Progress Report

Output 1: **Improved, small-seeded, bean germplasm resistant to major biotic and abiotic stresses and with greater nutritional and market value**

Activity 1.1 **Developing germplasm tolerant to abiotic stresses of drought and low soil fertility**

Highlights:

- More than 150 drought tolerant lines were identified from a second cycle of crosses to combine drought tolerance and resistance to BGYMV.
- The first group of lines with Brazilian grain types was selected. These will form the basis of crosses with high iron beans for north-east Brazil for the improvement of nutritional quality.
- Field evaluation of 36 promising bred lines and accessions over 2 seasons indicated that two accessions of *P. acutifolius* (G 40159 and G 40068) and two bred lines (RAB 650, SEA 23) were outstanding in their adaptation to water stress conditions. The superior performance of the two accessions of *P. acutifolius* under drought was associated with their ability to mobilize photosynthates to developing grain and to utilize the acquired N more efficiently for grain production.
- Field evaluation of 121 RILs of the cross MD 23-24 x SEA 5 indicated that two lines (MR 81, MR 25) were superior in their adaptation to drought stress conditions. The superior performance of these two lines was associated with higher values of pod harvest index and seed TNC content indicating the importance of mobilization of photosynthates to pods and seed in common bean under rainfed conditions.
- Greenhouse evaluation for differences in root distribution among 5 common bean genotypes grown in large soil cylinders indicated that SEA 5, BAT 477 and G 21212 were deep rooted compared with BAT 881 and MD 23-24.
- Field evaluation of 49 genotypes over 3 seasons indicated that two landraces (Carioca and G 21212) and two bred lines (MAR 1 and SEA 5) had the highest seed yield in Al-toxic soils. Significant negative relationship was observed between seed yield and seed P content indicating that greater seed filling and P use efficiency (g of seed produced per g of P uptake) contributed to higher yield in Al-toxic soils.
- Collaborative research with the University of Hannover showed that Al-induced callose formation is not a suitable parameter for assessing genotypic differences in Al resistance in common bean.
- Greenhouse screening of 66 RILs of the cross G 5273 x MAM 38 resulted in identification of four lines, HF14137-19, HF14137-26, HF14137-98 and HF14137-88 that were relatively more resistant to Al.
- Accessions of *Phaseolus coccineus* presented substantial vigor in a soil with high aluminum saturation, and may have important genes for tolerance to aluminum.
- Greenhouse screening of 30 RILs of the cross BAT 477 x DOR 364 resulted in identification of four lines BT21138-50, BT21138-83-1-3, BT21138-83-1-1 and BT21138-25 that were superior in their adaptation to low P supply based on total root length, number of root tips and total root biomass per plant.

- In Nicaragua and in Quilichao, tolerance to low soil fertility proved to be as important as drought tolerance or more so in confronting multiple abiotic stress.

1.1.1 Drought tolerance

1.1.1.1 Development and testing of lines and segregating populations combining drought tolerance and disease resistance in small red, small black, navy and Brazilian grain types

Rationale: Drought tolerance must be combined with other traits to be employed in commercial varieties. In most regions where drought is a problem in the Americas, bean golden yellow mosaic virus (BGYMV) is also a serious limitation. For the Central American region, small red and small black grain type is required. In Africa a more diverse range of grain types are acceptable, although BGYMV is not yet a problem. However, recessive resistance to BCMNV is highly desirable. In Brazil angular leaf spot and *Fusarium* resistance must be combined with drought tolerance for the northeast of the country. Populations were evaluated under drought to seek combinations of multiples traits.

Materials and Methods: Last year we reported on positive results with F_8 families that represented the end of our first cycle of breeding for drought tolerance. Experience in Central America in November, 2003 indicated that higher levels of resistance to BGYMV were needed in these selections. The materials tested in 2004 combine first cycle drought selections with additional sources of virus resistance. In 2004 we evaluated second cycle $F_{3.5}$ families in: three yield trials of red-seeded beans (each in 7 x 7 lattice design); two of black seeded beans (one 7 x 7 and one 6 x 6 lattice design); and a sixth of $F_{4.6}$ families with Brazilian grain types (10 x 10 lattice). Yet another trial of crosses of drought sources with parents for high iron will be reported under Output 1.4. Trials were planted on-station at CIAT-Palmira in June 2004, receiving only three irrigations, amounting to about 200 mm water. The last irrigation was applied three weeks after planting and an additional 10-20 mm of rain fell one week later. Common checks were included across trials: red seeded commercial cv. Tio Canela; black seeded commercial cv. DOR 390; and an African cowpea cultivar bred for stress conditions, Mouride.

Additional new populations were created to combine high mineral content with drought tolerance, low fertility tolerance, and disease resistance, using the best combining parents for these traits, based on experience with crosses in 2003. A large set of approximately 3500 $F_{1.2}$ families from 140 crosses was evaluated in the 2004 summer planting season.

Results and Discussion: During the crop cycle, day-time temperatures ranged from moderate ($28^{o}C$) on cloudy days, to quite high ($35^{o}C$) on clear days. Mid-day wilting was visible during the latter stages of flowering, and tensiometer readings increased gradually during pod filling, with readings of -700 millibars at the end of the crop cycle. Thus conditions resulted in severe terminal stress in nearly all plots, although it was evident that sections of fields with better soil structure suffered less stress. Although the DOR 390 check attained reasonable vegetative development, it produced very tiny, shriveled seed in most plots. However, check varieties yielded better than in past years in spite of drought, probably due to improved soil structure that permitted better root development, although grain quality was poor. As in past years, most selected lines produced commercial quality grain under even this level of stress.

Out of nearly 250 $F_{3.5}$ families, 194 were selected for additional selection and testing. These presented a yield advantage of as much as 60% over Tio Canela in the case of the reds (Table 1) and 100% over the commercial check, DOR 390 in the case of the blacks (Table 2). Very few lines yielded as well as the cowpea check, which gave higher yields than in previous years. The Nicaraguan national program bean breeder revised the nursery and selected 108 red seeded and 15 black seeded families for immediate testing and selection in Nicaragua. Another set has been shipped to Guatemala for local selection. Planting in Central America will permit evaluation for resistance to BGYMV. In particular the red seeded materials represent an important advance in meeting the commercial requirements for Central America.

Within the trial of Brazilian types, families with excellent tolerance, yield potential and grain type were identified for the first time in CIAT (Table 3). These include several lines with carioca and cream (mulatinho) grain type, and one family with rosinha grain. These form part of the collaboration with EMBRAPA under the Generation Challenge Program and will contribute to food security in the drought-prone north-eastern states of Brazil. The Brazilian counterpart has selected 34 of these for testing in Brazil.

F_2 populations: Among nearly 3500 F_1-derived F_2 families, 812 were selected for additional study. Of these 558 were derived from crosses combining high mineral parents with sources of agronomic traits including drought tolerance. Compared to results from last year, the quality of populations had improved markedly, due to the use of reselected parental stocks for several traits. These will be selected for disease resistance and mineral content in F_3 and will be analyzed for iron and zinc content in F_4, to be tested in 2005 under drought as $F_{3.5}$ families.

Table 1. Elite red seeded lines that yielded more than the check (P=0.05), identified in four separate yield trials.

Cross / Line	Yield as % checks[1]	Yield checks	Cross / Line	Yield as % checks	Yield check
(SXB 124 x (INB 35 x G23834E) x (SXB 123 x RIB 68)			**(SXB123 x EAP9503-32B) x SXB 113**		
MDSF 14742-3-MC-12Q-MQ	157	1191	SX 14816-66-MC-7P-MC	155	1191
(SXB 123 X EAP9653-16B-1) x SXB 125)			SX 14816-5-MC-15P-MC	154	1191
MDSX 14813-16-MC-6P-MQ	149	1530	SX 14816-5-MC-16P-MC	161	1417
MDSX 14813-37-MC-3P-MQ	143	1530	SX 14816-8-MC-34P-MC	159	1417
MDSX 14813-16-MC-18P-MQ	136	1530	SX 14816-8-MC-32P-MC	159	1417
MDSX 14813-61-MC-2P-MQ	143	1417	SX 14816-8-MC-21P-MC	153	1417
(SXB 123 x EAP9653-16B-1) x BFB 142			SX 14816-2-MC-1P-MC	149	1417
SXBF 14815-38-MC-2P-MQ	147	1417	SX 14816-66-MC-6P-MC	144	1197
SXBF 14815-10-MC-2P-MQ	145	1417	SX 14816-59-MC-20P-MC	143	1417
SXBF 14815-38-MC-3P-MQ	142	1417	SX 14816-8-MC-36P-MC	142	1417
SXBF 14815-21-MC-9P-MQ	139	1417	SX 14816-64-MC-9P-MC	138	1417
SXBF 14815-21-MC-6P-MQ	138	1417			

[1] Yields of lines were calculated as per cent of the yield of commercial check Tio Canela.

Table 2. Elite black seeded lines that yielded more than the check (P=0.05), identified in two separate yield trials.

Cross / Line	Yield as % checks[1]	Yield check	Cross / Line	Yield as % checks	Yield check
(SEA 18 x (FEB 192 x G21212)) x EAP9020-14			**(SXB 114 x EAP9503-32B) x SXB 124)**		
MR 14414-15-MC-1P-MQ-MC-55C-MC	211	863	MDSX 14808-24-MC-2P-MQ	160	1032
MR 14414-15-MC-1P-MQ-MC-54C-MC	192	863	**(SXB 114 x DOR 605) x SXB 123**		
MR 14414-15-MC-1P-MQ-MC-46C-MC	191	863	MDSX 14810-16-MC-20P-MQ	192	1032
(SXB 116 x DOR 677) x BFB 149			MDSX 14810-4-MC-9P-MQ	191	1032
SXBF 14799-1-MC-19P-MQ	192	1032	MDSX 14810-16-MC-10P-MQ	188	1032
SXBF 14799-11-MC-12P-MQ	173	1032	MDSX 14810-36-MC-12P-MQ	188	1032
SXBF 14799-1-MC-15P-MQ	169	1032	MDSX 14810-36-MC-5P-MQ	185	1032
SXBF 14799-9-MC-3P-MQ	163	1032	MDSX 14810-35-MC-5P-MQ	183	1032
SXBF 14799-11-MC-5P-MQ	147	1032			
(SXB 118 x EAP9503-32B) x SXB 111)			MDSX 14810-16-MC-14P-MQ	173	1032
MDSX 14801-31-MC-15P-MQ	230	1032	**(SXB 123 x EAP9653-16B-1) x SXB 125**		
MDSX 14801-25-MC-2P-MQ	201	1032	MDSX 14813-2-MC-3P-MQ	187	1032
MDSX 14801-31-MC-12P-MQ	198	1032			
MDSX 14801-43-MC-5P-MQ	197	1032	**(SXB 123 x DOR 677) x SEN 34**		
MDSX 14801-25-MC-1P-MQ	181	1032	SX 14820-37-MC-2P-MQ	181	1032
MDSX 14801-5-MC-1P-MQ	178	1032			
(SXB 118 x EAP9503-32B) x SEN 34			**(MAB 95 x EAP9653-16B-1) x SEN 34**		
MDSX 14802-46-MC-2P-MQ	180	1032	SXMA 14832-8-MC-10P-MQ	161	1032
(SXB 118 x EAP9503-32B) x BFB 140			**SEN 34 x (MAB 105 x EAP9020-14)**		
MDSX 14803-1-MC-4P-MQ	151	1032	SXMA 14833-39-MC-5P-MQ	202	1032
(SXB 114 x EAP9653-16B-1) x BFB145			SXMA 14833-39-MC-7P-MQ	178	1032
SXBF 14807-10-MC-7P-MQ	164	1032			

[1] Yields of lines were calculated as per cent of the yield of commercial check DOR 390.

Table 3. Elite drought tolerant lines that yielded more than the check (P=0.05) with cream striped, cream or pink grain color.

Cross / Line	Yield as % checks[1]	Yield check	Cross / Line	Yield as % checks	Yield check
(A686 x G21212) x (NXB 80 x Ligero)			**(SAM 6 x Ligero) x (NXB 80 x SEA 15)**		
BM 14521-3-MQ-MQ-5C-MC	160 n	1190	BM 14534-10-MQ-MQ-31C-MC	146 mu	1190
BM 14521-3-MQ-MQ-8C-MC	153 n	1190			
BM 14521-3-MQ-MQ-4C-MC	149 n	1190			
BM 14521-2-MQ-MQ-15C-MC	142	1190			
BM 14521-2-MQ-MQ-12C-MC	141 n	1190			
(A686 x A774) x (NXB80 x SEA 15)			**(TAR 4 x SEA 15) x (NXB 80 x Tio Canela)**		
BM 14524-3-MQ-MQ-8C-MC	161 mu	1190	BM 14535-10-MQ-MQ-3C-MC	152 car	1190
BM 14524-9-MQ-MQ-6C-MC	158 mu	1190	BM 14535-10-MQ-MQ-19C-MC	151 rs	1190
BM 14524-19-MQ-MQ-38C-MC	155 n	1190			
BM 14524-16-MQ-MQ-17C-MC	153 car	1190			
BM 14524-16-MQ-MQ-11C-MC	147 car	1190			
BM 14524-9-MQ-MQ-26C-MC	145 car	1190			
BM 14524-3-MQ-MQ-17C-MC	145 mu	1190			
BM 14524-16-MQ-MQ-35C-MC	145 mu	1190			
BM 14524-16-MQ-MQ-7C-MC	144 car	1190			
BM 14524-16-MQ-MQ-31C-MC	140 mu	1190			
BM 14524-16-MQ-MQ-25C-MC	139 mu	1190			
BM 14524-19-MQ-MQ-31C-MC	138	1190			
BM 14524-9-MQ-MQ-7C-MC	138	1190			

[1] Yields of lines were calculated as per cent of the yield of commercial check DOR 390.

Conclusions: Progress in drought tolerance continues with particular progress in F$_{3.5}$ families with regard to grain color in several grain classes. The quality of populations with high mineral parents improved substantially over those of a year ago.

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1.1.1.2 Evaluation of drought resistance and associated traits in advanced lines

Rationale: Development of drought adapted bean varieties is an important strategy to minimize crop failure and improve food security in bean growing regions. Previous research indicated that the superior performance of common bean genotypes under drought was associated with their ability to mobilize photosynthates to developing grain and to utilize the acquired N and P more efficiently for grain production. Among the plant traits evaluated, grain filling index and seed P content were identified as useful traits to consider in the breeding program in addition to grain yield for identifying bean genotypes that are better adapted to drought. We evaluated drought adaptation of 36 promising bred lines and accessions over 2 seasons and the average values for those 2 trials are reported here.

Materials and Methods: Two field trials were conducted at Palmira in 2001 and 2002 (June to September) to determine differences in tolerance to water stress conditions. Both trials included 36 bred lines and germplasm accessions. Two levels of water supply (irrigated and rainfed) were applied. A 6 x 6 partially balanced lattice design with 3 replications was used. Details on planting and management of the trial were similar to those reported before (CIAT, 1998). Experimental units consisted of 4 rows, 5 m long by 0.6 m wide. A number of plant attributes were measured at mid-podfilling in order to determine genotypic variation in drought resistance. These plant traits included leaf area index; canopy dry weight per plant; shoot nutrient (N, P, K, Ca and Mg) uptake; shoot and seed ash content; and shoot and seed TNC (total nonstructural carbohydrates). At the time of harvest, grain yield and yield components (number of pods per plant, number of seeds per pod, 100 seed weight) were determined. Seed N, P, ash content and TNC were measured. Pod harvest index (dry wt of pods/dry wt of total biomass at mid-podfill x 100) and grain filling index (100 seed weight of rainfed/100 seed weight of irrigated) were also determined.

Measurements of photosynthetic efficiency of recently fully expanded intact leaves were made with a portable Plant Efficiency Analyzer (Hansatech, King's Lynn, UK). Leaves were dark adapted for 20 min using leaf clips before a 5-s light pulse (1500 µmol m^{-2} s^{-1}) was supplied by an array of red light-emitting diodes. The rapid turn-on of the light-emitting diodes allowed the accurate determination of Fo (minimal fluorescence intensity with all photosystem II reaction centers open while the photosynthetic membrane is in the non-energized state in the dark) and, hence, Fv (maximum variable fluorescence in the state when all non-photochemical processes are at a minimum, i.e., Fm-Fo). The ratio of variable to maximal fluorescence (Fv/Fm = (Fm-Fo)/Fm) (Fm = fluorescence intensity with all photosystem II reaction centers closed) is a measure of the maximal photochemical efficiency of photosystem II. Leaf chlorophyll content of fully expanded leaves was measured by using a non-destructive, hand-held chlorophyll meter (SPAD-502 Chlorophyll Meter, Minolta Camera Co., Ltd., Japan). The principle is based on the difference in light attenuation at wavelengths 430 and 750 nm. From the difference in light attenuation, a numerical SPAD (Soil Plant Analysis Development) unit, ranging from 0 to 80 is calculated by the microprocessor in the SPAD-502 Chlorophyll Meter.

Results and Discussion:

Palmira – Soil, temperature, rainfall and evaporation: The soil is a Mollisol (Aquic Hapludoll) with no major fertility problems (pH = 7.7), and is estimated to permit storage of 130 mm of available water (assuming 1.0 m of effective root growth with –0.03 MPa and –1.5 MPa upper and lower limits for soil matric potential). During the crop-growing season, maximum and minimum air temperatures in 2001 were 30.5 and 18.7 °C and in 2002 were, 30.6 and 19.2 °C respectively. The incident solar radiation ranged from 4.6 to 25.0 MJ m^{-2} d^{-1} in 2001 and 11.2 to 24.7 MJ m^{-2} d^{-1} in 2002. Total rainfall during the active crop growth was 122.7 mm in 2001 and 83.9 mm in 2002. The potential pan evaporation was of 432 mm in 2001 and 441 mm in 2002. These data on rainfall and pan evaporation indicated that the crop suffered high level of drought stress during active growth and development.

Grain yield and physiological traits: Under water stress conditions in the field, the seed yield of 36 genotypes ranged from 448 to 1268 kg/ha (Table 4). Among the genotypes tested, two

accessions of *P. acutifolius* (G 40159 and G 40068) and two bred lines (RAB 650 and SEA 23) were outstanding in their adaptation to rainfed (water stress) conditions. There was response to irrigation in most of the genotypes but not in all genotypes as evident from the mean values (Table 4; Figure 1). This was mainly due to high incidence of a leafhopper pest (*Empoasca kraemeri*) during reproductive development in the rainfed treatment and attack of a pod-boring insect (*Epinotia opposita*) in the irrigated treatment that affected the performance of several genotypes including G 21212 in 2001 season. The relationship between grain yield of rainfed and irrigated treatments indicated that G 40159, RAB 650, SEA 23 and SEA 15 were not only adapted to water stress but also responsive to irrigation (Figure 1). Among the 36 genotypes tested, INB 36 was the most poorly adapted bred line under rainfed conditions.

Pod harvest index (PHI) is a measure of mobilization of photosynthates from leaves and stems to pod formation. Relationship between irrigated PHI and rainfed PHI showed that that the two accessions of *P. acutifolius* (G 40159 and G 40068) and Pinto Villa were outstanding in maintaining greater values of PHI under both rainfed and irrigated conditions (Figure 2). Grain filling index is a measure of mobilization of photosynthates to seed formation. Four genotypes (SEA 17, G 40068, RAB 650, RAB 609) were outstanding in their ability to fill the seed under rainfed conditions (Figure 3).

Under rainfed conditions, grain yield was not related to leaf area index (Table 4). This was because several genotypes (e.g., ICA Pijao, SEA 17) had greater leaf area values than the best performers such as the two accessions of *P. acutifolius* (G 40159 and G 40068) under rainfed conditions (Table 4). The superior performance of these two accessions could be due to better transport system for mobilizing photosynthates to developing grains as revealed by the greater levels of TNC in seed (Table 5). Under rainfed conditions, the two accessions of *P. acutifolius* (G 40159 and G 40068) yielded more than any line or accession of *P. vulgaris*, demonstrating the advantage that this species continues to display over *P. vulgaris*, and justifying our on-going breeding efforts in interspecific crosses. The red seeded breeding lines (sister lines RAB 650 and RAB 651) without directed selection for drought tolerance during their development performed well under rainfed conditions. It is important to note that seed TNC at harvest of the two *P. acutifolius* accessions (G 40068 and G 40159) under rainfed conditions was also greater than many genotypes (Figure 4).

Table 4. Influence of drought stress on grain yield, leaf area index and pod harvest index (dry wt of pods/dry wt of total biomass at mid-podfill x 100) of 36 genotypes evaluated in a Mollisol at Palmira over two seasons.

| Line or accession	Grain yield (kg/ha)		Leaf area index (m²/m²)		Pod harvest index (%)	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
G 40159 (*P. acutifolius*)	1700	1268	1.06	0.65	60	64
G 40068 (*P. acutifolius*)	1096	1264	1.14	1.14	57	64
RAB 650	1307	1112	1.8	1.82	48	44
SEA 23	1379	1036	1.79	1.33	49	46
SEA 15	1558	992	1.93	1.3	49	48
SEA 18	1128	968	1.81	1.92	43	39
RAB 651	1397	916	2.2	2.06	44	40
SEA 16	1253	904	2.88	2.39	39	33
RAB 632	1080	844	1.69	1.63	45	41
TIO CANELA 75	1264	820	1.66	1.7	43	39
SEA 5	1066	817	2.43	2	35	27
SEA 19	1204	808	2.09	1.92	46	43
BAT 477	1262	795	1.87	2.04	41	38
PINTO VILLA	1452	779	1.56	1.29	52	60
SEA 21	1223	761	1.8	1.24	49	54
SEA 20	829	747	1.92	1.68	40	37
RAB 619	1127	745	2	1.71	47	43
SEA 17	1014	741	2.67	2.28	40	35
RAB 618	1044	732	1.61	1.41	45	41
SEA 22	1251	716	1.93	1.5	47	43
G 21212	840	707	1.79	2.04	35	27
RAB 609	1248	703	1.86	1.77	42	39
RAB 636	839	698	1.81	1.54	45	41
G 1977	621	686	2.12	2.06	23	19
RAB 620	1047	679	1.39	1.61	40	35
APETITO	770	671	1.94	1.71	32	21
RJB 7	1048	645	2.11	1.71	40	38
INB 37	1027	637	2.16	1.82	44	40
INB 35	1143	635	2.87	2.13	33	26
INB 39	1183	622	2.16	1.78	33	24
INB 38	997	593	2.54	1.91	34	26
RAB 608	854	591	1.83	1.76	39	33
ICA QUIMBAYA	786	561	1.44	1.25	46	43
DOR 390	979	538	2	1.44	40	37
ICA PIJAO	1212	477	2.75	2.17	36	31
INB 36	939	448	2.09	1.7	35	30
Mean	**1116**	**769**	**1.97**	**1.71**	**48**	**37**
LSD (0.05)	**289**	**198**	**0.56**	**0.52**	**6**	**11**
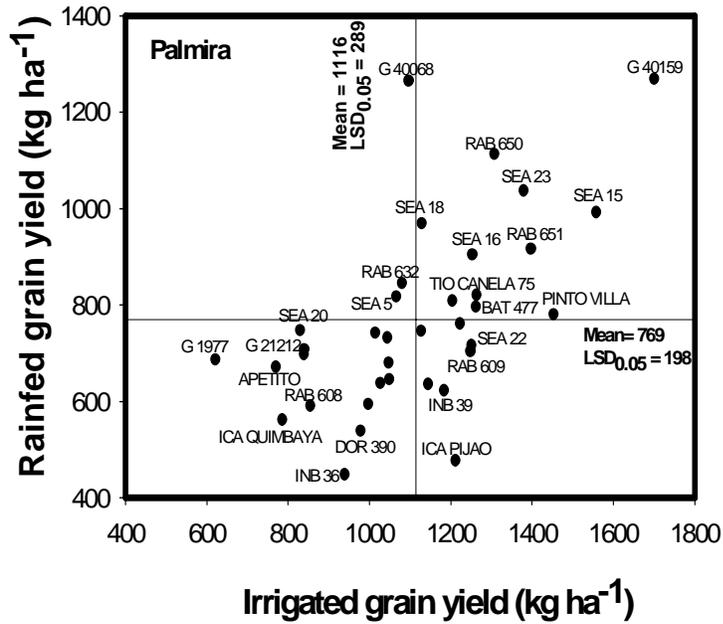
Figure 1. Identification of genotypes that are adapted to rainfed conditions and are responsive to irrigation to a Mollisol at Palmira. Genotypes that yielded superior with drought and were also responsive to irrigation were identified in the upper box of the right hand side.
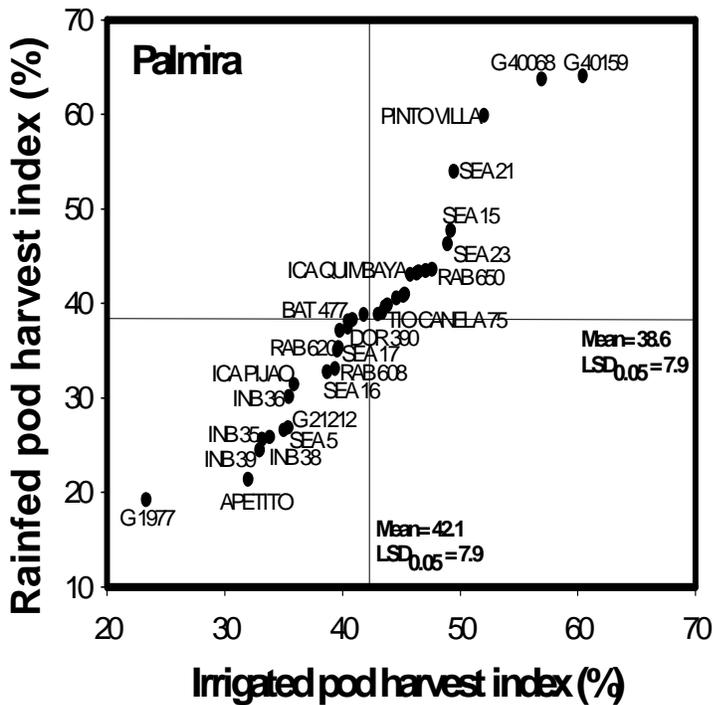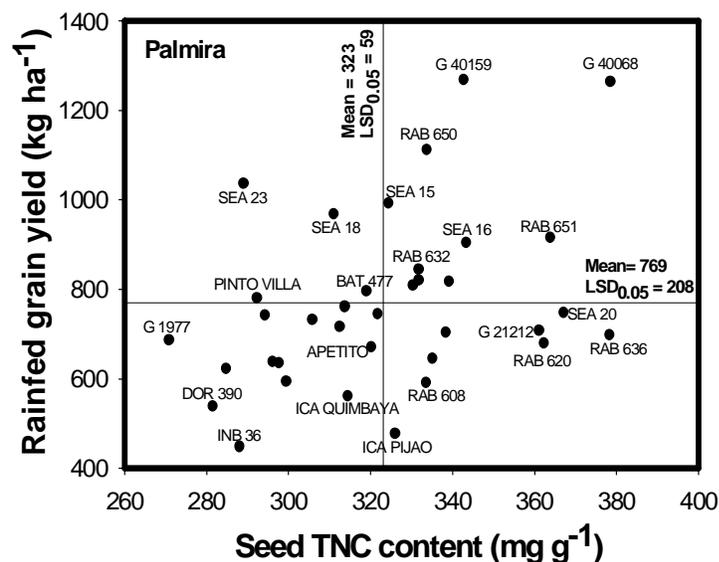

Figure 2. Identification of genotypes that are superior in mobilizing photosynthates to pod filling under both rainfed and irrigated conditions in a Mollisol at Palmira. Genotypes with greater pod harvest index (dry wt of pods/dry wt of total biomass at mid-podfill x 100) were identified in the upper box of the right hand side.

Table 5. Influence of drought stress on seed N, seed P and seed TNC content of 36 genotypes evaluated in a Mollisol at Palmira over two seasons.

Line or accession	Seed N content (%)		Seed P content (%)		Seed TNC content (mg g^{-1})	
	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed
G 40159 (*P. acutifolius*)	3.53	3.69	0.54	0.52	352	343
G 40068 (*P. acutifolius*)	3.93	3.76	0.58	0.57	357	379
RAB 650	3.67	3.78	0.56	0.59	370	334
SEA 23	4.02	4.12	0.62	0.63	342	289
SEA 15	3.99	3.86	0.64	0.55	357	324
SEA 18	3.25	3.92	0.61	0.53	360	311
RAB 651	3.80	4.01	0.60	0.54	337	364
SEA 16	3.78	3.85	0.58	0.5	317	343
RAB 632	4.20	4.15	0.63	0.65	341	332
TIO CANELA 75	3.82	4.02	0.58	0.56	319	332
SEA 5	3.99	4.02	0.66	0.52	359	339
SEA 19	3.79	4.05	0.54	0.54	368	330
BAT 477	4.44	4.28	0.64	0.57	343	319
PINTO VILLA	3.55	4.05	0.50	0.54	396	292
SEA 21	3.60	3.93	0.53	0.52	363	314
SEA 20	3.77	3.92	0.59	0.54	381	367
RAB 619	3.87	3.96	0.55	0.57	360	322
SEA 17	4.17	4.09	0.72	0.56	357	294
RAB 618	3.61	4.02	0.54	0.54	385	306
SEA 22	3.76	3.81	0.57	0.55	379	313
G 21212	3.76	4.03	0.58	0.55	320	361
RAB 609	3.84	3.98	0.62	0.56	363	338
RAB 636	3.70	3.99	0.46	0.53	423	378
G 1977	4.21	4.20	0.62	0.52	282	271
RAB 620	3.68	3.99	0.61	0.54	353	362
APETITO	3.79	3.8	0.59	0.56	367	320
RJB 7	3.80	4.05	0.61	0.57	367	335
INB 37	4.11	4.30	0.64	0.61	314	296
INB 35	4.19	4.24	0.63	0.53	329	298
INB 39	3.93	4.30	0.55	0.58	279	285
INB 38	3.81	4.17	0.61	0.51	311	300
RAB 608	3.78	4.17	0.62	0.65	329	334
ICA QUIMBAYA	3.66	4.09	0.61	0.63	354	315
DOR 390	4.03	4.17	0.63	0.52	341	282
ICA PIJAO	4.22	4.11	0.60	0.53	293	326
INB 36	3.98	4.29	0.66	0.55	295	288
Mean	**3.86**	**4.03**	**0.59**	**0.55**	**346**	**323**
LSD (0.05)	**0.43**	**0.26**	**0.06**	**0.06**	**76.6**	**59.7**
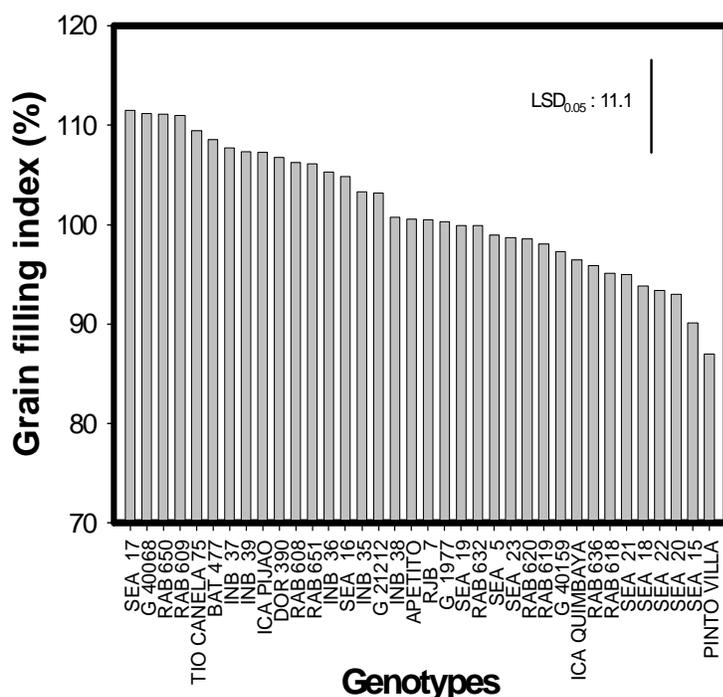
Figure 3. Genotypic differences in grain filling index (100 seed weight of rainfed/100 seed weight of irrigated) among 36 genotypes of common bean grown in in a Mollisol at Palmira.

Figure 4. Identification of genotypes that combine superior seed yield with higher TNC content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and higher seed TNC were identified in the upper box of the right hand side.

Previous research showed that the superior performance of certain bean genotypes under rainfed conditions could be related to lower seed P content. We tested this relationship further by measuring seed nutrient (N, P) content (Table 5) and seed ash (total mineral) content. Several superior performers combined high grain yield with lower seed N, P and ash content. The bred line SEA 23 was outstanding in combining greater yield with higher level of seed N (protein) under rainfed conditions (Table 5). The two *P. acutifolius* accessions (G 40068 and G 40159)

showed lower seed ash content than most of the genotypes tested under rainfed conditions. Although the two *P. acutifolius* accessions (G 40068 and G 40159) were outstanding in grain yield under rainfed conditions, these two accessions showed lower levels of seed N indicating greater N use efficiency (g of grain yield per g of N uptake). One of the bred lines RAB 650 was similar to the two *P. acutifolius* accessions in its outstanding ability to utilize acquired N and P for grain production.

Correlation coefficients between final grain yield and other shoot attributes indicated that greater seed yield under rainfed conditions was positively related to pod harvest index and negatively related with seed N and P content (Table 6). Leaf area production was negatively associated with seed yield under rainfed conditions indicating that mobilization of photosynthates to grain was limiting seed yield. Significant negative relationship was also observed between seed yield and seed N and P content under rainfed conditions. This observation indicates that the superior performers mobilized greater amounts of photosynthates to seed per unit amount of N and P in the seed. Seed yield under rainfed conditions was also negatively associated with the photosynthetic efficiency and total chlorophyll content of recently expanded leaves.

Table 6. Correlation coefficients (r) between final grain yield (kg ha^{-1}) and other plant attributes of 36 genotypes of common bean grown under rainfed and irrigated conditions over 2 seasons in a Mollisol at Palmira.

Plant traits	Irrigated	Rainfed
Leaf area index	0.431***	-0.205**
Seed N content	-0.295***	-0.454***
Seed P content	-0.337***	-0.193**
Seed TNC content	0.027	0.024
Seed Ash content	0.203**	-0.009
Pod harvest index	-0.192**	0.183**
Photosynthetic efficiency (Fv/Fm)	0.180**	-0.255**
Total chlorophyll content (SPAD)	0.184**	-0.185**

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

It appears that the two accessions of *P. acutifolius* (G 40068 and G 40159) and two bred lines (RAB 650 and SEA 23) could mobilize greater amounts of photosynthates from leaves and stems to grain during water stress. Use of these genotypes in the breeding program could contribute to greater yield potential under rainfed conditions due to greater utilization of photosynthates. The superior adaptation of these genotypes to drought was found to be due to their efficient utilization of N and P for grain production in addition to the mobilization of photosynthates.

Conclusions: This field study indicated that two accessions of *P. acutifolius* (G 40159 and G 40068) and two bred lines (RAB 650, SEA 23) were outstanding in their adaptation to water stress conditions. The superior performance of the two accessions of *P. acutifolius* under drought was associated with their ability to mobilize photosynthates to developing grain and to utilize the acquired N more efficiently for grain production.

References:

CIAT 1998. Bean Project Annual Report 1997. CIAT, Cali, CO. 197 p. (Working Doc. No. 177).

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1.1.1.3 Evaluation of drought resistance and associated traits in recombinant inbred lines (RILs)

Rationale: W evaluated 32 promising bred lines and 4 checks over 2 seasons for their adaptation to drought stress under field conditions. The results from these field studies indicated that two accessions of *P. acutifolius* (G 40068 and G 40159) and two bred lines (RAB 650 and SEA 23) were outstanding in their adaptation to water stress conditions. The superior performance of these four genotypes under drought was associated with their ability to mobilize photosynthates to developing grain and to utilize the acquired N and P more efficiently for grain production. We evaluated drought adaptation of 121 RILs of the cross MD 23-24 x SEA 5 to obtain phenotypic data for eventual gene tagging. The bred line SEA 5 is very well adapted to drought while MD 23-24 is superior in commercial grain quality.

Materials and Methods: The field trial was conducted at Palmira in 2003 (June to September) to determine differences in tolerance to water stress conditions. The trial included 121 RILs of MD 23-24 x SEA 5 along with 5 checks and 2 parents. An 11 x 11 partially balanced lattice design with 3 replicates was used. Two levels of water supply (irrigated and rainfed) were applied. Details on planting and management of the trial were similar to those reported before (CIAT, 1998). Experimental units consisted of 4 rows, 5 m long by 0.6 m wide. A number of plant attributes were measured at mid-podfilling in order to determine genotypic variation in drought resistance. These plant traits included leaf area index; canopy dry weight per plant; shoot nutrient (N, P, K, Ca and Mg) uptake; shoot and seed ash content; and shoot and seed TNC (total nonstructural carbohydrates). At the time of harvest, grain yield and yield components (number of pods per plant, number of seeds per pod, 100 seed weight) were determined. Seed N, P, ash content and TNC (total nonstructural carbohydrates) were also measured.

Results and Discussion:

Palmira – Soil, temperature, rainfall and evaporation: During the crop-growing season, maximum and minimum air temperatures were 34 and 15.8 °C, respectively, while incident solar radiation ranged from 11.2 to 24.7 MJ m^{-2} d^{-1} (Figure 5). The total rainfall during the active crop growth was 43.2 mm. The potential pan evaporation was of 337 mm. These data on rainfall and pan evaporation indicated that the crop suffered intermittent drought stress during active growth and development.

Under intermittent drought stress conditions in the field, the seed yield of 121 RILs ranged from 300 to 2250 kg/ha (Figure 6). Among the lines tested, two lines MR 81 and MR 25 were outstanding in their adaptation to rainfed (water stress) conditions. These two lines were also responsive to irrigation. The relationship between grain yield of rainfed and irrigated treatments indicated that several RILs lines were superior to the best parent, SEA 5 and the 5 checks.

Among the 121 lines tested, MR 114 was the most poorly adapted bredline under rainfed conditions.

Figure 5. Rainfall distribution, pan evaporation, maximum and minimum temperatures during crop growing period at Palmira.

Figure 6. Identification of genotypes that are adapted to rainfed conditions, and are responsive to irrigation in a Mollisol at Palmira. Genotypes that yielded superior with drought and were also responsive to irrigation were identified in the upper box of the right hand side.

Results on the relationship between irrigated pod harvest index (PHI) and rainfed PHI showed that several RILs were superior to the drought adapted parent, SEA 5 in mobilizing photosynthates to pods (Figure 7). The PHI values of cowpea were markedly lower than those of

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the common bean. Higher values of PHI indicate greater mobilization of photosynthates to pod formation while higher values of grain filling indicate greater mobilization to seed filling.
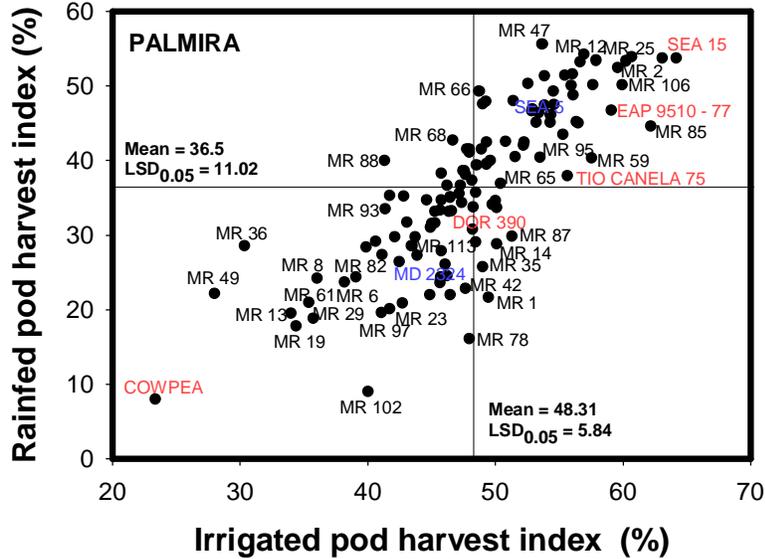
Figure 7. Identification of genotypes that are superior in mobilizing photosynthates to pod filling under both rainfed and irrigated conditions in a Mollisol at Palmira. Genotypes with greater pod harvest index (dry wt of pods/dry wt of total biomass at mid-podfill x 100) were identified in the upper box of the right hand side.

It is important to note that seed yield under rainfed conditions is positively associated with PHI, leaf area index and seed TNC content in this study (Table 7).

Table 7. Correlation coefficients (r) between final grain yield (kg ha^{-1}) and other plant attributes of RILs of common bean grown under irrigated and rainfed conditions in a Mollisol in Palmira.

Plant traits	Irrigated	Rainfed
Leaf area index	0.136**	0.394***
Seed P content	-0.041	0.013
Seed TNC content	0.026	0.176***
Seed ash content	-0.073	-0.024
Pod harvest index	0.113	0.437***

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

The relationship between rainfed seed yield and other plant attributes indicated that the superior performance of line MR 81 was associated with lower levels of seed P and higher level of seed TNC content. Lines MR 24 and MR 52 combined the attributes of greater level of seed TNC with seed yield under rainfed conditions (Figure 8).

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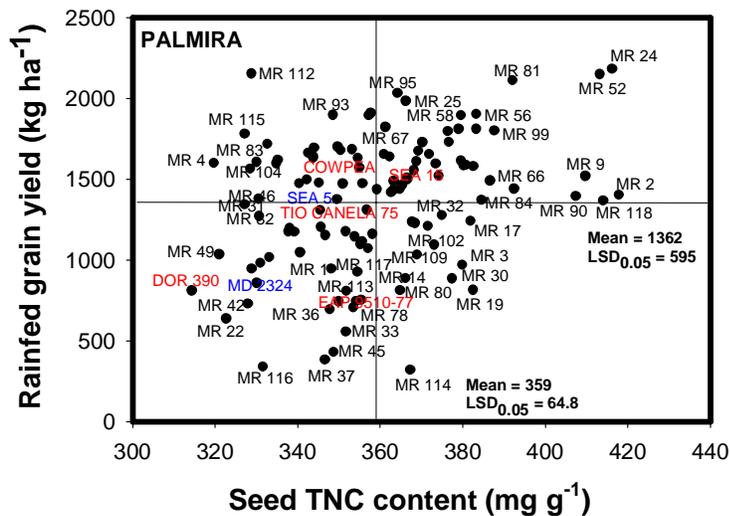
Figure 8. Identification of genotypes that combine superior seed yield with higher seed TNC when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and higher seed TNC were identified in the upper box of the left hand side.

Conclusions: Results from this field study indicated that among the 121 RILs of the cross MD 23-24 x SEA 5, two lines (MR 81, MR 25) were superior in their adaptation to drought stress conditions. The superior performance of these two lines was associated with higher values of pod harvest index and seed TNC content indicating the importance of mobilization of photosynthates to pods and seed in common bean under rainfed conditions. Therefore, these traits are candidates for gene tagging in a later phase of this work.

References:

CIAT 1998. Bean Project Annual Report 1997. CIAT, Cali, CO. 197 p. Working Document No. 177

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1.1.1.4 Genotypic differences in root distribution and drought tolerance of RIL parents

Rationale: Identification of shoot and root attributes that are associated with superior drought adaptation will help to develop rapid and reliable screening methods. These methods are needed to develop bean genotypes that combine drought adaptation with other desirable attributes. Field studies for the past few years indicated that one germplasm accession, G 21212 and two bred lines, SEA 5 and BAT 477 are superior in their adaptation to drought. RILs of BAT 881 x G 21212 and SEA 5 x MD 23-24 were also evaluated under field conditions to identify QTLs related to drought adaptation. A greenhouse study was conducted to characterize shoot and root responses to 3 different levels of water supply in these RIL parents using BAT 477 as a check for deep rooting ability and drought adaptation.

Materials and Methods: A greenhouse study was conducted using an andisol from Darién, Colombia. The trial comprises 5 entries, including 4 RIL parents (BAT 881, G 21212, SEA 5, MD 23-24) and a check (BAT 477). Plants were grown in large plastic cylinders (100 cm long and 15 cm diameter) covered with PVC tubes. The trial was planted as a randomized block in split-plot arrangement with three levels of water supply: 100% field capacity (well-watered),

60% field capacity (moderate drought stress) and 30% field capacity (severe drought stress) as main plots and genotypes as sub-plots. Soil was fertilized with an adequate level of nutrients (the equivalent in kg ha^{-1} of 80 N, 50 P, 100 K, 101 Ca, 29.4 Mg, 20 S, 2 Zn, 2 Cu, 0.1 B and 0.1 Mo). Treatments of water stress were imposed after two weeks of initial growth of plants established with seed. Water stress was maintained by weighing each cylinder every week and applying water to the soil at the top of the cylinder. After 7 weeks of stress treatment (at 85 days after germination), shoot biomass distribution, root biomass and root length distribution in different soil depths, and leaf and stem nutrient composition, ash content and TNC (total nonstructural carbohydrates) contents were determined.

Results and Discussion: Reducing the water supply to 30% of field capacity (FC) markedly decreased the leaf, stem and pod biomass of the five genotypes (Figure 9). Total biomass production was greater with 60% FC than at 100% FC. This appeared to be due to soil compaction and poor drainage at 100% FC.
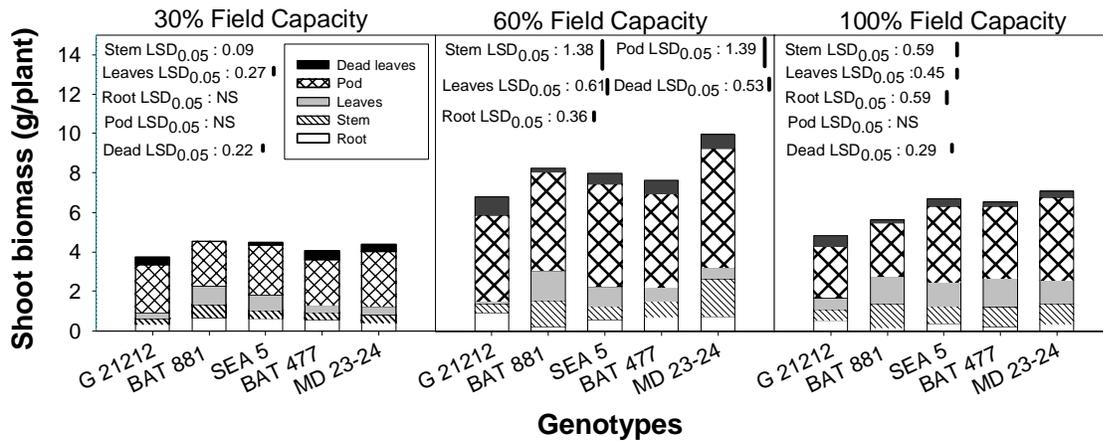
Figure 9. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on dry matter distribution among leaves, stem and roots of five common bean genotypes.

Results on root length distribution showed that G 21212 and MD 23-24 were relatively unaffected by drought stress (Figure 10). Water stress at 30% FC resulted in no penetration of roots beyond 50 cm soil depth in BAT 881 and MD 23-24. These 2 genotypes are sensitive to drought under field conditions. Among the 5 genotypes, SEA 5 showed greater root length distribution at 50 to 100 cm soil depth at all three levels of water supply indicating its deep rooting ability. We noted problems of compaction in some cylinders. Therefore we conducted some additional studies to overcome these problems and found that use of 2:1 of soil and sand in smaller plastic tubes (50 cm long and 5 cm diameter) could overcome some of the problems encountered with large cylinders. Use of small cylinders will also facilitate evaluation of a larger number of genotypes for root penetration in subsoil layers and root branching ability in subsoil.
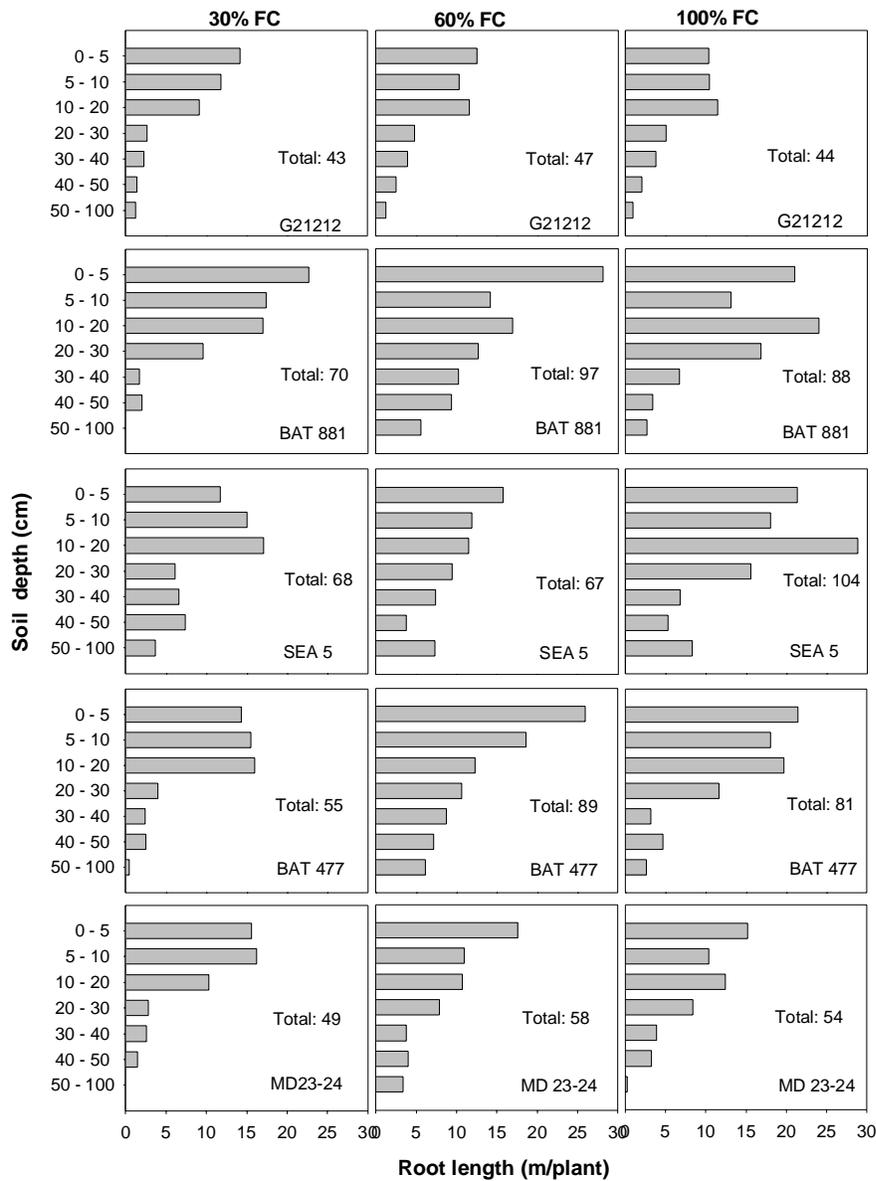
Figure 10. Influence of three levels of water supply (100%, 60% and 30% of field capacity) on root length distribution across soil depth in five common bean genotypes.

Results on the determination of N, P, ash (mineral) and TNC contents in leaves, stem and pod tissue indicated that water stress could markedly increase P content in pods. But leaf N, P, ash and TNC contents were only slightly influenced by water stress conditions.

Further work is in progress to evaluate the usefulness of fine root production in topsoil, root penetration in subsoil and root branching in subsoil as indicators of drought tolerance in common bean.

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1.1.2 Aluminum tolerance

### 1.1.2.1 Common bean (*Phaseolus vulgaris* L.) genotypes tolerant to aluminum-toxic soils in the tropics

Rationale: Toxicity of Al in acid soils in the tropics is a serious problem and amending soils with lime is difficult and prohibitively expensive. Common bean needs significant improvement in Al tolerance to reduce farmer dependence on lime and fertilizer. Field screening of 5000 germplasm accessions and breeding lines in Al-toxic soils with and without lime (65% Al saturation) indicated significant genotypic variation in seed yield (Rao, 2001). These genotypic differences in seed yield could be related to differences in tolerance to Al, acquisition and utilization of nutrients for transport of photoassimilates to developing seeds. Genotypes that are adapted to Al toxic soils are capable of acquiring essential nutrients in a low pH and high Al soils. Identification of plant traits that are linked to acid soil adaptation is fundamental to developing efficient screening procedures for genetic enhancement of common bean.

Materials and Methods: A set of 49 genotypes including germplasm accessions and breeding lines was evaluated at Quilichao (990 masl; Oxisol – Plinthic Kandiudox), Colombia for identification of plant attributes for adaptation to Al-toxic acid soils. A partially balanced lattice design with three replicates was used for three years. Two fertility levels were used. Plots with high fertilizer (HF) received banded application of P (40 kg/ha) in the form of triple super phosphate and two foliar application of urea (1 kg/ha). Plots with no fertilizer (NF) received no application of nutrients. Soil characterization data of NF plots showed toxic levels of exchangeable Al (66% Al saturation) and Mn (8 to 10 mg/kg) and low availability of Ca (1.4 $cmol_c$/kg) and Mg (0.51 $cmol_c$/kg) while P availability was adequate for plant growth and development. Plots of HF showed toxic levels of Mn (14 to 21 mg/kg) with very low levels of exchangeable Al. Leaf area index, shoot biomass, shoot nutrient (N, P, K, Ca and Mg) uptake and shoot total nonstructural carbohydrates (TNC) were measured at mid-podfilling (R8). Also, number of pods per plant, number of seeds per pod, 100 seed weight, seed yield and seed N, P and TNC contents were determined.

Results and Discussion: Among the 49 genotypes tested, two landraces (Carioca and G 21212) and two breeding lines (MAR 1 and SEA 5) were found to be higher yielding in Al-toxic soils (Figure 11). In NF environment, seed yield ranged from 21 to 399 kg/ha while in HF the range was from 93 to 1374 kg/ha. Relationship between seed yield in NF and HF indicated that VAX 1 was better adapted to both environments (Figure 11). In NF environment, BAT 477 exhibited greater leaf area index at mid-podfilling. This was mainly because of the ability of this genotype to acquire greater amounts of N, P, Ca and Mg from soil in the presence of toxic level of Al. Shoot Ca content of G 3593 was markedly greater than that of the most other genotypes.

Genotypes that were higher yielding in Al-toxic soils were also superior in their ability to acquire Ca and Mg from NF environment. This observation indicates the importance of Ca and Mg acquisition to seed yield when grown in Al-toxic soil. BAT 477 and SEA 5 were outstanding in combining greater seed yield with high content of N in seeds in NF environment. It appears that these two breeding lines are not only tolerant to toxic levels of Al in soil but also capable of mobilizing a greater proportion of shoot N to developing seeds. FEB 190 was also noteworthy

for its high N content in seed with moderate seed yield in NF environment. The breeding line A 785 and the landrace G 21212 were high yielding in NF but were moderate in seed N content.
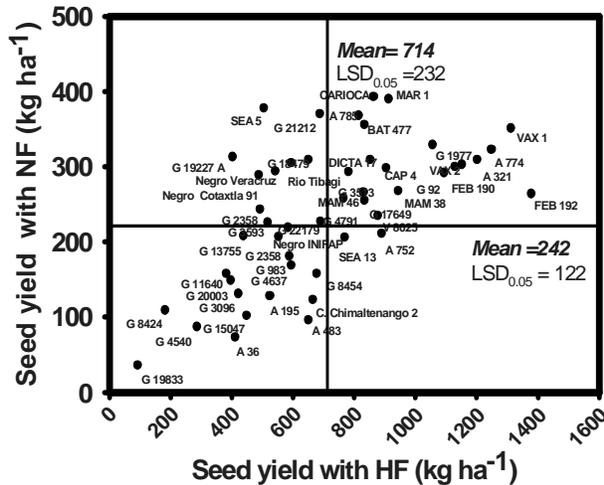
Figure 11. Identification of common bean genotypes that are adapted to Al toxic soil and are responsive to application of lime and P inputs to an Oxisol at Quilichao. Genotypes that yielded better with no fertilizer (NF) and were also responsive to application of high fertilizer (HF) were identified in the upper right hand quadrant.

Correlation coefficients between seed yield and other traits indicated that leaf area index was positively related to seed yield in NF environment (Table 8). Seed yield was also positively related to shoot nutrient uptake in both NF and HF environments. Seed yield in NF was also positively associated with shoot TNC content. A significant negative relationship was observed between seed yield and seed P and N content in NF indicating that greater P and N use efficiency contributed to superior adaptation to Al toxic soils. Results from these field studies in Quilichao indicate that two breeding lines (MAR 1 and SEA 5) and two landraces (Carioca and G 21212) were superior in their tolerance to Al-toxicity. This study also showed that it is possible to combine Al resistance with high seed N (protein) content.

Conclusions: Two landraces (Carioca and G 21212) and two breeding lines (MAR 1 and SEA 5) had the highest seed yield in Al-toxic soils. Significant negative relationship was observed between seed yield and seed P content indicating that greater seed filling and P use efficiency (g of seed produced per g of P uptake) contributed to higher yield in Al-toxic soils.

References

Rao IM 2001. Role of physiology in improving crop adaptation to abiotic stresses in the tropics: The case of common bean and tropical forages. In: M. Pessarakli (ed). *Handbook of Plant and Crop Physiology*. Marcel Dekker, Inc., New York, USA pp. 583-613.

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Table 8. Correlation coefficients (r) between seed yield (kg ha^{-1}) and other plant traits at mid-podfilling (R8) of 49 genotypes of common bean grown with no fertilizer (NF) or high fertilizer (HF) to an Oxisol at Quilichao.

| Plant traits	NF	HF
Leaf area index (m^2/m^2)	0.62***	0.44***
Shoot biomass (kg/ha)	0.66***	0.58***
Shoot N uptake (kg/ha)	0.61***	0.52***
Shoot P uptake (kg/ha)	0.64***	0.50***
Shoot K uptake (kg/ha)	0.56***	0.52***
Shoot Ca uptake (kg/ha)	0.61***	0.41***
Shoot Mg uptake (kg/ha)	0.63***	0.52***
Shoot N content (%)	-0.27***	-0.24***
Shoot P content (%)	-0.22***	-0.25***
Shoot Ca content (%)	0.36***	0.05
Shoot Mg content (%)	0.17***	-0.02
Seed N content (%)	-0.24***	-0.09
Seed P content (%)	-0.27***	-0.04
Shoot TNC (g/kg)	0.19***	0.17*
Seed TNC (g/kg)	0.05	0.003

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

1.1.2.2 Aluminium-induced callose formation is not a suitable parameter for assessing genotypic differences in aluminum resistance in *Phaseolus vulgaris*

Rationale: Poor growth of common bean in acid soils can be correlated to Al toxicity. However, other factors like toxicities of protons and Mn, and deficiencies of P, Ca and Mg may also play a role (CIAT, 1999). At pH values below 4.5 (required for Al screenings), protons may cause plant injury in proton-sensitive plant species such as common bean (Lazof and Holland, 1999). Proton toxicity can be ameliorated through an increased supply of calcium in the nutrient solution. Formation of callose is a common plant response to different stresses. Callose induction by Al in roots has been demonstrated for monocotyledonous and dicotyledonous plant species (Horst et al., 1997). In beans, clear differentiation in Al resistance has not been found (Massot et al., 1999). The present work aimed at determining the basic nutrient solution composition to be used in assessing genotypic differences in Al resistance of common bean independently of interactions with proton toxicity; and the possible use of Al-induced callose formation for assessing genotypic differences in Al resistance.

Materials and Methods: Seeds of common bean cultivars were germinated in peat substrate (pH 5.5) and precultured in 5 mM CaCl$_2$, 0.5 mM KCl and 8 µM H$_3$BO$_3$ under controlled environmental conditions. After pH adjustment, plants were treated with Al and solution pH was maintained at pH 4.5 ± 0.1. Root length was measured before and after 36 h of Al treatment, and was expressed in percentage of the controls without Al. Callose content in the 10 mm root apices was quantified according to standard methods.

Results and Discussion: The nutrient solution used successfully for the screening of maize for Al resistance (Horst et al., 1997) proved to be unsuitable for common bean because at pH 4.3 and 0.5 mM Ca^{2+} root elongation was greatly reduced making it difficult to select for Al resistance because of the lack of a proper control (Figure 12). However, proton toxicity could be avoided by adjusting the pH to 4.5, increasing the Ca supply to 5 mM Ca^{2+}, and adding 0.5 mM K^+. This did not affect the Al sensitivity of the plants.
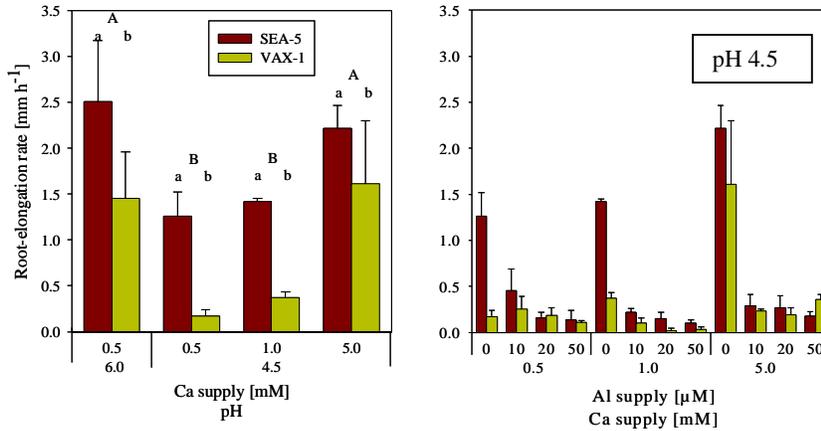

Figure 12. Root-elongation rate of two common bean grown in nutrient solution for 36 h as affected by Ca, and Al supply. Bars represent means ± SD, n = 4. Different letters indicate significant differenes (p < 0.05) between cultivars (a,b) or pH/Ca supply (A,B)

The thus modified treatment solution allowed clear separation of a larger set of genotypes for Al resistance using Al-induced inhibition of root elongation as a parameter. After 36 h of Al treatment large differences in Al resistance among the 28 cultivars tested could be identified (Figure 13).
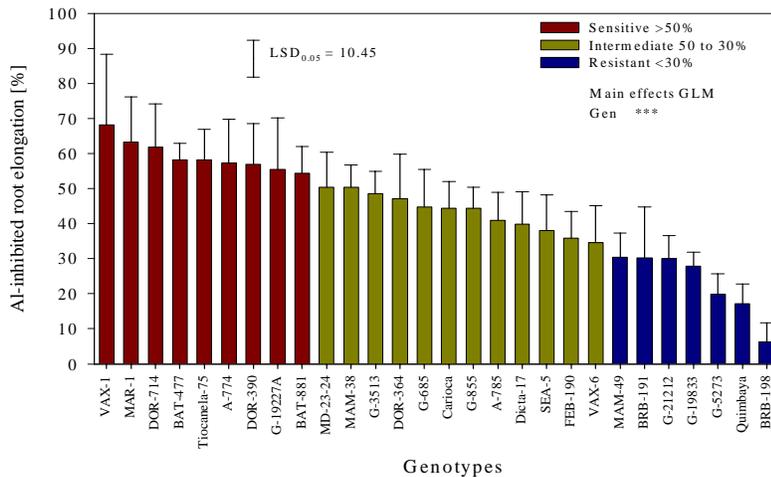
Figure 13.Aluminum-induced inhibition of root elongation of 28 common bean cultivars grown in a solution containing 5 mM $CaCl_2$, 0.5 mM KCl, and 8 μM H_3BO_3, pH 4.5 for 36h with or without 20 μM Al, pH 4.5. Bars represent means ± SD, n = 8.

As in maize (Horst et al., 1997) and soybean (Wissemeier and Horst, 1995), in common bean Al induced formation of callose in the root apices. However, maximum callose contents were found at 4 h of Al treatment and decreased thereafter. There was no clear differentiation between the cultivars that differed in Al resistance: no relationship existed between Al resistance as expressed by Al-induced inhibition of root elongation and callose formation (Figure 14)
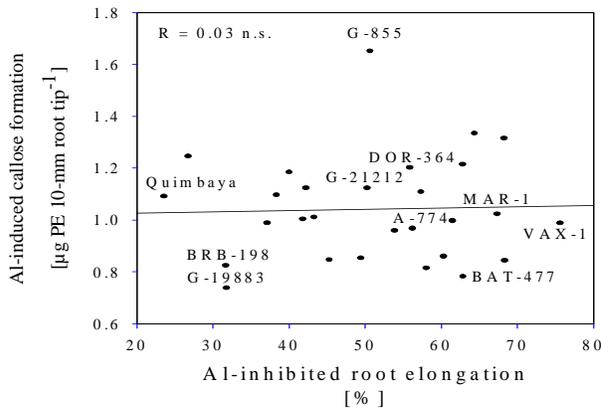
Figure 14. Relationship between Al-induced callose formation in 10 mm root apices after 4h of Al treatment, and Al-induced inhibition of root elongation after 36h of Al treatment. A total of 28 common bean cultivars were treated with 20 µM Al in a solution containing 5 mM $CaCl_2$, 0.5 mM KCl, and 8 µM H_3BO_3, pH 4.5.

The results suggest that in common bean Al-induced callose formation after short-term Al treatment leading to maximum callose contents in the root apices, is not a suitable parameters for assessing cultivar difference in Al resistance. These results are not in agreement with Massot et al. (1999). The main reason might be that common bean belongs to the pattern II plant species (Ma et al., 2001), where Al resistance mechanisms are only switched on after a lag phase of a few hours. Thus, after 4 h of Al treatment, all cultivars react as sensitive to Al as suggested by callose formation. The elucidation of Al resistance mechanisms in common bean requires a clear separation and better understanding of short and medium-term responses to Al.

Conclusions: Proton toxicity did not allow the screening of common bean genotypes using the established protocol for maize (0.5 mM $CaCl_2$, 8 µM H_3BO_3, pH 4.3). Increasing the pH to 4.5 and the Ca^{2+} concentration to 5 mM, and addition of 0.5 mM KCl fully prevented proton toxicity and allowed establishing large differences in Al resistance between 28 cultivars using inhibition of root elongation by treatment with 20 µM Al for 36 h as parameter of Al injury. Al induced callose formation in root apices with a maximum after 4 h of Al treatment. However, no relationship existed between Al-induced callose formation after 4h and inhibition of root elongation after 36.h. Therefore, Al-induced callose formation is not a suitable parameter for assessing genotypic differences in Al resistance in common bean.

References:
CIAT 1999: Meeting demand for beans in Sub-Saharan Africa in sustainable ways. Annual Report of the project IP-2. CIAT, Cali, Colombia.

Horst, W. J., Püschel, A. K., and Schmohl, N. 1997. Induction of callose formation is a sensitive marker for genotypic aluminium sensitivity in maize. Plant Soil. 192, 23-30.

Lazof, D. B. and Holland, J. M. 1999. Evaluation of the aluminium-induced root growth inhibition in isolation from low pH effects in *Glycine max*, *Pisum sativum* and *Phaseolus vulgaris*. Aust. J. Plant Physiol. 26, 147-157.

Ma, J. F., Ryan, P. R., and Delhaize, E. 2001. Aluminium tolerance in plants and the complexing role of organic acids. Trends in Plant Science. 6, 273-278.

Massot, N., Llugany, M., Poschenrieder, C., and Barcelo, J. 1999. Callose production as indicator of aluminium toxicity in bean cultivars. *J. Plant Nutr*. 22, 1-10.

Wissemeier, A. H. and Horst, W. J. 1995. Effect of calcium supply on aluminium-induced callose formation, its distribution and persistence in roots of soybean (Glycine max L. Merr.). J. Plant Physiol. 145, 470-476.

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1.1.2.3   Selection of families and lines of common bean with acid-soil tolerance

Rationale: As part of a restricted core project funded by BMZ-GTZ of Germany, field studies were carried out at Quilichao (990 masl; Oxisol – Plinthic Kandiudox) to select parental stocks and segregating populations for tolerance to an acid soil complex in which aluminum toxicity is a major component. Genetic differences have been observed for many years for the ability to produce grain under aluminum toxic conditions in Quilichao. With this effort we sought to exploit this variability and to increase levels of tolerance through conventional breeding techniques.

Materials and Methods: Germplasm that had presented superior performance in the low pH soil in Quilichao in previous years were selected for crossing (VAX 1, G24601, G21212, RAB 655, Apetito, SEA 15, A 774). In addition, Durango and Jalisco germplasm including Flor de Mayo accessions were tested both in Quilichao and in Rwanda, and elite accessions were identified (G19024, G1816, G2285, G21164, G11049, G13538, G19168, G22401). Physiological analysis subsequently demonstrated that some of these have a high number of root tips that favor calcium uptake, thus justifying their inclusion in crosses. Special attention was given to crosses that incorporate a small contribution of Andean genes (using BRB 191, BRB 198, and G5273) into a Mesoamerican background, to attempt to introduce the reaction expressed in Andean accession G5273 into Mesoamerican types. Crosses that combine even 25% of Andean genes in crosses with Mesoamerican types tend to be poorly adapted, so the Andean contribution was reduced to 6.25-12.5% in most cases.

Results and Discussion: Progress has been very slow, probably due to a combination of the narrow genetic variability and the very complexity of the acid soil problem (multiple problems that occur at low soil pH: besides aluminum *per se*, manganese toxicity and calcium and magnesium deficiencies). That said, several promising crosses (pending confirmation) combine an Andean parent in a small proportion with Mesoamerican parents (see Table 9). For example,

the cross ((G 24601 x (MAM 38 x BRB 198)F_1)F_1 x G 11015)F_1 X (MAM 38 x G 21212)F_1 produced promising progenies. Among the parents of this complex cross, BRB 198 is the only Andean type and here represents only one-sixteenth of the genetic constitution. Our expectation is that it is contributing genes for the same reaction that is observed in the greenhouse hydroponic test, and that these are combining with genes for vigorous root development from other parents. If this is confirmed, it would be a unique example in the case of common beans in which physiology led breeding in identifying useful traits that could not be recognized under conventional field breeding.

Conclusions: Progress in improving the tolerance of bean to aluminum toxicity has been extremely slow, due to narrow genetic diversity and the complexity of the acid soil problem. A definitive conclusion on degree of progress is pending.

Contributors: S. Beebe, I. Rao, C. Cajiao

Table 9. Families in F_3 to F_7 generations evaluated in Quilichao 2003B – 2004A. Andean parents appear in bold type.

| Code	Num	Identification	Families Evaluated Q03B	Families Selected Q03B	Ind. Plant sel. Q03B	Families Evaluated Q04A	Families Selected Q04A	F n
AL	14300	G 24601 X (G 22041 x **BRB 198**)F_2/-	5	3	5	3	1	F 3
AL	14709	G 21212 X (G 22041 x **BRB 198**)F_1/-	22	5	12	11	0	F 3
AL	14710	(G 1759 x **G 5273**)F_1 X G 21212/-	4	0	0	0	0	F 3
AL	14711	(SEA 15x **G 5273**)F_1 X G 21212/-	20	0	0	0	0	F 3
AL	14712	(VAX 1x **BRB 191**)F_1 X G 21212/-	25	8	32	27	8	F 3
AL	14713	(MAM 38xG 21212)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	10	3	14	12	3	F 3
AL	14714	(RAB 655xG 21212)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	14	8	49	41	6	F 3
AL	14715	(SEA 15x(A 774xG 21212)F_1)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	8	0	0	0	0	F 3
AL	14716	(MAM 49xG 21212)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	15	1	3	2	0	F 3
AL	14717	(SEA 15xDICTA 17)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	2	0	0	0	0	F 3
AL	14718	(G 1759xG 21212)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	5	1	4	4	0	F 3
AL	14719	(G 22041xG 21212)F_1 X (G 24601x(G 22041x **BRB 198**)F_2)F_1/-	27	4	15	14	0	F 3
AL	14722	((MAM 38xG 21212)F_1xG 19073)F_1 X (SEA 15xDICTA 17)F_1/-	7	2	11	9	0	F 3
AL	14723	((MAM 38xG 21212)F_1 X G 19073)F_1 X G 13637/-	9	2	7	5	0	F 3
		Total	173	37	152	128	18	**F 3**

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Table 9. cont'd.

Code	Num Identification	Families Evaluated Q03B	Families Selected Q03B	Ind. Plant sel. Q03B	Families Evaluated Q04A	Families Selected Q04A	F n
SF	13953 SEA 15 x (A 774xG 21212)F_1/-	1	**1**	4	4	0	F 4
AL	14300 G 24601 X (G 22041 x **BRB 198**)F_2/-	5	2	11	10	1	F 4
AL	14367 (MAM 38xG 21212)F_1 X G 19073/-	16	1	41	39	12	F 4
AL	14586 ((G 24601x(MAM 38 x **BRB 198**)F_1)F_1xG 11015)F_1 X (MAM 38xG 21212)F_1/-	13	5	40	39	12	F 4
AL	14587 ((G 24601x(MAM 38 x **BRB 198**)F_1)F_1xG 11015)F_1 X (9824-47-1/F_7xG 21212)F_1/-	5	1	11	11	3	F 4
AL	14588 ((RAB 655x(G 22041xRIB 66)F_1)F_1xG 11015)F_1 X (MAM 38xG 21212)F_1/-	9	2	4	4	1	F 4
AL	14595 ((MAM 38xRIB 66)F_1xG 11037)F_1 X (MAM 38xG 21212)F_1/-	11	4	31	27	3	F 4
AL	14589 ((G 24601x(MAM 38xRIB 66)F_1)F_1xG 19017)F_1 X (MAM 38xG 21212)F_1/-	1	0	0	0	0	F 4
AL	14591 ((RAB 655x(MAM 49xRIB 66)F_1)F_1xG 19017)F_1 X (MAM 38xG 21212)F_1/-	2	1	5	5	0	F 4
AL	14592 ((G 24601x(MAM 49xRIB 66)F_1)F_2xG 15989)F_1 X (MAM 38xG 21212)F_1/-	2	0	0	0	0	F 4
AL	14593 ((G 24601x(MAM 49xRIB 66)F_1)F_2xG 15989)F_1 X (SEA 15xDICTA 17)F_1/-	1	0	0	0	0	F 4
AL	14594 ((G 24601x(MAM 49xRIB 66)F_1)F_2xG 15989)F_1 X (9824-47-1/F_7xG 21212)F_1/-	4	2	18	15	4	F 4
	Total	70	19	165	154	36	**F 4**
AL	14300 G 24601 X (G 22041 x **BRB 198**)F_2/-	28	6	13	13	4	F 5
AL	14362 (RAB 655 X (G 22041xRIB 66)F_1)F_1 X G 11015/-	1	0	0	0	0	F 5
AL	14547 G 19017 X (SEA 15xDOR 714)F_1/-	1	0	0	0	0	F 5
AL	14557 G 15989 X (DOR 714 xMAM 49)F_1/-	1	0	0	0	0	F 5
AL	14544 G 11037 X (SEA 15xDOR 714)F_1/-	1	0	0	0	0	F 5
AL	14561 TIO CANELA 75 X (MUS 131xG 24601)F_1/-	1	1	13	9	2	F 5
SX	14508 (SEA 15xDOR 714)F_1 X (9824-47-1/F_7xG 21212)F_1/-	1	0	0	0	0	F 5
	Total	34	7	26	22	6	**F 5**
SF	13952 SEA 15 x (RAB 655 x G 21212)F_1/-	1	1	5	2	0	F 6
AL	14301 RAB 655 X (G 22041xRIB 66)F_1/-	1	0	0	0	0	F 6
AL	14304 RAB 655 X (MAM 49xRIB 66)F_1/-	2	2	15	12	4	F 6
	Total	4	3	20	14	4	**F 6**
	Grand Total	**281**	**66**	**363**	**318**	**64**	
	Number of crosses	**34**	**21**	**21**	**21**	**12**	

1.1.2.4 Screening of RILs of G 5273 x MAM 38 for resistance to aluminum using nutrient solution

Rationale: Previous research showed that an Andean germplasm accession, G 5273 is relatively resistant to Al in nutrient solution culture while MAM 38, a Mesoamerican advanced line, is sensitive based on the rate of tap root elongation in the presence of Al. Moreover, G 5273 is also known to be tolerant to both anthracnosis and angular leaf spot diseases. The objective of this study is to evaluate the recombinant inbred lines (RILs) of G 5273 x MAM 38 for their tolerance to Al in low ionic strength nutrient solution under greenhouse conditions using relative root elongation and root architecture. The results from this study are useful to identify QTLs associated with Al resistance in common bean.

Materials and Methods: The two parents and the 66 RILs (recombinant inbred lines) were evaluated using low ionic strength nutrient solution in the presence or absence of aluminum (50 μM) at pH 4.5. Each genotype is replicated 3 times using plastic containers with 20 L nutrient solution. The details on composition of nutrient solution were reported before (CIAT, 2002). Differences among genotypes in root architecture (length, diameter and number of root tips) were quantified using WinRhizo® software program after 3 days with or without Al treatment. Root system was stained with methelene blue or neutral red (0.1% in deionized water). Total dry wt of roots was determined by drying roots at 65 °C in an oven for 48 h. Specific root length was determined based on root dry wt and root length. The % decrease in total root length or average root diameter was determined as reported before (CIAT, 2003).

Results and Discussion: Based on total root length per plant and average root diameter per plant, the RILs HF14137-19, HF14137-104, HF14137-26, HF14137-101 and HF14137-98 were identified as outstanding in their resistance to Al for combining greater root length with finer root system while the RILs HF14137-97, HF14137-17, HF14137-1, HF14137-77 and HF14137-5 were identified as Al sensitive (Figure 15).
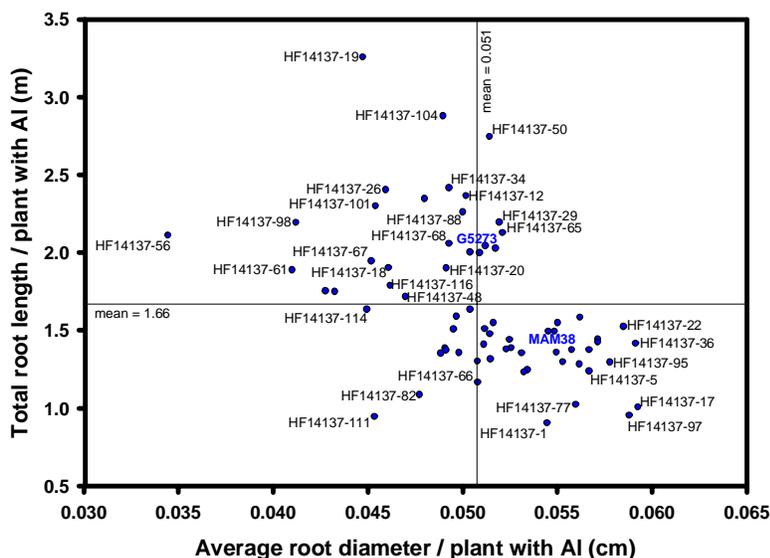
Figure 15. Relation between total root length and average root diameter with Al (50 µM) in nutrient solution for 3 days.

Three RILs HF14137-12, HF14137-98 and HF14137-101 were outstanding in terms of combining greater root length per plant with number of root tips per plant. The greater number of root tips found in these RILs could improve acquisition of Ca from infertile acid soils since root tips are the principal sites of Ca acquisition. On the other hand, five RILs HF14137-1, HF14137-82, HF14137-111, HF14137-97 and HF14137-17 showed lower values for these two attributes (Figure 16).

The increase in average root diameter per plant and the decrease in total root length per plant are indicators of the effect of Al toxicity as the Al resistant plants show longer and finer roots. Four RILs HF14137-19, HF14137-98, HF14137-88 and HF14137-26 showed lower values for these two attributes indicating greater level of Al resistance. Three RILs HF14137-7, HF14137-77 and HF14137-28 showed greater sensitivity to Al (Figure 17).
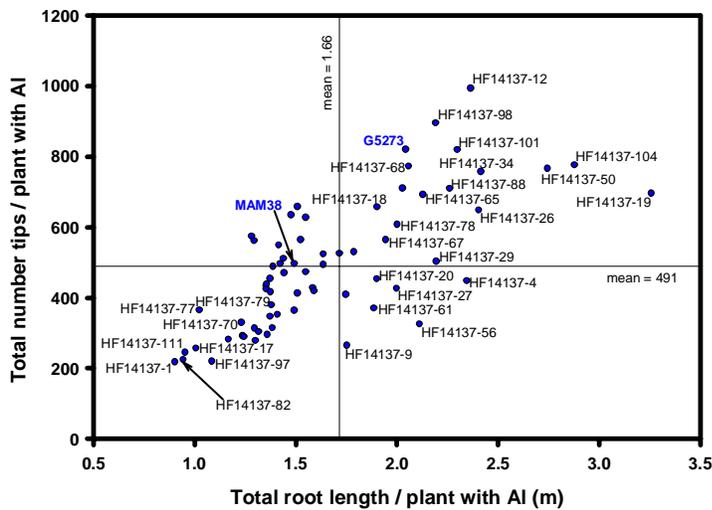
Figure 16. Relation between total number of root tips and total root length with aluminum (50 µM) in nutrient solution for 3 days.
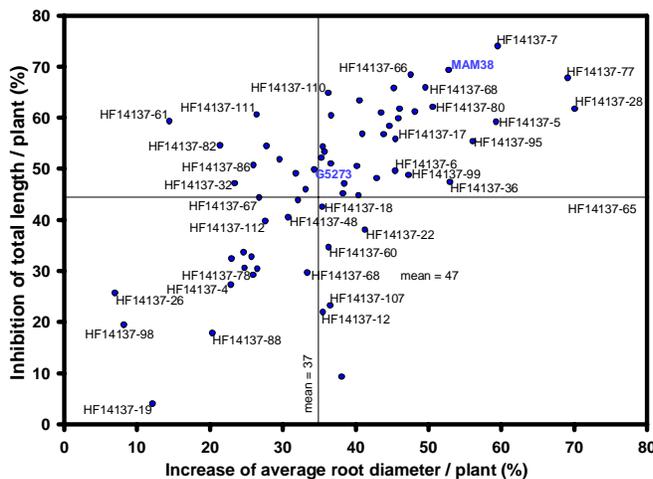
Figure 17. Relation between inhibition of total root length and increase of average root diameter in the presence of Al (50 µM) in nutrient solution for 3 days.

Conclusions: Results from this study indicate that four lines, HF14137-19, HF14137-26, HF14137-98 and HF14137-88 were relatively more resistant to Al.

References:
    CIAT. 2002. Annual Report 2002 Project IP-1. Bean improvement for the tropics. CIAT, Cali, CO.
    CIAT. 2003. Annual Report 2003 Project IP-1. Bean improvement for the tropics. CIAT, Cali, CO**.**

Contributors: G. Manrique, I.M. Rao and S. Beebe

1.1.2.5 Evaluation of a core collection of *Phaseolus coccineus* and *P. polyanthus* for field tolerance to aluminum

Rationale: In previous years, a core collection was established that is composed of sister species of the secondary gene pool of common bean. *P. coccineus* and *P. polyanthus* evolved on volcanic soils in moist highland environments. Although soil organic matter tends to be high in these soils, aluminum can be a problem of intermediate intensity. Both species are cross compatible with common bean, and *P. coccineus* in particular presents broad genetic variability.

Materials and Methods: Seed of 155 accessions of the core collection were obtained from the Genetic Resources Unit and planted in the field in Santander de Quilichao (990 masl; Oxisol – Plinthic Kandiudox) in unreplicated single row plots, to evaluate vegetative vigor under aluminum stress. A tolerant common bean check of VAX 1 was planted every 10 rows for comparison. Accessions were evaluated visually when the common bean check was entering flowering.

Results and Discussion: Wide variability in vigor was observed in the field, with several accessions accumulating far more biomass than the common bean checks. Although the core collection is approximately 50% *P. polyanthus*, only accessions of *P. coccineus* were identified as presenting unusual vigor. This tends to confirm that selection was not random and reflected genetic potential in adaptation to these conditions. Nineteen accessions were selected for additional study in both field and greenhouse hydroponic tests (Table 10). Some of these had been identified previously for their resistance to biotic constraints (BGYMV and Bean Stem Maggot). If the tolerance to aluminum is confirmed, this will represent a potential breakthrough for the common bean, as *P. coccineus* crosses readily with *P. vulgaris*. By using the greenhouse screening method that has been developed, it could be possible to backcross the respective genes to common bean with confidence, and recover the common bean phenotype with relative ease.

Conclusions: *P. coccineus* may offer a significant advantage over common bean in tolerance to aluminum. This possible advantage must be confirmed to determine if it is warranted to make interspecific crosses or to screen existing interspecific hybrid progeny.

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Table 10. Accessions of *P. coccineus* selected from the core collection of the secondary gene pool for adaptation to infertile soil with high aluminum saturation.

Accession	Country of Origin	Other traits[1]
G 35023	MEX	Res. to BSM
G 35025	TUR	
G 35066	MEX	
G 35103	MEX	
G 35105	MEX	
G 35157	MEX	
G 35172	RWA	Res. to BGYMV
G 35266	HND	
G 35341	MEX	Res. to BSM
G 35346	MEX	Res. to BSM
G 35369	CRI	
G 35405	MEX	
G 35448	MEX	
G 35464	MEX	
G 35609	MEX	
G 35621	MEX	
G 35777	GTM	
G 35884	YUG	
G 36022	ROM	

[1]BSM = Bean Stem Maggot, BGYMV = bean golden yellow mosaic virus

1.1.3 Tolerance to low phosphorus

1.1.3.1 Screening of RILs of BAT 477 x DOR 364 for tolerance to low phosphorus using nutrient solution

Rationale: Last year, we reported on a screening method to evaluate bean germplasm for low phosphorus supply in nutrient solution based on number of basal roots (CIAT, 2003). This year, we used the same screening method to evaluate 30 RILs of BAT 477 x DOR 364 for their root architecture using WinRhizo® software program. The main objective of this study was to determine differences in root architecture among RILs and use that information to identify QTLs for root architecture related to low P adaptation.

Materials and Methods: A total of 36 genotypes including 2 parents (BAT 477 and DOR 364), 2 non-nodulating lines of the parents (BAT 477NN and DOR 364NN), 30 RILs and 2 checks (G 21212 and V 8025), were evaluated at 2 levels of phosphorus in nutrient solution. The details on two levels of P (low and high) and the nutrient solution composition were reported last year (CIAT, 2003). Each genotype was replicated with 8 plants per each treatment of low and high P. After 9 days of treatment with low P, the plants were harvested and the root systems were scanned and the root architecture was analyzed using WinRhizo® software program. Shoot and root biomass was determined after drying the samples in an oven at 65 °C for 48 h.

Results and Discussion: Four RILs BT21138-4, BT21138-50, BT21138-115, BT21138-25 and an advanced line V 8025 were outstanding in their ability to produce greater root length per plant under both low and high P supply while three RILs BT21138-104, BT21138-3, BT21138-16 were less adapted to low P and less responsive to high P. The greater value of total root length per plant indicates that the plant could explore greater volume of soil to acquire P from low fertility soils that are deficient in P supply (Figure 18).
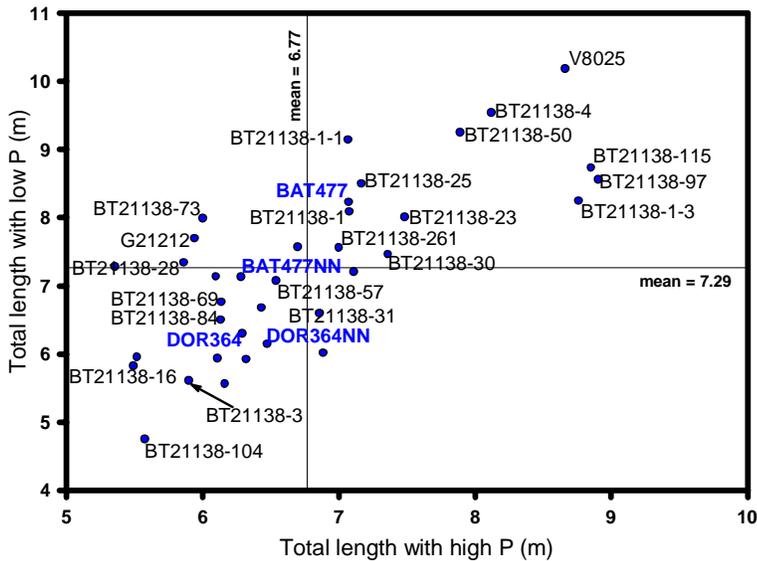
Figure 18. Relationship between the total root length with low and high P supply in nutrient solution.

We also found that six RILs BT21138-50, BT21138-83-1-1, BT21138-1-3, BT21138-1, BT21138-4 and BT21138-25 were superior in their root architecture by combining greater root length with greater number of root tips per plant with low P supply (Figure 19). This observation indicates that these genotypes could be capable acquiring greater amounts of P from soil with their branched root system. We identified five RILs BT21138-104, BT21138-124-1-2, BT21138-16, BT21138-3 and BT21138-31 that were markedly inferior in their ability to develop vigorous root system.
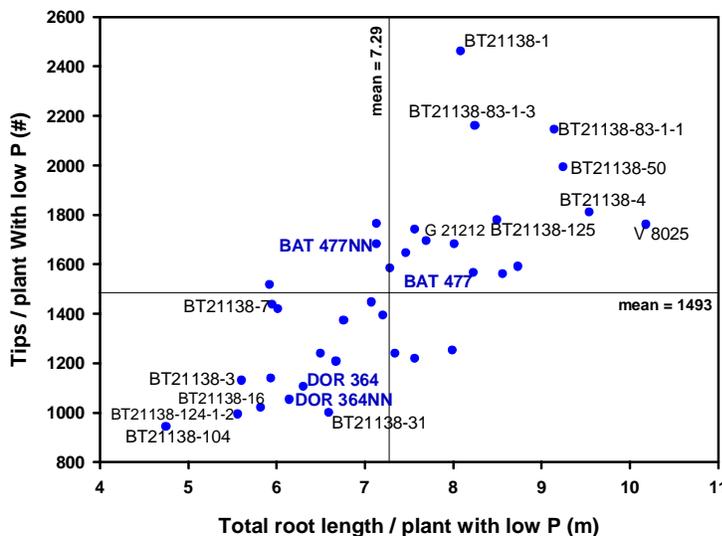
Figure 19. Relationship between the total root length per plant and the number of tips per plant in nutrient solution with low P

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The greater production of root biomass per plant with low P supply was observed with four RILs BT21138-83-1-1, BT21138-83-1-3, BT21138-50 and BT21138-115, while RILs BT21138-31, DOR364NN, BT21138-96 and BAT477NN were markedly inferior in their ability to produce root biomass (Figure 20).
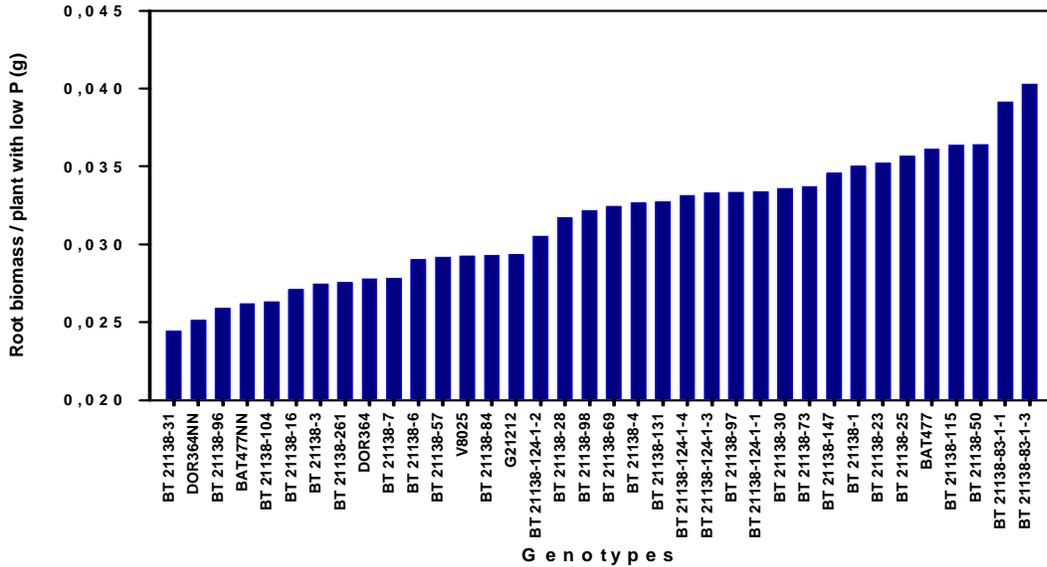
Figure 20.   Root biomass per plant in nutrient solution with low P

Conclusions: Results from this study indicated that four RILs BT21138-50, BT21138-83-1-3, BT21138-83-1-1 and BT21138-25 are superior in their adaptation to low P supply based on total root length, number of root tips and total root biomass per plant.

References:
   CIAT. 2003. Annual Report 2003 Project IP-1. Bean improvement for the tropics. CIAT, Cali, CO.

Contributors: G. Manrique, I.M. Rao and S. Beebe

1.1.3.2 **Effect of low P availability on shoot and root development of six common bean genotypes grown in nutrient solution**

**Rationale:** Last year we reported the effect of low P availability on total number of basal roots of RIL's of BAT 477 x DOR 364 (CIAT, 2003). We found that low P treatment for 9 days influenced basal root development but had limited effect on shoot development. This year, we conducted a study to evaluate the effect of low P supply for extended time of up to 3 weeks to determine the effects on both root and shoot development to determine the optimum time for screening under low P conditions.

Materials and Methods: A group of 6 genotypes including 2 landraces (G 19833 and G 21212) and 4 bred lines (DOR 364, DOR 390, BAT 477 and VAX 1) were evaluated with two P levels in nutrient solution at three intervals. The P levels were 0.25 µM for low and 75 µM for high and the evaluations were done at 1, 2 and 3 weeks after exposure to both P levels. Details on nutrient solution composition were reported last year (CIAT, 2003). Each genotype was replicated with three plants in each treatment with low or high P. After 7, 14 21 days of treatment, the plants were harvested and the root systems scanned and then analyzed with the WinRhizo® program. The shoot and root biomass were determined after drying in an oven at 65°C for 48 hours.

Results and Discussion: As reported before (CIAT, 2002 and 2003), the effects of the low P availability were evident from the first week on both total root length and leaf area production, which decreased around 15% and 35% respectively, with no significant genotypic differences among 6 genotypes (Figure 21; Table 11). After two weeks, the differences among the 6 genotypes increased showing reduction of up to 40% in both root length and leaf area production. After 3 weeks of low P treatment, total root length decreased up to 50% (Figure 21) while leaf area production diminished by 80% (Figure 21). Decrease in leaf area production was more marked between 2 and 3 weeks after treatment. After 3 weeks of low P treatment, two genotypes G 21212 and VAX 1 were superior to the other genotypes in their adaptation to low P supply in terms of total root length and leaf area production shoot growth. Among the 6 genotypes, DOR 390 was least adapted to low P supply. In general, all genotypes showed an increase in tap root length and a decrease in lateral root development with low P supply (Figure 22).

Low P treatment after 3 weeks markedly diminished the number of root tips per plant and increased the mean root diameter (Table 11). Total root length/leaf area increased markedly with low P treatment at the end of 3 weeks. VAX 1 was outstanding in its ability to produce greater number of root tips with low P treatment for 3 weeks. G 21212 responded better to high P supply by producing greater number of root tips. Genotypic differences in shoot and root traits became more evident at 3 weeks of low P supply than at 2 or 1 week of low P supply.
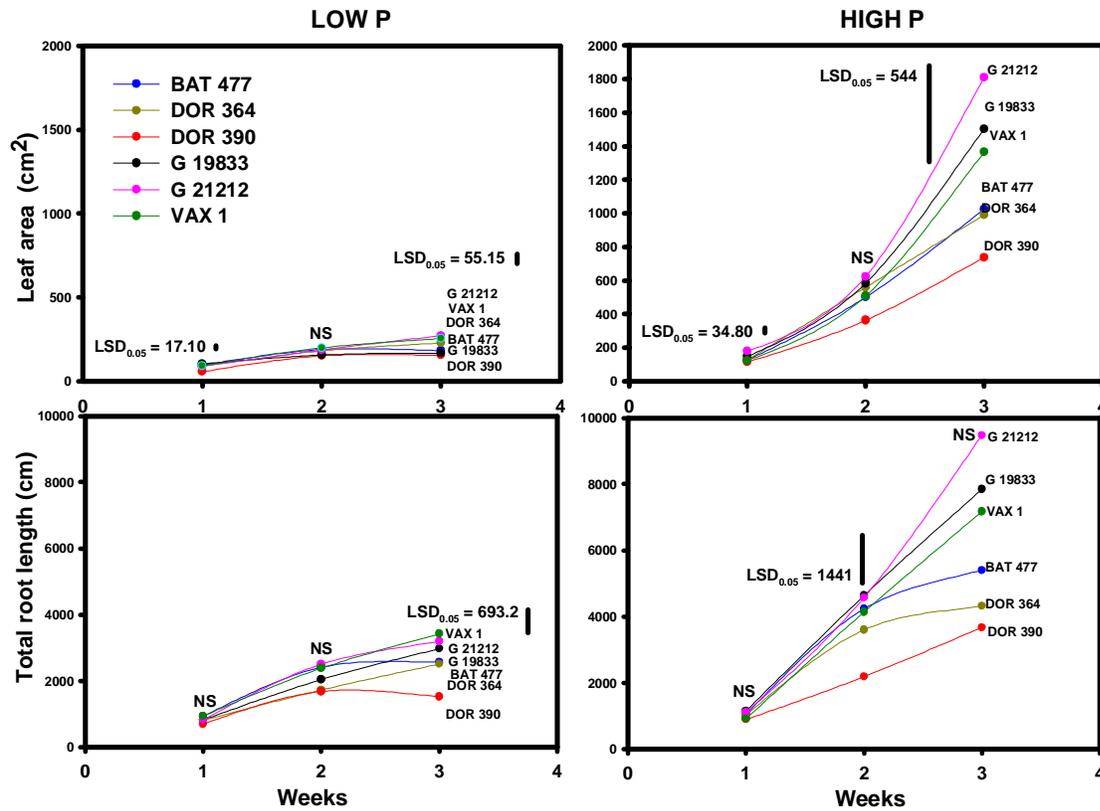
Figure 21. Effect of duration of low P or high P treatment on total root length and leaf area in 6 genotypes of common bean with low and high P in nutrient solution.

Figure 22. Common bean plants with three weeks of exposure to high and low P in nutrient solution.

Table 11. Effect of duration of low P or high P treatment on root traits of six genotypes.

Weeks of P treatment	Genotype	High P			Low P		
		Mean root diameter (mm)	Number of root tips	Root length / leaf area (m cm^{-2})	Mean root diameter (mm)	Number of root tips	Root length / leaf area (m cm^{-2})
1	BAT477	0.341	2055	0.083	0.352	2134	0.096
	DOR364	0.373	1868	0.081	0.363	1298	0.091
	DOR390	0.335	1994	0.079	0.299	1428	0.124
	G19833	0.370	2616	0.078	0.378	1517	0.080
	G21212	0.353	3268	0.062	0.336	1355	0.092
	VAX1	0.355	1998	0.074	0.330	1990	0.103
	Mean	**0.354**	**2300**	**0.076**	**0.343**	**1620**	**0.098**
	LSD$_{0.05}$	**NS**	NS	NS	0.030	NS	NS
2	BAT477	0.293	7024	0.085	0.338	3378	0.128
	DOR364	0.311	4886	0.064	0.361	3703	0.095
	DOR390	0.311	3064	0.060	0.342	2871	0.111
	G19833	0.318	8506	0.080	0.369	2871	0.131
	G21212	0.281	6217	0.073	0.333	4070	0.134
	VAX1	0.285	5880	0.082	0.340	3632	0.120
	Mean	**0.300**	**5929**	**0.074**	**0.347**	**3421**	**0.120**
	LSD$_{0.05}$	**NS**	3288	0.012	NS	NS	NS
3	BAT477	0.290	6409	0.053	0.335	2873	0.141
	DOR364	0.334	5953	0.044	0.356	3369	0.110
	DOR390	0.305	4926	0.050	0.370	2175	0.099
	G19833	0.262	7787	0.052	0.349	3323	0.176
	G21212	0.261	11108	0.052	0.346	4112	0.117
	VAX1	0.290	9706	0.053	0.336	4745	0.135
	Mean	**0.290**	**7648**	**0.051**	**0.349**	**3433**	**0.130**
	LSD$_{0.05}$	**0.047**	NS	NS	NS	1314	0.022

Conclusions: Results from this study indicate that the effects of low P supply on shoot and root growth parameters become more pronounced over time and screening for genotypic differences is possible at 3 weeks after low P treatment.

References:

CIAT. 2002. Annual Report 2002 Project IP-1. Bean improvement for the tropics. CIAT, Cali, CO.
CIAT. 2003. Annual Report 2003 Project IP-1. Bean improvement for the tropics. CIAT, Cali, CO.

Contributors: G. Manrique, I.M. Rao and S. Beebe

1.1.4 Testing of elite lines selected for low phosphorus tolerance or drought tolerance in multiple stress environments

Rationale: In small farmer production environments, crops often confront multiple stresses. Low soil fertility is a widespread limitation on production of common bean in LAC and Africa. Among several nutrients, soil phosphorus (P) is often especially limiting, due to low native P and also P fixing clays in soil that immobilize P in forms unavailable to plants. Drought stress is occasional and is exacerbated by infertile soils. Early plant vigor derived from low P tolerance might confer a degree of drought tolerance if it leads to better root development. The present trial was designed to test the relative value of low P tolerance and drought tolerance under conditions of single or multiple abiotic stresses.

Materials and Methods: Sixteen elite bean lines were selected based on their genetic potential, for evaluation under single or combined stress factors: A774, RAB 655 and MCD 2004 for low P tolerance; SEA's 5, 15, 21, 23 and RAB's 609, 612, and 651 for drought tolerance; G21212 and BAT 477 for multiple tolerance; BAT 881 for universal susceptibility; and DOR 364, EAP 9510-77, and Tio Canela as commercial checks. Trials were established in San Dionisio, Nicaragua in the second season of both 2002 and 2003, with no and moderate fertilization. In both years drought was a determining factor in the bean yields. In addition, the same trial was established in Santander de Quilichao in 2003 under high fertility and adequate rainfall; and under terminal drought that was created by a late planting date plus fertility stress. In 2004 three treatments were established in Quilichao: high fertility and adequate rainfall; low fertility and adequate rainfall; and terminal drought plus fertility stress.

Results and Discussion: There was evidence that the groups of genotypes formed according to their particular stress tolerance were performing differently under different conditions. In the combined analysis the group x treatment factor was significant (p<0.001). This interaction seemed to derive from the trials in Quilichao (where this term was significant) and not from Nicaragua (where it was not significant).

The ANOVA of results from Nicaragua, from Quilichao and the combined analysis suggested significant differences among the groups of genotypes, but principally between the commercial types and/or the susceptible check versus other materials (Table 12). Groups of genotypes selected for low fertility tolerance or multiple tolerance (i.e., drought plus low fertility tolerance) were consistently the best group in all of the analyses. This was expected in Quilichao where the stress seemed to be dominated by low soil fertility, and where several genotypes were actually selected on site for this trait. However, the same tendency was observed in Nicaragua. This suggests that in multiple stress conditions, some degree of tolerance to low soil fertility is required to assure plant vigor and adequate root development for the expression of drought tolerance.

Nonetheless, there was some difference between 2002 and 2003 in Nicaragua, where a slight (p = 0.05) interaction of year x group was detected. In 2003 when drought was more severe, SEA 15, which has excellent drought tolerance, was the best line in Nicaragua, and was the second best yielding line across all nine trials. It is likely that in 2003 in Nicaragua the effect of drought tolerance was more evident. Besides SEA 15, bred lines A774, RAB 655 and RAB 651 were all

consistently among the best yielders. EAP 9510-77 was the best commercial cultivar but was much less stable than the stress-selected lines.

Table 12.   Yields by groups of genotypes classed by adaptation to abiotic stress.

	Combined	Nicaragua	Quilichao
Low fertility tolerant (3)	1580a	1860a	1356a
Low fertility + drought tolerant (2)	1497a	1659a	1368a
Drought tolerant (7)	1388a	1699a	1140a
Commercial varieties (3)	1368a	1520a	1246a
Susceptible check (1)	871b	915b	835b

Conclusions: Multiple stresses occur on farm in many production regions. Drought tolerance alone may not be expressed adequately if soil fertility is inadequate. A degree of low fertility tolerance will likewise be required to improve yields on farm.

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Progress towards achieving output milestones:

- Tolerance to drought has been combined with a wider range of commercial grain types and highly desirable traits, and elite lines will form the basis for breeding improved nutritional status into agronomically superior varieties.

- Lines developed for drought tolerance also carry resistance to several diseases. Several selections identified this year carry recessive resistance to BCMNV. Some also carry resistance to the angular leaf spot pathogen.

- A few landraces and several advanced lines that were identified as better adapted to drought are currently being used in the breeding program to combine with biotic stress adaptation and commercial grain type.

- Greenhouse screening methods were implemented for phenotypic characterization for resistance to aluminum and tolerance to low P supply. Recombinant inbred lines are being evaluated to identify quantitative trait loci (QTLs) and candidate genes for further use in marker assisted selection.

- Breeders from national programs are using several parents/populations/lines to develop/identify genotypes tolerant to drought and low soil fertility conditions.

Activity 1.2   Developing germplasm with multiple resistance to diseases

Highlights:

- Four genotypes (G 22623; G 10909; G 3005 y G 1368) that combined resistance to pathogens causing angular leaf spot, anthracnose and ashy stem blight were identified.
- Ten genotypes from accessions identified as variable for tannins were identified that had high levels of resistance to ashy stem blight (*Macrophomina phaseolina*)
- The sources of resistance to aphid- and whitefly-transmitted viruses identified by CIAT and collaborating NARIs, continue to be effective to control common mosaic, black root and golden yellow mosaic in newly developed common bean cultivars.
- Lines with good drought tolerance and carrying the bc-3 gene for resistance to BCMNV have been recovered in small black and small red seeded phenotypes.
- Markers for two important genes for resistance to BGYMV have been multiplexed in the PCR amplification, increasing greatly the efficiency of MAS.
- Conducted successful screening for sources of resistance to the new virus disease affecting snap beans
- Three new rust resistant snap bean lines selected after four years of evaluation with farmers in Uganda and distributed for regional evaluation
- Four new snap bean varieties with improved pod quality, resistance to rust and yield potential and adaptation selected in national performance trials with farmers, exporters and certification agency in Kenya.
- New breeding populations of snap runner bean segregating for adaptation to short day tropical conditions developed in Eastern Africa.

1.2.1   Identification of genotypes with resistance to multiple constraints

1.2.1.1 Sources of multiple resistance

Rationale: Root rots are becoming a major bean production constraint, especially with declining farm sizes, lack of rotation and the increase in low soil fertility problems. Several pathogen cause root rots, and these often occur in complex. The prevalence of some pathogens is associated with high soil humidity and rain conditions (e.g. *Pythium* spp, *Fusarium* spp), while others are associated with water deficit and drought conditions (e.g. *Macrophomina phaseolina*). Identification of genotypes that are resistant to the largest number of root rot causing pathogens would be a major contribution towards the development of genotypes resistant to multiple constraints. Such materials would be useful as parents in breeding programs, or they can be deployed in areas where these pathogens are a major production constraint. Towards this goal, we have started evaluating genotypes from the core collection, that have previously been found to have resistance to other bean pathogens and pests, including *Colletotrichum lindemuthianum* and *Phaeoisariopsis griseola*.

Materials and Methods: Sixty genotypes representing a portion of the core collection known to have resistance to *P. griseola* and *C. lindemuthianum* were evaluated for *M. phaseolina* under greenhouse conditions. Ten plants were established for each genotype and were inoculated with a mixture of *M. phaseolina* from Quilichao, Darién and Palmira at the rate of 3grams of sclerotia

kg^{-1} of soil. Inoculation and plant evaluations were done as described previously (CIAT, 2002). The same genotypes were also evaluated for their reaction to *Rhizoctonia solani* using conditions described earlier (Abawi and Pastor-Corrales, 1989). In addition, accessions selected from the core collection for contrasting tannin content (41 genotypes) were also evaluated for their reaction to *M. phaseolina*.

Results and Discussion: Several genotypes had good levels of resistance to *M. phaseolina* (Table 13). However, no genotype had high levels of resistance to *R. solani*, only tolerance. However, these genotypes are also resistance to *M. phaseolina*, *P. griseola* and *C. lindemuthianum* and these genotypes are good candidates to use as parents in breeding programs. Among the 41 genotypes selected for tannins, 10 had high levels of resistance to *M. phaseolina* (Table 14). Efforts are underway to test these materials to as wide a range of bean pathogens as possible, in the hopes of identifying those that combine resistance to the greatest number of pathogens.

Conclusion: The following genotypes (G 22623; G 10909; G 3005 y G 1368) were identified to combine resistance to *M. phaseolina* and several races of *P. griseola* and *C. lindemuthianum*, and these should be considered in breeding programs as multiple constraint sources. Evaluation for resistance to other root rot causing pathogens (*Fusarium solani, Fusarium oxysporium, Pythium* spp*, Scletinia rolfsi* etc) is in progress.

References

   CIAT 2002. Annual Report, Bean Program 2002. CIAT, Cali, Colombia

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Table 13.   Response of 60 anthracnose and angular leaf spot resistant genotypes from the core collection, to inoculation with mixtures of *Macrophomina phaseolina* and *Rhizoctonia solani* isolates under greenhouse conditions.

Accession	Mp	*R. solani*	Accession	Mp	*R. solani*
G 22623	1.0	4.0	G 2494	6.9	3.9
G 15805	1.0	4.8	G 10979	7.2	3.5
G 19227 A	1.0	6.1	G 23778	7.4	3.2
G 1368	1.6	4.1	G 14675	7.4	4.9
G 16291	1.7	6.3	G 11780 D	7.6	4.1
G 2218	2.6	5.1	G 4380	7.7	4.2
G 10909	2.8	3.7	G 2328 C	7.7	5.5
G 3005	3.8	3.7	G 1727	7.7	
G 11785	4.2	3.1	G 21132	8.1	4.0
S 31476	4.2	3.9	G 14711	8.1	
G 19833	4.5	3.5	G 23806 A	8.1	3.3
G 18970	4.5	4.1	G 16145	8.2	3.6
G 12182	5.0	3.0	G 17172	8.2	4.1
G 11732	5.0	3.5	G 1373	8.2	5.0
G 3970	5.0	4.3	G 5476	8.2	6.0
G 6873	5.3	4.3	G 18141	8.2	
G 23578 A	5.6	4.6	G 13910	8.4	4.1
G 21135	5.6	5.6	G 18996	8.5	3.7
G 148	5.7	5.0	G 19235	8.5	4.5
G 7962	5.8	4.0	G 16374	8.5	6.3
G 2769	5.8	4.6	G 11728	8.6	3.1
G 2482	5.8	5.1	G 23777	9.0	2.9
G 17650	6.0	4.2	G 8209	9.0	4.0
G 20729	6.1	4.0	G 23614	9.0	4.0
G 20824	6.2	3.9	G 13054 B	9.0	4.1
G 3511	6.2	4.7	G 16267	9.0	4.7
G 4721	6.3	3.4	G 23814 D	9.0	4.8
G 2359	6.4	5.4	G 8925	9.0	6.3
G 14016	6.5	4.7	G 799	9.0	
G 19120	6.6	4.4	G 4278	9.0	
BAR 477	1.0				
A 70	9.0				
SEA 15	3.3				
Sanilac		4.8			
Ica Tui		3.0			
P.Sintetico		2.3			

Table 14. Response of 41 bean genotypes from the core collection to inoculation with mixtures of *Macrophomina phaseolina* under greenhouse conditions.

Genotype	Seed color	Seed size	Disease severity	Genotype	Seed color	Seed size	Disease severity
G 58	8	P	1.0	G 4756	2	M	6.6
G 2494	8	P	1.0	G 995	8	M	6.6
G 2769	8	P	1.0	G 16157	7M	G	6.6
G 5481	2M	G	1.0	G 11957	2M	G	7.2
G 22291	6M	P	1.0	G 7945	6M	M	7.3
G 19497	2R	P	1.2	G 11640	5	P	7.4
G 20592	2	P	1.8	G 1400	6	P	7.4
G 4495	8	P	2.8	G 18264	7	M	7.4
G 13778	7M	G	3.0	G 6639	8	G	7.4
G 16664	2	M	3.2	G 1083	2M	M	7.4
G 9384	2	P	3.6	G 12169	7M	G	7.4
G 3815	2M	M	3.7	G 6981	6	P	7.7
G 12171	6M	G	4.2	G 17166	6M	M	7.8
G 22365	7M	M	4.2	G 2906	5	M	7.9
G 4790	8	P	4.7	G 5285	6	M	8.0
G 22805	6	P	5.1	G 16072	2M	M	8.2
G 18244	7	M	5.9	G 13177	2M	M	8.2
G 14778	7R	G	6.0	G 23283	5	M	9.0
G 5758	8	P	6.3	G 4258	6	P	9.0
G 17913	2	G	6.5	G 3821	2M	M	9.0
G 2276	5	M	6.6				
Resistant controls				**Susceptible checks**			
DOR 500	8	P	1.4	CALIMA	6M	G	7.6
SEA 15		P	2.1	A 70	2M	P	6.7
NEP 2		P	2.6				

1.2.1.2 Selection of multiple resistance in Mesoamerican types for southern Africa

The program evaluated fixed lines of various market classes for adaptation, yield and reaction to diseases at Chitedze and Bembeke, and for tolerance to low soil fertility stress at Bembeke. The small seeded bean cultivars, although not very popular among the market classes in southern Africa, often feature among the mixtures at household level. They are mainly used at domestic level for food security, because of their high yielding potential and tolerance to diseases.

Materials and Methods: Nurseries for specific market classes included: carioca (50 lines) and small reds (44 lines). At Bembeke, a set of sugar and red kidney lines were also planted in a low soil fertility stress block. The soil fertility stress block has been used over years to identify potential bean lines, which can perform well under low P, low N and low pH complex conditions. The nurseries were planted during the second week of January, in single row plots

without replication. Data were collected on yield and reaction to diseases in both sets of nurseries, under with and without low soil fertility stress conditions.

Results and Discussion: The crop at Chitedze was adversely affected by the wide spread of virus-like symptoms, which appeared at early pod formation stage. This affected both, the expression of other diseases and the yield performance. As such the data from Chitedze were excluded from this report. This section therefore focused at data from Bembeke, a site, which continued to offer considerable disease pressure for ALS and floury leaf spot (FLS), but less of CBB, ascochyta (ASC) and anthracnose (ANT). This pattern was consistent across all nurseries. The data presented in the subsequent tables capture only the top 10 lines in each market class, which are compared to a control cultivar.

i. Small red lines

Among the top 10 lines, some like MR 13557-16-2, MR 13426-9-3 and MR 13508-7 had a good combination of high yield, ranging from 2800 to 4100 kg ha^{-1}, and good levels of resistance to ALS and FLS (Table 15).

Table 15.   Yield performance and reaction to diseases of top 10 small red lines at Bembeke, 2004.

Identity	Disease Score				Grain Yield (kg ha^{-1})
	ALS	**CBB**	**ASC**	**FLS**	
MR 13557-16-2	3	1	3	3	4107
MR 13568-5-4	4	3	3	5	3746
MR 13425-57-4	6	1	2	4	3386
MR 13557-16-7	4	2	2	5	3330
MR13456-12-3	2	1	2	7	3136
RAB 608	3	2	3	6	3108
MR 13426-9-3	3	2	2	4	3025
MR 135557-16-3	4	1	4	4	2969
MR 13508-7	3	2	2	4	2858
EAP-12-88 (DON)	4	1	2	4	2831
VAX 6 (Control)	6	2	3	2	777
Mean					**756**

ii. Carioca lines

Among the top 10 of the 50 carioca lines evaluated at Bembeke, were MN 13223-1, MC 12832-129-9, and MC 12832-129-5, all with good resistance to such diseases as ALS and FLS. Their yield levels were all above 4000 kg ha^{-1}, well above the control cultivar A286, 666 kg ha^{-1}, a carioca type released in many countries in southern Africa. The control cultivar was also below the site mean, 834 kg ha^{-1} (Table 16). Thus the regional breeding program has several better lines in the carioca market class that offer good diseases resistance to two diseases, but also give much higher yields.

Table 16.   Yield performance and reaction to diseases of top 10 carioca lines evaluated at Bembeke, 2004.

Identity	Disease Score					Grain Yield (kg ha^{-1})
	ALS	CBB	ASC	ANT	FLS	
MN 13223-1	2	1	2	1	1	4884
MC 12832-129-9	3	1	6	1	1	4440
MC 12832-129-5	3	1	5	1	1	4190
MC 12832-129-7	5	1	4	1	1	4190
MC 12832-129-2	3	1	7	1	1	4135
MC 12832-129-4	2	2	6	1	1	3941
MC 12832-129-10	2	1	7	1	1	3941
MC 12832-129-11	2	1	6	1	2	3830
RM 13387-17	2	1	6	1	1	3774
MA 12129-66	2	1	4	1	7	3691
Carioca (A 286-Control)	7	1	4	1	7	666
Mean						**834**

Conclusion: The regional breeding program continued to make progress in screening breeding materials for multiple attributes. Further progress has been achieved this season in selecting high yielding genotypes of various grain market classes that combine more than one attribute. Although Mesoamerican types are less popular than Andean types in southern Africa, .

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1.2.2   Resistance to viruses

1.2.2.1   Bean common mosaic and bean golden yellow mosaic viruses

This year, Bean Virology inoculated and evaluated 700 common bean germplasm accessions for their reaction to bean common mosaic and bean common mosaic necrosis (black root), bringing the total of BCMV/BCMNV-tested entries in the Common Bean Germplasm Collection of CIAT, to a total of 25,600 accessions. This information is critical to breeders, who must know the BCMV/BCMNV reaction of the parental materials used in their breeding projects, before making crosses.

A total of 157 small-seeded common bean entries belonging to different projects (e.g. drought, iron content, and other breeding materials identified as SEA) were also evaluated for their reaction to bean common mosaic and black root. Bean Virology also screens materials generated

by collaborators outside CIAT for their reaction to selected viruses. This year, 47 white-seeded materials from Peru were evaluated at CIAT.

Different whitefly-transmitted viruses (begomoviruses) affect common bean production in tropical America since the mid 1970s. CIAT pioneered the first international search for sources of begomovirus resistance and development of virus-resistant common bean cultivars, which led to the awarding of the prestigious King Baudouin Prize to CIAT in 1984. Since that year, Bean Virology has identified and characterized most of the sources of begomovirus resistance used to date. Based on these preliminary work, various other scientists and institutions have produced molecular markers to detect the resistance genes involved, and use these molecular tools in common bean improvement programs.

Thanks to these efforts, some NARIs and other international institutions, such as the Panamerican School (Zamorano) in Honduras, have been filling the gap left by the downsizing of CIAT's Bean Program, in terms of producing new common bean cultivars with resistance to bean golden yellow mosaic. Two good examples of these new cultivars produced by Dr. Juan Carlos Rosas in El Zamorano are: Tio Canela and CENTA-San Andrés. Bean Virology supported the evaluation and release of the latter cultivar in El Salvador, through the collaborative agreement subscribed on behalf of the Tropical Whitefly IPM Project financed by DFID. This new cultivar has recovered bean production in areas where this crop had been abandoned due to the high incidence of BGYMV, particularly during the prolonged dry season that affects Central America.

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1.2.2.2 Bean common mosaic virus (BCMV) and bean golden yellow mosaic virus (BGYMV) for Central America

Rationale: Central America is a priority region for poverty alleviation and for bean production. Therefore, the mainstream breeding program continues to focus on small red and small black beans, largely for Central America. Most of the improved varieties in the small red class have a darker tone of red than the landraces, which command better prices and are even preferred for export markets to the USA. Recovering the light red color has been a challenge since the inception of the bean team, due to a genetic linkage between the *I* gene for BCMV resistance and the dark red color. The solution to this problem lies in the deployment of the recessive *bc-3* gene for BCMV resistance, which in addition to not suffering linkage to poor color, also avoids the problem of a necrotic reaction with the Severe Mosaic virus that occurs with frequency in the region. The *bc-3* gene is also important for Africa to confer resistance to BCMNV which results in whole-plant necrosis when the *I* gene is present. Although the *bc-3* gene had been introduced into tropically adapted lines several years ago, this had not been accomplished specifically with an eye to recovering the soft red color of the Central American landraces.

Materials and Methods: Several breeding lines carrying the *bc-3* gene had been developed at CIAT and elsewhere. One of these obtained from the University of Puerto Rico was crossed to a

landrace from El Salvador, Rojo de Seda (G4090) and to a source of resistance to BGYMV in a triple cross. Grain color and the *bc-3* gene were selected phenotypically to obtain lines denominated RCB 136 and RCB 137 that were in turn crossed to sources of drought tolerance and another source of BGYMV resistance. Lines were selected for color, evaluated in the greenhouse for resistance to the necrotic strain of BCMNV, and evaluated for drought tolerance in the field. Additionally, a series of black seeded lines were evaluated in similar fashion.

Results and Discussion: Most families selected for grain color and adaptation in the field did not carry the *bc-3* gene. Out of 79 families with *bc-3* parents, only 7 expressed resistance to necrotic BCMV in most plants, and another 9 appeared to be segregating (Table 17). Although it has been particularly difficult to obtain *bc-3* in elite rustic materials, some of the selected lines (especially the black seeded) express a degree of drought tolerance. Among the red seeded lines several have the highly commercial grain color that is sought in Central America, and were selected by the a national program bean breeder for immediate testing in Nicaragua. MDSX 14826-13-MC-14P-MQ presented very good grain quality under drought and is the most promising among the reds. The elite lines have been incorporated into crosses for another round of selection.

Table 17. Small red and black seeded lines expressing a resistant phenotype or segregation for resistance to necrosis caused by BCMNV.

Pedigree	Color	Reaction to BCMNV Necrosis	Mosaic	None (*bc-3*)	Drought yield % check[1]	Check
(SXB 116 X EAP9653-16B-1) x RCB 136						
MDSX 14797-6-MC-2P-MQ	Red	1	-	15	103	1275
MDSX 14797-6-MC-6P-MQ	Red	-	12	5	96	1275
(SXB 123 x EAP9653-16B-1) x RCB 136						
MDSX 14814-7-MC-8P-MC	Red	-	-	18	103	1644
MDSX 14814-7-MC-11P-MC	Red	-	-	15	102	1644
MDSX 14814-8-MC-1P-MC	Red	8	-	4	101	1644
MDSX 14814-8-MC-4P-MC	Red	1	-	15	123	1191
MDSX 14814-8-MC-5P-MC	Red	-	-	20	124	1191
MDSX 14814-21-MC-3P-MC	Red	8	3	9	93	1275
(SXB 122 x EAP9653-16B-1) x RCB 137						
MDSX 14826-13-MC-14P-MQ	Red	1	-	13	124	1275
MDSX 14826-13-MC-31P-MQ	Red	5	-	10	90	1275
(SXB 118 X EAP9503-32B) x SEN 34						
MDSX 14802-38-MC-2P-MQ	Black	12	-	8	204	1032
(SXB 123 x DOR 677) x SEN 34						
SX 14820-37-MC-1P-MQ	Black	1	-	15	202	1032
SX 14820-37-MC-13P-MQ	Black	8	-	8	171	1032
SX 14820-37-MC-14P-MQ	Black	12	-	6	255	1032
SX 14820-37-MC-16P-MQ	Black	5	-	9	176	1032
SEN 34 x (MAB 105 X EAP9020-14)						
SXMA 14833-39-MC-2P-MQ	Black	1	-	14	163	1032

[1]The check for the red-seeded trials was Tio Canela, and for the black seeded trial, DOR 390. All black seeded families but no red seeded families yielded significantly more than the respective checks.

Conclusions: Families were obtained that represent significant advance in combining the *bc-3* gene with important agronomic traits for Central America and Africa in these grain types.

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1.2.2.3   Mainstream breeding applying MAS for two genes for resistance to BGYMV (*bgm-1* and W12)

Rationale: Bean golden yellow mosaic virus (BGYMV) continues to be an indispensable breeding priority for Central America. We have reported extensively on the use of a marker for *bgm-1* gene in the past. In addition, a second marker identified as W12 was converted to a SCAR by USDA-Prosser, Washington. Both markers were employed to evaluate the presence or absence of key viral resistance genes in F_1 plants of triple or double crosses.

Materials and Methods: This year we tested more than four thousand F_1 plants for the presence of the *bgm-1* and the W12 markers. A broader description of the derived families and their performance under drought pressure is reported in section 1.1.1.1 above. Here we deal with the results of the marker screening. Fine-tuning the amplification protocol permitted multiplexing the amplification of the two markers and streamlining the evaluation.

Results and Discussion: The *bgm-1* marker was present in 51% of the plants, and the W12 in 44%. The marker for *bgm-1* was detected in either the heterozygous or homozygous state in combination with the W-12 marker in 1182 out of 4498 total F_1 plants tested, or in 26% of plants (Table 18). This was the first time that we insisted in the combination of the two markers, with the expectation that this will improve the frequency of derived families with resistance. Of course, this resulted in a substantial reduction in the number of families that were tested in the drought nursery.

Table 18. Number of F_1 plants expressing or lacking two markers for resistance to BGYMV among 4498 plants that were evaluated.

		State of *bgm-1**		
		Homozygous	**Heterozygous**	**Absent**
State of W12	**Present**	1086	96	797
	Absent	1080	73	1366

*The *bgm-1* is codominant for the resistant and susceptible allele and thus permits defining heterogeneous class.

Conclusions: Marker assisted selection continues to be a pillar of our work to serve Central American breeding programs and bean producers. The large-scale evaluation that has been deployed is the result of continual improvements in routine laboratory protocols. Multiplexing markers in the amplification phase is a major achievement in this regard.

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1.2.3   Snap beans

1.2.3.1   Developing snap bean breeding lines resistant to Ascochyta blight and anthracnose

Rationale: Ascochyta and anthracnose are very destructive diseases of bean in the rainy higher altitude regions of the Andean zone and Africa.   Climbing snap beans are a high value horticultural crop with great potential in both the Andes and in Africa. Snap beans are especially susceptible to ascochyta, and an ascochyta attack early in the season is often the stimulus that triggers a vicious cycle of pesticide abuse. Thus, ascochyta resistance is an important component of an IPM strategy for snap beans. Anthracnose attacks both leaves and pods, and lesions on pods are unacceptable in the market. In other environments represented by Pradera in Valle de Cauca (~1000 masl) rust and occasionally angular leaf spot (ALS) are important limitations.

Materials and Methods: In previous years we reported on progress in implementation of resistance from interspecific crosses. While significant progress was made in combining resistance with snap bean characters, even the best selections did not present truly commercial snap bean pod type. Therefore, resistant families from previous semesters were incorporated into crosses with commercial varieties and improved lines. F_2 populations were planted in Popayán under natural attack of ascochyta and anthracnose in 2003, and in 2004 F_2-derived F_3 families were evaluated under similar conditions. Checks of Blue Lake (Lago Azul), the commercial snap bean, were planted every ten rows, together with rows of G685 (a Guatemalan climbing dry bean), ICTA Hunapú, and a resistant ASC bred line.

Results: In Popayán the commercial check 'Blue Lake' presented severe symptoms of both anthracnose and ascochyta, even without inoculation. The resistant checks presented far fewer symptoms than 'Blue Lake' for both ascochyta and anthracnose. Within the F_6 families most were either intermediate or moderately resistant to the two diseases. A drought occurred at flowering and for three weeks afterward, and this resulted in more fiber development in pods that we had seen in previous years. We therefore made an additional round of individual plant selections seeking to minimize pod fiber. A total of 113 individual plant selections were made in Popayán in F_6 for this purpose (Table 19).

Additionally, another 6 F_2 populations were selected in a second round of breeding, to continue to improve pod type in resistant lines (Table 20). A total of 161 individual plants were selected in F_2 and 221 in F_3 generations for resistance to cool season diseases. Meanwhile, crosses were initiated for resistance to gemini viruses that occur in production regions close to CIAT headquarters.

Table 19. F_5 and F_6 families of snap beans with climbing habit selected in Popayán, 2003B – 2004A.

Code	Cross number	Identification	F_5 families evaluated P03B	F_6 families evaluated P04A and selected for shipment	Individual F_7 selections P04A
SB	14565	HAV 129 X SBB 170/-	132	34	113
SB	14566	G 17647 X SBB 170/-	9	0	0
SB	14567	G 8992 X SBB 170/-	3	1	0
SB	14568	HAV 130 X SBB 171/-	1	0	0
		Total	**145**	**35**	**113**

Table 20. F_2 y F_3 families of snap beans with climbing habit selected in Popayán, 2003B – 2004A.

Code	Cross number	Identification	$F_{1,2}$ families evaluated P03B	F_3 families evaluated P04A	Individual F_4 selections P04A
SB	14896	(G 17723xHAV 124)F_1 X SBB 167/-	1	4	8
SB	14897	(G 17723xHAV 124)F_1 X SBB 168/-	5	22	42
SB	14898	(G 17723xHAV 124)F_1 X SBB 165/-	1	4	7
SB	14899	(G 17723xHAV 124)F_1 X SBB 166/-	1	3	6
SB	14900	HAV 129 X SBB 165/-	6	17	23
SB	14901	HAV 131 X SBB 166/-	26	111	135
		Total	**40**	**161**	**221**

Conclusion: In spite of the strict commercial criteria for pod characteristics of snap beans in Colombia, progress toward combining these pod characters with resistance to foliar diseases continues. We expect to distribute lines to collaborators in 2005.

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1.2.3.2   Breeding snap bean for smallholder production in East and Central Africa

Rationale: Snap bean is the probably the most important bean grown in East and Central Africa for export markets, and has been prioritized by ASARECA as a research priority. It is a major source of income for smallholder farmers especially in Kenya, Uganda, Sudan and Tanzania. There is growing interest to increase snap bean production for domestic and export markets in Rwanda, Ethiopia, Burundi, Madagascar and other countries in East and Central Africa. Snap bean is also grown by large commercial companies for export to overseas supermarkets and for canning industries. Yield of snap bean in smallholder farmers' fields varies from 2 to 8 t ha^{-1} (Ndegwa, 2002), compared to over 14 t ha^{-1} among large scale producers. Smallholder production is constrained by diseases especially rust, angular leaf spot, root rots, bean common mosaic virus and pests especially bean stem maggots, thrips and nematodes. The intensive nature of cultivation of this crop leads to high disease and insect pressure, and consequently excessive use of pesticides. Smallholder production is further constrained by high costs of seed because most of the varieties produced by private companies are protected by legislation. Thus seed produced by contract in the region is exported for processing and packaging, and re-imported for production. The few varieties developed by public institutions (especially in Kenya) are often susceptible to diseases and pests. Very little has been done to develop improved snap bean varieties freely accessible to smallholder farmers and informal seed producers (who supply over 90% of dry bean seed grown in the region) in the region. Due to the high quality demands, smallholder farmers rely on fungicides and insecticides to reduce production and post harvest losses associated with diseases and pests. This is no longer a viable option because recently instituted minimum residue levels, together with preference by importers to source produce from large scale producers, threaten to push smallholder farmers out of business. In East and Central Africa, production is based on determinate types. Unlike their counterparts in South America, East African farmers normally do not grow the indeterminate types, which are higher yielding and have longer harvest duration. Breeding for high yield, disease and pest resistance, tolerance to abiotic stresses, general adaptation to tropical conditions and acceptable market quality is a critical component of an integrated strategy to address constraints to snap bean production in the region. A regional program was therefore started in 2001 to support the development of improved snap bean varieties with high yield potential, resistance to biotic stresses and pod quality for smallholder production. This report highlights progress in this program.

Materials and Methods: The regional snap bean program is based at four institutions: one at Kawanda Research Institute in Uganda, and three in Kenya : Moi University in Eldoret; National Horticultural Research Centre, KARI-Thika, and a back-up program at the Department of Crop Science, University of Nairobi. Work at Kawanda has focused on screening snap bean varieties with farmers and developing production packages. At Moi University, crosses were made to develop locally adapted snap bean cultivars with improved pod yield, resistance to anthracnose and rust, and marketable pod quality (van Rheenen et al 2003). After six generations of selection, 23 lines were identified. Following preliminary evaluations in 2002, the number of lines was reduced to 12. In 2003, the 12 lines were evaluated in national performance trials at six locations (Eldoret, Thika, Kakamega, Marigat, Lanet and Njoro) in collaboration with Kenya Plant Health Inspectorate (KEPHIS), which represents the national variety release committee. The trial

included three commercial cultivars as checks. KARI-Thika focused on developing a working collection of snap and runner bean varieties and development of segregating populations. At the University of Nairobi, crosses were made to transfer rust resistance to popular commercial varieties, and development of short-day snap runner beans.

Results and Discussion: At Kawanda Agricultural Research Institute, three lines were finally selected after four years of evaluation with farmers. These were HAB 433, J12 and L3. Further evaluations of snap bean lines were conducted in DR Congo, Kenya and Sudan. Results are pending.

The characteristics of the 12 lines selected at Moi University in the national performance trials at six locations are presented in Table 21. Flowering was earliest at Thika (37 days) and latest at Njoro (50 days). Duration to first picking was shortest at Thika (56 days) and longest at Njoro (62 days). Picking period varied from 22.6 days at Marigat to 36 days at Njoro and 40 days at Thika. On an average, Lanet and Njoro showed better pod quality scores than Marigat and Eldoret. The pod quality at the latter location was poorest, probably due to the poor plant growth in general. The interaction between locations x trial entry was highly significant, suggesting that different entries respond differently to environmental conditions in respect of pod quality. The variety differences for reaction to rust were significant. The entry with the severest symptoms was No. 1, showing a mean score of 7.6. It differed significantly from those that had a score of 6.6 or less. Entry No. 7 had the lowest rust score of 1.2, differing significantly from those having a score of 2.2 or more. On an average, Marigat showed significantly more severe rust symptoms than other locations. The interaction between location x entry was significant, suggesting that possible differences in rust races occur. Variety differences for fresh pod yield were significant. The entry with the lowest yield was No. 3 with an average of 9.6 t ha^{-1}, followed by No.1 with 9.7 t ha^{-1}. These differed significantly only from those that had 12 t ha^{-1} or more. Entry No. 11 had the highest yield of 13.1 t ha^{-1}, differing significantly from those having a yield of 11 t ha^{-1} or less. The locations differed significantly pair-wise: Marigat and Njoro had the highest yields; Lanet and Kakamega were intermediate, and Eldoret and Thika lowest. The mean yields per location ranged from 3.1 t ha^{-1} at Thika to 19.7 t ha^{-1} at Marigat. No significant interaction between location and trial entry was observed which indicates that varieties had no differential response to environment. It suggests that the yield adaptation of the entries to different environments was similar.

Fifteen snap bean and five runner bean accessions were collected at KARI-Thika. All the snap bean accessions were determinate bush. Four of the runner bean accessions had a vigorous indeterminate growth habit and one was determinate. Success rate for crossing was higher for snap bean (80%) but lower for runner bean due to a high incidence of flower and pod abscission and seedless pods. Flower and pod abscission was higher for crosses done during the warmer period of the year. Twenty F$_4$ selections from a cross between a commercial variety and a locally improved rust resistant variety ('Kutuless') were made. Two promising F$_4$ lines showed a type 1 growth habit, good ground-pod clearance, high pod load (30 to 40 pods per plant), acceptable pod characteristics (pencil shaped, round cross section, smooth texture) and good snapping ability. These were advanced to F$_5$. At Kabete, five F$_3$ populations were developed from crosses between a long-day commercial runner snap variety and five short-day dry grain type varieties. Three F$_2$ populations were developed in crosses among three commercial, rust-

susceptible snap bean cultivars and two rust-resistant lines. Seed of 27 lines with combinations of *Ur* genes was increased.

Table 21. Days to flowering, first and last day of pod picking, pod quality, rust score and fresh pod yield of snap bean lines selected at Moi University, Eldoret, Kenya

Line*	Days to 50% flowering	Days to first pod picking	Days to last pod picking	Pod quality**	Rust score***	Fresh Pod yield t ha^{-1}
1	45.1	59.0	89.7	3.8	7.6	9.7
2	43.5	57.1	89.7	3.3	4.6	11.4
3	43.6	57.2	88.9	3.6	2.4	9.6
4	44.8	57.3	89.2	2.3	2.1	12.9
5	43.0	58.1	89.2	3.1	2.1	11.7
6	42.6	56.6	89.4	3.9	6.4	10.6
7	46.1	58.9	89.8	4.0	1.2	12.2
8	43.6	56.7	89.8	3.5	2.3	13.0
9	43.0	56.9	89.3	3.7	4.2	10.3
10	44.4	57.3	89.4	3.9	2.3	11.2
11	45.5	56.8	89.4	2.7	2.0	13.1
12	43.8	59.1	89.4	3.8	4.3	10.3
Mean	44.1	57.6	89.5	3.5	3.5	11.3
CV (%)	6.7	3.57	1.52	12.7	28.7	28.9
LSD $_{.05}$	1.94	1.35	NS	0.36	0.94	2.2

* Lines 1, 4, 7 and 11 were checks
** Pod quality on a scale of 1= best and 5=worst.
*** Rust on CIAT scale, 1-3 =resistant, 4-6= intermediate and 7-9=susceptible at three locations- Marigat, Lanet and Njoro.

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Collaborators: KEPHIS, KARI- Thika, Kakamega, Marigat, Lanet and Njoro, and S. Musaana (Kawanda Agric. Research Institute)

Progress towards achieving output milestones:

- Several accessions that combine resistance to several angular leaf spot and anthracnose races, *Macrophomina phaseolina* and tolerance to *Rhizoctonia solani* were identified. These constitute potential parents in programs breeding for improved resistance to foliar and soil-borne pathogens.

- Bean Virology has the responsibility to detect, characterize and manage plant viruses affecting common bean production around the world. Project milestones include the development of common bean cultivars that possess resistance to the three major bean viruses present in the Tropics: Bean common mosaic virus (BCMV) and the whitefly-borne Bean golden mosaic (BGMV) and Bean golden yellow mosaic viruses.

Activity 1.3   Developing germplasm with resistance to pests: *Zabrotes, Acanthoscelides, Empoasca, Apion, Thrips palmi*, leafhopper, pod weevil, and bruchids

Highlights:

- Resistance to the bean weevil (*Acanthoscelides obtectus*) was identified in *Phaseolus vulgaris* x *P. acutifolius* hybrids

- Finished studies on molecular markers for *Thrips palmi* resistance

- Progress was made in the development of molecular markers for resistance to the pod weevil (*Apion godmani*)

1.3.1   Screening for sources of resistance to major insect pests

Rationale: Identification of sources of resistance to major insect pests of beans is a continuous activity. Additional work is conducted trying to identify and characterize the mechanisms of resistance to specific major pests.

Materials and Methods: Bruchid nurseries are tested in the laboratory simulating normal storage conditions (20° C, 80% R.H., and 14 % seed humidity). Genotypes are tested using 3-5 replications of 50 seeds per genotype. Evaluation units (replicates) are infested with 7 pairs of *Z. subfasciatus* per 50 seeds or two eggs per seed in the case of *A. obtectus*. *T. palmi*, leafhopper and pod weevil nurseries are planted in the field under high levels of natural infestation, usually with 3-4 replicates per genotype in randomized complete block designs. Evaluations for resistance include damage and bean production ratings, insect counts, damage counts, and in some cases, yield components and yields.

Results and Discussion:

Bruchids

Acanthoscelides obtectus

Using a novel Double Congruity Backcross technique developed at CIAT, the Biotechnology Unit has been able to develop fertile interspecific *Phaseolus vulgaris* - *P. acutifolius* (common x tepary) bean hybrids using the tepary genotype NI576 (a genotype competent to *Agrobacterium*-mediated genetic transformation). Some of these crosses involve the tepary accession G 40199 an excellent source of resistance to the bean weevil, *Acanthoscelides obtectus*. In 2002 and 2003 we identified several progenies containing both *P. vulgaris* and *P. acutifolius* cytoplasm with very high levels of antibiosis resistance to *A. obtectus*. In 2004, emphasis was placed upon the reconfirmation of resistance in previously selected progenies. As shown in Table 22, one hybrid containing *P. vulgaris* cytoplasm and seven containing *P. acutifolius* cytoplasm showed high levels of resistance to the insect (< 20% adult emergence). Resistance in some cases was as high as that of G 40199, the resistant check.

Table 22. Resistance to *Acanthoscelides obtectus* in selected $F_{3.5}$ hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses

Code and generation	Cross	Percentage adult emergence	Days to adult emergence	Percentage seeds damaged
Interspecific *P. vulgaris* - *P. acutifolius* hybrids with *P. vulgaris* cytoplasm				
T7K – 2F F_3	V-DCBC5 x V-DCBC4	67.5	38.7	66.6
T7K – 2E F_3	V-DCBC5 x V-DCBC4	7.1	71.0	10.0
T7K – 2 8B F_4	V-DCBC5 x V-DCBC4	100.0	39.2	100.0
T7K – 2 8A F_4	V-DCBC4 x GNV	99.2	39.6	100.0
T7K – 2 - 6 F_5	V-DCBC5 x V-DCBC4	100.0	38.6	100.0
Interspecific *P. vulgaris* - *P. acutifolius* hybrids with *P. acutifolius* cytoplasm				
GKA – 12R F_3	A-DCBC7-2 x A6	54.8	53.9	86.1
GKA – 12R F_3	A-DCBC7-2 x A6	77.4	44.5	96.0
GKX – 6B F_3	A-DCBC8-2	12.7	48.0	33.3
GNVAV – 200A F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	12.2	44.4	20.7
GNVAV – 200B F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	88.2	42.9	100.0
GNVAV – 200D F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	2.7	41.0	8.7
GNVAV – 200G F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	1.1	67.0	3.3
GNVAV – 200H F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	0.0	N.E.	0.0
GVV – 101 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	55.9	47.1	76.7
GVV – 102 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	71.1	47.1	90.0
GVV – 104 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	55.6	49.4	79.3
GVV – 107 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	57.1	45.5	100.0
GVV - 108 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	11.1	56.6	36.1
GVV - 108 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	62.2	44.2	75.0
GVV - 110 F_3	{[(G40022 x NI576)x V5] x A3} x VS42-7	36.3	48.6	57.8
Checks				
G 12882 Arc 1	Susceptible wild *P. vulgaris* accession	78.3	35.8	100.0
G 12952 Arc 4	Susceptible wild *P. vulgaris* accession	75.0	46.3	100.0
G 40168	Susceptible *P. acutifolius* accession	88.3	41.8	100.0
G 25410	Susceptible *P. lunatus* accession	93.3	42.7	100.0
RAZ 44	Susceptible *P. vulgaris* line	98.3	36.9	100.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	96.6	31.7	100.0
G 40199	Resistant *P. acutifolius* accession	3.3	85.7	12.2
G 25042	Resistant *P. lunatus* accession	1.6	80.0	5.3

After multiplication of resistant seeds in the greenhouse, some of these hybrids again showed high resistance to *A. obtectus* (< 20% adult emergence) in replicated tests (Table 23).

Table 23. Resistance to *Acanthoscelides obtectus* in selected $F_{5,6}$ hybrid progenies derived from interspecific *Phaseolus vulgaris - P. acutifolius* crosses

Cross code	Hybrid number	Cross	Percentage adult emergence	Days to adult emergence	Percentage seeds damaged
		Hybrids			
GNVAV	200A9 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	18.7	54.0	32.6
GNVAV	200D21 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	47.9	51.2	52.6
GNVAV	200D22 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	31.9	52.7	63.8
GNVAV	200G16 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	14.4	58.1	31.3
GNVAV	200G17 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	30.6	52.0	45.2
GNVAV	200G18 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	0.6	71.0	1.8
GNVAV	200G19 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	13.0	56.0	25.5
GNVAV	200H5 F_6	{[(G40022 x NI576)x V5] x A3} x VS42-7	0.8	71.0	2.8
GVV	110G F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	5.2	58.0	18.8
GVV	110 I F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	21.1	48.9	36.6
GVV	108 N F_5	{[(G40022 x NI576)x V5] x A3} x VS42-7	14.6	55.7	37.0
		Checks			
G 12882 Arc1		Susceptible wild *P. vulgaris* accession	66.4	36.0	100.0
G 12952 Arc4		Susceptible wild *P. vulgaris* accession	60.0	47.6	100.0
G 40168		Susceptible *P. acutifolius* accession	65.2	43.4	100.0
G 25410		Susceptible *P. lunatus* accession	90.4	42.4	100.0
ICA Pijao		Susceptible *P. vulgaris* cultivar	93.9	30.6	100.0
G 40199		Resistant *P. acutifolius* accession	16.3	58.5	39.0
G 25042		Resistant *P. lunatus* accession	1.5	62.0	7.1

We also tested three interspecific hybrids with *P. vulgaris* cytoplasm (all susceptible) and eight with *P. acutifolius* cytoplasm, three of which (BWG–5N F_3, BWG–6Y F_3, and BWG–1F F_3) showed resistance (Table 24). After multiplication of selected seeds, replicated reconfirmation tests revealed intermediate resistance (20-50% adult emergence) in some of these hybrids (Table 25).

Table 24. Resistance to *Acanthoscelides obtectus* in selected segregating F$_3$ hybrid progenies derived from *Phaseolus vulgaris* - *P. acutifolius* crosses

Code and generation	Type of material	Percentage adult emergence	Days to adult emergence	Percentage seeds damaged
	Hybrids			
TZT – 4A3 F$_4$	Interspecific *P. vulgaris* - *P. acutifolius* hybrid with *P. vulgaris* cytoplasm	82.2	35.9	73.3
TZT – 4A1 F$_4$	" "	73.3	42.1	80.0
TZT – 1E F$_4$	" "	96.0	40.0	100.0
BWG – 5M F$_3$	" "	52.6	45.9	78.1
BWG – 5N F$_3$	Interspecific *P. vulgaris* - *P. acutifolius* hybrid with *P. acutifolius* cytoplasm	11.3	58.5	31.1
BWG – 5S F$_3$	" "	80.0	50.2	96.7
BWG – 6Y F$_3$	" "	48.8	52.8	85.0
BWG – 6W F$_3$	" "	94.5	41.9	100.0
BWG – 1F F$_3$	" "	39.8	49.3	67.0
BWG – 1A F$_3$	" "	64.4	46.3	76.7
BWG – 1G F$_3$	" "	95.6	39.2	93.3
	Checks			
G 12882 arc 1	Susceptible wild *P. vulgaris* accession	78.3	35.8	100.0
G 12952 arc 4	" "	75.0	46.3	100.0
G 40168	Susceptible *P. acutifolius* accession	88.3	41.8	100.0
G 25410	Susceptible *P. lunatus* accession	93.3	42.7	100.0
RAZ 44	Susceptible *P. vulgaris* line	98.3	36.9	100.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	96.6	31.7	100.0
G 40199	Resistant *P. acutifolius* accession	3.3	85.7	12.2
G 25042	Resistant *P. lunatus* accession	1.6	80.0	5.3

Table 25. Resistance to *Acanthoscelides obtectus* in selected segregating F₄ hybrid progenies derived from *Phaseolus vulgaris* - *P. acutifolius* crosses

Cross Code	Hybrid number	Percentage adult emergence	Days to adult emergence	Percentage seeds damaged
Hybrids				
BWG	1F7 F4	43.2	43.4	70.1
BWG	1F13 F4	45.4	46.4	90.5
BWG	1F14 F4	44.4	43.4	73.0
BWG	1F18 F4	25.6	43.7	53.8
BWG	5N1 F4	33.8	49.9	64.1
BWG	5N4 F4	24.4	55.3	55.1
BWG	6Y6 F4	29.5	50.7	64.8
BWG	6Y15 F4	17.5	51.4	35.1
Checks				
G 12882 Arc 1	Susceptible wild *P. vulgaris*	66.4	36.0	100.0
G 12952 Arc 4	" "	60.0	47.6	100.0
G 40168	Susceptible *P. acutifolius*	65.2	43.4	100.0
G 25410	Susceptible *P. lunatus* accession	90.4	42.4	100.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	93.9	30.6	100.0
G 40199	Resistant *P. acutifolius* accession	16.3	58.5	39.0
G 25042	Resistant *P. lunatus* accession	1.5	62.0	7.1

The tedious but important process of testing individual seeds to detect segregation in interspecific hybrids continued in 2004 (Table 26). Those selected for resistance were multiplied but the seed did not germinate. One intraspecific *P. lunatus* hybrid that did germinate (coded V5) showed a very high level of resistance comparable to that of the resistant accession G 25042. Two double congruent hybrids with *P. acutifolius* cytoplasm (GKVGAG 1B 4D F₅ and GKVGAG 1E 2C F₅) were selected for further testing (Table 27).

Table 26. Reconfirmation of resistance to *Acanthoscelides obtec*tus in pre-selected segregating hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses and intraspecific *Phaseolus lunatus* crosses

Code and generation	Type of material	Number of seeds evaluated	Number of resistant seeds	Days to adult emergence
Hybrids				
GNVAV-21 F_3	Interspecific *P. vulgaris* - *P. acutifolius* hybrid with *P. acutifolius* cytoplasm	4	3	N.E[a]
GKA 11 F_2	" "	15	3	N.E
Z99ZX6 F_2	Double congruent hybrid with *P. acutifolius* cytoplasm	6	1	N.E
Z99ZX–1A F_3	Double congruent hybrid with *P. acutifolius* cytoplasm	20	8	N.E[1]
Z99ZX–11A F_3	" "	7	1	N.E
ZX99-15 F_3	" "	7	3	N.E
ZXTG31-4-10 F_4	" "	12	5	N.E
GKVGAG-1A F_3	" "	31	31	N.E
A6 F_2	Intraspecific *P. lunatus* hybrid	9	7	N.E
VS42-14 F_2	" "	5	4	N.E
V5 F_2	" "	31	31	N.E.
VS42-7 F_2	" "	6	6	N.E
Checks				
G 40168	Susceptible *P. acutifolius* accession	15	0	42.6
G 25410	Susceptible *P. lunatus* accession	15	0	44.4
RAZ 44	Susceptible *P. vulgaris* line	15	0	38.0
ICA Pijao	Susceptible *P. vulgaris* cultivar	15	0	31.9
G 40199	Resistant *P. acutifolius* accession	15	9	N.E
G 25042	Resistant *P. lunatus* accession	15	9	N.E
G 25713	Resistant *P. lunatus* accession	26	24	N.E

[a] N.E., no adult emergence from resistant seeds

Table 27. Reconfirmation of resistance to *Acanthoscelides obtec*tus in pre-selected segregating F$_5$ hybrid progenies derived from interspecific *Phaseolus vulgaris* x *P. acutifolius* crosses

| Code and generation	Type of material	Number of seeds evaluated	Number of resistant seeds	Days to adult emergence
	Hybrids			
GKVGAG 1B 4D F$_5$	Double congruent hybrid with *P. acutifolius* cytoplasm	25	22	N.E[a]
GKVGAG 1E 2C F$_5$	" "	59	58	N.E
	Checks			
G 12882 ARC1	Susceptible wild *P. vulgaris* accession	20	0	37.5
G 12952 ARC 4	" "	20	0	53.3
G 40168	Susceptible *P. acutifolius* accession	20	0	45.2
G 25410	Susceptible *P. lunatus* accession	19	0	43.7
RAZ 44	Susceptible *P. vulgaris* line	20	0	37.3
ICA Pijao	Susceptible *P. vulgaris* cultivar	20	0	30.5
G 40199	Resistant *P. acutifolius* accession	19	19	N.E
G 25042	Resistant *P. lunatus* accession	20	20	N.E

[a]N.E., no adult emergence from resistant seeds

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Pod weevil (*Apion godmani*)

Rationale: The pod weevil is one of the most important pests of beans in Mexico and Central America. As indicated in previous reports, we are attempting to develop a molecular marker for *Apion* resistance. This work has been conducted in close collaboration with Dr. Ramón Garza from INIFAP. In order to support the molecular work, new phenotypic data were obtained by testing for resistance in the field a set of 54 recombinant inbred lines (RILs) developed in 2002. The lines are derived from a cross between Jamapa (a susceptible cultivar) and J-117 (a highly resistant Mexican landrace). The materials were tested at two locations (Santa Lucía de Prías in Mexico State and Atotonilco in Hidalgo State) in replicated nurseries using three replications per material in a randomized complete block design. The infestation in Santa Lucía was low and unreliable for proper resistance evaluation. That in Atotonilco was high and reliable to discriminate between susceptible and resistance genotypes.

The population of RILs was normally distributed for *Apion* resistance (Figure 23), suggesting that the inheritance of resistance to the pod weevil may be governed by more than a single major resistance gene. Even though overall levels of infestation in 2003 were higher than in 2002, there was a significant correlation (r = 0.423; $P < 0.01$) between damage scores obtained in 2002 and in 2003 (Figure 24). The phenotypic data obtained in 2003 is being used in the development of a molecular marker for pod weevil resistance (for details see SB-2 report).
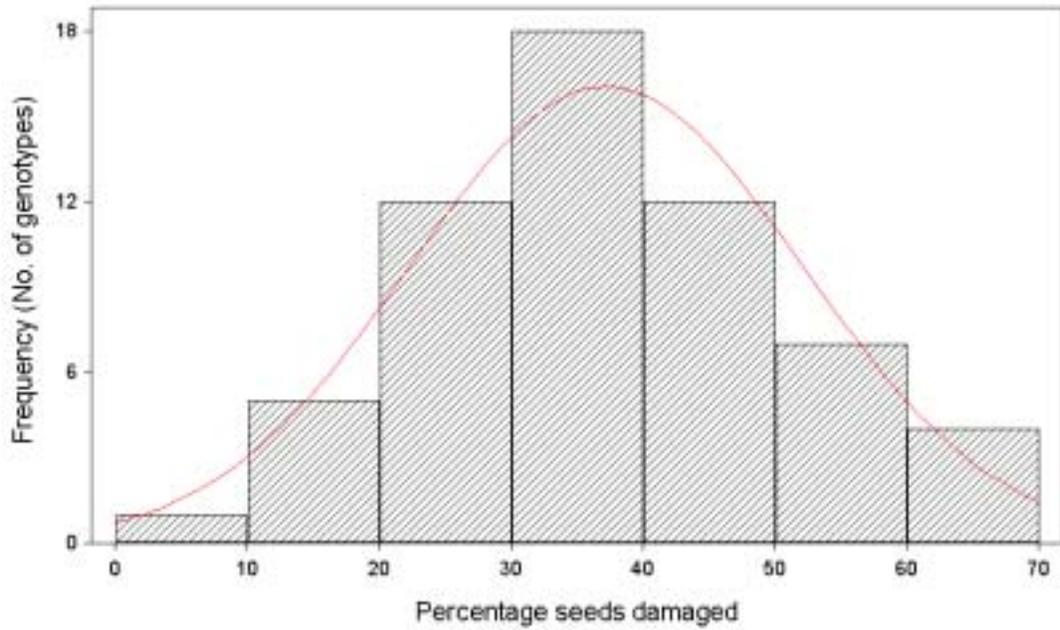
Figure 23. Frequency histogram of percentage seeds damaged by the pod weevil (*Apion godmani*) in a population of 54 recombinant inbred lines derived from a cross between Jamapa (a highly susceptible cultivar) and J-117 (a highly resistant Mexican landrace). The lines were screened under field conditions and high insect populations in a replicated nursery. Atotonilco, Hidalgo State, Mexico, 2003B.
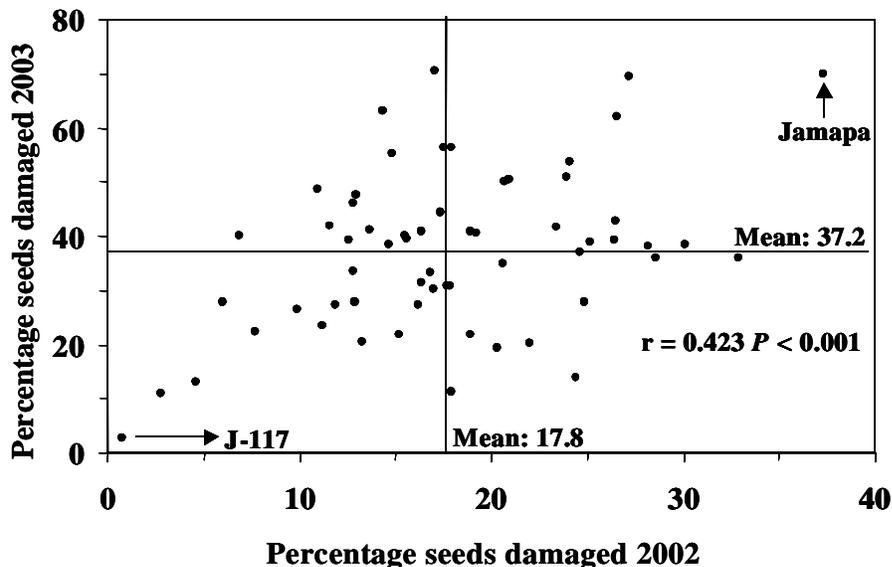
Figure 24. Percentage seed damage in 54 recombinant inbred lines (RILs) tested for resistance to the pod weevil (*Apion godmani*) in two consecutive trials. RILs derived from a cross between Jamapa (a susceptible cultivar) and the resistance source J-117 (a Mexican landrace). Tests

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Leafhopper (*Empoasca kraemeri*)

In 2004 we screened a total of 549 bean germplasm accessions for resistance to the leafhopper. Those selected in 2003 (33) were reconfirmed in replicated nurseries. Of these, 21 were selected for further testing in 2004. We also gave support to the mainstream breeding activities of the Bean Project by screening a series of nurseries. These included 29 selections made in 2003 individual plant selections in Andean crosses performed with selected EMP lines as parents. Thirteen were yield-tested in 2004. Other yield tests included 13 lines derived from crosses with EMP 250 and lines from crosses with Saladin and 16 Andean lines.

We will highlight the work on evaluation of interspecific *P. vulgaris - P. acutifolius* hybrids. Similar to the work with bruchids these progenies were obtained by means of the Double Congruity Backcross technique developed at CIAT. We tested 189 progenies (F_2 and F_3) of crosses made with the tepary sources of resistance to leafhopper G 40019 and G 40036. Selected progenies and their reaction to leafhopper are shown in Tables 28-30. In general, the best lines show an intermediate level of resistance comparable to that found in the tolerant check, ICA Pijao. It can also be said that resistance to leafhopper in interspecific hybrids is not as good as the resistance found in *P. acutifolius* accessions G 40036, G40019, and G 40019.

Contributors: J. M. Bueno, C. Cardona, A. Mejía, J. Tohme.

Table 28. Resistance to *Empoasca kraemeri* in selected $F_{2.5}$ progenies derived from interspecific *Phaseolus vulgaris* - *P. acutifolius* crosses

Code	Pedigree[a]	Damage scores[b]	Reproductive adaptation scores[c]	Overall rating
Hybrids				
KKQ-11 F_5	V-DCBC x V-DCBC	6.8	3.8	Intermediate
A99Y-15 F_4	V-DCBC x (G40199 X A-DCBC)	6.0	5.3	Resistant
A19Y-103 F_5	V-DCBC x (G40019 X A-DCBC)	6.3	4.0	Intermediate
A19Y-117 F_4	V-DCBC x (G40019 X A-DCBC)	6.4	4.8	Intermediate
A36Y-42 F_5	V-DCBC x (G40036 X A-DCBC)	6.1	4.3	Intermediate
A99Y-86 F_4	V-DCBC x (G40199 X A-DCBC)	6.4	4.8	Intermediate
A99Y-90 F_4	V-DCBC x (G40199 X A-DCBC)	5.9	5.0	Resistant
A99Y-91 F_4	V-DCBC x (G40199 X A-DCBC)	6.3	4.3	Intermediate
ANIY-101 F_4	V-DCBC x A-DCBC	6.9	4.0	Intermediate
A36Y-42 F_4	T-6FB x G36NGP-3FL	6.5	-	Intermediate
EMPZ-2 F_3	A99Y-90 x ZXTGS21-9	6.2	-	Intermediate
EMPZ-5 F_3	A36Y-42 x ZXTGS-21-11	6.7	-	Intermediate
EMPZ-8 F_3	A99Y-103 x ZXTGS49-8	7.0	-	Intermediate
EMPZ-9 F_3	A99Y-103 x ZXTGS49-8	7.0	-	Intermediate
TZTE - 9F F_2	TZT-12FL x EMPZ-3FB	7.0	-	Intermediate
TZTE – 11F_2	TZT-3FL x EMPZ-2FB	7.0	-	Intermediate
TZTE - 20B F_2	TZT-3FL x EMPZ-3FB	6.5	-	Intermediate
TZTE - 71B F_2	TZT-4FL x EMPZ-3FB	5.7	-	Intermediate
Checks				
BAT 41	Susceptible *P. vulgaris* line	8.8	3.5	Susceptible
EMP 250	Tolerant *P. vulgaris* line	6.4	6.0	Intermediate
EMP 508	Tolerant *P. vulgaris* line	6.2	6.3	Intermediate
EMP 512	Tolerant *P. vulgaris* line	6.0	5.8	Resistant
G40016	Susceptible *P. acutifolius* accession	8.6	3.0	Susceptible
G40019	Resistant *P. acutifolius* accession	5.6	5.5	Resistant
G40036	Resistant *P. acutifolius* accession	5.3	6.0	Resistant
G40056	Susceptible *P. acutifolius* accession	8.8	2.5	Susceptible
G40065	Susceptible *P. acutifolius* accession	8.1	4.8	Susceptible
G40119	Resistant *P. acutifolius* accession	5.1	6.0	Resistant
ICA Pijao	Tolerant *P. vulgaris* cultivar	7.3	6.0	Intermediate
NI576	Susceptible *P. acutifolius* line	8.8	2.0	Susceptible

[a]V-DCBC = Double congruent hybrid with *P. vulgaris* cytoplasm; A-DCBC = Double congruent hybrid with *P. acutifolius* cytoplasm
[b]On a 1-9 visual scale (1, no damage; 9, severe damage)
[c]On a 1-9 visual scale (1, no yield, no pod formation; 9, excellent pod formation and filling, excellent yield).

Table 29. Resistance to *Empoasca kraemeri* in selected $F_{3.6}$ progenies derived from interspecific *Phaseolus vulgaris - P. acutifolius* crosses

Code	Pedigree[a]	Damage scores[b]	Reproductive adaptation scores[c]	Overall rating
		Hybrids		
A99Y-15 F_5	V-DCBC x (G40199 X A-DCBC)	6.8	4.5	Intermediate
A19Y-103 F_6	V-DCBC x (G40019 X A-DCBC)	6.8	4.0	Intermediate
A19Y-117 F_5	V-DCBC x (G40019 X A-DCBC)	7.2	4.0	Intermediate
A99Y-86 F_5	V-DCBC x (G40199 X A-DCBC)	6.8	4.5	Intermediate
A99Y-90 F_5	V-DCBC x (G40199 X A-DCBC)	6.6	4.5	Intermediate
A99Y-91 F_5	V-DCBC x (G40199 X A-DCBC)	6.8	4.3	Intermediate
ANIY-101 F_5	V-DCBC x A-DCBC	7.2	3.5	Intermediate
A36Y-42 F_5	T-6FB x G36NGP-3FL	7.0	4.5	Intermediate
EMPZ-8 F_4	A99Y-103 x ZXTGS49-8	7.2	3.5	Intermediate
EMPZ-9 F_4	A99Y-103 x ZXTGS49-8	7.0	3.8	Intermediate
TZTE - 20B F_3	TZT-3FL x EMPZ-3FB	7.4	3.8	Intermediate
EMPZ-2 F_4	A99Y-90 x ZXTGS21-9	6.4	5.0	Resistant
EMPZ-5 F_4	A36Y-42 x ZXTGS-21-11	6.8	4.5	Intermediate
TZTE - 71B F_3	TZT-4FL x EMPZ-3FB	6.5	4.0	Intermediate
		Checks		
G40019	Resistant *P. acutifolius* accession	5.6	5.5	Resistant
G40036	Resistant *P. acutifolius* accession	5.6	5.8	Resistant
NI576	Susceptible *P. acutifolius* line	7.8	2.8	Susceptible
G40033	Susceptible *P. acutifolius* accession	8.8	2.3	Susceptible
G40119	Resistant *P. acutifolius* accession	5.7	5.0	Resistant
EMP 512	Tolerant *P. vulgaris* line	6.1	5.3	Resistant
EMP 508	Tolerant *P. vulgaris* line	6.5	4.3	Intermediate
EMP 250	Tolerant *P. vulgaris* line	6.8	4.3	Intermediate
BAT 41	Susceptible *P. vulgaris* line	9.0	2.0	Susceptible
ICA Pijao	Tolerant *P. vulgaris* cultivar	6.8	4.3	Intermediate

[a] V-DCBC = Double congruent hybrid with *P. vulgaris* cytoplasm; A-DCBC = Double congruent hybrid with *P. acutifolius* cytoplasm

[b] On a 1-9 visual scale (1, no damage; 9, severe damage)

[c] On a 1-9 visual scale (1, no yield, no pod formation; 9, excellent pod formation and filling, excellent yield)

Table 30. Resistance to *Empoasca kraemeri* in selected $F_{3.6}$ progenies derived from interspecific *Phaseolus vulgaris* - *P. acutifolius* crosses

| Code[a]	Pedigree	Damage scores[b]	Reproductive adaptation scores[c]	Overall rating
		Hybrids		
TSC	TZTA-1A2L FB x Row 3 FB (A36Y 42)	6.3	4.0	Intermediate
TSC	TZTA-1A2L FB x Row 3 FB (A36Y 42)	6.0	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2N FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTA-1A2L FB x Row 3 FB (A36Y 42)	6.3	4.3	Intermediate
TSC	TZTTZ-85R FB x Row 10 FB (EMPZ2)	6.3	4.3	Intermediate
TSC	TZTTZ78M FB x Row 30 FB (TZTE-71)	6.3	4.3	Intermediate
ZXTGS	ZXTG6FB x Row 49 Entry 70FL	6.3	4.3	Intermediate
ZXTGS	ZXTG6FB x Row 49 Entry 70FL	6.3	4.3	Intermediate
ZXTGS	ZXTG6FB x Row 21 FLG36NGP-$3F_2$	5.7	4.3	Resistant
SCO	ZXTG6FB x Row 49 Entry 70FL	6.3	4.7	Intermediate
SCO	ZXTG6FB x Row 21 FLG36NGP-$3F_2$	5.7	4.7	Resistant
TSC	TZTA-1A2L FB	6.3	5.5	Resistant
TSC	TZTTZ-98B FL	6.0	5.0	Resistant
		Checks		
G40019	Resistant *P. acutifolius* accession	4.5	6.0	Resistant
G40036	Resistant *P. acutifolius* accession	4.3	6.1	Resistant
NI576	Susceptible *P. acutifolius* line	8.0	2.8	Susceptible
G40033	Susceptible *P. acutifolius* accession	8.8	2.3	Susceptible
G40119	Resistant *P. acutifolius* accession	4.6	5.6	Resistant
EMP 512	Tolerant *P. vulgaris* line	5.9	6.0	Resistant
EMP 508	Tolerant *P. vulgaris* line	6.5	4.3	Intermediate
EMP 250	Tolerant *P. vulgaris* line	6.2	6.0	Intermediate
BAT 41	Susceptible *P. vulgaris* line	8.3	2.6	Susceptible
ICA Pijao	Tolerant *P. vulgaris* cultivar	6.7	5.3	Intermediate

[a] TSC = Double congruent hybrid with *P. vulgaris* cytoplasm; A-DCBC = Double congruent hybrid with *P. acutifolius* cytoplasm; both ZXTGS and SCO possess *P. acutifolius* cytoplasm
[b] On a 1-9 visual scale (1, no damage; 9, severe damage)
[c] On a 1-9 visual scale (1, no yield, no pod formation; 9, excellent pod formation and filling, excellent yield)

1.3.2   Studies on progress in tolerance to leafhopper

In 2004 we finished our studies on progress in incorporating tolerance to leafhopper. We performed the combined analysis of variance for the five consecutive trials aimed at measuring the response of EMP lines (bred for leafhopper resistance) and checks to two levels of infestation (3 and 6 nymphs per leaf). These were obtained by exercising chemical control at pre-established action levels. There was not a significant interaction between trials and treatments. At all levels of infestation, EMP 250, EMP 542, EMP 544, and EMP 588 yielded significantly better than the susceptible check BAT 41 and EMP 124. None performed better than ICA Pijao, the tolerant check (Figure 25). However, in terms of percentage yield losses, new lines (the EMP 500 series) performed better at all levels of infestation than the improved checks EMP 124 and EMP 250, and, in some cases, better than the standard tolerant check, ICA Pijao. At very high levels of infestation (6 nymphs per leaf) average yield losses in EMP lines was above the 30% level, meaning that even tolerant materials would benefit from integration with chemical control exercised at pre-established action levels.
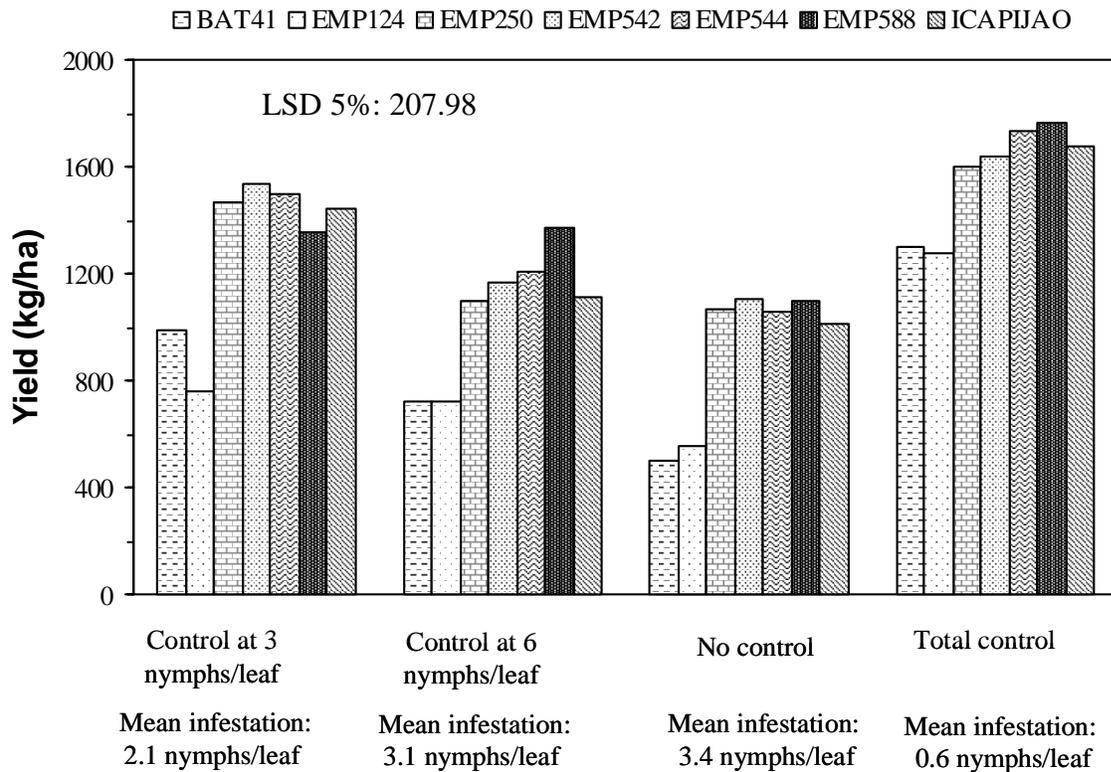
Figure 25. Yields of selected EMP lines and checks (BAT 41, ICA Pijao) at different levels of Infestation with the leafhopper *Empoasca kraemeri*. Means of five trials

Contributors: J.M. Bueno, C. Cardona

Progress toward achieving output milestones:

- The identification of resistant progeny from interspecific crosses between *P. vulgaris* and *P. acutifolius* suggests that resistance is a heritable trait, although most resistant lines carry cytoplasm of *P. acutifolius.*

- Obtaining a second year of phenotypic data for the reaction of RILs to the pod weevil will permit gene tagging. The development of molecular markers for pod weevil, thrips, and bruchids should facilitate breeding for resistance.

Activity 1.4   Developing more nutritious small seeded bean varieties

Highlights:

- Lines combining a moderate level of drought tolerance with 50% higher level of iron have been recovered. These are still deficient in grain type and other agronomic traits but will form the basis for another cycle of recurrent selection.
- A study of G x E across CIAT's research sites suggests that some factor associated with low pH results in low seed iron concentration.
- Two varieties (Gofta and Roba-1) show high levels of iron and zinc concentration across locations and seasons
- Significant interaction of genotypes, soil conditions and P application can be exploited to enhance grain iron and zinc concentration
- Accessions of *Phaseolus coccineus* and *Phaseolus polyanthus* present levels of iron as much as 60% above checks and zinc only narrowly above checks in this trial. Confirmation of a wider advantage is necessary to justify interspecific crosses to improve iron concentration.
- New bean lines high in iron and zinc concentration identified in Eastern Africa and evaluated for agronomic characteristics by more than 20 farmers.

1.4.1   Evaluation of F_3-derived F_5 families to combine high iron with agronomic traits

Rationale: Improvement of nutritional quality must go hand in hand with improvement of agronomic characters. Eventual adoption and cultivation by farmers will depend on some visible advantage in terms of yield, market value, or some other trait that induces farmers to grow beans. Past advances must be maintained and additional agronomic traits must be added. For many bean growing areas, drought tolerance is a trait that is needed for food security and will be very attractive to farmers. Combining drought tolerance with higher mineral concentration will serve to accelerate the diffusion of varieties with improved nutritional value.

Materials and Methods: Multiple crosses were created among parents with diverse traits: resistance to BGYMV, which is now a requisite for varietal release in most areas of Central America; resistance to angular leaf spot, which is important in both Central America and Africa; drought tolerance; and high iron concentration. Multiple parents of high iron were employed to combine genes for this trait from various sources. In the F_2 generation about 1500 F_1-derived F_2 families were evaluated under drought, and 250 were advanced and selected under pressure of anthracnose and angular leaf spot is successive generations. Twenty-seven F_3-derived F_5 families were again tested under drought pressure in 2004. This group represents a sub-set of those reported in section 1.1.1 above.

Results and Discussion: Our short term goal (i.e., in year 1 of the HarvestPlus program) for the improvement of iron and zinc concentration calls for an increase of 50% in mineral content in some ten F_3-derived F_5 families with tolerance to drought. This would be the equivalent of about 65-70 mg/kg^{-1} iron in the conditions of this particular trial in Quilichao in which the checks presented less than 45 mg kg^{-1} of iron. A few families meet this criterion (Table 31) when the standard against which drought tolerance is compared is Tio Canela, a widely used commercial

variety in Central America, although their advantage in drought tolerance is modest. However, no improvement in zinc concentration of the selected lines was detected. Nonetheless, the recovery of even this small number of lines with a positive combination of traits suggests that higher mineral concentration can be obtained in agronomically superior materials. For example, MDSF 14729-23-MC-4Q-MQ presented a 17% yield advantage over the check in a replicated trial, and from 74 mg kg^{-1} iron in an unreplicated assay to 80 mg kg^{-1} in a replicated trial. This bodes well for future work, and a second round of populations with high mineral parents appears to be more promising in its adaptation to drought conditions.

Table 31. Lines derived from crosses to combine superior mineral concentration with drought tolerance.

Cross / Line	Yield as % checks[1]	Yield checks	[Fe] in F_4 families (mg kg^{-1})	[Zn] in F_4 families (mg kg^{-1})
(SXB 111 x (INB35 x G23834E)) x (MAB 85 x EAP9653-16B-1)				
MDSF 14729-23-MC-2Q-MQ	140	1191	70	31
MDSF 14729-23-MC-3Q-MQ	113	1191	75	31
MDSF 14729-23-MC-4Q-MQ	117	1191	74	33
(SXB 127 x (MIB 81 x G23823E)) x (MAB 87 x EAP9653-16B-1)				
MDSF 14734-31-MC-35Q-MQ	123	1191	75	23
MDSF 14734-31-MC-22Q-MQ	98	1191	83	23
MDSF 14734-31-MC-37Q-MQ	89	1191	97	22
(SXB 124 x (INB 35 x G23834E) x (SXB 123 x RIB 68)				
MDSF 14742-3-MC-12Q-MQ	157	1191	65	32
MDSF 14742-3-MC-27Q-MQ	113	1191	65	28
(SXB 122 x (INB 36 x G23818B)) x (MAB 95 x EAP9653-16B-1)				
MDSF 14743-27-MC-23Q-MQ	106	1191	75	30
MDSF 14743-51-MC-3Q-MQ	129	1191	75	29
(SXB 122 x (INB 36 x G23818B)) x BFB 146				
MDSF 14744-15-MC-2Q-MQ	145	1191	87	30
DOR 500			42	28
CAL 96			44	24

[1] Yields of lines were calculated as a per cent of the yield of commercial check Tio Canela.

Conclusion: Combining higher mineral concentration with acceptable agronomic type is a particular challenge for the warmer tropical environments in which the sources of higher mineral concentration are poorly adapted. However, progress in this regard indicates that this is possible.

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                    O. Mosquera (Analytical Laboratory)

1.4.2 A study of G x E interaction across CIAT's research sites with lines bred for high mineral content

Rationale: Genotype x Environment interaction confounds the expected performance of selected materials over seasons or in environments other than the selection sites *per se*. Our original evaluation of gene bank accessions in the core collection was carried out in Popayán and in Darién where most landrace germplasm adapts reasonably well. However, breeding for warm tropical environments requires selection at other sites, and in the case of CIAT, this implies selection at Palmira and/or Santander de Quilichao, both of which have soils that contrast with the those at Popayán or Darién.

Materials and Methods: A nursery of 31 lines and sources was composed of original sources (gene bank accessions) and lines selected from crosses among sources. This was planted at three sites with contrasting soil characteristics: Popayán (inceptisol with high organic matter and a modified pH of 5.6); Quilichao (oxisol with a modified pH of 4.5-5.0); and Palmira (molisol with a pH of 7.2). Single row plots were used in three replications. The primary purpose was to verify the effect of G x E on mineral concentration, but yield was measured as well.

Results and Discussion: Mineral content was strongly affected by location, genotype and genotype x location interaction (Tables 32 and 33). Palmira gave the highest levels of iron, while Popayán gave the highest levels of zinc. The effect of location could have been due to several factors. Palmira and Quilichao have similar temperatures but resulted in widely differing mineral concentrations in grain. On the other hand Quilichao and Popayán have very different temperatures but both present relatively low mineral concentrations. Thus, temperature differences do not appear to explain differences in mineral concentration over sites. The most obvious common factor that Quilichao and Popayán have in common and that distinguishes both from Palmira is an acid soil pH and other possibly associated soil characteristics such as manganese toxicity. However, an acid soil would not be expected to give lower iron concentration. Quite the contrary, an acid soil should have more available iron than a neutral to alkaline soil like that in Palmira. However, another trial with a partial set of genotypes in Quilichao in a lot with pH of 6.2 presented higher levels of iron than the Quilichao soil at pH 4.5 – 5.0, thus tending to confirm that more iron is accumulated in grain at higher soil pH. Hypotheses to explain this behavior include a pH-sensitive plant response to activate uptake mechanisms at higher pH; or interference of manganese with iron absorption at low pH. Both Quilichao and Popayán have high manganese content in the soil. In spite of G x E effects, correlations across sites for iron concentration were high, ranging from $r = 0.71$ to 0.86, whereas correlations with zinc ranged from 0.47 to 0.74. Thus, selection would still be effective across sites, especially for iron.

Table 32.   Significance of effects in ANOVA for mineral concentration and yield of 31 genotypes over three sites.

Source	Df	[Fe]	[Zn]	Yield
Locality	2	***	***	***
Genotype	30	***	***	***
Locality x Genotype	60	***	***	***
Rep (Locality)	6	**	NS	***

Table 33. Iron and zinc concentrations in selected lines, gene bank accessions and controls in three sites and under different soil pH values.

Tmt.	Identification	Iron					Zinc				
		Pal. pH 7.2	Pop. pH 5.6	Quil. pH 4.5	Ave.	Quil. pH 6.2	Pal. pH 7.2	Pop. pH 5.6	Quil. pH 4.5	Ave.	Quil. pH 6.2
4	MIB 151	87	68	75	77	94	27	42	34	34	43
5	MIB 152	90	59	81	77	89	26	37	31	32	42
6	MIB 153	82	60	72	71	92	30	34	31	32	40
8	MIB 154	82	68	63	71	89	30	35	29	31	38
10	MIB 155	105	66	70	80	97	35	33	32	33	38
11	MIB 156	81	50	51	60	77	27	30	25	27	35
12	MIB 157	105	72	77	85	93	40	32	29	34	38
13	MIB 158	104	78	77	86	93	37	33	28	33	38
23	G23818B	92	87	79	86		35	38	36	36	
24	G23823E	95	96	92	94		36	35	30	33	
25	G23834E	119	91	87	99		30	32	29	30	
26	DICTA 17	58	45	48	50		23	28	28	26	
27	DOR 500	58	48	52	53	53	24	29	24	26	27
28	CAL 96	50	51	50	50	46	19	23	23	22	22
	Trial mean	**78**	**61**	**64**	**68**	**82**	**27**	**31**	**28**	**29**	**36**

Compared to mineral concentration of local checks DOR 500 and CAL 96, the advantage of the lines bred for high minerals was reduced by about 50% under pH 5.6 and lower. However, implications for targeting of high iron materials and the effect of G x E on mineral content may not be serious for many bean growing areas. The bred lines maintained a broad advantage over checks at pH 6.2 in Quilichao, and this pH would more typical of most soils in Central America and the Caribbean. Very low pH soils would be a greater concern for Costa Rica, southern Mexico and Central Africa, especially Congo and western Rwanda.

Although yield was measured on single row plots, highly significant effects were detected for materials, locations, and materials x locations. A tendency emerged of a negative correlation between yield and mineral concentration, reflecting the poor adaptation of the original sources. Among the gene bank accessions, all performed very poorly at Palmira and Quilichao, but much better in Popayán under cooler temperatures, where G23834E produced 3428 kg/ha. This highlights the difficulty of breeding high mineral beans for warmer environments with these sources, and the need for recurrent selection.

Conclusion: The effect of pH on mineral content of bean seed requires additional study. But in spite of G x E effects, ranking of genotypes across sites tends to be similar and to permit selection of high mineral cultivars.

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1.4.3 Influence of P application on grain iron, zinc and protein concentration

Rationale: Micronutrient deficiency is a world-wide health problem affecting more than 1 billion people. Major deficiencies occur in iron, zinc and vitamin A. Alternative strategies of reducing micronutrient deficiencies such as supplementation and fortification have had limited success in the developing world, where the problem is most acute, because of limited coverage, underdeveloped food industry and difficulties in patient compliance and access. Dietary improvement is probably the most effective and sustainable strategy for reducing micronutrient deficiencies in Africa. This approach aims to increase dietary availability, regular access and consumption of mineral-rich foods in at - risk and micronutrient-deficient groups of populations. It involves development and promoting enhanced consumption of culturally acceptable, mineral rich grains and vegetables. Common bean offers unique opportunities for improved micronutrient nutrition and food security because it is widely grown (3.7 million ha annually in Africa) and consumed; it is rich in protein (>20%), minerals and calories, and is relatively cheap and highly marketable. We previously reported that genetic variation exists in common bean to facilitate improvement of the grain iron concentration by 80% and zinc level by 50%. However, the stability of expression of this trait may be influenced by environmental factors and interactions with genotypes. Rengel (1999) in an extensive review of literature concluded that fertilization with inorganic and organic forms of micronutrients has potential to increase their concentration in the grain. Effectiveness of various agricultural measures in increasing micronutrient density depends on soil type, crop, cultivar, and rotation, and environmental and other factors, thus necessitating development of a specific set of measures for individual regions. However, little is known on the role applying macronutrients such as P, N and K on the grain iron and zinc concentration in common bean and other staple crops. In this report we highlight results of a study on the effect of P application on seed iron, zinc and protein.

Materials and Methods: Four trials were conducted to evaluate the effect of levels of P application on seed iron, zinc and protein in 27 bean lines. The trials were conducted at Kabete (1860m) and Thika (1500m) for two seasons. The seasons were the long rain season (April-August) of 2002 and short rain season (November 2002 to February 2003). Soil at Kabete and Thika are acidic to slightly acidic nitosols (pH between 4.7 and 6). However, Kabete soils are of medium fertility. Soils at Thika are deficient in P and N. A basal rate of 100 kg calcium ammonium nitrate (21% N) was applied at the two sites. P was applied was applied at 0, 50 and 100 kg ha^{-1}. Source of P was triple super phosphate (45% P_2O_5). A plot had four, 5 m rows. Spacing was 10 cm within rows and 45 cm between rows. The experimental design was a split plot with three replicates. P levels were the main plots and bean lines as the subplots. Seed for mineral analysis was harvested from the inner two rows. Mineral concentration was determined with atomic absorption following standard procedures at CIAT, Colombia. Data was analyzed using SAS statistical software. Some plots were lost due susceptibility to diseases (especially root rot, black root) and therefore were excluded from the analysis.

Results and Discussion: Combined analysis of the data showed that there were highly significant differences in grain iron concentration (P<0.01) due to season, location, and genotypic effects. Significant season x P level, season x location and location x season x P levels were also detected (Table 34). There were significant interactions between genotypes and locations, genotypes and seasons and genotypes with P application levels. Significant second

order interactions were detected among genotypes, locations, seasons and P levels for grain iron concentration. Mean grain iron concentration varied with seasons. It was higher during the short rain season (76.8 mg kg^{-1}) compared to long rain season (68.4 mg kg^{-1}). Mean iron concentration was higher at Thika (75 mg kg^{-1}) than Kabete (70.1 mg kg^{-1}). Grain iron concentration increased modestly with P application. The average concentration was 72, 72 and 73.6 mg kg^{-1} at 0, 50 and 100 kg P ha^{-1}, respectively over the two locations, seasons and genotypes. However, iron concentration varied with locations and P levels. Mean iron concentration was highest at 50 kg P ha^{-1} at Thika (77 mg kg^{-1}) and lowest at same P level at Kabete (66.7 mg kg^{-1}). Gofta had the highest seed iron concentration (79.7 mg kg^{-1}) across sites, seasons and P levels, confirming previous reports. It was followed by Roba-1 (78 mg kg^{-1}). Genotypic responses were influenced by location, season and P level. The highest iron concentration was for Zebra at 50 kg P ha^{-1} (90 mg kg^{-1}), Gofta and MCM 2001 at 100 kg P ha^{-1} at Thika (89 mg kg^{-1}) during the 2002 long rain season. Red Wolaita (66 mg kg^{-1}) and Ranjonoby (64 mg kg^{-1}) had the lowest seed iron concentration at 0 kg P ha^{-1} in the same site and season. These results suggest that P application enhances seed iron concentration. The seasonal effect may be due to moisture availability, since P is a relatively immobile element, and translocation efficiency of individual genotypes.

Table 34.   Mean squares for seed iron, zinc and protein concentration of 15 bean lines grown at Kabete and Thika (Kenya) over two seasons under different P treatments.

Source	Df	Mean Squares		
		Iron (mg kg^{-1})	Zinc (mg kg^{-1})	Protein (%)
Seasons (S)	1	9423.68**	1822.48**	508.99**
Locations (L)	1	3318.22**	575.93**	1565.56**
Locations x Seasons	1	21667.01**	65.06**	744.94**
Reps (L x S)	8	360.23**	57.46**	57.31**
P levels	2	166.57NS	117.89**	305.92**
S x P levels	2	1937.69**	322.29**	469.99**
L x P levels	2	1173.53**	22.77NS	85.17**
L x S x P levels	2	3387.87**	105.47**	99.66**
Reps x P levels(L x S)	16	122.96**	16.43*	74.53**
Genotypes (G)	14	590.46**	78.80**	41.33**
G x S	14	261.83**	41.40**	22.20**
G x L	14	234.52**	40.74**	18.06**
G x S x L	14	104.98NS	22.64**	8.60NS
G x P levels	28	103.84*	10.86NS	5.57NS
G x S x P levels	28	127.80**	10.81NS	5.14NS
G x P levels x L	28	137.46**	8.31NS	9.18*
G x P levels x S x L	28	137.46**	6.52NS	4.5NS
Pooled error	536	66.76	8.60	5.38

*, **= Significant at 5% and 1% probability levels, respectively; NS= not significant.

There were highly significant season, location, P level and genotypic effects on the seed zinc concentration (Table 34). Zinc concentration was higher during the short rain season (37.4 mg kg^{-1}) compared with the long rain season (33.4 mg kg^{-1}). In contrast with iron, grain zinc concentration was higher at Kabete (36.6 mg kg^{-1}) than at Thika (34.5 mg kg^{-1}). Zinc concentration varied with P levels. The concentrations were 34.7, 36.3 and 35.6 mg kg^{-1} with application of 0, 50 and 100 kg P ha^{-1}, respectively. Mean zinc concentration varied from 33 mg kg^{-1} for TY 3396, Ranjonoby and K131 to 38.8 mg kg^{-1} for ICA Pijao (Table 35). However, the genotypic responses varied with locations and P levels. The highest Zn levels were recorded at Kabete with application of 50 kg P ha^{-1}. Gofta had the highest Zn concentration (35.5 mg kg^{-1}) at Thika. ICA Pijao (42.7 mg kg^{-1}) and Ituri matata (42.5 mg kg^{-1}) had the highest zinc concentration at 100 kg P ha^{-1}. Gofta had a mean Zn concentration of 36.8 mg kg^{-1} at 100 kg ha^{-1} across sites.

Table 35. Mean seed iron, zinc and protein concentration of 15 bean lines grown at Kabete and Thika (Kenya) for two seasons.

Line	Iron (mg kg^{-1})	Zinc (mg kg^{-1})	Protein (%)
Atndaba	69.1	35.7	20.1
Gofta	79.7	35.3	21.2
ICA Pijao	73.8	38.8	20.7
Ituri Matata	70.6	36.7	20.7
K131	69.0	33.8	19.8
Lingot Blanc	71.5	35.1	21.6
Maasai Red	71.3	37.3	21.4
MCM 2001	71.5	35.7	20.4
Mexican 142	74.6	37.3	21.8
Ranjonoby	63.8	33.8	20.6
Red Wolaita	70.6	36.0	21.5
Roba-1	78.5	34.6	21.1
Simama	73.8	34.1	19.9
TY 3396-12	73.8	33.8	20.1
Zebra	77.1	35.2	19.9
Mean	72.6	35.6	20.7
CV (%)	19.2	12.6	13.1

There were significant season, location, P level and genotypic effects on the seed protein. Seed protein concentration was higher during the short rain season (21.3%) compared with long rain season (20.1%). Seed protein was higher at Kabete (21.8%) compared to Thika (19.6%). Seed protein increased with levels of P application. The mean protein concentration across sites, season and genotypes was 19.9, 20.6 and 21.6% with 0, 50 and 100 kg P application, respectively. Mexican 142, Maasai Red and Lingot Blanc had the highest mean protein concentration (21.8 %) across sites and seasons. K131 and TY 3396-12 (both carioca grain type) had the lowest seed protein concentration (19.9%). Gofta had the highest seed protein concentration at Thika (20.2%).

These results suggest that application of P may be enhance the seed iron, zinc and protein concentration. However, the magnitude of the effect will vary with locations and seasonal factors.

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1.4.4 Identification of high mineral accessions in sister species of common bean

Rationale: Common bean, *Phaseolus vulgaris*, is one of five domesticated species in the genus Phaseolus, and is readily crossed with *P. coccineus* and *P. polyanthus*. Several years ago a core collection was designed to represent the eco-geographical variability in the sites of origin of the accessions of *P. coccineus* and *P. polyanthus* held in the CIAT gene bank. Core collections offer a rapid assessment of the potential of a species for any given trait about which little is known for that species.

Materials and Methods: The core collection of approximately 160 accessions was planted and harvested in Popayán, and evaluated for iron and zinc concentration. Promising accessions were planted again in Popayán for the evaluation of single plant harvest. Both species are allogamous and accessions are likely to be variable from plant to plant. The harvest of individual plants was likewise analyzed for iron and zinc.

Results and Discussion: In both the initial evaluation and in the validation based on individual plants, *P. polyanthus* tended to express higher levels of iron than *P. coccineus* (Table 36). Both species presented values higher than those normally found in *P. vulgaris*, although the values for the common bean checks, DOR 390 and CAL 96, were somewhat higher than normal. Although in the initial evaluation some high values of zinc were found, in the subsequent evaluation these fell within values that have been observed in better common bean lines.

Conclusion: While interspecific crosses are a long-term solution to any problem, in some cases they can offer significant genetic gain. It is important to verify how wide an advantage the sister species enjoy over the common bean to determine if interspecific crosses are warranted for the improvement of mineral content. However, both species could have value in and of themselves in moist highland areas where they would be expected to adapt.

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Table 36. Concentration of iron and zinc in accessions of *P. polyanthus* and *P. coccineus* in two successive evaluations of seed harvested in Popayán.

| | | | Core evaluation values (mg kg⁻¹) | | Individual plant values (mg kg⁻¹) | | | |
| | | | | | Fe | | Zn | |
G number	Origin	Species*	Fe	Zn	Ave	Range	Ave	Range
G 35155	GBR	COCC	82	50	76	69-99	36	24-44
G 35243	PRT	COCC	80	52	93	69-115	41	30-56
G 35382	MEX	COCC	86	52	83	73-124	35	30-43
G 35573	CRI	PLAN	99	52	121	107-134	38	37-38
G 35575	MEX	PLAN	95	54	111	90-142	41	35-44
G 35595	GTM	PLAN	94	50	104	78-131	40	28-49
G 35597	GTM	COCC	104	54	106	94-119	39	35-44
G 35623	YUG	COCC	66	52	96	86-110	49	38-55
G 35755	GTM	PLAN	114	50	92	70-131	46	41-54
G 35999	COL	COCC	87	63	103	71-128	42	32-48
G 36011	YUG	COCC	70	51	75	57-84	36	27-41
DOR 390	Check				74		37	
CAL 96	Check				80		32	

* COCC = *P. coccineus*; PLAN = *P. polyanthus*

Progress towards achieving output milestones:

- Mid-term milestones call for an increase of 50% iron concentration in bred materials. This level has been attained in several bred lines with a moderate level of drought tolerance but agronomic quality for other traits is still deficient. These materials will used to create another cycle of crosses, to improve both mineral concentration and agronomic value.

- Studies of G x E demonstrate that environmental and agronomic effects influence mineral content greatly, but that ranking of materials is similar in spite of G x E.