

Progress Report

Output 1: Improved small-seeded Middle American bean germplasm with less dependence on inputs

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

Highlights:

- Drought tolerant lines selected at CIAT-Palmira were tested in an international trial and the tolerance of several of these was confirmed.
- More than 160 crosses and 7500 F₁-derived F₂ families were evaluated for drought, identifying at least 15 elite crosses and 260 elite families.
- The superior performance of two bred lines (SEA 5 and A 801) and two landraces (Carioca and G 21212) under drought stress was shown to be associated with resistance to soil-borne pathogens combined with their ability to mobilize photosynthates to developing grain and to utilize the acquired N and P more efficiently for grain production.
- Field evaluation of recombinant inbred lines for drought resistance resulted in identification of several lines superior to their parents and this superior performance was associated with low content of seed phosphorus and high content of total nonstructural carbohydrates in seeds.
- Two bred lines (MAR 1 and MAM 46) and two landraces (G 92 and Carioca) were shown to be superior in their resistance to Al and this superior performance was related to greater concentrations of Ca and Mg in the shoot tissue.
- A reliable screening method was used to identify Calima and G 21212 as resistant landraces to toxic levels of manganese based on shoot growth and leaf expansion.
- Among the four genotypes (G 21212, VAX 2, “Negro Cotaxtla 91”, and MAM 38) that were superior in their tolerance to low P supply in soil, G 21212 was particularly outstanding in its ability to utilize phosphorus and nitrogen for grain production.

1.1.1 International testing of drought-tolerant selections

Rationale: Drought is one of the most widespread risks of agriculture and is estimated to affect perhaps 60% of bean production. Several regions of endemic poverty that are of particular interest for CIAT’s priorities are especially subject to water deficits, such as Central America and southern Africa. Thus, it is especially urgent to address this problem with a strategy that includes drought-tolerant bean varieties. As often occurs with physiological stress, drought stress presents interactions with other environmental factors such as soil depth and soil fertility. Thus, it is important to evaluate drought tolerance sources across environments to assure that the tolerance

is expressed in the appropriate environments. In the past, an international nursery had been evaluated and at least one material, BAT 477, presented some degree of tolerance in most environments. The present nursery was distributed to obtain data on materials identified more recently as having a degree of tolerance at CIAT-Palmira.

Materials and Methods: Last year we reported on the evaluation of breeding lines developed from drought-tolerant parents, and of red-seeded breeding lines with no previous history of selection for drought tolerance, and of inter-specific progeny from crosses of *Phaseolus vulgaris* and *P. acutifolius*. The best of these were identified for the international nursery. Thirty-six genotypes were included in the trial in a 6 x 6 lattice design. Trials were distributed to Haiti, Honduras (Zamorano), and Guatemala, and were planted at CIAT-Palmira.

Results and Discussion: Data have not been received from all sites as yet, nor are all data analyzed. A more complete report will be presented in the future, but preliminary results are worthy of comment in light of results with breeding in CIAT (see section 1.1.2.). In the trial planted in Honduras, the effect of local adaptation was evident, as the local checks “Milenio” and “EAP 9510-77” outperformed slightly (not significantly) the drought selections (coded as SEA lines), with the exception of SEA 23 (Table 1).

Table 1. Yields of selected entries in the International Drought Nursery at the Escuela Agrícola Panamericana (EAP), Zamorano, April 2001, Honduras.

Line or accession	EAP-Zamorano		CIAT-Palmira
	Irrigated yield (kg ha ⁻¹)	Drought yield (kg ha ⁻¹)	Drought yield (kg ha ⁻¹)
SEA 15	3023	639	999
SEA 18	2946	415	879
SEA 21	2710	618	648
SEA 23	2881	735	996
RAB 650	1949	111	1155
RAB 651	2352	69	961
G 21212	2533	508	503
G 40068 (<i>Phaseolus acutifolius</i>)	2375	1233	1272
G 40159 (<i>P. acutifolius</i>)	2421	1368	1264
SEA 5	2680	470	817
BAT 477	1829	336	801
Check 1 ^a	2365	764	437
Check 2 ^b	2886	732	508
Tío Canela (commercial)	2644	318	804
DOR 390 (commercial)	2960	195	442
Average	2392	382	677
LSD (<i>P</i> = 0.05)	717	315	273

a. Check 1 = “Milenio” at Zamorano, “ICA Pijao” at CIAT.

b. Check 2 = EAP 9510-77 at Zamorano, “ICA Quimbaya” at CIAT.

However, the ranking of the SEA lines was similar to that obtained at CIAT-Palmira. In particular, SEA 15 performed relatively well at both sites, and SEA 21 did well at Zamorano. Accession G 21212 yielded well at Zamorano, but slightly less than the SEA lines cited above. At Zamorano, both local checks and several SEA lines out yielded regional commercial varieties Tío

Canela (318 kg ha⁻¹) and DOR 390 (195 kg ha⁻¹), which are more representative of the level of tolerance that many farmers have in hand at present. In general, the data indicate a tendency for improvement in drought tolerance, in relation both to commercial checks and to the tolerant check, SEA 5. Accessions of *P. acutifolius* yielded significantly more than any line or accession of *P. vulgaris*, demonstrating the advantage that this species continues to display over *P. vulgaris*, and justifying our continued interest in interspecific crosses. The red-seeded breeding lines without directed selection for drought tolerance during their development did not perform well in Honduras, although RABs 650 and 651 yielded well at CIAT.

Conclusions: In general, there appears to be reasonably good correspondence between results in Honduras and previous experience at CIAT-Palmira with the drought-selected SEA lines. As data are analyzed from additional sites, we will determine if this conclusion holds more widely. Although preliminary, these results are useful in identifying the most promising parental materials for improvement of beans for Central America.

1.1.2 Development and testing of segregating populations combining drought tolerance and disease resistance in small red and small black 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 black-seeded grain type is required. In Africa, a more diverse range of grain types is acceptable, although BGYMV is not yet a problem. However, recessive resistance to bean common mosaic virus (BCMV) is highly desirable.

Materials and Methods: Based upon results at CIAT-Palmira, parental materials were identified among bred lines that had been coded as SEA lines. Emphasis was placed on lines that had grain color that was closer to commercial type (i.e., red- or black-seeded). As many as 15 parents were used as sources of tolerance (SEA lines 15, 16, 18, 20, 21, 22, and 24; G 21212; RAB lines 608, 609, 612, 619, 651, 623, and 630). These were combined with sources of resistance to BGYMV, common bacterial blight (CBB), necrotic BCMV strains, and tolerance to low soil fertility. Sources of heat tolerance obtained from the Escuela Agrícola Panamericana (EAP)-Zamorano were also included in the crosses. Between September and December 2000, the first simple crosses were made, and multiple crosses were executed between January and March 2001. The F₁ seeds of about 160 multiple crosses were planted in April 2001, and crosses that were variable for the *bgm-1* resistance gene were evaluated with the sequence characterized amplified region molecular markers (SCAR marker) as in previous years (see section 1.2.1 below). F₁ plants that carried the *bgm-1* marker were harvested individually to form about 7500 F₁-derived F₂ populations, using the gamete selection scheme.

Seeds of F₂ populations were planted in the first week of July, and were irrigated to germinate. A rain fell soon after and served to assure germination, and another irrigation was applied 3 weeks later. No additional water was applied and there was no significant rainfall for another 7 weeks, when the crop was in physiological maturity. Thus, the crop received a total of about 70 mm of water in the entire crop cycle. Populations were evaluated for vegetative vigor at flowering, pod load at about 2 weeks after flowering, and pod filling at maturity. After selection of families and seed harvest, seed was reviewed for grain quality and many families were discarded based on

poor grain filling. A tolerant check of SEA 5 was planted every 10 rows, and the respective commercial parental genotypes used in each cross were planted at the beginning of each cross.

Results and Discussion: During the crop cycle, daytime temperatures ranged from moderate (28 °C) on cloudy days to relatively high (35 °C) on clear days. Tensiometer readings taken in the sixth week after the last irrigation (i.e., in the ninth week of crop growth) registered more than 700 millibars of water tension in the soil, when normally irrigations are carried out when soil water tension reaches 230 millibars. Daily evapotranspiration potential reached as high as 10 mm per day on the hottest days.

Drought stress was especially severe in this season, to the degree that the tolerant check, SEA 5 (bred on-station at CIAT and considered one of the best materials for drought to date) presented severe drought symptoms in some plots (reduced vegetative development, ovule abortion, and delayed maturity). Among commercial parents, black-seeded DORs 390 and 500 were especially sensitive, in both pod load and grain filling. Red-seeded “Tío Canela” and MD 23-24 (“Bri-bri” in Costa Rica) presented reduced vegetative growth and delayed pod setting, but seed filling in the few pods that formed was quite good, especially in MD 23-24. “ICTA Ostua” was superior to the black-seeded DOR lines in pod setting, but inferior to the red-seeded varieties in grain filling. “ICTA Ligerito” escaped the worst effects of drought because of extreme earliness. The behavior of the commercial parental materials was reflected quite consistently in their progeny, and affected the quality of the respective populations.

Large differences were observed among crosses and among F₁-derived F₂ families within crosses. Drought tolerance was expressed as vegetative vigor, as the ability to set pods, and as the ability to fill pods and grain. A number of families appeared to be as good or better than the SEA 5 check. Despite severe drought stress in this season, many families produced uniformly well-filled seed, a trait that is especially important to maintain market quality of grain. Among the several parents that were used, purple-seeded SEA 15 followed by pink-seeded SEA 21 proved to be especially effective in transmitting drought tolerance to their progeny. It is highly significant that these were among the best of the SEA lines in the drought trial in Honduras. Black-seeded SEA 18 worked well in some combinations. RAB lines 609, 651, 623, and sometimes G 21212, gave good grain filling, although G 21212 did not function as well as hoped and in almost no case was it an adequate parent by itself. Among the commercial materials with BGYMV resistance, ICTA Ligerito, Tío Canela and MD 23-24, proved to be the best parents, as well as some of the heat-tolerant lines from EAP-Zamorano. Sources of the *bc-3* gene for resistance to BCMV did not combine well for drought tolerance in most cases.

The most successful SEA lines combine several drought-tolerant parents. SEA 15 is a progeny from cross SX 12293 {(SEA5 x Apetito) x [(BAT 477 x G 17341) x (XAN 309 x G 17722)]}. Its purple or “roxo” grain color reflects the influence of Apetito, a Mexican landrace, and combined relatively easily with commercial red and black colors. SEA 21 is derived from cross SX 12010 [(BAT 477 x G 17341) x (XAN 309 x G 17722)] and therefore is related to SEA 15. SEA 18 is derived from SX 12008 [(Sea 5 x Apetito) x (Sea 9 x N Durango)]. Apetito, G 17341, and Negro Durango are all from race Durango, and SEA 5 displays several Durango traits derived in turn from G 2618. Thus, Durango genes were well represented in these lines, which combine race Durango with race Mesoamerica parents such as BAT 477. Among the RAB lines that combined well, RABs 623 and 651 are derived from Nicaraguan landrace “Orgullosito”, which has been a

useful parent in the past, and the good performance of the RAB lines may reflect its influence. A commercial variety from Honduras, DICTA 122, proved to be relatively drought tolerant. DICTA 122 is resistant to *Apion godmani* and for 2 years was the highest yielding line in regional yield trials in Central America. This yield advantage might reflect drought tolerance in some degree. DICTA 122 also has a Durango parent in its pedigree.

In total, about 10% of the F₁-derived F₂ populations were selected. Fifteen multiple crosses proved to be especially tolerant, within which 270 populations were selected (Table 2). Another six simple crosses were identified as promising. Two hundred and seventy six populations were therefore selected for seed increase to the F₄ generation, to be shared with national programs and other collaborators in Central America in May 2002.

Table 2. The most successful crosses combining drought tolerance with disease resistance, and number of selected families among the total number of families in the cross.

Cross	No. families selected	Total no. families in cross
Principally black seeded:		
(FEB 192 x G 21212) x Ligero	11	21
(RJB 10 x Tío Canela) x (SEA 18 x (FEB 192 x G 21212))	21	62
(SEA15 x Ostua) x (Tío Canela x (FEB 192 x G 21212))	9	14
(SEA 15 x (A 774 x G 21212)) x (NEB 31 x (G 21212 x Ostua))	6	17
(TAR 4 x SEA 18) x Tío Canela	23	40
SEA 15 x Ligero	1	1
Principally red seeded:		
(RAB 619 x Tío Canela) x ((RAB 655 x G 21212) x SEA 21)	16	46
(RAB 651 x Tío Canela) x (RAB 608 x SEA 15)	27	45
(SEA 21 x RAB 623) x 9653-16B-1	51	96
(RAB 623 x MD 23-24) x (SEA 15 x (RAB 655 x G 21212))	6	12
(MD 23-24 x (TLP 35 x G 21212)) x (SEA 21 x RAB 612)	13	27
(SEA 15 x MD 23-24) x (Tío Canela x G 21212)	12	24
(SEA 21 x RAB 609) x DOR 582	41	95
(RAB 623 x DICTA 17) x (RAB 630 x SEA 21)	8	23
RAB 630 x SEA 21	1	1
SEA 15 x MD 23-24	1	1
SEA 21 x RAB 623	1	1
Tío Canela x SEA 21	1	1
SEA 22 x DOR 364	1	1
Red seeded, segregating resistance to <i>Apion godmani</i> :		
DICTA 122 x (RAB 651 x (VAX 1 x RAB 655))	17	89
DICTA 113 x (RAB 609 x DICTA 17)	5	109
DICTA 113 x (RAB 612 x (A 774 x G 21212))	4	46
Total	276	772

Conclusions: Although this was our first attempt to combine drought tolerance with other agronomic traits in commercial varieties, an apparently high level of tolerance was obtained in families that were also segregating acceptable grain type, and resistance to important diseases, especially BGYMV.

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1.1.3 Drought resistance

1.1.3.1 Identification of traits associated with drought resistance

Rationale: A set of 49 genotypes was evaluated for tolerance to low soil fertility conditions. Several of the genotypes included in this set of materials were known to be good performers under drought conditions. Last year we reported results from a field trial that evaluated genotypic differences in adaptation to drought stress among 49 genotypes. Based on the evaluation of these 49 genotypes for drought or low soil fertility conditions, a smaller set of 36 genotypes was assembled for evaluation to drought and low soil fertility conditions. This year we report results obtained from these 36 genotypes for their adaptation to drought stress. This group of 36 materials included several parents that were used to generate recombinant inbred lines (RILs). These RILs are particularly useful to identify quantitative trait locus (QTLs) involved in drought adaptation.

Materials and Methods: A field trial was conducted at Palmira in 2000 (June to September) to determine differences in tolerance to water stress conditions. The field trial included 36 bean genotypes. Details on planting and management of the trial were similar to those reported in CIAT (1998), p. 33-34. Experimental units consisted of four rows, 5 m long by 0.6 m wide. The incidence of *Macrophomina phaseolina* and *Sclerotium rolfsii* was recorded at physiological maturity. 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 ash content, and shoot total nonstructural carbohydrates (TNC). 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 also measured.

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 upper limit and -1.5 MPa lower limit for soil matric potential). During the crop-growing season, maximum air temperature was 29.6 °C and minimum air temperature was 18.8 °C, while incident solar radiation ranged from 9 to 24.5 MJ m⁻² d⁻¹. The total rainfall during the active crop growth was 125.6 mm (57.7mm of which fell during flowering and pod filling). The potential pan evaporation was 371.3 mm. These data on rainfall and pan evaporation indicated that the crop suffered a moderate 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 0 to 1235 kg ha⁻¹ (Figure 1). Among the genotypes tested, two bred lines (SEA 5 and A 801) and two landraces (Carioca and G 21212) were outstanding in their adaptation to water stress conditions. The relationship between grain yield of rainfed and irrigated treatments indicated that SEA 5, A 801, and Carioca were not only adapted to water stress, but also responsive to irrigation (Figure 2). SEA-5 was also found to be very well adapted to moderate and severe water stress conditions (CIAT 1998, p. 32-39; CIAT 1999, p. 27-29).

Among the 36 genotypes tested, AFR 699 and G 19842 were the most poorly adapted. Under rainfed conditions, AFR 699 completely failed to produce any grain.

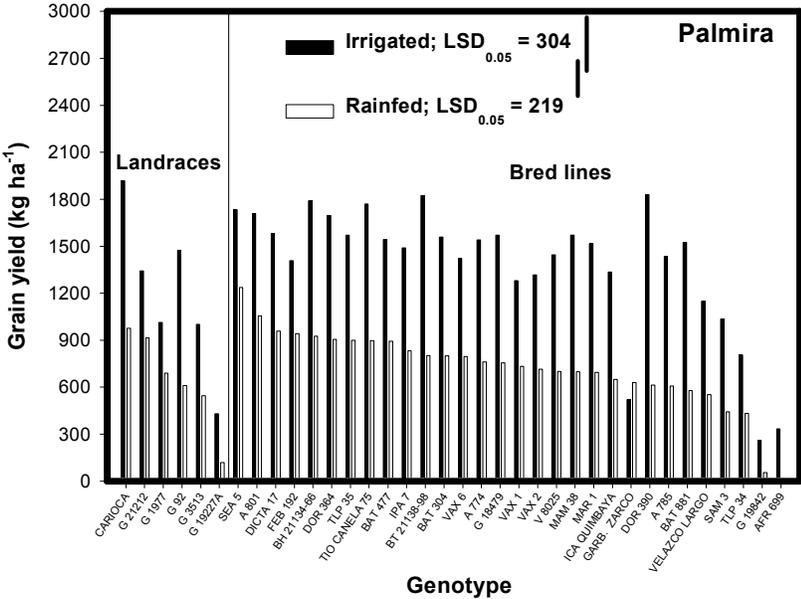


Figure 1. Genotypic variation in adaptation to rainfed and irrigated conditions of 36 common bean genotypes grown in a Mollisol at Palmira, Colombia.

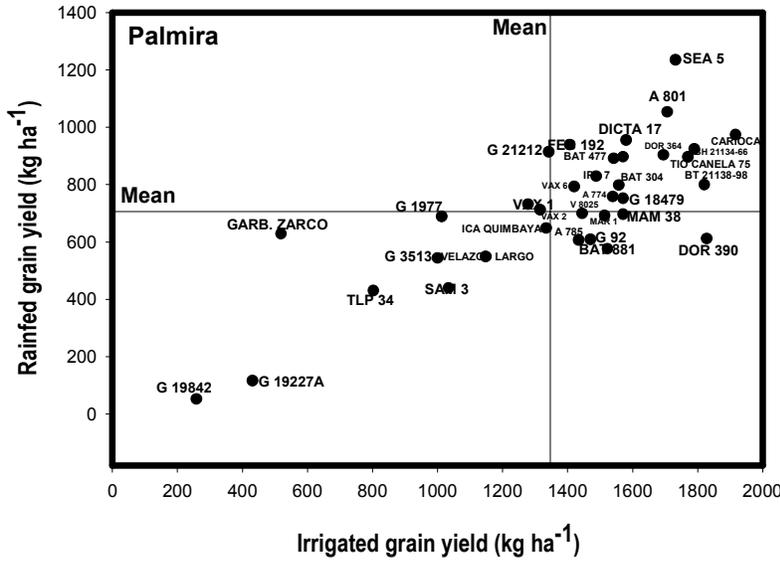


Figure 2. Identification of genotypes that are adapted to rainfed conditions and are responsive to irrigation to a Mollisol at Palmira, Colombia. Genotypes that yielded superior with drought and were also responsive to irrigation were identified in the upper box of the right hand side.

Several genotypes that yielded well under rainfed conditions were also found to be less affected by the incidence of soil-borne pathogens (*Macrophomina phaseolina* and *Sclerotium rolfsii*) as determined by the percentage of infected plants (Figure 3). Among the well-adapted genotypes, G 21212 and SEA-5 were particularly less affected by the incidence of soil-borne pathogens. Three out of 36 genotypes including DOR 390, FEB 192, and MAR 1 showed greater sensitivity to soil-borne pathogens. Two bred lines, FEB 192 and BAT 477, yielded well under rainfed conditions even with relatively higher incidence of soil-borne pathogens.

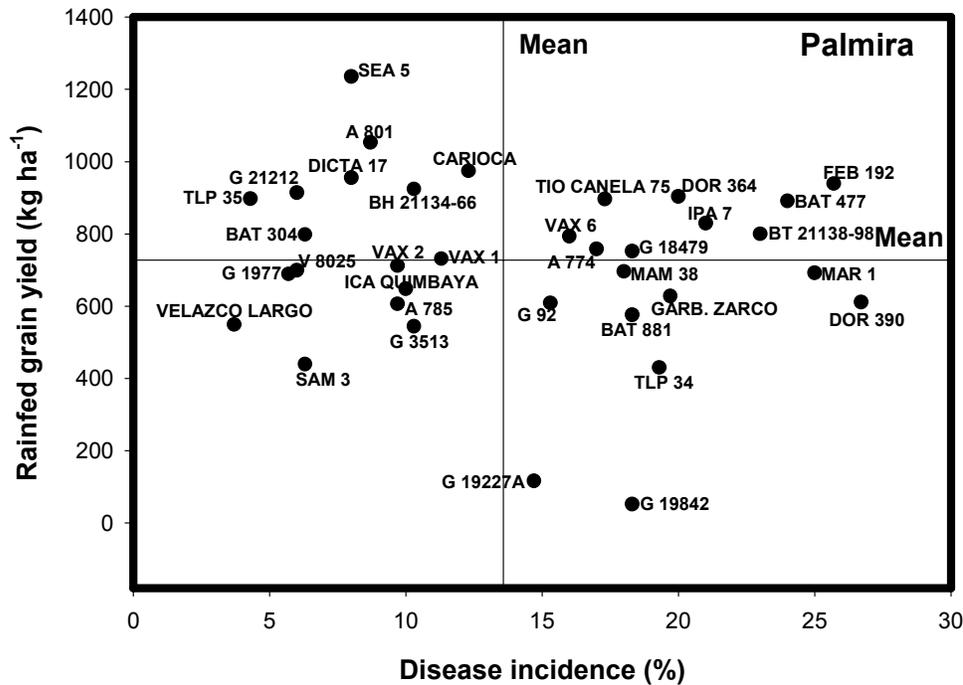


Figure 3. Identification of genotypes that are adapted to rainfed conditions and are less sensitive to soil-borne pathogens in a Mollisol at Palmira. Genotypes that yielded superior with drought and were less sensitive to soil-borne pathogens (*Macrophomina phaseolina* and *Sclerotium rolfsii*) were identified in the upper box of the left hand side.

Both under rainfed and irrigated conditions, grain yield was not related to leaf area index (Table 3). Several genotypes had greater leaf area values than the best performers such as SEA 5, Carioca, and G 21212 under rainfed conditions (Figure 4). The superior performance of these genotypes could be attributed to better transport system for mobilizing photosynthates to developing grains.

Table 3. Correlation coefficients (r) between final grain yield (kg ha^{-1}) and other plant attributes of 49 genotypes of common bean grown under rainfed and irrigated conditions in a Mollisol at Palmira, Colombia.

Plant traits ^a	Rainfed ^b	Irrigated ^b
Leaf area index	-0.07	0.03
Shoot biomass	0.32***	0.32***
Shoot N uptake	0.39***	0.23*
Shoot P uptake	0.30**	0.14
Shoot K uptake	0.29**	0.18
Shoot Ca uptake	-0.02	0.24*
Shoot Mg uptake	0.21*	0.28**
Shoot TNC content	0.12	0.11
Shoot ash content	0.01	-0.25*
Seed N content	-0.34***	-0.38***
Seed P content	-0.33***	-0.18*
Seed K content	-0.22*	-0.53***
Seed TNC content	0.08	0.17
Seed ash content	-0.01	0.11

- a. TNC = total nonstructural carbohydrates.
 b. * = significant at $P = 0.05$, ** at $P = 0.01$, and *** at $P = 0.001$.

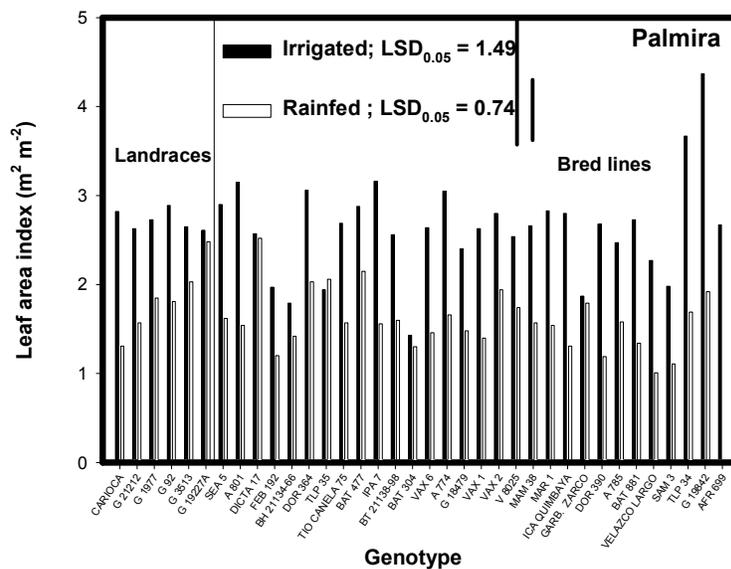


Figure 4. Genotypic variation in leaf area index of 36 genotypes of common bean grown in a Mollisol at Palmira, Colombia.

We found that the superior performance of SEA 5 could be related to lower seed P content (CIAT 2001, p. 12-13). We tested this relationship further by measuring seed nutrient (N, P, K, Ca, and Mg) content and seed ash (total mineral) content. There was no relationship between rainfed grain yield and seed ash content (Table 3). But the landrace Carioca and the bred line A 801 were outstanding in combining low seed ash content with high grain yield under water stress conditions (Figure 5). The seed ash content of the bred line SEA 5 was intermediate, while with Tío Canela 75 it was very high. Thus, similar to the results reported last year, seed ash may not be a very useful indicator of water stress resistance in common bean (Table 3). Nevertheless, seed P content showed significant negative relationship with grain yield under water stress conditions (Table 3). Several superior performers combined high seed yield with lower seed P content (Figure 6).

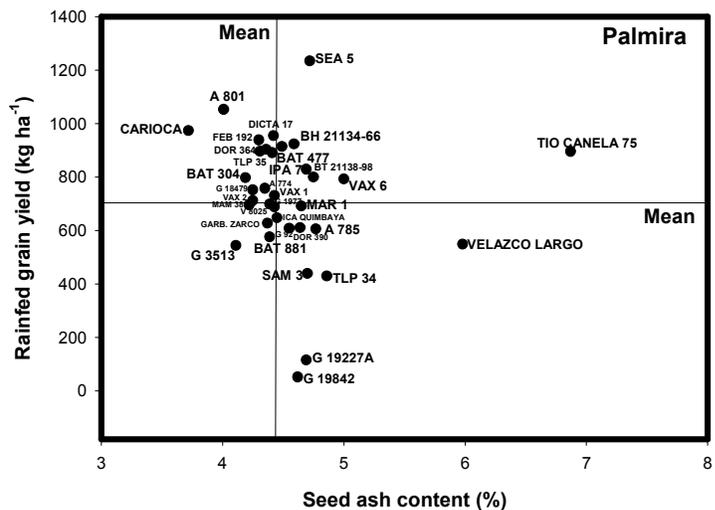


Figure 5. Identification of genotypes that combine superior seed yield with lower ash (mineral) content in seed when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Genotypes that were superior in grain yield and lower in seed ash were identified in the upper box of the left hand side.

Relationship between seed N (protein) and grain yield under rainfed conditions indicated that BAT 477 was superior in combining greater seed N content with high seed yield (Figure 7). It is important to note that SEA 5 showed moderate level of seed N. Under rainfed conditions, grain yield was greater and shoot TNC content at mid podfilling was also greater for SEA 5 and G 21212 (Figure 8). It appears that these two genotypes could mobilize photosynthates better than the other genotypes tested (Figure 9). One bred line, A 801, showed very high level of seed TNC combined with greater yield under rainfed conditions (Figure 9). Use of this bred line in the breeding program could contribute to greater yield potential under rainfed conditions. The superior adaptation of two bred lines, A 801 and BH 21134-66, to drought was found to be because of their efficient utilization of N and P for grain production (Figure 10) in addition to the mobilization of photosynthates.

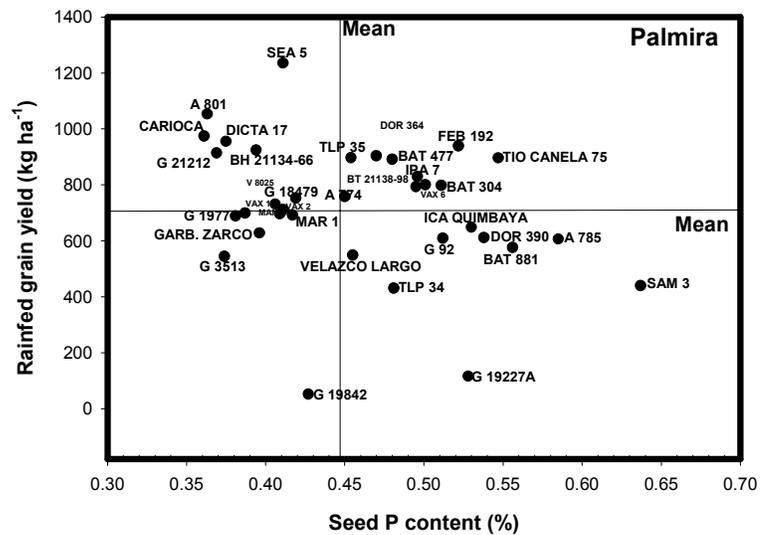


Figure 6. Identification of genotypes that combine superior seed yield with lower P content in seed when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Genotypes that were superior in grain yield and lower in seed P were identified in the upper box of the left hand side.

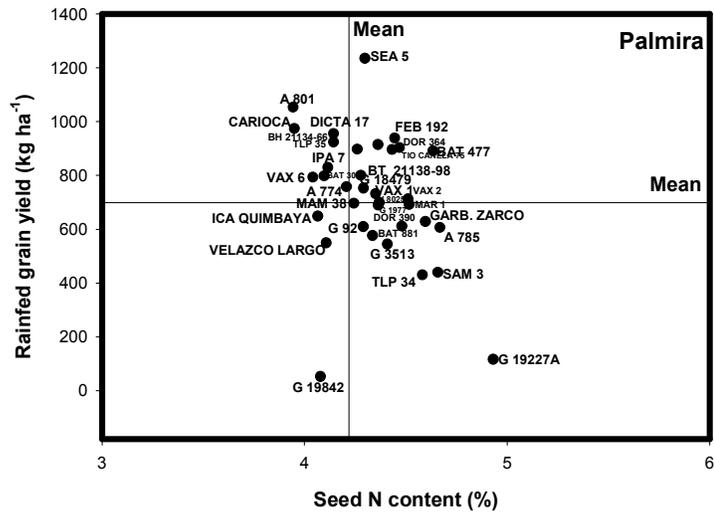


Figure 7. Identification of genotypes that combine superior seed yield with greater N content in seed when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Genotypes that were superior in grain yield and higher in seed N were identified in the upper box of the right hand side.

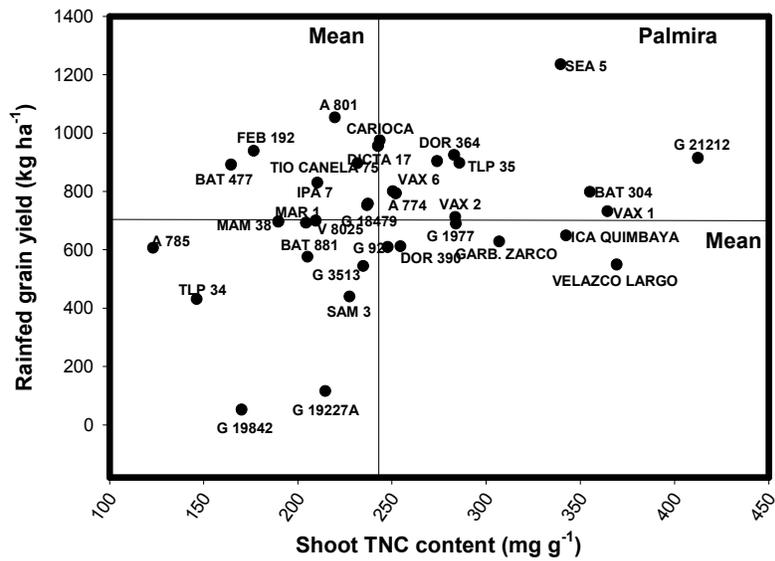


Figure 8. Identification of genotypes that combine superior seed yield with greater amount of total nonstructural carbohydrates (TNC) in the shoot when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Genotypes that were superior in grain yield and higher in shoot TNC were identified in the upper box of the right hand side.

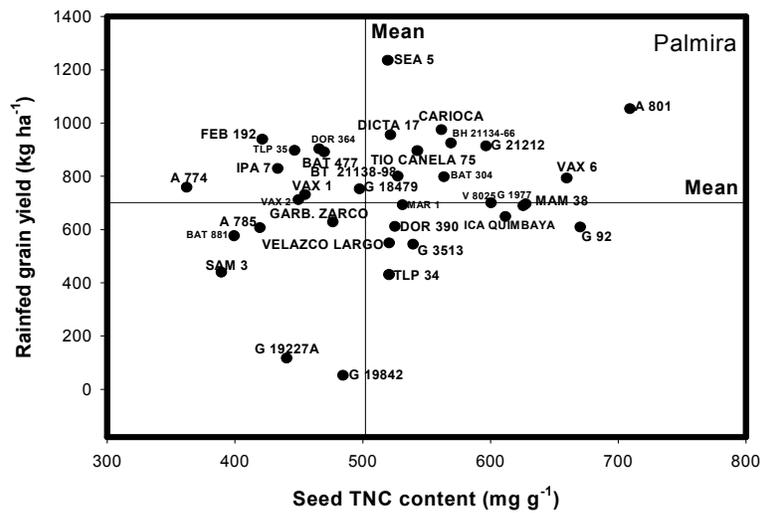


Figure 9. Identification of genotypes that combine superior seed yield with greater amount of total nonstructural carbohydrates (TNC) in the seed when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Genotypes that were superior in grain yield and higher in seed TNC were identified in the upper box of the right hand side.

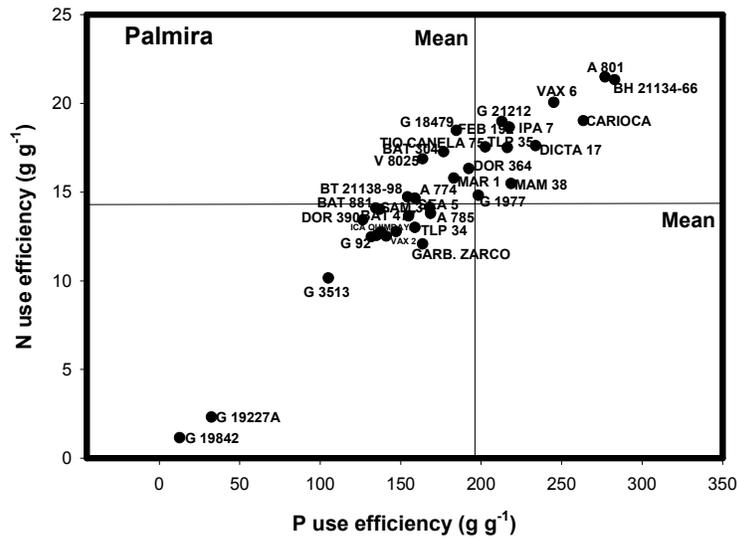


Figure 10. Identification of genotypes that are efficient in the utilization of N and P to produce greater seed yield when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Genotypes that were superior in both N and P use efficiency were identified in the upper box of the right hand side.

Correlation coefficients between final grain yield and other shoot attributes indicated that leaf area production was not related to yield under both rainfed and irrigated conditions (Table 3). This observation is in contrast with the previous observations for rainfed conditions. The lack of relationship is because of lack of climatic adaptation of certain genotypes that produced a lot of leaf area, but not enough grain. However, shoot biomass production was found to be highly related to grain yield under both rainfed and irrigated conditions. Shoot nutrient uptake, particularly N uptake, was highly related to seed yield with rainfed conditions (Table 3). 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. There was no relationship between grain yield and seed ash or TNC content both under rainfed and irrigated conditions.

Conclusions: This field study indicated that two bred lines (SEA 5 and A 801) and two landraces (Carioca and G 21212) were very well adapted to drought stress. The superior performance of these four genotypes under drought was associated with resistance to soil-borne pathogens combined with their ability to mobilize photosynthates to developing grain and to utilize the acquired N and P more efficiently for grain production. This study also provided further evidence for the usefulness of lower level of seed P as a selection method in addition to grain yield for identifying bean genotypes that are better adapted to drought.

References:

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CIAT. 1999. Bean Project Annual Report 1998. CIAT, Cali, CO. 202 p. (Working doc. no. 179)
CIAT. 2001. Annual Report 2001 Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

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1.1.3.2 Development and screening of lines for drought resistance

Rationale: Several years of field evaluation for drought resistance resulted in the identification of a number of elite germplasm accessions and landraces. One germplasm accession, G 21212, showed adaptation to low P soils and to drought. This landrace was crossed with a bred line, BAT 881, which showed sensitivity to low P soils and drought. Recombinant inbred lines were generated for genetic studies and for eventual marking of drought resistance genes with DNA markers. We used these RILs to test the usefulness of seed P content as one of the selection parameters for improved drought adaptation.

Materials and Methods: A field trial was conducted at Palmira in 2000 (June to September) to determine differences among RILs of the cross G 21212 x BAT 881 for their tolerance to drought stress. The field trial included 100 RILs along with the two parents. Details on planting and management of the trial were similar to those reported in activity 1.1.3.1. A number of plant attributes, including shoot TNC, seed N, P, and ash content, were measured at mid podfilling. Seed yield was measured at maturity.

Results and Discussion: Several lines were identified that out-yielded the parents under rainfed conditions (Figure 11). The relationship between rainfed grain yield and shoot TNC indicated that several lines were superior in adaptation to drought and maintained greater amounts of TNC in the shoot (Figure 12). These lines may take advantage of late rains to yield better by mobilizing photosynthates to grain. Several lines that showed greater adaptation to drought also showed lower amounts of seed ash content, but several lines also showed lower seed yield with lower seed ash content (Figure 13). Thus, seed ash content alone may not be very useful as a selection parameter for assessing drought adaptation. In contrast to seed ash content, lower amounts of seed N and seed P showed greater relationship with seed yield under rainfed conditions (Figures 14 and 15). It is important to note that several RILs showed greater seed yield under rainfed conditions combined with greater level of seed N (protein). The relationship between seed P and rainfed grain yield indicates that the two parents used (G 21212 and BAT 881) had the contrast in seed P content and several RILs were identified with lower seed P and greater grain yield than either of the two parents.

Correlation coefficients between final grain yield and other shoot attributes indicated that shoot biomass production was highly related to yield under both rainfed and irrigated conditions (Table 4). Superior performance under rainfed conditions was associated with greater amounts of seed TNC content. Significant negative relationship was also observed between seed yield and seed P content under rainfed conditions. This observation indicates that the superior performers mobilized greater amounts of photosynthates to seed per unit amount of P in the seed. There was no relationship between grain yield and seed ash content under rainfed conditions. A positive

association of seed ash content with seed yield under irrigated conditions indicated that the superior performers mobilized greater amounts of minerals to grain.

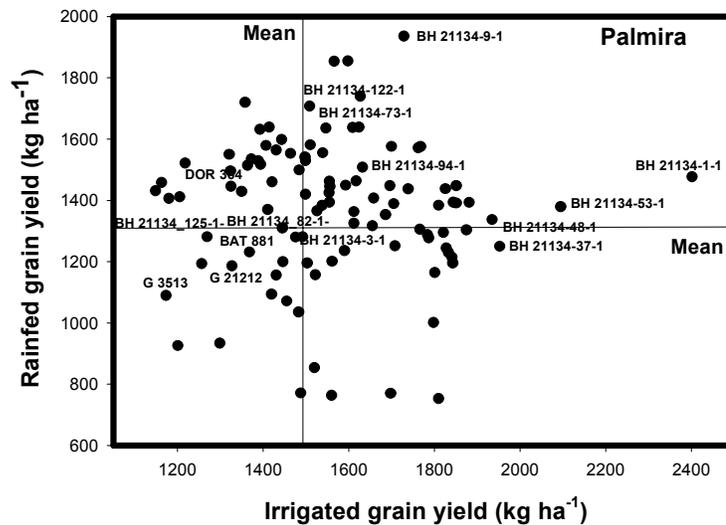


Figure 11. Identification of recombinant inbred lines (RILs) that are adapted to rainfed conditions and are responsive to irrigation to a Mollisol at Palmira, Colombia. Those RILs that yielded superior with drought and were also responsive to irrigation were identified in the upper box of the right hand side.

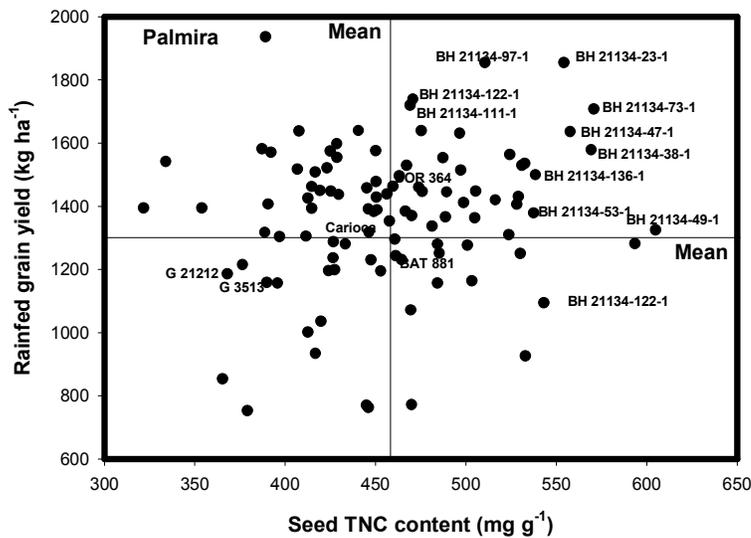


Figure 12. Identification of recombinant inbred lines (RILs) that combine superior seed yield with greater amount of total nonstructural carbohydrates (TNC) in the shoot when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Those RILs that were superior in grain yield and higher in shoot TNC were identified in the upper box of the right hand side.

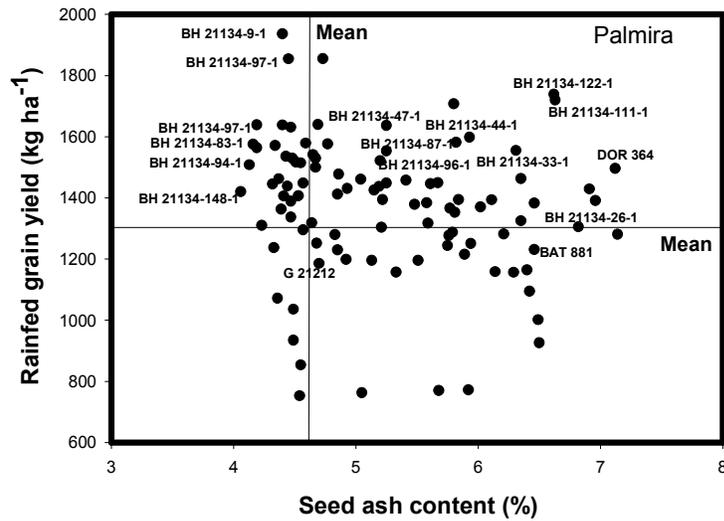


Figure 13. Identification of recombinant inbred lines (RILs) that combine superior seed yield with lower ash (mineral) content in seed when grown under rainfed conditions in a Mollisol at Palmira. Genotypes that were superior in grain yield and lower in seed ash were identified in the upper box of the left hand side.

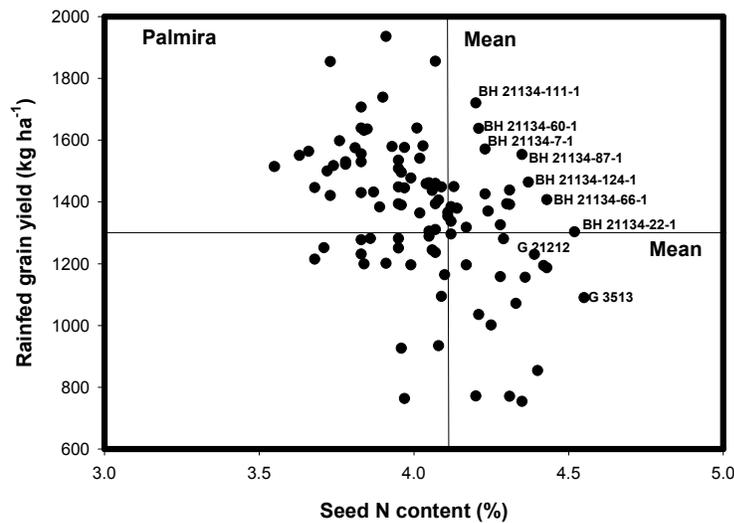


Figure 14. Identification of recombinant inbred lines (RILs) that combine superior seed yield with greater N content in seed when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Those RILs that were superior in grain yield and higher in seed N were identified in the upper box of the right hand side.

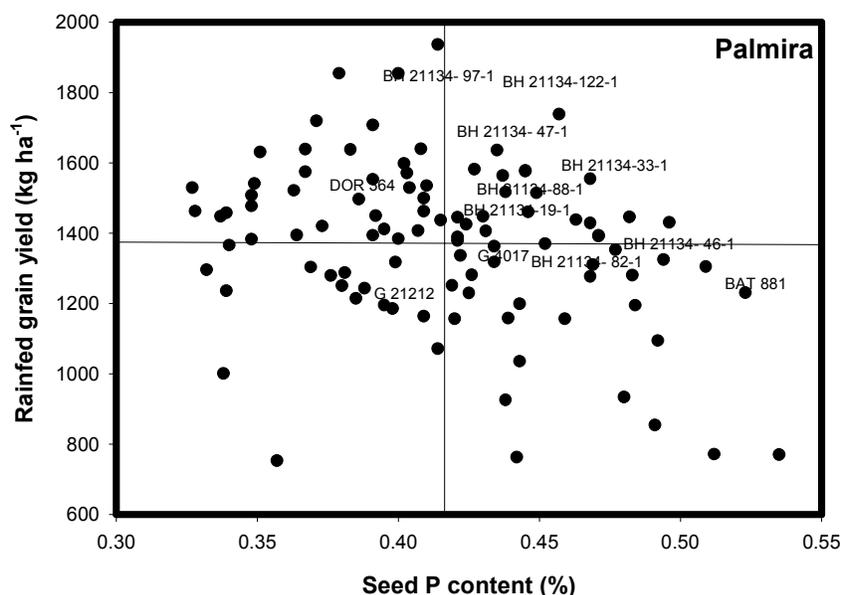


Figure 15. Identification of recombinant inbred lines (RILs) that combine superior seed yield with lower P content in seed when grown under rainfed conditions in a Mollisol at Palmira, Colombia. Those RILs that were superior in grain yield and lower in seed P were identified in the upper box of the left hand side.

Table 4. Correlation coefficients (r) between final grain yield and other plant attributes of recombinant inbred lines of common bean grown under rainfed and irrigated conditions in a Mollisol at Palmira, Colombia.

Plant traits ^a	Rainfed ^b	Irrigated ^b
Shoot biomass	0.30***	0.49***
Seed N content	-0.06	0.15**
Seed P content	-0.11*	-0.06
Seed K content	-0.09	0.16**
Seed TNC content	0.17**	0.09
Seed ash content	0.05	0.12*

a. TNC = Total nonstructural carbohydrates.

b. * = significant at $P = 0.05$, ** at $P = 0.01$, and *** at $P = 0.001$.

Conclusions: This field study indicates that seed P content may be an additional plant trait for selection of advanced lines that are adapted to drought conditions. Further work is needed to identify QTLs linked to seed P content.

Contributors: I.M. Rao, S. Beebe, J. Ricaurte, H. Terán, R. García

1.1.4 Identification of traits associated with aluminum resistance

Rationale: As part of a restricted core project funded by the Federal Ministry for Economic Cooperation and Development (BMZ) and the German Agency for Technical Cooperation (GTZ), field studies were continued at Santander de Quilichao (990 m, Oxisol – Plinthic Kandiodox) to identify Al-resistant genotypes. Toxicity of Al in subsoils is a serious problem and amending subsoils with lime is both difficult and prohibitively expensive for resource-poor farmers. Last year, we reported results from a field evaluation of 49 genotypes for resistance to Al-toxic soils. We repeated the evaluation of the same 49 genotypes. Genotypic differences in seed yield in Al-toxic soils could be related to differences in resistance to Al, acquisition of nutrients, and utilization of nutrients for transport of photoassimilates to developing seeds.

Materials and Methods: *Santander de Quilichao* – A set of 49 genotypes including germplasm accessions and bred lines was evaluated in the field for identification of plant attributes for adaptation to infertile acid soil conditions. Two levels of fertilizer (high and nil) input were applied. Plots with high fertilizer input (HFI) received banded application of P (40 kg ha⁻¹) in the form of triple super phosphate and foliar application (twice) of urea (1 kg ha⁻¹). Plots with no fertilizer input (NFI) received no application of nutrients. Soil characterization data of NFI plots showed toxic levels of exchangeable Al and Mn and low availability of Ca and Mg, while P availability was more than adequate for plant growth and development (CIAT 2001, p. 23-29).

A number of plant attributes were measured at mid podfilling in order to determine genotypic variation in tolerance to toxic level of Al in soil. These plant traits included leaf area index, canopy dry weight per plant, and shoot nutrient (N, P, K, Ca, and Mg) uptake. 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 and P contents were also determined.

Results and Discussion: Among the 49 genotypes tested, two landraces (G 92 and Carioca) and two bred lines (MAR 1 and MAM 46) were found to be outstanding in their adaptation to Al-toxic soil conditions (Figure 16). With NFI treatment, grain yield ranged from 0 to 480 kg ha⁻¹, while with HFI the range was from 38 to 1773 kg ha⁻¹. Grain yield of bred lines in general was greater than that of landraces (Figure 16).

Relationship between grain yield with NFI and grain yield with HFI indicated that MAR 1 and MAM 46 were better adapted to both low and high input of fertilizer (Figure 17). The superior performance of MAM 46 is in contrast with the results reported last year. Several bred lines (VAX 1, A 774, A 321, and FEB 190) responded to HFI treatment. The superior performance of MAR 1 and VAX 1 with NFI treatment was associated with greater Ca content in shoot tissue (Figure 18). Shoot Mg content of MAR 1 was also greater with NFI treatment (Figure 19). In contrast with shoot Ca and Mg content, shoot K content of MAR 1 was moderate with NFI treatment (Figure 20). The superior performers, MAR 1 and MAM 46, also showed lower seed N (protein) compared with other genotypes (Figure 21). Seed N content of SEA 5 and G 21212 was markedly superior to that of MAR 1 and MAM 46. None of the 49 genotypes tested showed a combination of greater grain yield with greater seed N with NFI treatment.

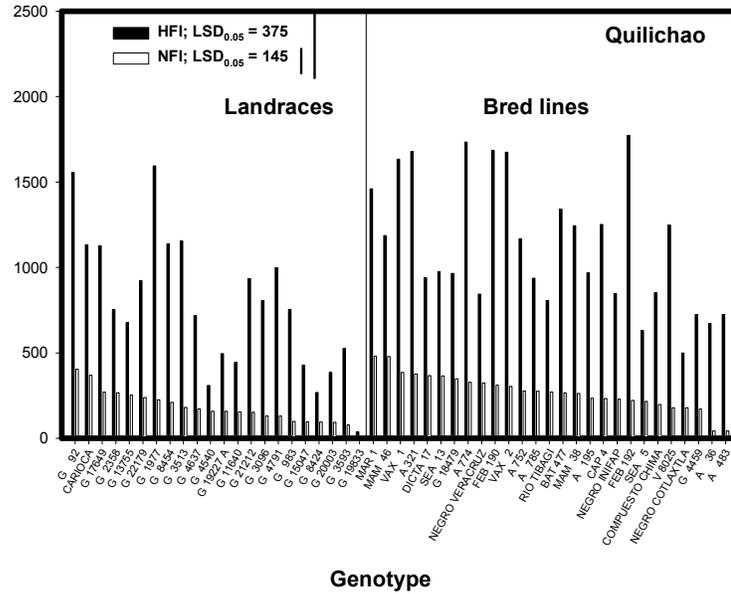


Figure 16. Genotypic variation in adaptation to Al-toxic soil at Santander de Quilichao, Colombia. No fertilizer input (NFI); high fertilizer input (HFI).

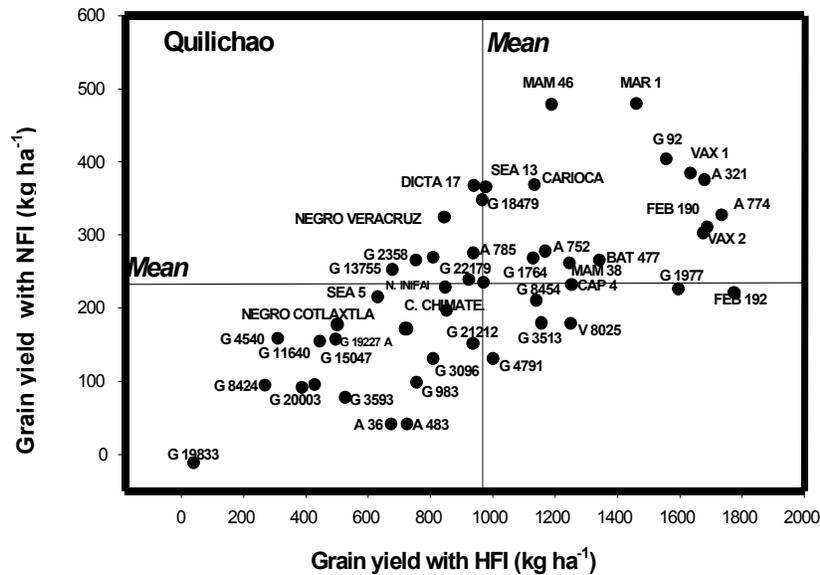


Figure 17. Identification of genotypes that are adapted to Al-toxic soil and are responsive to application of lime and P inputs to an Oxisol at Santander de Quilichao, Colombia. Genotypes that yielded superior with no fertilizer inputs (NFI) and were also responsive to application of high fertilizer inputs (HFI) were identified in the upper box of the right hand side.

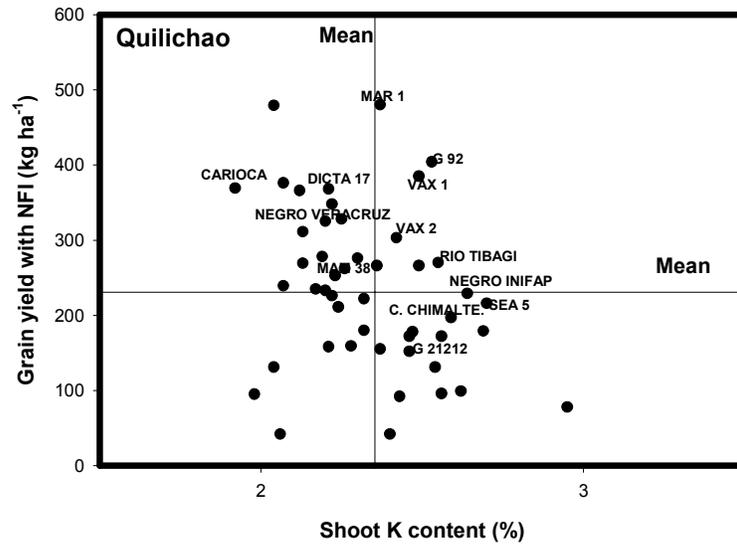


Figure 20. Identification of genotypes that combine superior seed yield with greater K content in the shoot tissue when grown with no fertilizer input (NFI) to an Oxisol at Santander de Quilichao, Colombia. Genotypes that were superior in grain yield and shoot K content were identified in the upper box of the right hand side.

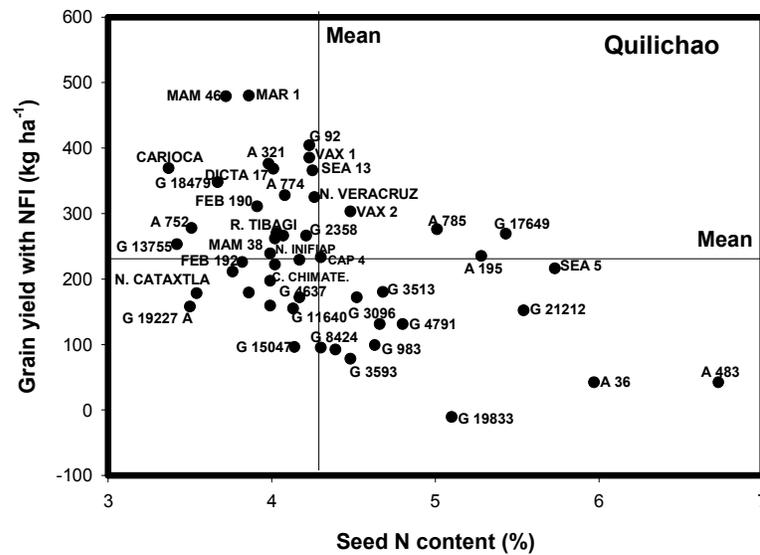


Figure 21. Identification of genotypes that combine superior seed yield with greater N content in seed when grown with no fertilizer input (NFI) to an Oxisol at Santander de Quilichao, Colombia. Genotypes that were superior in grain yield and seed N were identified in the upper box of the right hand side.

Correlation coefficients between final grain yield and other plant attributes indicated that stem and pod biomass production, shoot TNC content, and seed ash content were highly related to seed yield with NFI treatment (Table 5). Seed yield showed strong negative relationship with shoot N and Al concentration, and seed N content with NFI treatment. Significant negative relationship was also observed between seed yield and seed N content for both treatments (NFI and HFI) indicating that greater N use efficiency could contribute not only for superior adaptation to Al-toxic soil conditions, but also for responsiveness to high fertilizer input.

Table 5. Correlation coefficients (*r*) between final grain yield and other plant attributes at mid podfilling of 49 genotypes of common bean grown with no fertilizer input (NFI) or high fertilizer input (HFI) to an Oxisol at Santander de Quilichao, Colombia.

Plant traits ^a	NFI ^b	HFI ^b
Stem biomass	0.62***	0.51***
Pod biomass	0.61***	0.42***
Shoot N content	-0.49***	0.01
Shoot P content	-0.13	-0.29***
Shoot K content	-0.22**	0.16
Shoot Ca content	-0.16	0.14
Shoot Mg content	0.03	-0.04
Shoot Al content	-0.29***	-0.21*
Shoot TNC content	0.27***	0.09
Seed N content	-0.44***	-0.28***
Seed P content	-0.13	-0.35***
Seed ash content	0.35***	0.22

a. TNC = Total nonstructural carbohydrates.

b. * = significant at $P = 0.05$, ** at $P = 0.01$, and *** at $P = 0.001$.

Conclusions: Results from this field study at Santander de Quilichao indicate that two bred lines (MAR 1 and MAM 46) and two germplasm accessions (G 92 and Carioca) are superior in their resistance to Al, and this superior performance was related to greater concentrations of Ca and Mg in the shoot tissue. Specific physiological and biochemical mechanisms contributing to Al resistance need to be investigated.

Reference:

CIAT. 2001. Annual Report 2000 Project IP-1. CIAT, Cali, CO. (Working doc. no. 186)

Contributors: I.M. Rao, S. Beebe, J. Ricaurte, J. Manuel Osorno, H. Terán, R. García

1.1.5 Identification of genotypes resistant to toxic level of manganese

Rationale: Manganese (Mn) toxicity is an important constraint to bean productivity in tropical soils. Amending soils with lime is often not feasible for resource-poor farmers. Genetic variation in tolerance to toxic levels of Mn exists in bean germplasm. Previous research indicated that screening of genotypes in solution culture is useful to identify sources of tolerance to Mn toxicity

(Gonzalez and Lynch, 1999a). We used solution culture technique to identify bean genotypes with Mn resistance.

Materials and Methods: Thirteen genotypes were evaluated in the greenhouse at CIAT-Palmira using solution culture technique (Gonzalez and Lynch, 1999a). Nine-day-old seedlings were transplanted to 12-L plastic tanks containing nutrient solution with the following composition ($\mu\text{mol L}^{-1}$): 2000 K; 4300 NO_3 ; 1000 NH_4 ; 1200 Ca; 1000 Mg; 200 PO_4 ; 1600 SO_4 ; 20 Fe-EDTA, 1.5 Zn; 1.5 Mn; 0.5 Cu; 0.5 B; and 0.14 Mo. Manganese was applied as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ 4 days after transplanting to a final concentration of either 1.5 μM (adequate) or 125 μM (toxic level). Each tank held three plants each of three genotypes. Each treatment was replicated three times in each experiment. The experiment was replicated twice in a greenhouse. Solution pH was daily adjusted to 5.5 using KOH. The nutrient solution was replaced twice during the week. Plants were harvested 11 days after Mn treatment was initiated. At harvest, dry matter distribution into leaves and stems, leaf area, and leaf Mn content per unit dry weight were recorded.

Results and Discussion: Among the 13 genotypes tested, six showed greater level of Mn resistance than the widely used cultivar, Carioca (Figure 22). Among the six genotypes, Calima and G 21212 showed greater level of Mn resistance based on shoot dry weight, leaf dry weight, and stem dry weight. Leaf expansion of Calima, G 21212, and G 3513 was not affected by Mn toxicity, while it was markedly reduced with sensitive genotypes (Figure 23). The degree of sensitivity found with VAX 1 indicates that this bred line, although well adapted to Al-toxic soil conditions at Santander de Quilichao, may have problems dealing with toxic levels of Mn. The percentage inhibition of leaf expansion may serve as a useful selection parameter for Mn sensitivity. As expected, high Mn treatment markedly increased leaf Mn content, but the extent of increase was similar for both resistant and sensitive genotypes (Figure 23). Therefore, tissue Mn content is not a useful selection parameter. The greater resistance of the cultivar Calima to toxic level of Mn was found to be related compartmentation of Mn in epidermal cells of mature and immature leaves (Gonzalez and Lynch, 1999b).

Conclusions: Results from this glasshouse study indicate that the screening method used is reliable for identifying sources of Mn resistance and the method is suitable to screen RILs to identify QTLs for resistance to Mn in common bean.

References:

- Gonzalez, A.; Lynch, J. 1999a. Tolerance of tropical common bean genotypes to manganese toxicity: Performance under different growing conditions. *J Plant Nutr* 22: 511-525.
- Gonzalez, A.; Lynch, J. 1999b. Subcellular and tissue Mn compartmentation in bean leaves under Mn toxicity stress. *Aust J Plant Physiol* 26: 811-822.

Contributors: I.M. Rao, S. Beebe, J. Ricaurte, R. García

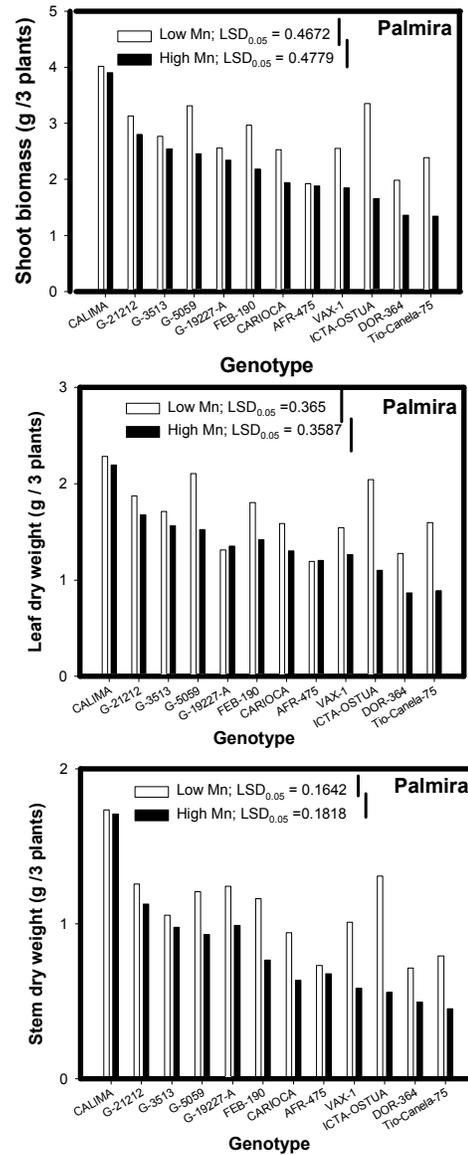


Figure 22. Genotypic variation among 13 common bean genotypes in shoot biomass, leaf dry weight, and stem dry weight as influenced by low and high levels of Mn in nutrient solution. Plants were exposed to Mn stress for 11 days under greenhouse conditions at Palmira, Colombia.

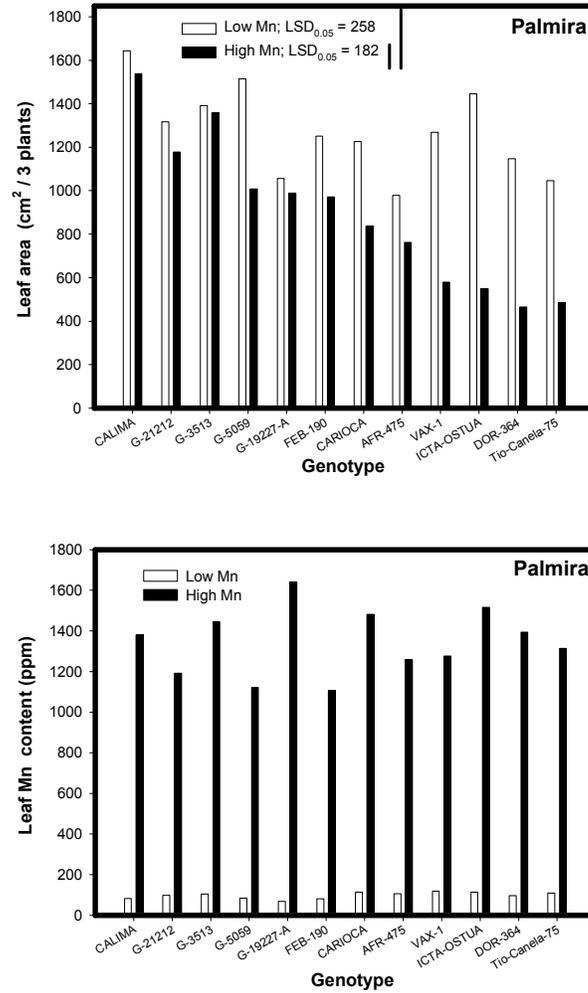


Figure 23. Genotypic variation among 13 common bean genotypes in leaf area production and leaf Mn content per unit leaf dry weight as influenced by low and high levels of Mn in nutrient solution. Plants were exposed to Mn stress for 11 days under greenhouse conditions at Palmira, Colombia.

1.1.6 Identification of traits associated with phosphorus efficiency

Rationale: Last year, we reported results from a field evaluation of 49 genotypes for tolerance to low nutrient supply, particularly to low phosphorus and micronutrients from a site at Popayán (1750 m, Inceptisol – Typic Dystropept). The same set of 49 genotypes were tested with very low P supply at a field site at Darién for further studies.

Materials and Methods: Darién – The trial at Santander de Quilichao was duplicated at Darién with the same genotypes at low and high P supply. Details on P treatments and measurements were described before (CIAT, 1998; p 18-28). A number of plant attributes were measured at mid podfilling in order to determine genotypic variation in tolerance to low P supply in soil. These plant traits included leaf area index, canopy dry weight per plant, shoot P uptake, and shoot TNC. At the time of harvest, grain yield and yield components (number of pods per plant, number of seeds per pod, and 100 seed weight) were determined. Seed N and P contents and seed TNC were also determined.

Results and Discussion: Among the 49 genotypes tested, four (G 21212, VAX 2, ‘Negro Cotaxtla 91’, and MAM 38) were outstanding not only in their tolerance to low P supply in soil, but also in their responsiveness to high P supply (Figures 24 and 25). With low P supply, grain yield ranged from 368 to 1198 kg ha⁻¹, while with high P supply the range was from 899 to 3522 kg ha⁻¹. With low P treatment, grain yield of G 21212 was 2.4-fold greater than that of a widely adapted genotype, Carioca. The highest yield of 3522 kg ha⁻¹ with high P application was obtained with the bred line A 774. Several landraces and bred lines were superior to Carioca in their adaptation to low P supply.

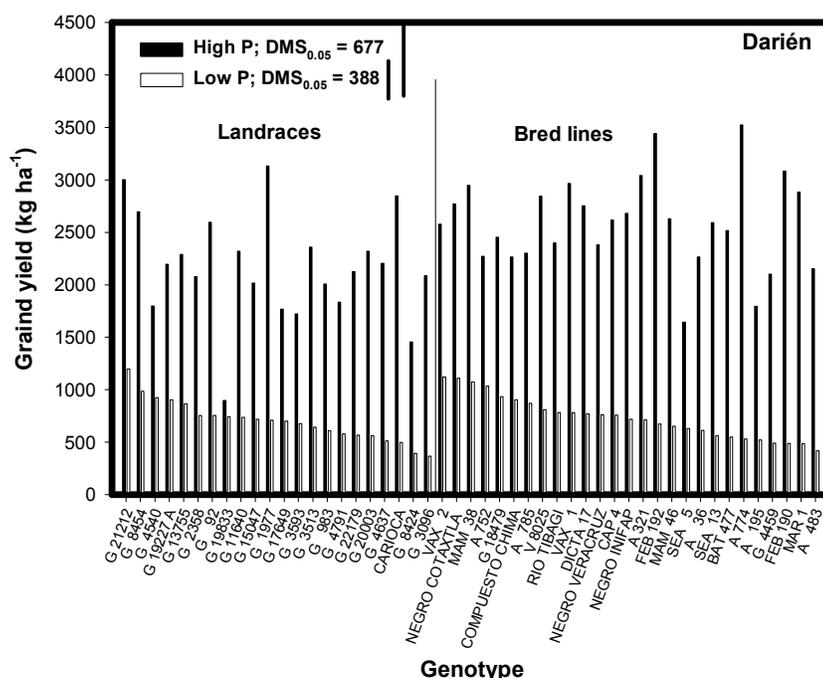


Figure 24. Genotypic variation in adaptation to low phosphorus supply in an Andisol at Darién, Colombia.

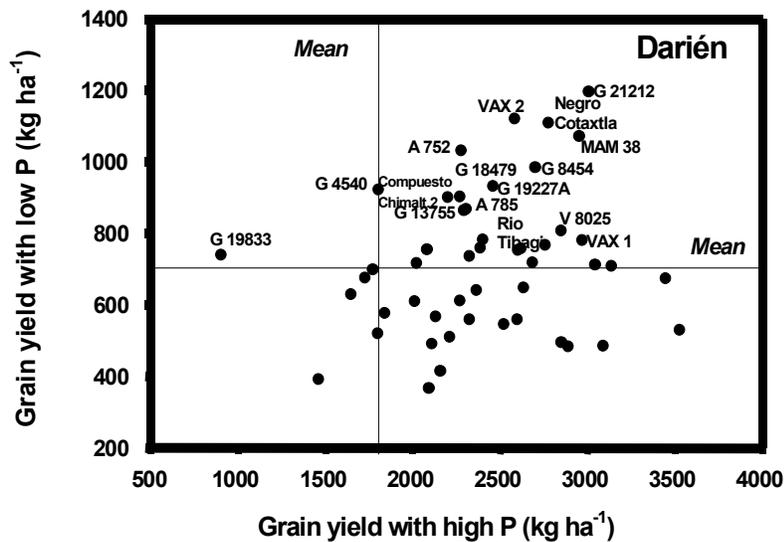


Figure 25. Identification of genotypes that are adapted to low P supply in soil and are responsive to application of P input to an Andisol at Darién, Colombia. Genotypes that yielded superior with low P and were also responsive to P application were identified in the upper box of the right hand side.

Relationship between seed yield with low P and leaf area index with low P treatment indicated that the interspecific bred line, VAX 2, was outstanding both in production of leaf area and in grain yield (Figure 26). But it is interesting to note that G 21212 could produce greater seed yield with moderate amounts of leaf area indicating its superior ability to mobilize photosynthates to grain.

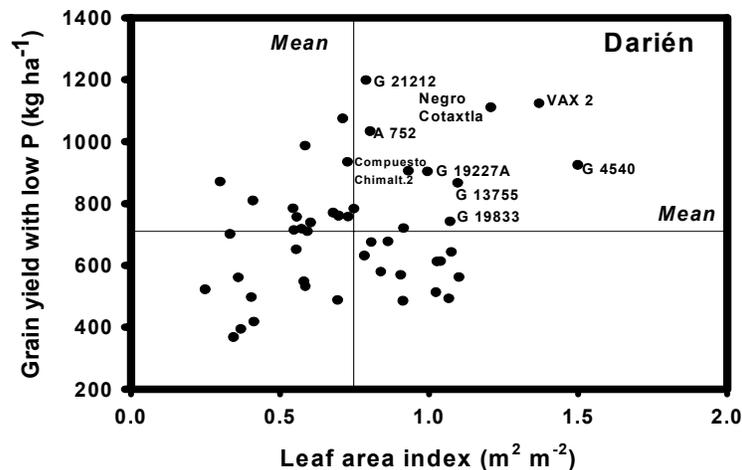


Figure 26. Identification of genotypes that are adapted to low P supply in soil and are vigorous in leaf area development when grown in an Andisol at Darién, Colombia. Genotypes that yielded superior with low P and were also superior in leaf area development were identified in the upper box of the right hand side.

Acquisition of P by G 21212 was also moderate, but seed yield was greater than that of the other genotypes with low P treatment (Figure 27). Among the 49 genotypes, VAX 2 was outstanding in acquiring P from low P soil. But as observed before, P as well as N use efficiency of G 21212 was outstanding with low P supply in soil (Figure 28). It is important to note that because of its greater ability to use N and P for grain production, seed N content of this genotype was markedly lower than most of the genotypes (Figure 29). However, we found that VAX 2 could combine higher seed yield with moderate seed N content. Thus it should be possible to genetically recombine greater seed yield with moderate level of seed protein when grown in low P soils.

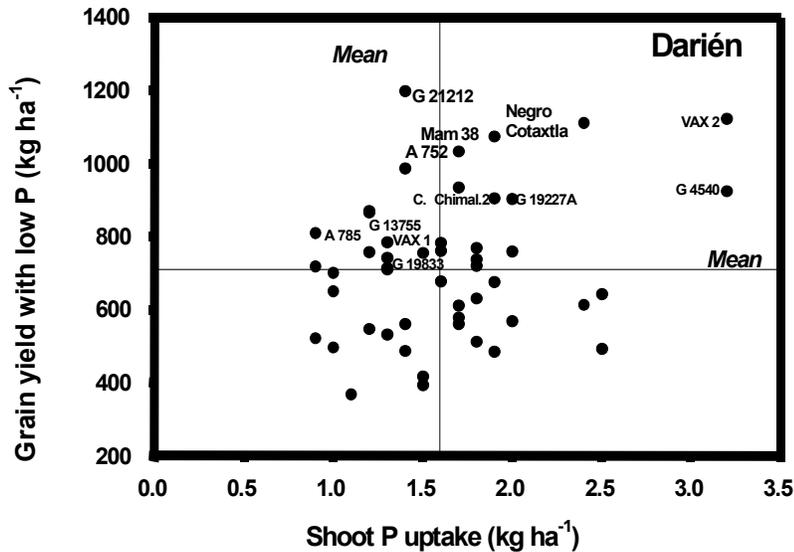


Figure 27. Identification of P-acquiring genotypes from soil with low P supply to an Andisol at Darién, Colombia. Genotypes that yielded superior with low P supply and were also superior in acquiring P from low P soil were identified in the upper box of the right hand side.

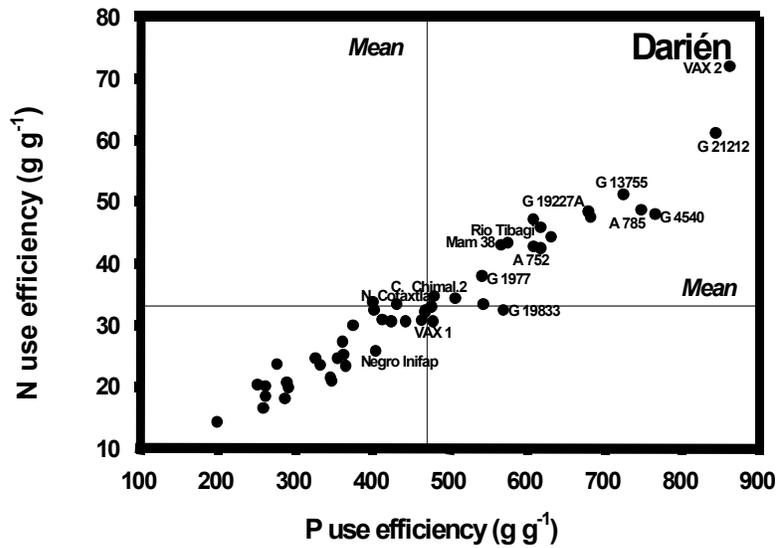


Figure 28. Identification of genotypes that are efficient in the utilization of N and P to produce greater seed yield when grown in low P soil at Darién, Colombia. Genotypes that were efficient in N and P utilization were identified in the upper box of the right hand side.

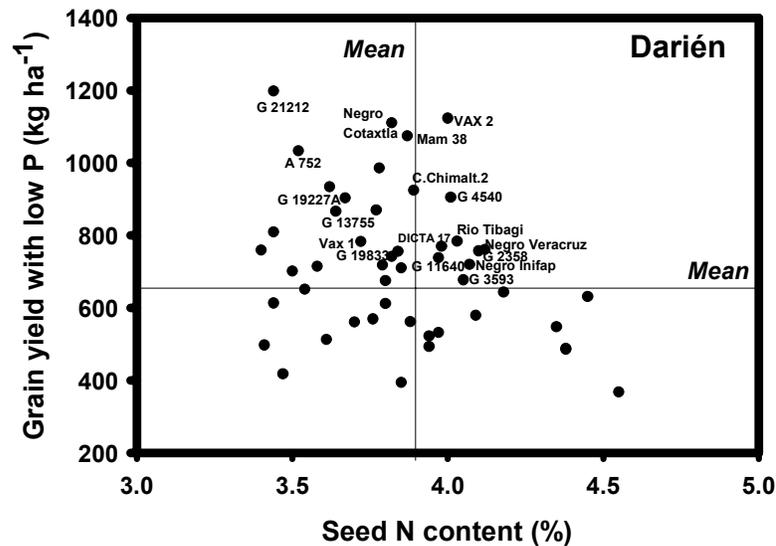


Figure 29. Identification of genotypes that combine superior seed yield with greater N content in seed when grown with low P supply in an Andisol at Darién, Colombia. Genotypes that were superior in grain yield and seed N were identified in the upper box of the right hand side.

Correlation coefficients between final grain yield and other plant attributes indicated that leaf area production, leaf, stem, and pod biomass production, total shoot biomass production, shoot N uptake, and shoot P uptake were all highly related to seed yield with low P treatment (Table 6). None of these shoot traits were related to seed yield with high P treatment. Significant negative relationship was observed between seed yield and seed P content indicating that greater P use efficiency has contributed to superior adaptation to low P soil conditions. A significant positive association was observed between TNC content in seed with seed yield under both low and high P treatments indicating that greater ability to mobilize photosynthates from vegetative growth to grain production is an important plant attribute for improving yield potential.

Table 6. Correlation coefficients (*r*) between final grain yield (kg ha⁻¹) and other plant attributes of 49 genotypes of common bean grown with low or high P supply to an Andisol at Darién, Colombia.

Plant traits ^a	Low P ^b	High P ^b
Leaf area index	0.49***	-0.04
Leaf biomass	0.47***	0.01
Stem biomass	0.44***	0.07
Pod biomass	0.31***	0.04
Shoot biomass	0.47***	0.06
Shoot N uptake	0.47***	0.11
Shoot P uptake	0.50***	0.08
N content in shoot biomass	0.00	0.12
P content in shoot biomass	0.11	0.10
TNC in shoot biomass	0.05	-0.16*
Seed N content	-0.11	-0.20*
Seed P content	-0.40***	-0.09
TNC in seed	0.23**	0.16*

a. TNC = Total nonstructural carbohydrates.

b. * = significant at $P = 0.05$, ** at $P = 0.01$, and *** at $P = 0.001$.

Conclusions: This field study indicated that four genotypes (G 21212, VAX 2, Negro Cotaxtla 91, and MAM 38) were outstanding both in their tolerance to low P supply in soil, and in their responsiveness to high P supply. Among the four genotypes, G 21212 was particularly outstanding in its ability to utilize P and N for grain production.

Reference:

CIAT. 1998. Bean Project Annual Report 1997. CIAT, Cali, CO. (Working document no. 177)

Contributors: I.M. Rao, S. Beebe, J. Ricaurte, H. Terán, R. García

Progress towards achieving output milestones:

Parents/populations/lines tolerant to drought/low soil fertility available

- Twelve nurseries with the most advanced drought tolerant lines were distributed to national programs. The information from these nurseries has already served as feedback to the breeding program.
- More than 170 F₂ populations were evaluated under severe drought pressure, permitting identification of the most promising for future selection.
- A number of landraces and advanced lines that are better adapted to drought were identified. These are currently being used in the breeding program to combine with biotic stress adaptation and commercial grain type.
- Several landraces and advanced lines with superior adaptation to low P supply in soil were identified for further improvement.
- Breeders from national programs are evaluating several of these parents/populations/lines for tolerance to drought and low soil fertility conditions.

Activity 1.2 Developing germplasm with multiple resistance to diseases

Highlights:

- The selection of the *bgm-1* gene was applied to about 7000 individual plants in a single season, more than doubling the capacity to screen for this gene.
- Highest levels of bean golden yellow mosaic virus (BGYMV) resistance ever recorded were observed in 2000-2001.
- The proportion of bean common mosaic virus (BCMV)-resistant materials in the Andean region has increased, although this trend seems associated with the cultivation of more medium- and small-seeded bean cultivars in the region, as compared to the proportion of large-seeded beans currently managed by the Andean bean project.
- Lines developed for angular leaf spot (ALS) resistance with multiple sources maintained their resistance at two different sites with contrasting isolates.
- Greenhouse evaluation of potential sources of ALS resulted in identification of 12 accessions and two bred lines (RWR 222 and AND 277) as highly resistant to race 63-63.
- *Ascochyta* resistance and possibly anthracnose resistance has been transferred into snap bean breeding lines that can serve as future parental material.
- From the low fertility trial, nine genotypes were identified that combine resistance to *Macrophomina phaseolina* and *Sclerotium rolfsii*.
- The accessions G 4495, G 2494, G 20592, G 6981, and G 13778 were shown to be highly resistant to both *Macrophomina phaseolina* and *Sclerotium rolfsii*.

1.2.1 Mainstream breeding including *bgm-1* selection

Rationale: Central America is a priority region for poverty alleviation and for bean production. Therefore, the mainstream breeding program continued to focus on small red and small black beans, largely for Central America. Bean golden yellow mosaic virus continues to be an indispensable breeding priority for this region.

Materials and Methods: Selection of the *bgm-1* gene for resistance to bean golden mosaic virus (BGMV) continued as reported last year, as part of the gamete selection scheme with the purpose of identifying F₁ plants that carry the gene. In March 2001, another nursery of F₁ multiple cross hybrids was planted. Although in other plantings our maximum output had been data on some 3000 plants, in this opportunity, high throughput marker assisted selection (MAS) was improved with the evaluation of the BGYMV SCAR in a nursery of 7085 plants.

Results and Discussion: A total of 3253 (46%) plants were found to have the *bgm-1* marker and selected for drought evaluation in the next generation. A team of workers completed the

evaluation in 28.7 days (Table 7). In order to compare the efficiency of screening twice the usual number of plants at a time, and because a different number of people is involved in each trial, we defined person-day (p-d) as the amount of time (days) that only one person would spend during each activity (number of persons X number of days). We found that the screening of a set of 7000 plants took 55.2 p-d (or 23.7 p-d for 3000 plants). Thus, in comparison with a previous trial of 3000 plants that required 30.7 p-d, the p-ds for the evaluation of the BGMV SCAR was reduced significantly. This was accomplished through using high technology equipment, for example electronic pipettes (Finnpipette Biocontrol) and the Hydra 96 micro dispenser (Robbins Scientific) often used for large-scale genomics and high throughput screening, which accelerated all laboratory processes.

Table 7. Improving large-scale screening for selecting *bgm-1* marker.

Task	Previous trial (n = 3000)			Last trial (n = 7000)		
	Persons (no.)	Time (days)	(p-d) ^a	Persons (no.)	Time (days)	(p-d) ^a
Preparation of stickers and labels	4	2.0	8.0	4	3.0	12.0
Labeling plants in the field	3	2.0	6.0	6	2.0	12.0
Field sampling	7	0.6	4.2	6	1.5	9.0
DNA extraction	1	2.0	2.0	1	3.2	3.2
DNA dilution	1	2.0	2.0	1	2.7	2.7
Polymerase chain reaction (PCR)	1	2.0	2.0	1	5.1	5.1
Electrophoreses	2	3.0	6.0	1	8.5	8.5
Reading gels	1	0.5	0.5	1	2.7	2.7
Total		14.1	30.7		28.7	55.2

a. p-d = person-day, or the amount of time (days) that one person would spend during each activity.

It should be emphasized that the use of markers as described here represents an emerging strategy in the breeding program. This strategy involves:

- (1) Characterization of resistance genes and identification of key genes of wide utility;
- (2) Tagging of these genes with robust, stable markers with potential for mass application;
- (3) Selection of these by MAS in breeding populations in early (F₁) or advanced (F₅ or F₆) generations; and
- (4) Increasing emphasis on abiotic stress tolerance in the fieldwork.

Thus, the use of markers assures that we maintain the advances that have been made in disease resistance (e.g., in resistance to BGYMV), while we assume new challenges of moving abiotic stress tolerance into acceptable commercial varieties. The breeding work described in section 1.1.2 is an example of this, and is made practical by increases in efficiency of MAS as presented here. This strategy must advance with the use of more key resistance genes for BGMV, for ALS, and for recessive BCMV resistance (these latter two priorities in support of Africa).

Conclusions: It has been possible to improve significantly the efficiency of MAS in the selection of the *bgm-1* resistance gene for BGMV. This is a model for the use of markers in breeding that

needs to be expanded to permit us to manipulate resistance genes efficiently in the breeding program while we confront new challenges, especially the deployment of abiotic stress tolerance.

Contributors: S. Beebe, G. Mahuku, H. Terán, C. Jara, C. Cajiao (IP-1); C. Quintero, J. Tohme (SB-2)

1.2.2 Bean golden yellow mosaic virus

Viral diseases constitute an important constraint to bean production in all of the Mesoamerican bean-producing regions where the Bean Project works. The lowlands and mid-altitude valleys of tropical America are particularly affected by whitefly-transmitted viruses, such as BGMV, BGYMV, and bean calico mosaic virus. Beetle-transmitted viruses are also important in these regions, particularly where beans are associated with maize (the latter being a good host of beetle vectors of plant viruses).

The Bean Project, through the regional Proyecto Regional de Frijol para Centro América, México y el Caribe (PROFRIJOL)-Swiss Development Cooperation (SDC) Project for Central America, Mexico, and the Caribbean, and national programs, have greatly contributed to the development of resistance to whitefly-transmitted viruses (geminiviruses) in the main small-seeded Mesoamerican bean cultivars. Basically, the materials (DOR lines) developed and selected in the 1990s by CIAT and national program breeders for their resistance to geminiviruses, are the sources of resistance in most of the crosses made today by different institutions in the region. For instance, in Guatemala, the bean varieties released for the lowlands in the period 1987-1999 (e.g., ICTA Chapina, ICTA Costeña, ICTA Santa Gertrudis, DOR ICTA, and ICTA Ligerito) are based on BGYMV-resistant genotypes identified in previous years. We tested ICTA-Ligerito (DOR 385 X JU-90-4) this year for its reaction to BGYMV under artificial conditions, and it was shown to possess one of the highest levels of virus resistance ever recorded. In El Salvador and Nicaragua, DOR 364 has made possible the cultivation of beans in BGYMV-affected areas in the past decade. Currently, El Salvador is benefiting from the active bean breeding project in Honduras, financed by SDC and the Collaborative Research Support Project (CRSP)-TXII (United States Agency for International Development [USAID]). New red-seeded bean cultivars, such as Tío Canela (derived from DOR lines 391 and 483) and the new line EAP-9510-77, produced by the EAP in Honduras, should soon replace DOR 364.

Rationale and Methodology: Bean virology supports all breeding activities related to the improvement of bean germplasm for resistance to viruses. Germplasm evaluations are conducted under controlled glasshouse conditions to standardize the inoculum and infection process, and to prevent contamination problems.

Results and Discussion: This year, breeders advanced the BGYMV-resistant lines selected in previous years, and the evaluation of breeding materials reverted to the screening of bred genotypes selected for different desirable traits against biotic and abiotic constraints. This year, 137 lines were evaluated again for their reaction to BCMV, the number one constraint of bean production around the world. Of these Mesoamerican genotypes, 8.7% were susceptible to the virus, and 2.9% were still segregating for their resistance/susceptibility to BCMV. The remaining 88.4% were homozygous resistant to the virus. Maintaining the resistance to this virus in Mesoamerica is critical to avoid significant yield losses caused by this virus, particularly in

combination with other bean viruses, such as cowpea chlorotic mottle, as we shall discuss later on in this report.

Contributor: M. Castaño

1.2.3 Bean common mosaic virus

Bean cultivars in the Andean region are not attacked by viruses to the extent that mid-altitude and lowland cultivars are. In the past decade, however, the aphid-borne BCMV has been increasing its incidence in the Andean highlands because of more favorable climatic conditions for the insect vector (drier and warmer climate changes). The BCMV screening of the Vivero del Equipo de Frijol (VEF) this year, showed that out of 75 bean genotypes deployed in the Andean region, 24 (32%) were homozygous resistant to BCMV, 3 (4%) were still segregating, and 64% of the genotypes screened were still susceptible to BCMV. Moreover, of the 24 bean genotypes found to be resistant to BCMV, only 2 (2.6%) were large seeded. Thus, there seems to be a trend to grow medium- and small-seeded bean cultivars in the Andean region, which should facilitate the introduction of BCMV resistance in the region.

Rationale: As mentioned earlier, the incidence of plant viruses in the Andean zone is increasing because of climate change, including the “El Niño” phenomenon.

Materials and Methods: The incorporation of BCMV resistance into large-seeded Andean materials is still based on the dominant monogenic necrosis gene. The screening methodology is done under controlled glasshouse conditions using the bean common mosaic necrosis virus (BCMNV) strain, NL3.

Results and Discussion: Results are mentioned above. They suggest that more attention should be paid to the genetic improvement of Andean bean genotypes for their resistance to BCMV. This research, however, necessitates a change in breeding strategy from monogenic dominant resistance to durable recessive resistance (*bc-3*), mainly to avoid seed color-BCMV susceptibility linkages.

Contributor: M. Castaño

1.2.4 Developing sources of angular leaf spot resistance through breeding

Rationale: Angular leaf spot is considered the most important foliar pathogen of bean in Africa and Brazil, and has become increasingly important in Central America. The development of agronomically acceptable lines that can serve as sources of resistance is an urgent need of breeding programs for these regions.

Materials and Methods: Five populations were created with several sources of resistance. Some populations possess only Mesoamerican sources of resistance (particularly Cornell 49-242, which appears in MAR 1), while other sources are derived from the Andean gene pool (G 5686; G 9603, or Jalo EEP 558, an Andean genotype from Brazil selected for ALS resistance; AND 279, a bred Andean type). G 4032 (Vainica Ahumado Chirripo) is a climber from Costa Rica.

XR 12546 = VAX 1 x ((MAR 1 x A 429) x (G 17341 x G 3017))
MA 12129 = VAX 1 x ((MAR 1 x G 5686) x (MAR 3 x G 5653))
MN 12688 = MAR 2 x NAB 69
BM 12721 = FEB 216 x (A 806 x ((MAR 1 x G 4032) x (A 240 x G 5686)))
BM 12722 = A 686 x (A 801 x ((A 247 x G 9603) x (AND 279 x G 5207)))

These populations were inoculated under field conditions and were selected for resistance at Santander de Quilichao for several generations. Subsequently they were submitted to the VEF (see Output 4) where they were inoculated with local isolates at both Santander de Quilichao and Darién.

Results: Among the five crosses represented from the ALS project at Santander de Quilichao, three (XR 12546, MA 12129, and MN 12688) gave very high resistance at Santander de Quilichao, but only showed susceptibility at Darién (see VEF data in Table 59). The parents in these crosses are all quite typical race Mesoamerica types. The other two crosses (BM 12721 and BM 12722) resulted in slightly lower (but still very good) resistance at Santander de Quilichao, but intermediate resistance at Darién as well. These crosses include as parents Carioca types selected at Santander de Quilichao (A 686 and FEB 216) and crossed with more divergent germplasm. As noted above, G 5686, G 9603, and AND 279 are Andean genotypes. G 4032, the climber from Costa Rica, likely pertains to race G, in which resistance to ALS occurs frequently. The presence of this diverse germplasm in the crosses likely confers more ample resistance on the progeny.

Conclusions: Breeding for ALS resistance has been successful in the Santander de Quilichao environment, but the selected lines must now be confronted with races from other regions. Materials that are resistant at Santander de Quilichao often fail at Darién, possibly because of the failure of a gene from Cornell 49-242. However, the use of divergent parents from the Andean gene pool and/or other Middle American races results in more stable resistance. This is a result that confirms our expectations and is relevant for both Latin America (Central America, Brazil, Bolivia, and Argentina) and especially Africa where ALS is the number one biotic constraint.

Contributors: S. Beebe, G. Mahuku, H. Terán, C. Jara, C. Cajiao (IP-1); C. Quintero, J. Tohme (SB-2)

1.2.5 Identifying common bean genotypes with resistance to angular leaf spot

Rationale: *Phaeoisariopsis griseola* is a highly variable pathogen and many races of this fungus have been described. Because of the incredible pathotypic diversity, cultivars resistant in one year or location may become susceptible in another. This incredible genetic diversity complicates the development and use of resistant varieties to manage the disease. Therefore, identifying and verifying suitability of potential sources of resistance is a continuous activity. One of the activities of the CIAT bean pathology program is to identify potential sources of resistance, put together a nursery, and distribute to collaborating partners in national programs for testing under local conditions. The objective of this work was to test the previously described sources of resistance that were part of the Bean Angular Leaf Spot International Trial nursery (BALSIT) with the most virulent race that we have identified, in the hopes of identifying those materials with the

greatest resistance. The final goal is to evaluate these materials under different bean production zones where ALS is a problem.

Materials and Methods: Under greenhouse conditions, 100 common bean lines that were part of the 1999 BALSIT nursery were evaluated with race 63-63. Nine plants of each line were sown in plastic pots, three per pot, and the first fully expanded trifoliolate inoculated. Plants were incubated in a humid chamber (90%-100% relative humidity) for 4 days, after which they were put on benches. Disease ratings were taken starting 8 days after inoculation up to 18 days after inoculation.

In addition, 104 lines that had previously been found to have resistance to *P. griseola* were screened under field conditions with a mixture of races (races 63-0, 31-47, 5-47, and 63-15) at Darién and (races 63-0, 31-55, 13-63, 1-55, and 31-47) at Santander de Quilichao. The latter is at about 900 m altitude, while Darién is at 1300 m. The aim was to identify resistant materials that would be combined with materials from evaluations of the BALSIT nursery to have a new nursery.

Results and Discussion: Of the 100 materials evaluated, 19 were resistant to race 63-63, while 11 had an intermediate reaction (Table 8). The remaining materials (70) were susceptible. This result demonstrates that we need to add new sources of resistance to create a new BALSIT nursery that can be sent out to different partners for testing under their local conditions. We have put up a nursery (BALSIT 2001) that is ready for distribution to partners. Disease expression was better at Darién than at Santander de Quilichao. The prevailing conditions in the latter were not conducive to disease development. Only seven genotypes were resistant at Darién compared to 56 at Santander de Quilichao (Figure 30). However, most of the genotypes tested were intermediate. The resistant and intermediate genotypes can be included in an ALS nursery to be sent to collaborators. The intermediate materials might form part of a population constituting stable forms of resistance to ALS.

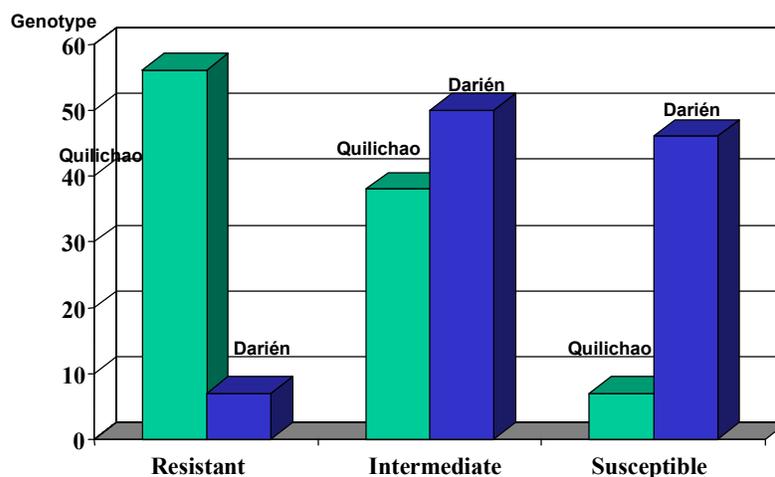


Figure 30. Response of 104 common bean genotypes to inoculation with a mixture of *Phaeoisariopsis griseola* races under field conditions.

Table 8. Response of genotypes from the bean angular leaf spot nursery to inoculations with *Phaeoisariopsis griseola* race 63-63.

Accession / Entry	Growth habit ^a	Seed color ^b	Seed size ^c	Response to race 63-63 ^d
G 5207	II	8	s	1.0
G 22447	III	3R	m	1.0
G 18842	I	2	m	1.9
G 22267	III	6	l	1.9
G 20939	I	3	l	2.0
G 20949	I	3	m	2.0
RWR 222	III	5	m	2.0
G 18587	III	3	m	2.1
G 20743	III	6	l	2.4
G 20818	II	3	m	3.0
G 5686	II	4M	l	3.0
G 6727	III	3M	s	3.0
G 19115	IV	5	m	3.0
AND 277	I	6M	m	3.1
G 18224	III	6	s	3.1
G 20523	III	6	l	3.1
BAT 496	II	2	s	3.2
G 9282	II	5	s	3.2
A 673	III	2M	m	3.4
AND 829	I	6	m	4.1
G 1845	III	8	s	4.3
AND 279	I	6M	l	4.6
G 14656	II	1	s	4.8
G 6861	III	9	s	4.8
A 82	III	2R	s	5.0
G 13835	III	2	m	5.7
G 19939	IV	6	m	5.7
A 193	II	6	s	6.0
NIC 159	II	6	m	6.0
G 9462	III	8	s	6.0

- a. Growth habit: I = determinate bush, II = indeterminate upright bush, III = indeterminate prostrate, and IV = indeterminate, possessing strong climbing ability.
- b. Seed color: 1 = white, 2 = cream, 3 = yellow, 4 = brown, 5 = pink, 6 = red, 8 = black, 9 = other, M = mottled, and R = striped.
- c. Seed size: s = small, m = medium, and l = large.
- d. Response on a scale of 1-9, where 1 is resistant and 9 is susceptible.

Conclusion: The resistant and intermediate materials identified in this study are being planted out together with other materials identified from other studies to constitute a new BALSIT 2002 nursery. Seed has been multiplied and this nursery of 70 materials will be distributed to collaborating partners in Central and South America, and Africa.

Contributors: G. Mahuku, C. Jara

1.2.6 Developing snap bean breeding lines resistant to *Ascochyta* blight

Rationale: *Ascochyta* is a highly destructive disease of bean in the rainy higher altitude regions of the Andean zone and Africa. In these areas, *Ascochyta* severely limits the potential use of bean. Climbing snap beans are a high-value horticultural crop in the Andean zone, and are often produced by small-scale farmers in hillsides in a labor-intensive system. However, they are usually produced under very heavy pesticide use, with weekly applications of mixtures including highly toxic insecticides. Furthermore, 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 integrated pest management (IPM) strategy for snap beans.

Materials and Methods: In previous years we reported on the progress in implementation of resistance from interspecific crosses. Additionally, a bred variety from Guatemala, ICTA-Hunapú, has proved to be an excellent source. Crosses were created among sources and between sources of resistance and commercial snap bean, and F₃ families were selected in previous semesters for both resistance and pod type. F₄ families were planted in single-row plots at the Popayán station in April 2001 without inoculation, but without protection. Checks of Blue Lake (Lago Azul), the commercial snap bean, were planted every 10 rows, together with rows of G 685 (a Guatemalan climbing dry bean), ICTA Hunapú, and a resistant ASC bred line.

Results: The commercial check, Blue Lake, presented severe symptoms of both *Ascochyta* and anthracnose shortly after flowering (in the range of 6-8 on a 9-point scale for both diseases), even without inoculation. The resistant checks, G 685, and almost all of the bred lines presented far fewer symptoms than did Blue Lake (in the range of 2-3 for most lines) for both *Ascochyta* and anthracnose. Because these were F₄ families, many were still segregating for pod type, and within a few families nearly commercial pod type was observed (Table 9). However, this observation must be taken with caution because the market demands for pod type are so stringent that the Blue Lake type must be replicated indistinguishably in the lines. These lines must be considered essentially as potential parents for future crosses. Nonetheless, they represent a significant advance in the development of disease-resistant snap beans.

Table 9. Crosses, number of selected families, and individual selections within families, of snap bean breeding lines resistant to *Ascochyta* and anthracnose.

Cross	Number of families selected	Number of individual selections within families
Blue Lake x (G 685 x (ASC 72 x ICTA Hunapú))	5	14
Blue Lake x (G 685 x (ASC 73 x ICTA Hunapú))	7	13
Blue Lake x (G 685 x (ASC 74 x ICTA Hunapú))	1	1
Blue Lake x (ICTA Hunapú x (ASC 74 x ASC 77))	1	2
G 685 x (ASC 73 x ICTA Hunapú)	6	39

Conclusion: For the first time, a level of resistance to *Ascochyta* is available in snap bean types that can permit genetic gain against this disease. This resistance has been recovered with resistance to certain races of anthracnose as well, thus their use in snap bean breeding is likely to be highly useful as a contribution to IPM and the reduction of pesticide use.

Contributors: S. Beebe, G. Mahuku, H. Terán, C. Jara, C. Cajiao (IP-1); C. Quintero, J. Tohme (SB-2)

1.2.7 Identifying root rot resistant bean genotypes

Rationale: Root rots are caused by a complex of soil-borne pathogens that include *Fusarium* root rot (*Fusarium solani* f.sp. *phaseoli*), *Rhizoctonia* root rot (*Rhizoctonia solani*), southern blight (*Sclerotinium rolfsii*), *Pythium* root rots (*Pythium* spp.), and charcoal rot (*Macrophomina phaseolina*). The incidence of these pathogens is influenced by soil fertility and prevailing environmental conditions. For example, incidence of *Macrophomina phaseolicola* can increase from as low as 9% under adequate water conditions to as high as 80% under water stress. However, the contrary is true for other pathogens, such as *Pythium* spp. and *Fusarium solani*, which become important when there is excess soil moisture. Depending on prevailing conditions, losses from root rots can be complete. In light of the recent increases in the incidence of soil-borne pathogens, coupled to decrease in soil fertility, we screened the materials from the low soil fertility trial for their reaction to different soil-borne pathogens. In addition, we screened bean genotypes from the different root rot nurseries that the pathology program previously established. This is an ongoing activity whose objective is to identify materials resistant to the majority of soil-borne pathogens for use as parents in breeding programs.

Materials and Methods: Common bean materials representing the low soil fertility trial (36) and accessions from the core collection for tannins (47) were planted in trays in the greenhouse. Ten seeds of each material were inoculated with either *M. phaseolicola* or *S. rolfsii* using the methods described by Abawi and Pastor-Corrales (1990). For each pathogen, two isolates were used; one isolate from Santander de Quilichao and the other from Palmira. These isolates had been found to have different molecular profiles following random amplified microsatellite (RAMS) analysis of their DNA. For *M. phaseolicola*, the trays were kept relatively dry to simulate water deficit conditions, while for *S. rolfsii*, the trays were kept wet to simulate excess moisture conditions. Ratings were done 21 days after planting using the rating scale reported by Abawi and Pastor-Corrales (1990).

Field Evaluations: The same materials were evaluated during two seasons under field conditions at Santander de Quilichao. The low fertility nursery was also evaluated in the field at Palmira. The field at Santander de Quilichao has a known history of root rots. For the past four seasons, this portion of the field has been planted to susceptible materials to increase root rot inoculum level, as evidenced by the increase in disease incidences for the susceptible materials used. During wet conditions, high incidences of *Fusarium* spp., *Rhizoctonia*, and *S. rolfsii* are noticed, while under dry conditions, *M. phaseolicola* is the prevalent pathogen. The materials were sown in two rows of 3 meters each, replicated three times. Evaluations were done 15 days after emergence (plant counts) and at harvest.

In addition, four nurseries (International *Fusarium oxysporium* nursery (VIMFO), International *Rhizoctonia* nursery (VIPRPS), International *Macrophomina* nursery (VIMP), and Bean root rot resistant materials nursery (BRRIN) were evaluated at Santander de Quilichao. An additional 14 materials, which included the VAX lines (VAX 1 – VAX 6) and materials reported by other programs as having resistance to root rots, were also included. These nurseries contain potential sources of resistance to different root rot pathogens.

Results:

Response to *M. phaseolina*: The response of the genotypes from the low fertility trial varied for the two seasons at Santander de Quilichao. The first semester was extremely dry and *M. phaseolina* was the predominant pathogen. The second semester was very wet and the predominant pathogens were *F. solani* and *S. rolf sii*. In this complex, DOR 390, A774, VAX 1, VAX 6, G 3513, BAT 447, and G 21212, among others, were highly resistant. G 21212 has been reported to yield well under water stress conditions and this has been attributed to tolerance to *M. phaseolina* and *S. rolf sii*. The results reported here seem to suggest that the performance of G 21212 under water stress is related to its resistance to *S. rolf sii*, rather than *M. phaseolicola*. When materials from the low soil fertility trial were compared between Palmira and Santander de Quilichao, nine materials including SEA 5 were resistant at both locations (Figure 31). However, G 21212 was resistant at Palmira (site known to have a high incidence of *S. rolf sii*), but susceptible at Santander de Quilichao (site where *M. phaseolina* predominates). Only six materials were susceptible in both locations, while the rest were resistant in one location and not the other.

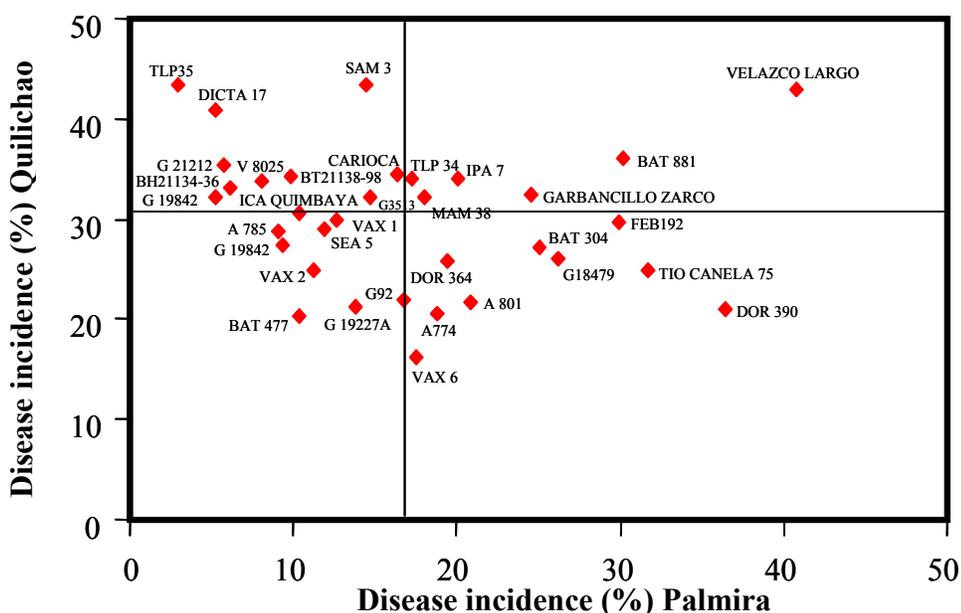


Figure 31. Response of genotypes from the low fertility trial to *Macrophomina phaseolina* under field conditions at Palmira and Santander de Quilichao, Colombia.

Greenhouse evaluations with *S. rolf sii* confirmed field observations that G 21212 has a susceptible reaction to *M. phaseolina*, while it is highly resistant to *S. rolf sii* (Figures 32 and 33). Another genotype that performs well under water stress conditions is SEA 5. This genotype is highly resistant to *M. phaseolina* under both field and greenhouse conditions, but is tolerant to susceptible when challenged with *S. rolf sii*. Other genotypes that do well under heavy pressure of *M. phaseolina* are BAT 477, G 3513, and A 785. Only four genotypes from the low fertility trial (DOR 390, A 785, Velazco Largo, and BAT 447) were resistant to both *M. phaseolina* and *S. rolf sii* under greenhouse conditions.

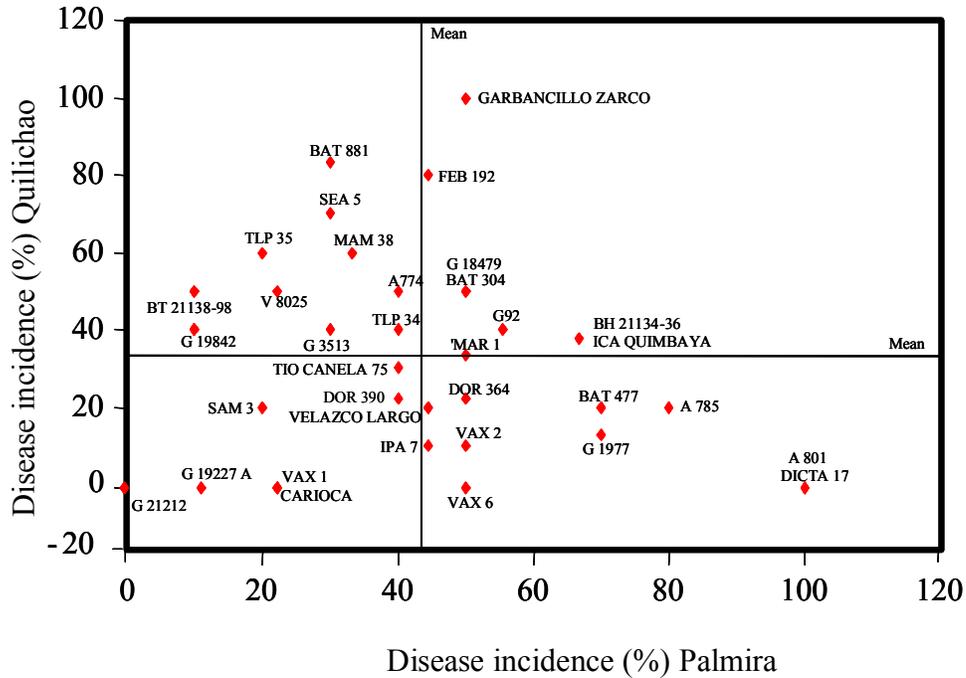


Figure 32 Response of materials from the low fertility nursery to inoculations with *Sclerotium rolfsii* isolates from Palmira and Santander de Quilichao.

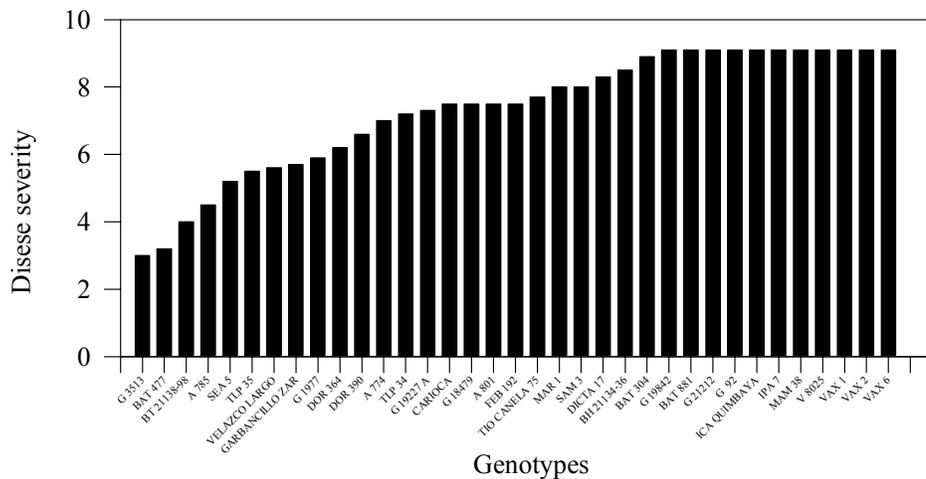


Figure 33. Response of bean genotypes from the low fertility nursery to inoculations with *Macrophomina phaseolina* under greenhouse conditions. Disease severity is on a scale of 1-9, where 1 = resistant and 9 = susceptible.

Response of *S. rolfsii*: Seven genotypes (G 21212, G 19227A, Carioca, SAM 3, VAX 1, DOR 390, and Tío Canela) from the low soil fertility nursery were resistant to both the Santander de Quilichao and Palmira isolates of *S. rolfsii*, while 10 genotypes were susceptible to both isolates (Figure 32). Some genotypes (e.g., SEA 5 and G 3513) were resistant to the Palmira isolate and

susceptible to the Santander de Quilichao isolate, revealing possible differential interactions. Of the 47 accessions that are part of the tannin core collection, 10 were resistant to both the Palmira and Quilichao *S. rolf sii* isolates (Figure 34), whereas seven accessions were susceptible to both isolates. Of the resistant accessions, five (G 4495, G 2494, G 20592, G 6981, and G 13778) have been found to be highly resistant to *M. phaseolina* under greenhouse conditions. The rest of the accessions were resistant to only one isolate (six for Palmira and 10 for Santander de Quilichao isolates), again demonstrating differential interaction.

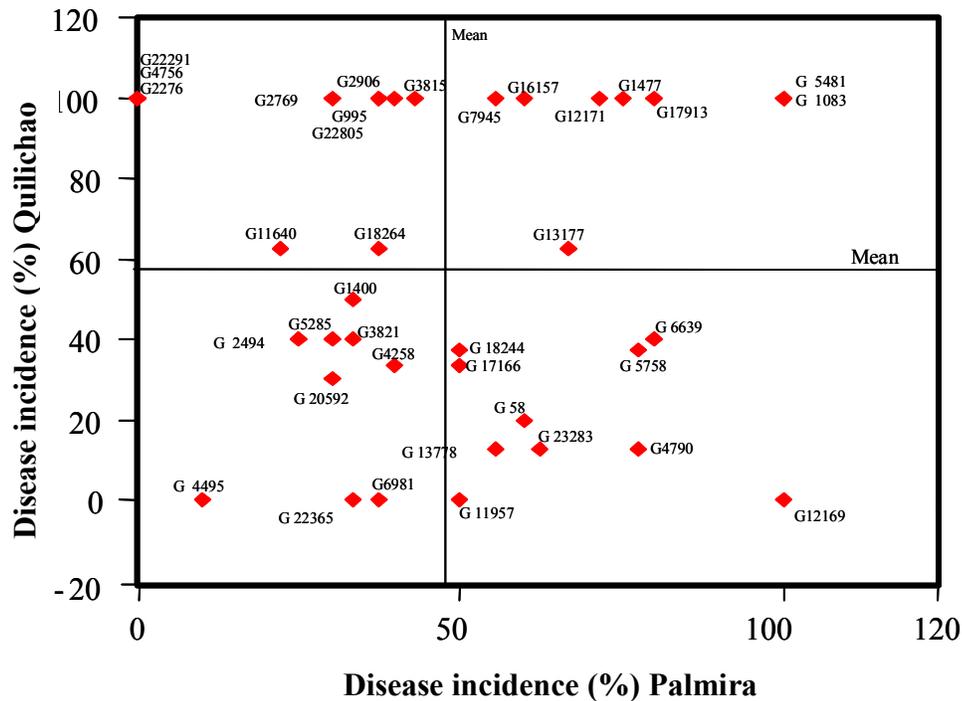


Figure 34. Response of common bean genotypes with *Sclerotium rolf sii* isolates from Palmira and Santander de Quilichao, Colombia under greenhouse conditions.

Evaluation of nurseries: Most of the materials from the *M. phaseolina* nursery (Figure 35) and bean root rot resistant genotypes (Figure 36) were resistant. Among the genotypes that performed well during both seasons were WAF 4, Sanilac, BAT 1400, A 195, Diacol Calima, MCD 254, AND 286, Bayo Rio Grande, XAN 195, A 211, EMP 81, A 197, Rio Tibagi, VAX 1, VAX 3, VAX 4, VAX 5, BAT 1293, WAF 9, Pico de Ouro, A 300, XAN 112, EMP 86, G 92, and G 19227A. The promising materials from all the nurseries have been put together to constitute a new nursery that will be screened against several root rot pathogens (e.g., *F. solani*, *Pythium* spp., *Rhizoctonia* spp., and *S. rolf sii*) under greenhouse conditions.

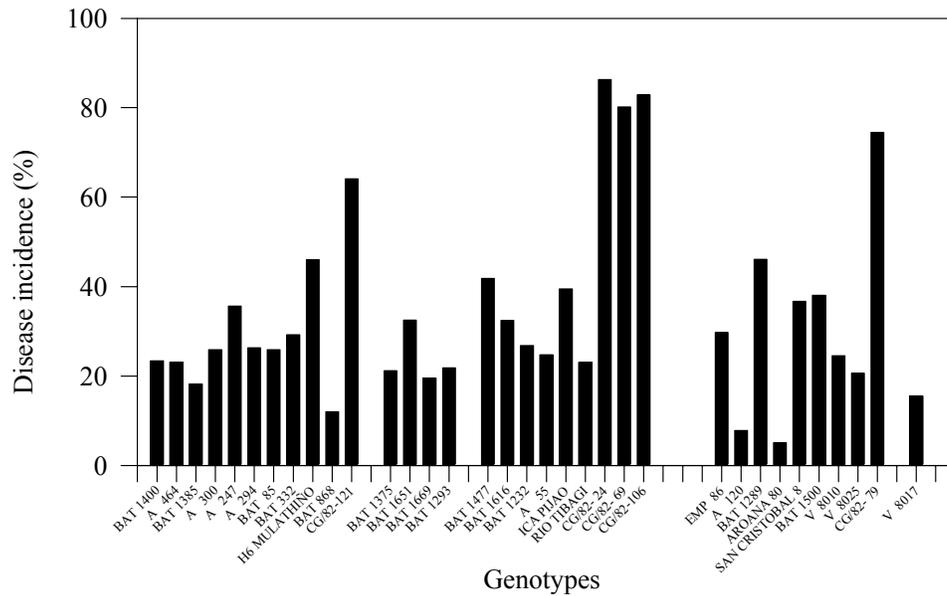


Figure 35. Response of the materials from the *Macrophomina phaseolicola* nursery to root rot evaluations under field conditions.

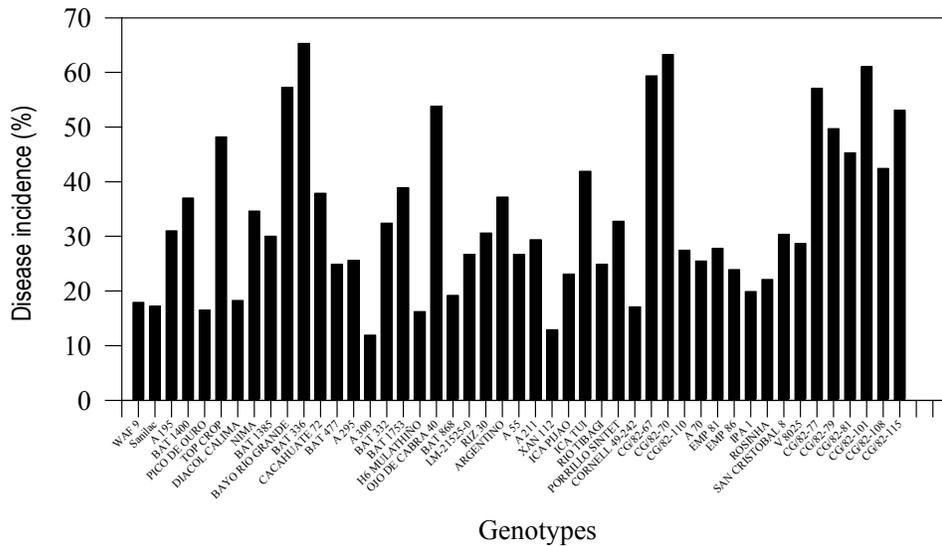


Figure 36. Response of the materials from the bean root rot nursery to root rot evaluations under field conditions.

Conclusion: Materials that are resistant to *M. phaseolina* and *S. rolfsii* were identified among the different nurseries evaluated in this study. These materials have been put together to constitute a new nursery that is currently being screened with other root rot pathogens under controlled conditions. Although this study is ongoing, preliminary results clearly show that differential interaction exists for *S. rolfsii* and isolates from different places should be used to confirm

identified sources of resistance. The associations between water stress tolerance and *S. rolfii* and or *M. phaseolina* resistance need to be studied further. Results from this study seem to suggest that the performance of G 21212 under water stress might be linked to its resistance to *S. rolfii*.

Reference:

Abawi, G.S.; Pastor Corrales, M.A. 1990. Root rots of beans in Latin America and Africa: Diagnosis, research methodologies, and management strategies. CIAT, Cali, CO. 114 p.

Contributors: G. Mahuku, C. Jara, G. Castellanos

Progress towards achieving output milestones:

Parents / lines with stable resistance to multiple constraints identified or developed

- More than 70 new red- and black-seeded lines with multiple resistance were distributed to Honduras, Costa Rica, Nicaragua, and Argentina.
- Forty red-seeded lines that were distributed in 2000 were sent to the Hillsides Project in Honduras and Nicaragua for local evaluation with farmers.
- Marker assisted selection was scaled up even further, in preparation for the selection of a greater number of genes in the future.
- Lines combining resistance to BGYMV and other diseases are being distributed in Central America.
- Levels of BCMV resistance are increasing in the Andean region.
- Several landraces and bred lines with resistance to several ALS races were identified. These have been put together to constitute a nursery to be distributed for evaluation by national scientists
- A number of landraces and advanced lines with high levels of resistance to root rot pathogens were identified. These are being evaluated against other pathogens and production constraints.

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

Highlights:

- New sources of resistance to *Thrips palmi*, *Empoasca kraemeri*, *Zabrotes subfasciatus*, and *Acanthoscelides obtectus* were identified.
- New lines incorporating insect resistance were selected.
- Studies on mechanisms of resistance to *T. palmi* were initiated.
- Progress was made in the development of molecular markers for resistance to *Apion godmani*.

1.3.1 Screening for sources of resistance to major pests

Rationale: Identification of sources of resistance to major bean pests is a continuous activity. For some major pests, identification of the mechanisms underlying resistance is important for the development of appropriate breeding strategies. Major emphasis in 2001 was given to studies on the resistance to *T. palmi*.

Materials and Methods: Leafhopper and *T. palmi* nurseries are planted in the field under high levels of natural infestation, usually with three to four replications per genotype. Evaluations for resistance include damage and reproductive adaptation scores, insect counts, and in some cases, yields. Bruchid nurseries are tested in the laboratory using three to five replications of 50 seeds per genotype. Infestation levels are seven pairs of *Z. subfasciatus* per 50 seeds, or two eggs per seed in the case of *A. obtectus*.

Results and Discussion:

***Thrips palmi*:** Intensive work on resistance to this new pest of beans continued in 2001. Repeated testing at two sites revealed significant differences between resistant and susceptible genotypes. The commercial variety, Brunca, and the elite breeding lines, FEB 115 and EMP 486, consistently showed significantly lower damage levels and higher reproductive adaptation scores than the susceptible checks PVA 773, APN 18, and RAZ 136 (Table 10).

Although damage ratings and reproductive adaptation scores varied with varying levels of infestation (which in these trials ranged from five adults per leaflet in trial 2 to 17 adults per leaflet in trial 4), genotype responses and resistance ratings were consistent. In general, correlations between visual damage scores and reproductive adaptation scores were high ($r = -0.876$, $P < 0.001$, $n = 72$) meaning that selection for damage is useful in the selection of genotypes that may have tolerance as a mechanism of resistance. Overall, resistance levels in beans can be considered as moderate because none of the genotypes tested received damage scores of less than 3 and none was ever rated as highly resistant in terms of reproductive adaptation scores. Adult and larval populations varied significantly between several genotypes in

three consecutive trials. Consistently, lowest adult populations occurred on FEB 115. In two out of three trials, this genotype also exhibited the lowest larval populations (Table 11).

Table 10. Damage and reproductive adaptation scores^a of selected bean genotypes exposed to natural infestations of *Thrips palmi*.

Genotype	Damage scores ^b				Reproductive adaptation scores ^c		
	Trial 1 (CIAT)	Trial 2 (Pradera)	Trial 3 (Pradera)	Trial 4 (CIAT)	Trial 1 (CIAT)	Trial 2 (Pradera)	Trial 3 (Pradera)
PVA 773	8.2 ± 0.44 a	4.8 ± 0.69 ab	7.8 ± 0.17 a	7.9 ± 0.36 a	1.0 ± 0 c	2.0 ± 0.82 cd	2.0 ± 0 c
APN 18	7.7 ± 0.66 a	5.1 ± 0.68 a	6.9 ± 0.43 b	7.9 ± 0.34 a	2.0 ± 0.58 bc	4.5 ± 0.41 b	3.8 ± 0.55 b
RAZ 136	7.3 ± 0.86 a	6.5 ± 0.41 a	7.9 ± 0.09 a	7.8 ± 0.36 a	1.0 ± 0 c	1.0 ± 0 d	2.3 ± 0.29 c
EMP 486	6.7 ± 0.67 ab	5.1 ± 0.12 a	4.9 ± 0.48 c	5.4 ± 0.62 b	3.3 ± 0.67 abc	6.5 ± 0.41 a	4.3 ± 0.87 b
FEB 115	5.4 ± 0.38 b	6.3 ± 0.53 a	4.6 ± 0.32 c	5.6 ± 0.57 b	3.7 ± 0.33 ab	6.0 ± 0 a	5.8 ± 0.29 a
Brunca	5.0 ± 0.87 b	3.0 ± 0 b	4.6 ± 0.19 c	6.4 ± 0.46 b	4.0 ± 0.45 a	6.0 ± 0 a	6.3 ± 0.29 a

- Means ± SEM of three replications per genotype in trial 1, two replications in trial 2, and four replications in trials 3 and 4. Means within a column followed by the same letter are not significantly different by LSD.
- On a 1 to 9 scale, where 1 = no damage and 9 = very severe damage.
- On a 1 to 9 scale, where 1 = no pod setting and 9 = excellent pod setting.

Table 11. Adult and larval populations found in bean genotypes tested for resistance to *Thrips palmi*.^a

Genotype	Cumulative no. of adults per leaflet ^b			Cumulative no. of larvae per leaflet ^c		
	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3
EMP 514	-	91.8 ± 2.1 a	41.2 ± 3.3 a	-	190.9 ± 13.8 a	52.4 ± 5.2 a
APN 18	-	65.6 ± 3.7 bc	39.0 ± 8.9 ab	-	111.5 ± 3.9 e	53.1 ± 8.7 a
BAT 41	-	44.0 ± 5.8 d	34.2 ± 3.5 ab	-	130.3 ± 5.5 de	60.7 ± 11.4 a
BAT 477	64.8 ± 5.7 a	72.9 ± 5.2 b	39.9 ± 4.3 ab	87.7 ± 13.6 a	173.3 ± 9.3 ab	50.6 ± 12.8 a
Brunca	44.7 ± 3.4 ab	58.4 ± 4.1 c	32.6 ± 7.7 bc	65.7 ± 16.5 ab	152.0 ± 4.0 bcd	53.2 ± 14.7 a
BH-5	-	72.1 ± 4.7 b	31.5 ± 7.4 bc	-	164.8 ± 10.1 abc	43.3 ± 8.3 a
EMP 486	58.8 ± 10.8 a	67.6 ± 4.8 bc	38.0 ± 5.7 ab	129.9 ± 18.6 a	146.2 ± 7.4 cd	62.2 ± 8.0 a
FEB 115	33.1 ± 6.7 b	43.1 ± 3.3 d	24.8 ± 3.2 c	41.4 ± 12.6 b	119.0 ± 9.7 e	41.8 ± 4.9 a
BH-60	-	61.3 ± 2.4 bc	31.6 ± 3.7 abc	-	143.8 ± 15.6 cd	66.7 ± 9.6 a

- Means ± SEM of four replications per genotype, six leaflets per replication. Means within a column followed by different letters are significantly different by LSD.
- Counts made weekly from 7 to 63 days after planting.
- Counts made weekly from 15 to 63 days after planting.

The lower levels of infestation on FEB 115 could be a reflection of either of two mechanisms (antixenosis or antibiosis), or a combination of both, which are impossible to distinguish with the experimental procedures used in this study.

In a yield trial conducted under high levels of infestation (19.8 adults per leaflet, 33.7 larvae per leaflet at 56 days after planting), highly significant differences were found among genotypes. Brunca, FEB 115, and BAT 477 showed intermediate levels of damage, while EMP 486, and the RILs, BH-60 and BH-5, were rated as resistant (Table 12). Thrips damage had a significant impact on yields. FEB 115, the resistant check, significantly out yielded all other genotypes tested. With respect to FEB 115, yield losses ranged from 22% in resistant lines BH-60 and BH-

5, to 81% in the susceptible check APN 18, and 78% in the susceptible check BAT 41. In general, yields under the high level of infestation recorded in this trial can be considered low, an indication of the very significant impact of thrips damage on bean yields.

Table 12. Damage scores, percentage of empty pods, and yields of selected bean genotypes^a tested for resistance to *Thrips palmi*.

Genotype	Damage scores 56 days after planting	Percentage of empty pods	Yield (kg ha ⁻¹)	Percentage yield reduction ^b
APN 18	7.5 ± 0.86 a	50.0 ± 4.8 a	209 ± 51.3 d	81.2
BAT 41	7.2 ± 1.03 ab	37.3 ± 4.1 a	240 ± 85.3 d	78.5
EMP 514	6.2 ± 0.75 bc	30.6 ± 2.9 a	297 ± 65.3 d	73.4
Brunca	6.2 ± 0.47 bc	13.7 ± 2.1 b	535 ± 121.8 c	52.0
BAT 477	6.0 ± 0.40 c	17.6 ± 5.6 b	546 ± 98.6 c	51.0
EMP 486	4.5 ± 0.86 d	7.3 ± 2.0 b	744 ± 75.4 bc	33.3
BH-60	4.5 ± 0.64 d	15.8 ± 1.3 b	874 ± 123.0 b	21.6
BH-5	4.2 ± 0.62 d	14.7 ± 3.3 b	859 ± 90.1 b	22.9
FEB 115	4.2 ± 0.47 d	16.9 ± 2.9 b	1115 ± 116.3 a	-

a. Means ± SEM of four replications per genotype. Means within a column followed by different letters are significantly different by LSD.

b. With respect to the best treatment.

As stated in 2000, further studies on resistance to thrips were conducted using RILs derived from the elite cross BAT 881 x G 21212, two genotypes that in preliminary screenings showed intermediate levels of resistance to the melon thrips. This cross is being used by CIAT for diverse genetic studies. BAT 881 is a source of resistance to ALS, while G 21212 is a source of tolerance to low P conditions. The unreplicated initial testing of 139 RILs and parents showed an array of responses that fit a distribution skewed towards susceptibility (CIAT 2001, p. 63-66). The correlation between damage scores and reproductive adaptation scores was significant ($r = -0.801$, $P < 0.001$, $n = 141$). When these materials were tested again using four replications per genotype, damage scores ranged from 3 to 9, with some materials showing relatively high levels of resistance. Again, damage scores and reproductive adaptation scores were significantly correlated ($r = -0.881$, $P < 0.001$, $n = 423$), meaning that damage scores tend to predict yield responses. The population of RILs was normally distributed for thrips resistance suggesting that the inheritance of resistance to the melon thrips may be quantitative. It is also possible that the two parents may possess distinct genes for resistance to thrips because their progeny resulted in transgressive segregation. The rank correlation coefficient between unreplicated and replicated nurseries was significant and high (0.823, $P < 0.001$, $n = 131$) suggesting that genetic differences tend to show consistency across seasons and infestation levels.

Yield testing of selected RILs in comparison with previously selected resistant and susceptible genotypes showed highly significant differences among genotypes for damage scores, reproductive adaptation scores, and yields (Table 13).

As in previous trials, correlations between damage scores and reproductive adaptation scores were significant ($r = -0.771$, $P < 0.001$, $n = 48$). Also significant were the correlations between damage scores and yields ($r = -0.862$, $P < 0.001$, $n = 48$) and between reproductive adaptation

scores and yields ($r = -0.873$, $P < 0.001$, $n = 48$). Some of the RILs were as resistant as the resistant checks EMP 486 and FEB 115, and several out yielded the variety Brunca. As compared to FEB 162, the highest yielding genotype in this trial, several of the RILs suffered negligible losses, while losses in the susceptible checks were APN 18 = 74%, PVA 773 = 77%, and RAZ 136 = 93%.

Table 13. Resistance levels and yields of selected bean genotypes^a tested for resistance to *Thrips palmi*.

Genotype	Damage scores ^b	Reproductive adaptation scores ^c	Yield (g per plant)	Percentage yield reduction ^d
RAZ 136	9.0 ± 0 a	1.0 ± 0 f	0.4 ± 0.12 f	93.0
PVA 773	8.0 ± 0 a	2.3 ± 0.33 de	1.3 ± 0.14 ef	77.2
APN 18	8.0 ± 0 a	2.0 ± 0 ef	1.5 ± 0.06 def	73.7
FEB 115	6.7 ± 0.32 b	3.3 ± 0.33 cd	3.8 ± 0.39 abc	33.3
Brunca	6.3 ± 0.66 b	3.3 ± 0.33 cd	4.2 ± 0.63 abc	26.3
BH-5	6.0 ± 0 ab	4.0 ± 0.57 bc	3.2 ± 0.23 cde	43.8
BH-130	6.0 ± 0 ab	3.7 ± 0.67 bc	3.4 ± 0.62 bcd	40.3
BH-37	5.0 ± 0.57 bc	4.7 ± 0.68 ab	5.6 ± 0.63 a	1.7
BH-46	5.0 ± 0.56 bc	4.7 ± 0.67 ab	4.9 ± 0.44 abc	14.0
BH-53	5.0 ± 0.56 bc	4.0 ± 0.57 bc	5.2 ± 0.65 abc	8.8
G 3569	5.0 ± 0.42 bc	4.0 ± 0 bc	4.9 ± 0.17 abc	14.0
EMP 486	4.7 ± 0.33 c	4.0 ± 0.57 bc	4.5 ± 0.39 abc	21.0
BH-139	4.7 ± 0.33 c	4.7 ± 0.33 ab	5.3 ± 0.67 ab	7.0
FEB 161	4.7 ± 0.33 c	4.7 ± 0.88 ab	5.3 ± 0.36 ab	7.0
BH-84	4.7 ± 0.33 c	4.3 ± 0.33 abc	4.9 ± 0.60 abc	14.0
FEB 162	4.3 ± 0.33 c	5.3 ± 0.67 a	5.7 ± 0.71 a	-

- Means ± SEM of three replications per genotype. Means within a column followed by the same letter are not significantly different by LSD ($P < 0.001$).
- On a 1 to 9 scale, where 1 = no damage and 9 = very severe damage.
- On a 1 to 9 scale, where 1 = no pod setting and 9 = excellent pod setting.
- With respect to the best treatment.

An additional yield trial was conducted in which we compared 38 RILs and their parents with selected elite breeding lines and susceptible and resistant checks. Table 14 shows results.

In terms of yield under a moderate seasonal infestation level of 6.3 adults per leaflet, RILs outperformed elite breeding lines and susceptible checks and yielded as well as the resistant checks. In terms of damage, differences were consistent with previously reported resistance ratings; all groups compared being significantly different from the susceptible checks. The parents BAT 881 and G 21212 showed intermediate resistance suggesting that transgressive segregation may be the explanation for higher levels of resistance in some of the RILs tested.

The correlation between damage scores and yields was high and significant ($r = -0.735$, $P < 0.001$, $n = 285$). Using damage scores and yield responses as estimates of tolerance to damage, it would be possible to facilitate selection among large numbers of genotypes. As shown in Figure 37, genotypes falling in the upper left quadrant would be selected for further testing and for identification of potential parents. Using damage scores as first criterion, one could discard all

genotypes showing high damage scores, and then measure yields of those with low damage scores. All genotypes falling in other quadrants would be discarded.

Table 14. Yields and damage scores of selected bean genotypes tested for resistance to *Thrips palmi*^a. Best recombinant inbred lines and elite breeding lines were chosen on the basis of yields and are here compared to resistant and susceptible checks.

Genotypes	Yield (g per plant)	Damage scores ^b	
		30 days after planting	50 days after planting
Recombinant inbred lines :			
BH-37	5.6 ± 0.63	6.0 ± 0	5.0 ± 0.57
BH-139	5.3 ± 0.87	4.7 ± 0.33	4.7 ± 0.33
BH-53	5.2 ± 0.36	5.3 ± 0.66	5.0 ± 0.57
BH-46	4.9 ± 0.44	5.7 ± 0.33	5.0 ± 0.57
BH-84	4.9 ± 0.60	5.0 ± 0.57	4.7 ± 0.33
Mean 38 lines	4.0 ± 0.73 a	5.9 ± 0.52 b	5.8 ± 0.52 b
Parents:			
BAT 881	2.1 ± 0.52	6.7 ± 0.33	7.0 ± 0
G 21212	3.8 ± 0.89	6.0 ± 0.57	6.3 ± 0.88
Mean	2.9 ± 0.64 c	6.3 ± 0.42 b	6.6 ± 0.86 b
Elite breeding lines and commercial varieties:			
FEB 162	5.7 ± 0.71	5.7 ± 0.33	4.3 ± 0.33
FEB 161	5.3 ± 0.36	5.0 ± 0.57	4.7 ± 0.57
G 3569	4.9 ± 0.17	5.3 ± 0.33	5.0 ± 0.33
VAX 5	4.9 ± 0.47	5.0 ± 0.57	5.3 ± 0.57
A 788	4.4 ± 0.55	6.0 ± 0	5.7 ± 0.33
Mean 49 genotypes	3.2 ± 0.68 b	6.3 ± 0.48 b	6.2 ± 0.54 b
Resistant checks:			
EMP 486	4.5 ± 0.39	6.0 ± 0.57	5.0 ± 0.57
FEB 115	4.4 ± 0.67	5.7 ± 0.33	6.0 ± 0
Brunca	4.2 ± 0.63	5.7 ± 0.33	6.3 ± 0.67
Mean	4.4 ± 0.51 a	5.8 ± 0.38 b	5.8 ± 0.56 b
Susceptible checks:			
APN 18	1.5 ± 0.06	7.7 ± 0.33	8.0 ± 0
PVA 773	1.4 ± 0.14	7.3 ± 0.33	8.0 ± 0
RAZ 136	0.4 ± 0.12	8.7 ± 0.33	9.0 ± 0
Mean	1.1 ± 0.32 d	7.9 ± 0.45 a	8.3 ± 0.29 a

- a. Means ± SEM of three replications per genotype. Means within a column followed by different letters are significantly different, separation by Scheffe's *F* method of significance testing for arbitrary linear contrasts with 188 df ($P < 0.05$).
- b. On a 1-9 scale, where 1 = no damage and 9 = very severe damage.

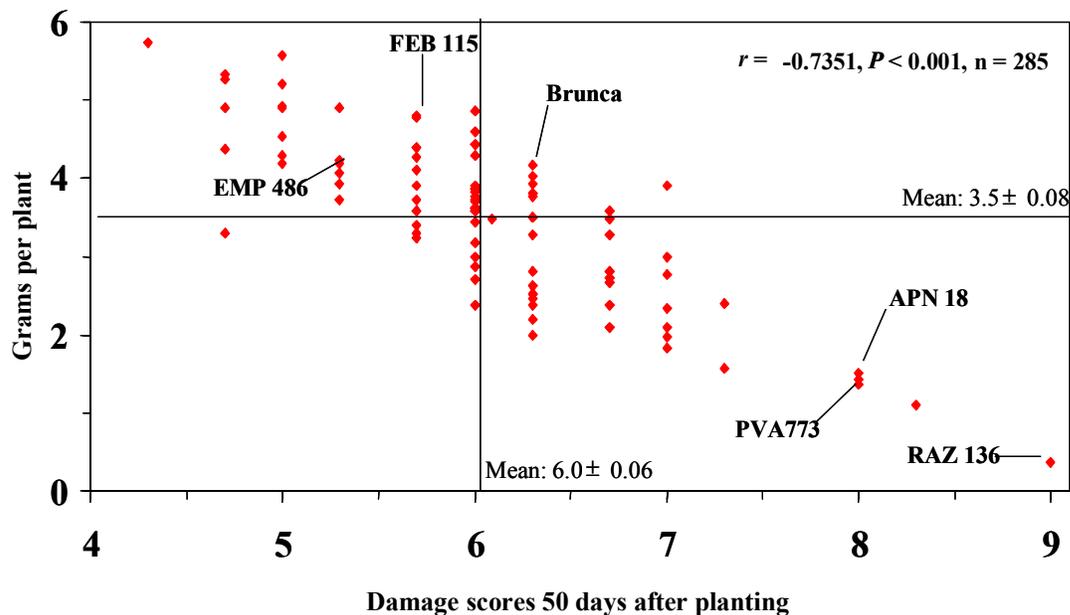


Figure 37. The relationship between damage scores and yield per plant in 92 bean genotypes tested for resistance to *Thrips palmi*. Damage scores are on a 1-9 scale, where 1 = no damage and 9 = very severe damage. Resistant and susceptible checks are labeled.

Reference:

CIAT. 2001. Annual Report Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

Contributors: C. Cardona, J.M. Bueno, A. Frei, J. Díaz, S. Beebe

Further studies on resistance to *T. palmi* were conducted with a set of 114 F₂ and F₃ selections derived from eight crosses made for BGMV resistance. The unreplicated testing of these materials under very high insect pressure (19.8 adults per leaflet seasonal average) revealed that nine selections were resistant, 51 were intermediate, and 54 were susceptible. Table 15 shows the best levels of resistance. The resistant and intermediate genotypes are being reevaluated at the Pradera site.

We also evaluated a set of 97 RILs derived from a cross between DOR 364 and BAT 477. Under a moderate level of infestation (7.9 adults per leaflet), seven genotypes were classified as resistant, 60 were intermediate, and 30 were susceptible. As in other trials, there was a significant, negative correlation ($r = -0.70, P < 0.001$) between damage scores and reproductive adaptation scores (Figure 38).

Table 15. Best levels of resistance to *Thrips palmi* in populations derived from crosses made for resistance to bean golden mosaic virus.

Code	Identification	Damage scores ^a
MN 13638-14	(BH 21134-5 x BAT 477)F ₁ x (DOR 390 x (DOR 390 x (DOR 390 x SAM 1)F ₁)F ₁)F ₁ /-	4.7
MN 13638-26	(BH 21134-5 x BAT 477)F ₁ x (DOR 390 x (DOR 390 x (DOR 390 x SAM 1)F ₁)F ₁)F ₁ /-	5.0
MN 13638-28	(BH 21134-5 x BAT 477)F ₁ x (DOR 390 x (DOR 390 x (DOR 390 x SAM 1)F ₁)F ₁)F ₁ /-	5.0
MN 13641-2	(BH 21134-5 x TLP 35)F ₁ x (DOR 364 x (DOR 364 x (DOR 364 x SAM 1)F ₁)F ₁)F ₁ /-	5.0
MN 13641-3	(BH 21134-5 x TLP 35)F ₁ x (DOR 364 x (DOR 364 x (DOR 364 x SAM 1)F ₁)F ₁)F ₁ /-	4.0
MN 13641-4	(BH 21134-5 x TLP 35)F ₁ x (DOR 364 x (DOR 364 x (DOR 364 x SAM 1)F ₁)F ₁)F ₁ /-	4.7
MN 13641-5	(BH 21134-5 x TLP 35)F ₁ x (DOR 364 x (DOR 364 x (DOR 364 x SAM 1)F ₁)F ₁)F ₁ /-	5.0
MN 13641-7	(BH 21134-5 x TLP 35)F ₁ x (DOR 364 x (DOR 364 x (DOR 364 x SAM 1)F ₁)F ₁)F ₁ /-	5.0
MN 13640-11	(BH 21134-59 x BAT 477)F ₁ x (DOR 364 x (DOR 364 x (DOR 364 x SAM 1) F ₁)F ₁₀ F ₁ /-	5.0
BAT 304 ^b		6.6
DOR 364		8.2
PVA 773 ^c		7.5
ICA Pijao		7.6
BAT 41		8.2
RAZ 136 ^c		8.9

- a. On a scale of 1-9, where 1 = no damage, and 9 = very severe damage.
- b. Resistant check.
- c. Susceptible check.

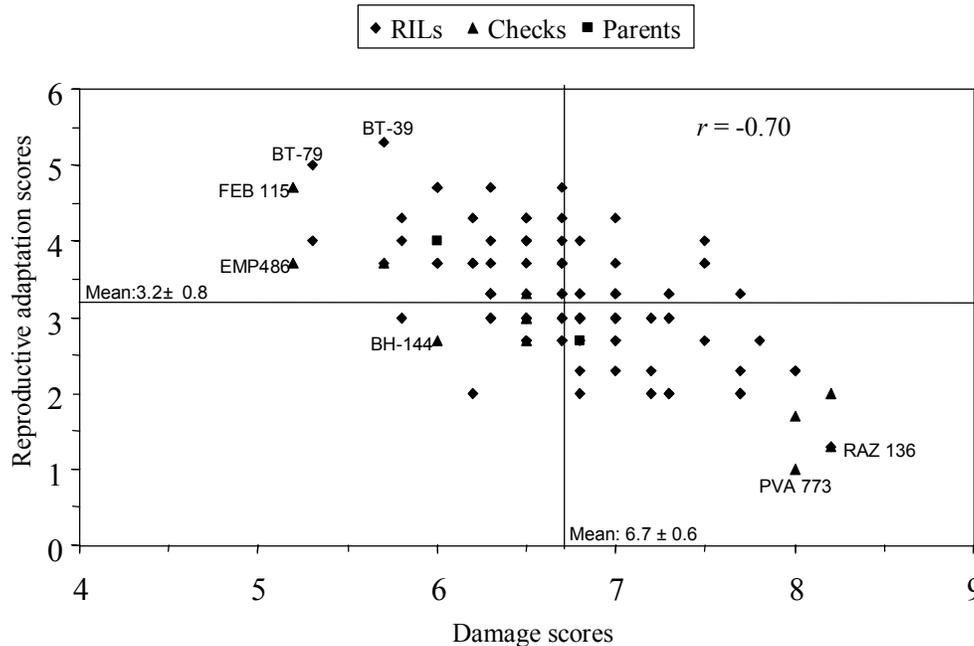


Figure 38. The relationship between damage scores and reproductive adaptation scores in 97 recombinant inbred lines (RILs) derived from a DOR 364 x BAT 477 cross-tested for resistance to *Thrips palmi*. Damage scores are on a scale of 1-9, where 1 = no damage and 9 = very severe damage. Reproductive adaptation scores are on a scale of 1-9, where 1 = no pod setting and 9 = excellent pod setting.

Contributors: C. Cardona, S. Beebe, J.M. Bueno

Additional sources of resistance to *T. palmi* were identified when a set of 73 EMP lines were evaluated at CIAT and Pradera under high levels of infestation (up to 19.7 adults per leaflet). Table 16 shows best genotypes.

Table 16. Resistance to *Thrips palmi* in selected lines (coded EMP) bred for resistance to the leafhopper *Empoasca kraemeri*.

Genotype	Damage scores ^a	Reproductive adaptation scores ^b
EMP 127	6.2	2.7
EMP 135	6.3	3.0
EMP 151	7.0	3.7
EMP 155	5.7	3.0
EMP 165	6.7	2.3
EMP 169	6.0	2.3
EMP 174	5.7	3.0
EMP 175	6.2	2.3
Resistant checks:		
BH 1134-5	5.8	2.3
BH 1134-60	5.3	4.3
BH 1134-144	5.5	3.0
FEB 115	5.5	3.0
EMP 486	5.3	3.7
BAT 304 (Brunca)	6.0	3.3
Susceptible checks:		
APN 18	7.3	2.0
PVA 773	8.7	1.0
BAT 41	8.3	1.0
RAZ 136	9.0	1.0
PVA 773	8.7	1.0

a. Damage scores on a scale of 1-9, where 1 = no damage and 9 = severe damage.

b. Reproductive adaptation scores on a scale of 1-9, where 1 = no pod setting and 9 = excellent pod setting.

Contributors: C. Cardona, J.M. Bueno.

Mechanisms of resistance to *T. palmi*: Intensive work on mechanisms of resistance to *T. palmi* was initiated in 2000 and continued through 2001. These studies are part of Miss Andrea Frei's Ph. D. thesis conducted at CIAT as part of the continuous collaboration with the Eidgenössische Technische Hochschule-Zentrum (ETHZ) Institute in Zurich, Switzerland. A set of previously selected resistant and susceptible bean genotypes are being used to study antixenosis, antibiosis, and tolerance under greenhouse conditions. Results of two independent multiple choice tests in the greenhouse showed clear preference of thrips for the bean variety EMP 486. At the same time, adult colonization was highly reduced on the variety FEB 115, when alternatives were available to the insect (Table 17).

Results so far suggest different resistance mechanisms in the chosen bean varieties. Although all four resistant varieties showed low damage and good reproductive adaptation in previous field studies (conducted to choose the varieties), different levels of insect infestation and different

preferences were observed. The variety EMP 486 showed high infestation and low damage, tolerance being the possible mechanism of resistance. FEB 115 showed low damage and low infestation, with antixenosis as a possible mechanism. In the other varieties, a combination of different mechanism or just tolerance may be responsible for resistance.

Table 17. Multiple choice test, percentage (mean \pm SEM)^a of observations of *Thrips palmi* found per bean variety, in two independent trials (n = 20 each).

Bean variety	Trial 1	Trial 2
	Insects per plant (%)	Insects per plant (%)
Brunca	19.2 \pm 1.6 b	15.6 \pm 0.9 c
BH-130	20.0 \pm 1.3 b	20.2 \pm 1.1 b
EMP 486	24.1 \pm 1.3 a	26.3 \pm 1.3 a
FEB 115	15.0 \pm 1.4 c	17.5 \pm 1.4 c
APN 18 (susceptible)	21.3 \pm 1.2 b	20.5 \pm 1.5 b
<i>P</i> -value variety	<0.0001	<0.001

a. Means (\pm SEM) within a column followed by the same letter are not significantly different ($P < 0.01$, Fisher's Protected LSD test on arcsine transformed data).

Antibiosis studies did not reveal significant differences in immature development of thrips on different bean varieties. Duration of the different immature stages (i.e., egg, larva, prepupa, and pupa) (Table 18) and adult characteristics such as body length were very similar for all genotypes tested.

Table 18. Immature development. Mean duration (\pm SEM) of life stages of insects reared on different bean varieties in antibiosis life cycle study.

Variety	Duration (days \pm SEM)				
	Egg	Larva	Prepupa	Pupa	Immatures
Brunca	4.7 \pm 0.08	4.6 \pm 0.10	1.3 \pm 0.06	3.1 \pm 0.06	13.7 \pm 0.11
BH-130	4.9 \pm 0.06	4.4 \pm 0.08	1.2 \pm 0.05	3.0 \pm 0.06	13.5 \pm 0.08
EMP 486	4.5 \pm 0.07	4.4 \pm 0.09	1.3 \pm 0.05	3.0 \pm 0.07	13.3 \pm 0.10
FEB 115	4.7 \pm 0.07	4.3 \pm 0.08	1.3 \pm 0.05	3.1 \pm 0.07	13.4 \pm 0.07
APN 18 (susceptible)	4.6 \pm 0.07	4.5 \pm 0.07	1.2 \pm 0.05	3.0 \pm 0.07	13.3 \pm 0.07

No antibiosis effects were found in life cycle studies and in studies on adult survival and oviposition rates. Work is in progress to measure tolerance under greenhouse and field conditions, as well as in the development of molecular markers for resistance to *T. palmi*. Results will be presented in 2002.

Contributor: A. Frei

Leafhopper: In 2001, 506 germplasm bank accessions were screened for resistance to *Empoasca kraemeri*. Those selected (169) will be re-evaluated in replicated nurseries in 2002. We also screened 24 F₈ lines derived from crosses attempting to combine leafhopper and BGMV resistance, but none was selected for resistance to leafhopper. Other screenings for resistance

included the evaluation of progenies obtained from 27 individual plant selections performed in a Berna x EMP 419 population. These materials were developed as part of our collaborative work with the University of Guelph in Canada. Eleven were selected and tested again in 2001. Four were then selected for superior performance under high leafhopper infestation (10.5 nymphs per leaf at 50 days after planting) and are being yield-tested at present.

In continuation of studies aimed at measuring progress in breeding for multiple insect resistance, we compared the performance of lines derived from crosses with EMP 250 with that of other superior EMP lines. EMP 250 is one of the best parents for leafhopper resistance used by the Bean Project. Results clearly indicated that strict selection for higher yield under protected and non-protected conditions (Figure 39) has resulted in a number of lines that outperform not only the tolerant check (ICA Pijao), but also superior lines such as EMP 250.

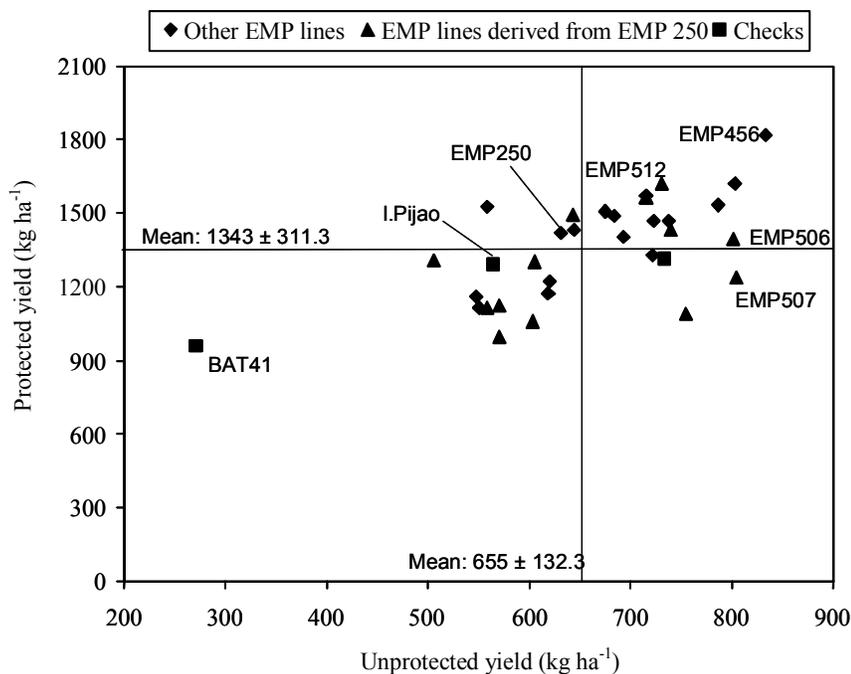


Figure 39. The relationship between protected and non-protected yields in bean lines developed for resistance to the leafhopper *Empoasca kraemeri*. BAT 41 is the susceptible check; ICA Pijao is the tolerant check.

A highly important activity in 2001 was the evaluation of the VEF for leafhopper resistance. This nursery was reactivated by the Bean Project (see Section 4.1.2) in order to focus the team's attention on a set of common genotypes and to gauge the state of the art of genetic advance. Out of 287 entries evaluated, 28 were classified as resistant (damage scores of 6 or less in a 1-9 scale): 13 EMP lines, 13 BM lines, MAM 38, and Tío Canela (Table 19).

Table 19. Leafhopper damage scores in best entries of the 2001 Bean Team Nursery (VEF).

Entry no.	Line	Seed color ^a	Seed size ^b	Leafhopper damage score ^c
190	EMP 589	6	s	3.5
191	EMP 590	6	s	3.7
209	EMP 486	6	s	4.2
136	BM 12722-133-24	2 R	s	4.5
135	BM 12722-133-7	2 R	s	4.7
184	EMP 488	6	s	4.7
117	BM 12722-49-7	2 R	s	4.8
133	BM 12722-129-10	2 R	s	4.8
130	BM 12722-126-7	2 R	s	4.8
180	EMP 415	8	s	5.2
118	BM 12722-49-8	2 R	s	5.2
182	EMP 423	8	s	5.2
121	BM 12722-57-26	2 R	s	5.3
181	EMP 419	1	s	5.5
122	BM 12722-60-10	2 R	s	5.5
186	EMP 512	2 R	s	5.5
114	BM 12722-44-2	2 R	s	5.7
109	BM 12722-34-2	2 R	s	5.8
128	BM 12722-84-7	2 R	s	5.8
179	EMP 400	2	s	5.8
62	Tío Canela 75	6	s	6.0
178	EMP 387	6	s	6.0
188	EMP 539	2 R	s	6.0
86	MAM 38	5 sp	m	6.0
115	BM 12722-44-4	2 R / 4	s	6.0
124	BM 12722-77-11	2 R	s	6.0
176	EMP 124	1	s	6.0
177	EMP 250	2 R	s	6.0
66	ICA Pijao ^d	8	s	6.8
290	BAT 41 ^e	6	s	9.0

- a. Seed color: 1 = white, 2 = cream, 4 = brown, 5 = pink, 6 = red, 8 = black, sp = speckled, and R = striped.
b. Seed size: s = small and m = medium.
c. On a 1 – 9 score scale, where 1 = no damage and 9 = severe damage.
d. Tolerant check.
e. Susceptible check.

Contributors: C. Cardona, J.M. Bueno

Bruchids: A total of 179 *Phaseolus* spp. accessions were screened for resistance to *Acanthoscelides obtectus*. These included 16 wild *P. vulgaris*, none of which was resistant. G 40177B was the only one resistant among eight *P. acutifolius* studied. In contrast, 120 of 152 *P. lunatus* evaluated were highly resistant to *A. obtectus*. This conforms previous results (CIAT 2001, p. 67-68) on the prevalence of resistance to the bean weevil in lima beans.

Reference:

CIAT. 2001. Annual Report 2000 Project IP-1. CIAT, Cali, CO. 188 p. (Working doc. no. 186)

Contributors: C. Cardona, J.F. Valor

Zabrotes

Rationale: *Zabrotes* (or the Mexican bean weevil) is the second most important storage pests of bean. Unusual seed storage proteins called arcelin are derived from wild bean and confer resistance. In previous reports we have described the selection of arcelin in complex crosses with sources of agronomic traits.

Materials and Methods: Complex crosses segregating for arcelin and for the *bgm-1* gene were created 3 years ago. The F₁ plants of these crosses were selected first for arcelin with electrophoretic gels and then for the *bgm-1* gene with the SCAR marker. Thus, two important resistance genes were selected by indirect methods to create populations with both genes. F₁-derived F₂ families were selected at Santander de Quilichao, and F₃ families at Popayán, under moderate fertility stress and disease pressure. F₄ families were planted at Santander de Quilichao and individual plants were selected.

Results: Individual plant selections (147 red seeded and 266 black seeded) have been made that are now in the F₅ generation. F₅ families were planted at Darién in low phosphorus conditions. Simultaneously, an individual seed of each selection has been delivered to Bean Entomology for the evaluation for arcelin, and these data will be taken into account in the harvest of the F₅ families. Conclusions are pending.

Contributors: S. Beebe, C. Cardona, J.F. Valor

Apion godmani

Rationale: *Apion godmani*, the bean pod weevil, is normally controlled in Central America with the use of extremely dangerous pesticides, although resistant varieties DICTA 113 and 122 are reportedly reducing the use of pesticides in Honduras. However, at present there is no longer an active breeding program for resistance to this pest. Because the pest does not exist in Colombia, selection cannot be practiced here either. An alternative strategy is to improve the DICTA varieties by backcrossing other traits into them, thus maintaining their level of *Apion* resistance.

Materials and Methods: The two DICTA varieties are derived from BGMV-resistant cultivar DOR 364 and present an intermediate reaction to BGMV. To fortify the value of these varieties, they were included in the *bgm-1* backcross program. The introduction of *bgm-1* will make these varieties more viable and attractive for farmers who face BGMV as well as *Apion*.

Results: The backcross program with the DICTA lines is in the F₃BC₂ phase. Families were evaluated in the drought nursery and many derived from DICTA 122 proved to be quite tolerant to drought, exhibiting good pod load, but only moderate grain filling. Several of the drought populations referred to in section 1.1.2 are derived from DICTA 122 and these will be delivered to national programs in Central America for selection.

Conclusions: The progeny of DICTA 113 and 122 will soon be purified for the *bgm-1* gene and will be distributed to national programs, especially that of Honduras where they were originally selected. The fact that DICTA 122 displays drought tolerance may explain in part why it performed so well in regional yield trials, and increases its value as a parental material. The development of molecular markers for *Apion* resistance was initiated in 2001. Results are reported in SB-2 Annual Report.

Contributor: S. Beebe

1.3.2 Developing germplasm resistant to insects

Resistance to leafhopper: As reported in Section 2.2.1, several different breeding nurseries were generated and evaluated for leafhopper resistance in 2001. The main objective of breeding in this case is the pyramiding of genes for leafhopper resistance. For details on breeding activities and results, see Section 2.2.1.

Contributors: M.W. Blair, C. Cardona, J.M. Bueno

Progress towards achieving output milestones:

- Studies on insect resistance mechanisms, identification of sources of resistance to insects, and development of parental lines with multiple disease and insect resistance contribute to the mainstream breeding efforts of the project.
- Insect resistance to thrips may be a basic IPM component for management of this very difficult pest of beans.

Activity 1.4 Incorporating wider genetic diversity into beans

Highlight:

- The high iron trait found in a wild bean was successfully transferred through two cycles of backcrossing.

1.4.1 Wild quantitative trait locus pursued in population of DOR 390 and G 10022

Rationale: Wild relatives of several crops have been investigated as sources of QTL for complex traits, including yield. Wild *P. vulgaris* has been demonstrated to have much broader genetic variability than cultivated bean, and feasibly could serve as a source of useful variability for broadening the base of the cultivated bean.

Materials and Methods: Several populations involving wild bean have been explored using the advanced backcross or backcross selfing method, whereby a large number of lines are generated by backcrossing a wild x cultivated hybrid twice to the cultivated parent. Yield and mineral analyses were initiated in the advanced backcross population of DOR 390 and G 10022.

DOR 390 is a widely used Mesoamerican, small, black-seeded cultivar resistant to BGYMV, and G 10022 is a wild bean from Mexico that presents Mesoamerican DNA patterns and high iron concentration in seed. Yield trials were established in the field at Santander de Quilichao under moderate fertility stress in the spring of 2001. A population of 144 lines was planted in a lattice of 12 x 12.

Results: In the field, no line presented a statistically significant yield advantage over DOR 390. However, three lines presented iron levels above the rest of the population (Table 20), and one of

these had iron almost at the level of the wild parent (98 mg kg⁻¹), which is about 78% above the average for common bean varieties. Unfortunately, this line did not recover all of the desirable traits of the cultivated parent, although its yield was only slightly below that of DOR 390. Thus, it was possible to transfer the high iron trait from the wild bean to a much more acceptable genetic background for future use in the breeding program.

Table 20. Yield and iron concentration of advanced backcross progeny of DOR 390 and G 10022.

Line (NH 21153-...)	Iron (mg kg ⁻¹)	Yield (kg ha ⁻¹)
67-1-1	98	1407
11-1-1	85	1098
13-1-1	81	1059
31-1-1	62	1647
31-2-1	66	1626
37-1-1	66	1647
37-1-2	63	1783
DOR 390	62	1569
LSD (<i>P</i> = 0.05)		365

Correlations among relevant parameters in this group of lines appear in Table 21. The only significant correlation was between seed size and yield. Although mineral content showed a very slight negative tendency in relation to other critical traits, these correlations were very weak and non significant. Thus, there should not be any problem with recovering high mineral content with acceptable grain size and yield.

Table 21. Linear correlations among yield, seed size, and mineral content in advanced backcross lines of DOR 390 and G 10022.

Traits	Correlation ^a
Yield - seed size	+0.27 *
Yield – iron	-0.13 NS
Yield – zinc	-0.19 NS
Seed size – iron	-0.07 NS
Seed size – zinc	-0.11 NS

a. * = significant at *P* = 0.05, NS = not significant.

Conclusions: Although we did not achieve a line that had all of the desirable traits of the cultivated DOR 390 with high iron, we confirmed in this population that the high iron trait can be transferred from the wild bean. Line NH 21153-67-1-1 ought to be a useful parent for additional crosses to increase iron concentration of grain. Given the fact that mineral content was not correlated with yield, it ought to be possible to recover high iron and high yield.

Contributors: S. Beebe, H. Terán, C. Cajiao (IP-1); M.W. Blair (SB-2)

Progress towards achieving output milestones:

- A breeding line with about 78% above-average iron concentration was recovered from crosses of wild x cultivated bean.