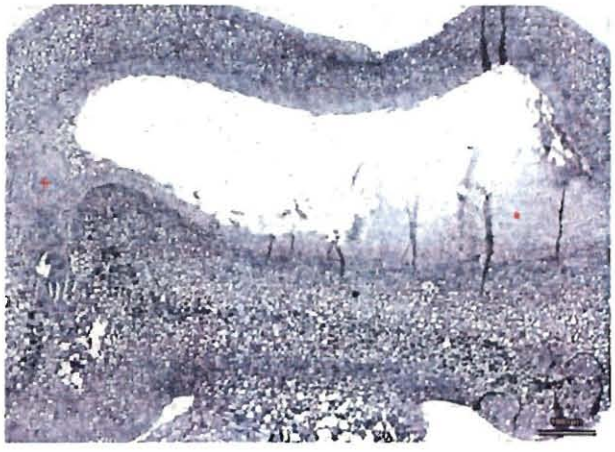


Figure 43. Section at D+6 showing a hybrid embryo at globular stage (+). His development is parallel to the proliferated tissue of endothelium origin (*).

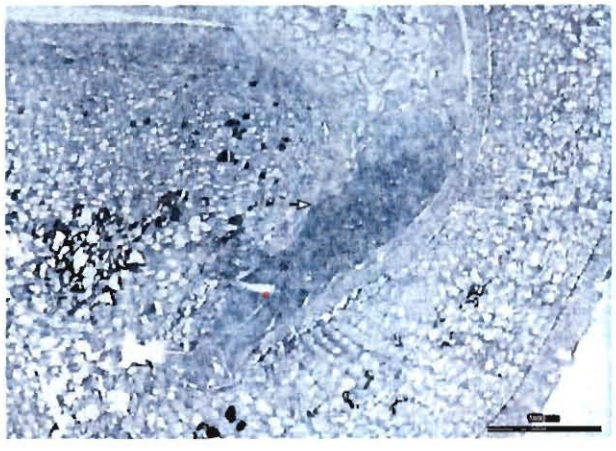
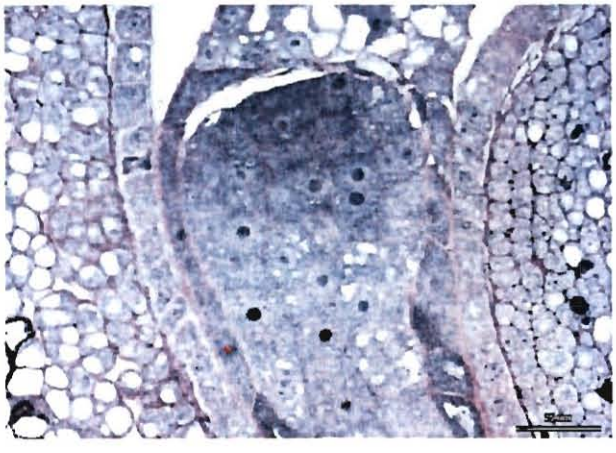


Figure 44. Section at D+7 showing that the hybrid embryo is still at globular stage. Suspensor is loosely attached to the embryo (*). Cells division of the embryo-proper is irregular and cells grow into the integuments (arrowhead). Therefore, cellular endosperm does not surround completely the embryo.

Median longitudinal sections in normal seeds of ***P. vulgaris*** **(G 21245).**



At D5, initiation of cotyledons begins. Cells division is regular providing an embryo-proper completely surrounded by cellular endosperm (*).

3.1.5.2.3 Conclusions and perspectives

The study of abortion processes in *Phaseolus* interspecific hybrids is essential to overcome incompatibility barriers. Indeed, improvement of common bean *Phaseolus vulgaris* for major biotic and abiotic constraints has been so far limited due to the incompatibility barriers encountered between interesting genotypes, mainly from different species. A critical

case study is the introgression of Ascochyta blight resistance from *Phaseolus polyanthus* into the common bean (Baudoin 1992). Some interspecific crosses have been attempted by Lecomte (1997) between *Phaseolus polyanthus* (♀) x *P vulgaris* but, although fertilized ovules were obtained, up to 60 % of globular embryos failed to develop.

In order to study the problems of the hybrid embryo development within *Phaseolus* it was important to review the existing literature on *Phaseolus sp.* Although some works have been carried out on *Phaseolus* embryogenesis, histological illustrations in *Phaseolus vulgaris* are very limited and restricted to embryos not less than 4 days old after pollination. Nothing was found on *P. polyanthus* histology. Considering this state of knowledge, it was essential to examine the different developmental stages of *Phaseolus* embryos, especially during the first days after anthesis.

Histology of early embryogenesis within *Phaseolus* was reviewed using a histological method for small and relatively soft plant material (10 to 30 mm^3 depending on the shape) that has been improved in our laboratory during the first two years of the project. Emphasis was placed on the functions played by the different parts of the developing embryo and of their interactions with the surrounding cells and tissues.

From this base work, we investigated the development of hybrid embryos in order to identify the incompatibility barriers between *P. polyanthus* (♀) x *P. vulgaris*. Genotypes were selected according to results obtained during the second year of investigations. In particular, the wild genotype G 21245 was considered for *P. vulgaris* while two genotypes (NI 1015 and G 35348) were identified for *P. polyanthus*.

We studied first the problems of abortion in the reciprocal cross using *P. vulgaris* as female. Indeed, developing hybrid seeds when using G 21245 as female were more easily obtained. Moreover, the differences in the expression of incompatibility barriers in hybrids between the two reciprocal crosses (*P. polyanthus* used as female or male), enable us to identify more precisely the most important changes related to abortion in *P. polyanthus* (♀) x *P. vulgaris* crosses. The study of *P. polyanthus* (♀) x *P. vulgaris* crosses is on progress.

Our study on reciprocal cross using *P. vulgaris* as female shows that, soon after fertilization, endosperm cells disintegrate and a tissue from the endothelium proliferate into the embryo sac. In parallel to these changes, xylem elements and, later, also the nucellus, show hypertrophied cells. We suppose that cell hypertrophy of the vascular elements, causing a deficiency in nutrient transport, could be due to the export of substances produced by the degenerating endosperm and/or by the proliferating tissue of the endothelium, and, as a consequence, leads to a weak development of the embryo. Some hybrid embryos evolved to

maturity and provide hybrid seeds. Germination of one seed has given an hybrid plant. The other hybrid seeds were dried and preserved for later studies.

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

Previously we reported:

- Crosses with common bean were created using sources of resistance identified in the previous year.
- Interspecific progeny were distributed to Africa for Bean Fly evaluation.

For 1998-99 we report the following:

- Populations among interspecific progeny and *P. vulgaris* (PV) were screened for resistance.
- More than 90 selections were obtained in segregating F_2 populations and will soon be planted for evaluation as families.
- Additional populations for both snap and dry bean types are in F_1 and F_2 generations, for advance and subsequent field screening. Thirty-two F_2 populations are ready for planting in the second semester of 1999.
- Segregating populations involving a local Guatemalan cultivar were shared with the Guatemalan national program for selection in the highlands.

The crosses that were obtained among resistant interspecific progeny, and between these and PV, were advanced to the F_2 generation and were evaluated under extremely severe disease pressure in the April planting season in Popayán. Such was the disease pressure in this season that several neighboring trials of common bean were lost. Within the segregating populations, resistant plants were observed in a small minority of the population, thus tending to confirm that the resistance is probably recessive in its expression, or multigenic. Nonetheless, more than 90 individual selections were obtained and will soon be planted for evaluation as families (**Table XVII**). The most promising populations were those that combined the Guatemalan variety, ICTA Hunapú, with the interspecific lines. Hunapú is bred variety that was developed under natural attack of ASC and expresses and intermediate resistance. It is hoped that the progeny will combine genes from Hunapú and the interspecific lines, to attain superior levels. However, it was noted that the most resistant selections were

normally quite late to mature, thus plant maturity may still be contributing to the apparent resistance of selections. It will be necessary to consciously seek plants that mature within an acceptable period, and express the maximal resistance possible within that maturity range, although this may not be the best resistance observed.

Segregating populations involving Hunapú were shared with the Guatemalan national program for selection in the highlands of that country (**Table II**). This a region in which resistance to ASC would be a welcome contribution to small farmer agriculture.

Another segregating populations were planted in which resistance sources represented only one-fourth of the parentage [G685 x (G17723 x ICTA Hunapú)]. In these populations no individual plants could be selected (**Table XVII**). It is clear that this strategy of complex multiple crosses is not adequate to transfer resistance to ASC.

Table XVII. Populations that involve the best interspecific selections for ascochyta resistance, inoculated and evaluated in field conditions. Popayán 1999 A.

Obs	Identification (*)	F_2 Families 1998 B	F_2 Plants Evaluated – 1999 A	F_3 Families Selected (**) – 1999 B
1	[249-(8)A-F11 x 346-3 A-F_4]F_1	11	234	7
2	[249-(8)A-F11 x ICTA HUNAPU]F_1	5	235	11
3	[282-(2)A-F12 x 346-3 A-F_4]F_1	10	250	14
4	[282-(2)A-F12 x ICTA HUNAPU]F_1	19	420	16
5	[283-(8)A-F12 x 346-3 A-F_4]F_1	4	160	21
6	[283-(8)A-F12 x ICTA HUNAPU]F_1	19	475	48
7	G 685 x (G17723 x ICTA HUNAPU)F_1	7	350	0
	TOTAL	75	2124	117

(*)
239-(8)A-F_{11} = BAT 338 x G35252
249-(8)A-F_{11} = BAT 338 x G35252
282-(2)A-F_{12} = G15428 x G35174
283-(6)A-F_{12} = G15428 x G35174
346-F_4 = ((G35876 x G 3807) x G35182) x KABANIMA

(**)
Includes some susceptible selections for molecular marker studies.
Partial data. To date (Sep 8/99) the harvest has not been finalized.

Additional populations for both snap and dry bean types are in F_1 generation and will be advanced to F_2 and screened for ASC in the present year (**Table XVIII**). These involve the same hybrids of Hunapú and the interspecifics crossed to susceptible parents. Time will tell if

this strategy permits the transfer of adequate resistance.

Table XVIII. Crosses for the improvement of *P. vulgaris* that involve the best interspecific selections for resistance to Ascochyta. Popayán 1998B – 1999 A.

Obs	Female	x	Male (*)	F_1 Seed 1998 B	F_1 seed 1999 A	F_2 Families 1999 A §
1.	G 685	x	[239-(8)A-F_{11} x 346-3 A-F_4]F_1	60		16
2.	G 685	x	[239-(8)A-F_{11} x ICTA HUNAPU]F_1	156		29
3.	G 685	x	[249-(8)A-F_{11} x 346-3 A-F_4]F_1	102		24
4.	G 685	x	[249-(8)A-F_{11} x ICTA HUNAPU]F_1	111		30
5.	G 685	x	[282-(2)A-F_{12} x 346-3 A-F_4]F_1	92		22
6.	G 685	x	[282-(2)A-F_{12} x ICTA HUNAPU]F_1	88		35
7.	G 685	x	[283-(8)A-F_{12} x 346-3 A-F_4]F_1	83		25
8.	G 685	x	[283-(8)A-F_{12} x ICTA HUNAPU]F_1	92		35
9.	G17723	x	(AB 136 x G10859)F_1	128		35
10.	G17723	x	(AB 136 x ICTA HUNAPU)F_1	59		29
11.	G17723	x	[239-(8)A-F_{11} x 346-3 A-F_4]F_1	10		5
12.	G17723	x	[239-(8)A-F_{11} x ICTA HUNAPU]F_1	133		40
13.	G17723	x	[249-(8)A-F_{11} x 346-3 A-F_4]F_1	36		4
14.	G17723	x	[249-(8)A-F_{11} x ICTA HUNAPU]F_1	67		32
15.	G17723	x	[282-(2)A-F_{12} x 346-3 A-F_4]F_1	69		33
16.	G17723	x	[282-(2)A-F_{12} x ICTA HUNAPU]F_1	85		32
17.	G17723	x	[283-(8)A-F_{12} x ICTA HUNAPU]F_1	184		23
18.	G17723	x	[G 685 x (G17723 x ICTA HUNAPU)F_1]F_1	107		38
19.	G17723	x	[G 685 x (G17723 x ICTA TEXEL)F_1]F_1	36		15
20.	ICA CAUCAYA	x	[239-(8)A-F_{11} x 346-3 A-F_4]F_1	5		2
21.	ICA CAUCAYA	x	[239-(8)A-F_{11} x ICTA HUNAPU]F_1	46		22
22.	ICA CAUCAYA	x	[249-(8)A-F_{11} x 346-3 A-F_4]F_1	9		2
23.	ICA CAUCAYA	x	[249-(8)A-F_{11} x ICTA HUNAPU]F_1	27		14
24.	ICA CAUCAYA	x	[282-(2)A-F_{12} x 346-3 A-F_4]F_1	15		4
25.	ICA CAUCAYA	x	[282-(2)A-F_{12} x ICTA HUNAPU]F_1	32		9
26.	ICA CAUCAYA	x	[283-(8)A-F_{12} x ICTA HUNAPU]F_1	44		22
27.	ICTA HUNAPU	x	[239-(8)A-F_{11} x 346-3 A-F_4]F_1	95		38
28.	ICTA HUNAPU	x	[239-(8)A-F_{11} x ICTA HUNAPU]F_1	182		30
29.	ICTA HUNAPU	x	[249-(8)A-F_{11} x 346-3 A-F_4]F_1	92		32
30.	ICTA	x	[249-(8)A-F_{11} x ICTA HUNAPU]F_1	116		34

Obs	Female	x	Male (*)	F_1 Seed 1998 B	F_1 seed 1999 A	F_2 Families 1999 A §
	HUNAPU					
31.	ICTA HUNAPU	x	[282-(2)A-F_{12} x 346-3 A-F_4]F_1	123		36
32.	ICTA HUNAPU	x	[282-(2)A-F_{12} x ICTA HUNAPU]F_1	73		30
33.	ICTA HUNAPU	x	[283-(8)A-F_{12} x 346-3 A-F4]F1	97		34
34.	ICTA HUNAPU	x	[283-(8)A-F_{12} x ICTA HUNAPU]F_1	83		32
35.	G 2337	x	G17723 x (AB 136 x ICTA HUNAPU)F_1		102	
36.	G 2337	x	G17723 x [282-(2)A-F_{12} x ICTA HUNAPU]F_1		157	
37.	G 2337	x	G17723 x [283-(8)A-F_{12} x ICTA HUNAPU]F_1		128	
38.	G 5702	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A-F_4]F_1		105	
39.	G 5702	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		126	
40.	G 6619	x	G 685 x [282-(2)A-F_{12} x 346-3 A- F_4]F_1		12	
41.	G 6619	x	G 685 x [283-(8)A-F_{12} x ICTA HUNAPU]F_1		46	
42.	G14614	x	G 685 x [282-(2)A-F_{12} x ICTA HUNAPU]F_1		81	
43.	G17723	x	G 685 x [239-(8)A-F_{11} x 346-3 A- F_4]F_1		189	
44.	G17723	x	G 685 x [239-(8)A-F_{11} x ICTA HUNAPU]F_1		95	
45.	G17723	x	G 685 x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		132	
46.	G17723	x	G 685 x [249-(8)A-F_{11} x ICTA HUNAPU]F_1		95	
47.	G17723	x	G 685 x [282-(2)A-F_{12} x 346-3 A- F_4]F_1		142	
48.	G17723	x	G 685 x [282-(2)A-F_{12} x ICTA HUNAPU]F_1		68	
49.	G17723	x	G 685 x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		136	
50.	G17723	x	G 685 x [283-(8)A-F_{12} x ICTA HUNAPU]F_1		122	
51.	G17723	x	ICTA HUNAPU x [239-(8)A-F_{11} x 346-3 A- F_4]F_1		25	
52.	G17723	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		107	
53.	G17723	x	ICTA HUNAPU x [282-(2)A-F_{12} x 346-3 A- F_4]F_1		54	
54.	G17723	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		132	
55.	G20523	x	ICTA HUNAPU x [239-(8)A-F_{11} x 346-3 A- F_4]F_1		90	
56.	G20523	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		95	
57.	G20523	x	ICTA HUNAPU x [282-(2)A-F_{12} x 346-3 A- F_4]F_1		29	
58.	G20523	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		125	
59.	G22041	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		94	
60.	G22041	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		156	
61.	ICTA ALTENSE	x	ICTA HUNAPU x [239-(8)A-F_{11} x 346-3 A- F_4]F_1		58	
62.	ICTA ALTENSE	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		72	
63.	ICTA ALTENSE	x	ICTA HUNAPU x [282-(2)A-F_{12} x 346-3 A- F_4]F_1		78	
64.	ICTA ALTENSE	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		91	

Obs	Female	x	Male (*)	F_1 Seed 1998 B	F_1 seed 1999 A	F_2 Families 1999 A §
65.	OBO A-061	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		117	
66.	OBO A-061	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		67	
67.	OBO A-075	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		82	
68.	OBO A-075	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		97	
69.	OBO A-088	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		25	
70.	OBO A-088	x	ICTA HUNAPU x [282-(2)A-F_{12} x 346-3 A- F_4]F_1		88	
71.	TIB 3042	x	ICTA HUNAPU x [249-(8)A-F_{11} x 346-3 A- F_4]F_1		50	
72.	TIB 3042	x	ICTA HUNAPU x [283-(8)A-F_{12} x 346-3 A- F_4]F_1		25	
			TOTAL			**843**

(*)
239-(8)A-F_{11} = BAT 338 x G35252
249-(8)A-F_{11} = BAT 338 x G35252
282-(2)A-F_{12} = G15428 x G35174
283-(6)A-F_{12} = G15428 x G35174
346-F4 = ((G35876 x G 3807) x G35182) x KABANIMA

§ : Reaction to Ascochyta was evaluated under inoculations in the field in Popayán in 1999B.

Additional crosses with PC and PP for BGMV and BSM were also obtained (**Table XIX**). These crosses will be advanced, while those for BSM will be held as F_2 populations, still pending information from Tanzania. Crosses for BGMV will be given a treatment of congruity backcross for one more complete cycle (crossing to the PC parent and then again to the PV parent), to increase genetic recombination.

Table XIX. Crosses that involve *P. vulgaris* and *P. coccineus* or *P. polyanthus* . Popayán 1998B – 1999 A.

Obs	Female	x	Male	Purpose	F_1 Seed 1998 B	F_1 Seed 1999 A	F_2 Families 1999 A
1	G 4090	x	G35171	BGMV	50 §		18 §§
2	G 4090	x	G35172	BGMV	27 §		2 §§
3	G 4090	x	(G 4090 x G35171)F_1	BGMV		115	
4	G 4090	x	(G 4090 x G35172)F_1	BGMV		49	
5	TALAMANCA	x	G35171	BGMV	43 §		3 §§
6	TALAMANCA	x	G35172	BGMV	177 §		29 §§
7	TALAMANCA	x	(TALAMANCA x G35171)F_1	BGMV		109	
8	TALAMANCA	x	(TALAMANCA x G35172)F_1	BGMV		76	
9	G 2525	x	G35345	BSM	24		
10	G 2525	x	G35348	BSM	30		
11	G 2525	x	G35350	BSM	14		

12	G 3410	x	G35345	BSM	35
13	G 3410	x	G35348	BSM	43
14	G 3410	x	G35350	BSM	21
15	G20523	x	G35345	BSM	59
16	G20523	x	G35348	BSM	28
17	G20523	x	G35350	BSM	36
18	G22036	x	G35345	BSM	17
19	G22036	x	G35348	BSM	13
20	G22036	x	G35350	BSM	27
21	G22041	x	G35345	BSM	17
22	G22041	x	G35348	BSM	12
23	G22041	x	G35350	BSM	25

§ : Reported in the annual report of 1998.
§§ : Partial data. To date (Sep 8/99) the harvest is not finalized.

4. Conclusion and perspectives

Progress has been substantial toward understanding the underlying causes of hybrid infertility in crosses between *P. vulgaris* and *P. polyanthus*. We expect that these problems can be addressed effectively through the studies on embryo culture, which are a breakthrough in embryo rescue at the earliest stages of development. These results will have impact when they are deployed in breeding programs. If the methods are as promising as they appear, they could feasibly be applied on populations of several hundred progeny, permitting their ample use in breeding of common bean. Thus, the new methodologies are very timely, since they are becoming available through the University of Gembloux as the breeding program in CIAT is focussing more on transfer of traits to the common bean. The formation of a core collection in CIAT using agroecological data to select accessions from a broad range of environments has created a useful research tool to explore the potential of these two species. Data obtained with the core collection are beginning to shed more light on the value of these genetic resources. Results in CIAT suggest that *P. coccineus* and *P. polyanthus* may have more disease resistance than previously recognized, and if genes can be transferred more readily among these species, common bean could benefit significantly. We look forward to employing the methodologies developed in Gembloux when they are fully refined, on a broader scale in the future, to fulfill the objectives of the project and overcome production constraints of small farmers.

III. Coordination of Gembloux and CIAT

In order to ensure close coordination between the activities carried out at Gembloux and at CIAT, Ir. Pascal Geerts traveled to CIAT (September 1999). Two seminars were presented in collaboration with Dr S. Beebe (Head of Bean Germplasm Characterization section), and Dr W. Roca, head of the Biotechnology Unit and an expert in tissue culture techniques. Furthermore, consultations were held with Dr D. Debouck (Head of the Genetic Resources Unit) in order to finalize the Third Annual Report.

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