Output 2: Improved large-seeded Andean bean germplasm with less dependence on inputs

Activity 2.1 Developing germplasm resistant to diseases

Highlights:

- Common bacterial blight and ALS resistance were incorporated into advanced Andean breeding lines and tested in inoculated yield trials.
- Marker assisted selection applied to introgress bean golden mosaic virus (BGMV) resistance genes into advanced breeding lines with red-mottled seed type.
- Popping beans (ñuña) were crossed with anthracnose and BCMV resistance sources to develop new bush-type and climbing varieties of ñuñas.
- Forty-nine interspecific lines derived from simple and complex crosses of *Phaseolus vulgaris* and *P. coccineous* or *P. polyanthus* were shown to combine high levels of resistance to anthracnose and ALS.

2.1.1 Screening for common bacterial blight resistance in Andean breeding lines

Rationale: Common bacterial blight is an important foliar and seed-borne disease of Andean beans grown in tropical lowland and mid-elevation areas of Africa, Central America, and the Caribbean. The disease is also important in the subtropical and temperate regions of the Americas and Africa during hot, humid summer weather. The disease is caused by the pathogen *Xanthomonas campestris* pv. *Phaseoli* (XCP), which is widespread and part of a complex of Xanthomonas bacterial pathogens attacking many broadleaf and vegetable crops. Races of the XCP pathogen are not recognized. The current level of CBB resistance in Andean genotypes is insufficient and most current Andean varieties and advanced lines are highly susceptible. For example, in a previous study of 75 Andean lines from the International Bean Nursery (IBN) 2000 tested at Palmira for CBB resistance, only six had moderate resistance to this disease—all others were susceptible. In the past 2 years we have been advancing a series of populations derived from multiple crosses with good sources of resistance to CBB. We are using mainly VAX and XAN lines developed at CIAT to breed for CBB resistance. The VAX lines and some of the XAN lines (such as XAN 159 and XAN 309) are derived from interspecific hybridization between common bean (*P. vulgaris* L.) and tepary bean (*P. acutifolius* Gray). The source of the CBB resistance in XAN 159 and XAN 309 was originally derived from a tepary bean accession that was crossed into GN Sel 27 and later used at CIAT in the early 1980s. Breeders in the United States have used this resistance independently to create a number of CBB-resistant varieties, such as Jules and Tara. Our objective for this research was to test advanced lines from multiple parent crosses derived from Mesoamerican and interspecific sources of CBB resistance to see if their level of resistance was better than that of currently available Andean beans.

Materials and Methods: Four experiments were established in 2001A to compare the CBB resistance in varying numbers of advanced lines from the following seed classes:

(1) Red and purple mottled (Calima, Jose Beta, Pompadour – 170 F_7, F_8, and F_9 lines from 25 crosses);
(2) Cream mottled (Sugar, Cranberry – 71 F_6 and F_7 lines from 14 crosses);
(3) Yellow and white (Canario, White Kidney – 23 F_7 selections from 14 crosses); and
(4) Cream and red mottled (137 F_6 selections from seven crosses).

The first three groups of lines had been selected from a yield trial conducted at Palmira in 2000B, while the last group originated from single-plant selections made at Santander de Quilichao in 2000A. Table 22 gives the number of selections derived from a varying number of gametes where gamete selection was used for each cross, and its pedigree. The treatments were planted in single-row hill plots that were 3 m long. The crop was fertilized once with a foliar micronutrient application of boron-zinc. Three replications per experiment were used, along with a randomized complete block design. The CBB trial was inoculated with local isolates of XCP from Palmira at 3-weekly intervals starting at 20 days after planting (DAP). The CBB resistance was evaluated at 50 DAP during pod filling, using a standard 1-9 scale (where 1 = resistant and 9 = susceptible) according to CIAT evaluation methods (CIAT, 1987). VAX 3, a small, red-seeded breeding line, was used as the resistant check, while BAT 41, a cream-colored genotype, was used as the susceptible check. The resistant and susceptible checks were planted every 20 rows. Local checks were different for each of the experiments depending on the seed class being tested. Red-mottled checks were A 36, AFR 699, AFR 735, AND 1090, CAL 96, and G 4494; cream-mottled checks were SUG 47, SEQ 1027, and COS 16; the yellow-seeded check was A 797; and the white-seeded one G 5273. The lines were harvested and the yield calculated on a per plant and per hectare basis. Un-inoculated plots of the same genotypes were grown in a different field in the same season to produce clean seed for shipment and distribution.

Table 22. Replicated common bacterial blight (CBB) screening and yield trials at Palmira, Colombia, 2001A.

Population code	Pedigree	Gametes (no.)	Selection (no.)	CBB score[a]
Red-mottled (F_7, F_8, F_9) – 25 crosses – 170 selections:				
RX 21216	((A 384 x APN 277) x (VAX 3 x PVA 800A))	na	3	6.3
RG 12964	(A 483 X Montcalm) X ((MAM 48 X A 486) X (VAX 2 X Kaboon))	1	3	4.3
RG 12651	(AFR 188 X Montcalm) X (Montcalm X (XAN 309 X A 193) X (MAR 3 X G 5653))	2	4	5.0
RG 12639	(Chocho X Montcalm) X (XR 12307-1 X AND 277)	1	1	5.0
RX 21211	(G 6416 x RAZ 105) x (PVA 800A x VAX 3)	na	5	5.1
RG 12669	(PVA 800 A X AND 277) X (PVA 800 A X ((XAN 309 X A193) X (MAR 3 X G 5653)))	3	14	6.3
CT 13122	(VAX 3 x COS 16) x ((PVA 800A x Araucano 85) x (VAX 3 x Araucano 85))	1	1	2.0
RG 13130	(VAX 3 x PVA 773) x (A 483 x G 6416)	1	12	4.9
RX21214	(VAX 4 x G 5659) x (PVA 773 x G 23342)	na	11	4.8
RG 12944	(VAX 3 X PVA 773) X (A 483 X Montcalm)	2	5	4.1
RG 12943	(VAX 3 X PVA 773) X (PVA 800A X Araucano 85 INIA)	2	4	4.4
RG 12641	(XR 12308-1 X AND 277) X (EMP 355 X Montcalm)	2	4	4.0
RX 21221	A 193 x ((MAR 1 x G 6416) x (VAX 3 x G 9603))	na	6	4.8
RX 21213	A 483 x ((VAX 1 x G 6416) x (G 14016 x G 23342))	na	8	6.3

Continued.

Table 22. Continued.

Population code	Pedigree	Gametes (no.)	Selection (no.)	CBB score[a]
Red-mottled (F_7, F_8, F_9) – 25 crosses – 170 selections:				
RX 21223	AFR 619 x ((VAX 3 x AFR 298) x (G 6416 x RAZ 105))	na	3	3.9
RX 21212	AFR 619 x ((VAX 3 x G 4494) x (Catrachita x A 483))	na	8	4.8
RG 12655	BRB 190 X (PVA 800 A X ((XAN 309 X A 193) X (MAR 3 X G 5653)))	3	8	5.7
RG 13018	CAL 143 x ((A 483 x G 6416) x (VAX 3 x AFR 298))	na	6	3.7
RG 13128	CAL 143 x ((VAX 3 x PVA 773) x (PVA 800A x Araucano 85))	1	12	4.8
RX21215	G 23383 x ((TIF 1 x VAX 3) x (G 6416 x RAZ 105))	na	2	6.7
RG 13131	PVA 773 x ((G 6416 x G 4494) x (VAX 4 x G 4449))	1	1	7.0
RX 21227	PVA 773 x ((VAX 3 x G 4494) x (Catrachita x A 483))	na	14	4.3
RX 21225	PVA 773 x (VAX 1 x CAL 143)	na	30	6.0
RG 12966	PVA 773 X ((ICA Tundama X Edmund) X (VAX 3 X PVA 773))	1	4	4.2
RG 13024	PVA 800A x ((VAX 3 x AFR 298) x (Araucano 85 x G 6416))	na	1	8.0
Cream-mottled (F_6, F_7) - 14 crosses – 71 selections:				
CT 11976	(COS 16 X XAN 159) X (TIF 1 X Taylor)	1	2	4.8
CT 21220	(G 23383 x TIF 1) x (VAX 3 x COS 16)	na	4	3.0
CT 12949	(PVA 800A X Araucano 85 INIA) X (VAX 3 X Araucano 85 INIA)	2	5	3.0
CT 12950	(Taylor X A 483) X (VAX 3 X Araucano 85 INIA)	2	13	5.2
CT 12947	(TIF 1 X Montcalm) X (VAX 3 X Araucano 85 INIA)	4	14	3.0
CT 13122	(VAX 3 x COS 16) x ((PVA 800A x Araucano 85) x (VAX 3 x Araucano 85))	na	4	4.3
RG 12943	(VAX 3 X PVA 773) X (PVA 800A X Araucano 85 INIA)	1	4	3.9
CT 12953	Cardinal X ((COS 16 X VAX 3) X (TIF 1 X A A 193))	1	3	4.6
CT 12954	COS 16 X ((A 483 X MAM 38) X (Araucano 85 INIA X Wilkinson 2))	1	4	3.1
RX 21219	G 23383 x ((TIF 1 x VAX 3) x (G 6416 x RAZ 105))	na	1	2.7
RG 13024	PVA 800A x ((VAX 3 x AFR 298) x (Araucano 85 x G 6416))	na	1	7.0
RX 21217	SEQ 1027 x ((G 23383 x TIF 1) x (VAX 3 x COS 16))	na	12	3.1
RX 21218	SEQ 1040 x ((TIF 1 x G 6416) x (VAX 3 x COS 16))	na	2	3.2
CT 12957	Taylor X ((COS 16 X VAX 3) X (TIF 1 X AA 193))	1	2	3.0
White and yellow (F_7) - 14 crosses – 23 selections:				
WA 12965	(97RS-326 X VAX 3) X ((G 22263 X WAF 9) X (Edmund X AND 279))	4	4	3.4
CT 12954	COS 16 X ((A 483 X MAM 38) X (Araucano 85 INIA X Wilkinson 2))	1	2	3.0
CN 12971	ARA 18 X ((PVA 800 A X JALO EEP 558) X (IND. Jamaica Red X Wilkinson 2)	1	1	4.3
CN 12973	JALO EEP 558 X ((CAP 4 X BAYO MEX) X (VAX 3 X AND 279))	4	16	3.7
Cream and red-mottled (F_6) – 7 crosses – 137 selections made at Santander de Quilichao in 2000A:				
	(G 6416 x RAZ 105) x (PVA 800A x VAX 3)	1	1	5.7
	(RAZ 105 X VAX 3) X (PVA 800A X Montcalm)	12	85	3.0
CT 13413	(TIF 1 X Montcalm) X (VAX 3 X COS 16)	3	10	3.3
RG 13410	(VAX 3 X Calima) X (Catrachita X A 483)	2	7	3.7
CT 13414	(VAX 3 X COS 16) X (COS 16 X Local of Khomein)	1	11	2.3
RG 13409	(VAX 3 X Line DB 202 /5) X (RAZ 105 X PVA 800A)	3	19	3.6
CT 13412	(VAX 4 X Araucano 85) X (TIF 1 X Montcalm)	1	4	3.3

a. CBB score on a scale of 1-9, where 1 = resistant and 9 = susceptible.

Results and Discussion: Good levels of resistance were found in some of the advanced lines in each class. Table 22 shows the number of gametes and selection made per cross and the average CBB score of the cross. The percentage contribution of CBB-resistant parent genome to the pedigrees from which lines were selected ranged from 6.7% to 37.5%. Common bacterial blight

resistance of the pedigrees was associated with the parental contribution, although resistant lines could be selected from pedigrees where the contribution of the CBB resistant parent was small. The source used also had an effect on the overall level of resistance of lines derived from the cross. VAX lines produced more resistant progeny on the average than did XAN 159, XAN 309, Wilkinson 2, or XR 12308-1. VAX 3, with small red seed, was the VAX line that was used most often in the crosses for all four seed types. However, VAX 1 (Carioca), as well as VAX 2 and VAX 4 (both small cream-colored beans), also produced lines with CBB resistance in the red-mottled seed class. The disease had strong effects on the yield potential of the red- and cream-mottled genotypes. Yield was positively correlated with CBB resistance in the red-mottled (r = 0.223) and the cream-mottled (r = 0.428) lines, but not in the yellow and white lines, where yields were generally lower. Average yield and CBB resistance were substantially higher for the resistant check, VAX 3 (yield = 3075 kg ha^{-1}, CBB score = 2.0), than for the susceptible check, BAT 41 (yield = 1842 kg ha^{-1}, CBB score = 7.1), in the first three trials. Among the check varieties, most were moderately to highly susceptible, while among the lines there were a few with resistance similar to the resistant check and resistance scores that were intermediate as described above (Table 23).

Table 23. Average common bacterial blight (CBB) scores[a] and yields (kg ha^{-1}) of advanced lines and resistant, susceptible, and commercial checks that were compared against in four replicated trials grown at Palmira, Colombia, 2001A.

Population code	Red-mottled		Cream-mottled		Yellow - white		F_6 trial	
	CBB	Yield	CBB	Yield	CBB	Yield	CBB	Yield
Average lines	5.2	1398.5	3.9	1473.5	3.6	945.4	3.0	967.8
BAT 41	7.8	1734.5	6.8	1967.9	6.8	1826.7	6.0	1502.3
VAX 3	2.0	3031.6	2.0	3135.9	2.0	3061.1	2.5	1933.3
AND 1090	2.7	1324.0	---	---	---	---	2.7	918.5
AFR 699	4.3	1525.9	---	---	---	---	2.0	1358.3
AFR 735	4.3	1246.3	---	---	---	---	3.0	547.2
G 4494	7.0	1333.3	---	---	---	---	5.7	957.4
A 36	7.3	861.1	---	---	---	---	4.5	952.8
CAL 96	6.3	1303.7	---	---	---	---	4.5	655.6
COS 16	---	---	4.0	1827.8	---	---	---	---
SUG 47	---	---	6.0	1720.4	---	---	---	---
SEQ 1027	---	---	2.7	2259.3	---	---	---	---
A 797	---	---	---	---	5.0	1014.8	---	---
G 5273	---	---	---	---	4.0	877.8	---	---
PVA 773	---	---	---	---	---	---	3.0	686.1

a. CBB scores on a 1-9 scale, where 1 = resistant, and 9 = susceptible.

Conclusions and Future Plans: Resistance to CBB was successfully transferred to several Andean seed classes. Given the large number of resistant lines obtained, even in multiple parent crosses, it is likely that a few well-placed genes are instrumental in encoding the CBB resistance that was transferred. This bodes well for the breeding of elite lines with CBB resistance for the Caribbean and lowland areas of Africa and Latin America. In addition to the yield trials, 15 F_5 lines each were selected from two populations (G 4494 x VAX 6 and Wilkinson 2 x G 5686) and tested for CBB resistance. Both populations used sources of CBB resistance (Wilkinson 2 or VAX 6) to improve the red-mottled seed class; G 4494 being the original Calima variety released

by ICA and G 5686 being a source of ALS resistance. Meanwhile, a program to introgress CBB resistance more directly from the best Mesoamerican sources such as VAX6 through backcrossing is underway. The VAX 6 source is advantageous for use in marker-assisted breeding because the resistance gene in this variety has been tagged with a useful SCAR marker (SU 91) by our laboratory. We plan to implement high throughput MAS for CBB resistance in Andean beans in the upcoming year.

Reference:
CIAT. 1987. Bean Program Annual Report 1987. CIAT, Cali, CO. 352 p. (Working doc. no. 39)

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2.1.2 Screening for angular leaf spot resistance in Calima-type, red-mottled beans

Rationale: ALS is a serious disease in humid, low- and mid-elevation, bean production areas. The disease is caused by a fungal pathogen, *Phaeoisariopsis griseola*, which is well known for its pathogenic variability. Resistance is often location-specific depending on the races prevalent in a given area. The disease reaction exhibited by leaves and pods from the same genotype can be different, suggesting that separate genes can control resistance in these organs. Therefore it is important to pyramid various sources of resistance when trying to develop new varieties that will be field resistant to the widest array of races in many bean-growing regions. The objective of this research was to test a set of advanced lines that were developed from multiple crosses of ALS-resistant parents, for their level of field resistance to the disease.

Materials and Methods: Two ALS experiments were established for two different sets of advanced, Andean breeding lines. Both experiments were planted in Darién, the first in the 2000B season and the second in the 2001A season. The 2000B trial consisted of 175 lines, all of which were red mottled (Calima, Andino, and Mortiño types); while the 2001A trials consisted of 84 lines, of which 22 were red flecked (Flor de Mayo types) and the remaining 62 lines were red mottled. Table 24 gives the pedigree of each cross that was tested in the 2000B season, and Table 25 gives the number of gametes and selections in the 2001A season. Sixteen Andean check varieties were analyzed in both experiments (Table 26). Of these, A36 and Montcalm are considered susceptible checks, while AFR 735, AND 277, AND 279, CAL 96, and CAL 143 present resistance in greenhouse trials to individual Andean isolates of the disease and therefore were considered resistant checks for the field trials. The trials were designed as split-plot, randomized complete block experiments with inoculated and un-inoculated treatments and three replications each. The plots consisted of single-row, 2-m hill plots. The crop was fertilized with 100 kg ha^{-1} of super phosphate at planting and a foliar application of recommended zinc-boron chelate micronutrient. Weed control and other cultural practices were as recommended. A protectant fungicide was used once in the un-inoculated plots only to control ALS and other fungal diseases such as *Ascochyta* blight. The ALS disease treatments were spray inoculated with a suspension of conidia from local isolates of ALS pathogen at 3-weekly intervals starting at 25 days after planting. The lines were evaluated for ALS resistance at 40 (after flowering), 48 (during pod fill), and 68 (near physiological maturity) days after planting with a standard 1-9 scale (where 1 = resistant and 9 = susceptible) according to CIAT evaluation methods (CIAT, 1987). The lines in both the inoculated and un-inoculated experiments were harvested and the yield calculated on a per plant and per hectare basis.

Table 24. Origin of advanced red-mottled lines tested at Darién, Colombia, 2000B season for angular leaf spot resistance.

Cross	Pedigree	Gamete (no.)	Selection (no.)	Average yield (kg ha^{-1})
NM 12801	AND 279 X ((MAM 38 X CAL 143) X (PVA 800A X AND 277))	12	93	1443
NM 12802	(AND 279 X PVA 800 A) X ((Cornell 49-242 X G5686) X (Montcalm X CAL 143))	3	5	1385
CT 12809	(Cardinal X PVA 800 A) X ((Catrachita X PVA 773) X (PVA 800 A X AND 277))	2	4	1484
RG 12666	PVA 773 X (PVA 800 A X ((XAN 309 X A 193) X (MAR 3 X G 5653)))	7	13	1661
RG 12667	(PVA773 X ICA Tundama) X (PVA 800 A X ((XAN 309 X A193) X (MAR 3 X G 5653)))	17	31	1776
NM 12806	PVA 800 A X ((San Cristobal 83 X ICA Quimbaya) X (PVA 800 A X AND 277))	9	20	1945
RG 12978	I-414 x ((PVA 800A x G 5896) x (CAP 4 x Wilkinson 2))	1	3	1839
RG 12980	PVA 1441 x ((MAM 49 x Bola 60 Dias) x (PVA 800A x G 5896))	1	2	1754
RG 13023	PVA 773 x ((AND 1005 x (Catrachita x Bola 60 Dias)) x (MAM 13 x G 6416))	na	1	817
RG 13025	(PVA 800A x PVA 1441) x ((MAM 49 x Bola 60 Dias) x (PVA 800A x G 5896))	na	3	1671

Table 25. Origin of advanced lines, red-mottled, and Flor de Mayo lines tested at Darién, Colombia in 2001A for angular leaf spot resistance.

Cross	Pedigree	Gamete (no.)	Selection (no.)	Yield[a] (kg ha^{-1})	
				I	U
CN 13027	ARA 18 X (I 414 X ((PVA 800A X BAYO MEX) X (CAP 4 X Wilkinson 2)))	na	1	3612	3597
NM 12801	AND 279 X ((MAM 38 X CAL 143) X (PVA 800A X AND 277))[b]	3	22	3060	2557
RG 12631	ICA Quimbaya X A 483/	1	6	2560	3560
RG 12647	(MAM 48 X AFR 612) X (PI 150414 X PVA 800A)	1	1	1728	2826
RG 12649	(San Cristobal 83 X ICA Quimbaya) X (PVA 800 A X AND 277)	2	13	2626	3212
RG 12657	(Calima X (MAM48 X A483)) X (PVA800 A X ((EMP 376 X A193) X (NW 63 X A 429)	3	14	2076	2407
RG 12661	(DRK 138 X Pompadour J) X (PVA 800 A X ((DOR 708 X G 1344) X (A 429 XA 193)))	1	4	3173	3177
RG 12663	EMP 355 X Montcalm) X (A 193 X (XAN 309 X San Cristobal 83))	1	2	2842	2569
RG 12668	(PVA 800 A X AND 277) X (PVA 773 X ((EMP 385 X A 483) X (NW 63 X A 429)))	1	2	3424	3290
RG 12977	AND 1005 X ((Catrachita X Bola 60 Dias) X (MAM 13 X Montcalm))	5	8	2359	3512
RG 13025	PVA 800A X (PVA 1441 X ((MAM 49 X Bola 60 Dias) X (PVA 800A X Bayo MEX)))	na	9	2569	2706
RG 13026	PVA 1441 X (PVA 1441 X ((MAM 49 X Bola 60 Dias) X (PVA 800A X Bayo MEX)))	na	1	1736	2253

a. I = inoculated and U = uninoculated.
b. Pedigree giving rise to 22 Flor de Mayo types.

Table 26. Average yields (kg ha^{-1}) and angular leaf spot (ALS) resistance score[a] for lines and check genotypes in two experiments grown at Darién, Colombia in 2000B and 2001A.

Entry Check genotypes	2000B - ALS trial		2001A - ALS trial				
	ALS 48 DAP	Average yield	ALS 40 DAP	ALS 48 DAP	ALS 68 DAP	Average yield[b] I	 U
A 36	4.0	2940.9	7.5	6.0	7.0	2364.0	3525.3
A 193	3.0	1537.0	7.0	5.0	7.0	1792.0	2272.0
A 483	0.0	1749.6	na	na	na	na	na
AFR 298	0.0	1153.3	na	na	na	na	na
AFR 612	2.0	1089.7	2.0	4.0	2.0	3452.0	3712.0
AFR 619	2.0	1802.1	7.0	4.0	7.5	2968.0	2885.3
AFR 735	2.0	1618.6	2.0	2.0	3.0	2760.0	2525.3
AND 277	2.0	1034.3	2.5	3.5	2.5	3916.0	3373.3
AND 279	2.0	1556.0	3.5	4.0	3.0	2888.0	3640.0
AND 1005	3.0	1952.0	7.5	4.5	6.5	2060.0	2765.3
AND 1090	3.0	1796.6	7.0	5.5	7.5	984.0	2669.3
CAL 96	2.0	867.0	7.5	5.0	8.5	1432.0	2818.7
CAL 143	2.0	1956.9	2.5	2.0	2.0	3376.0	2592.0
POA 12	na	1858.3	na	na	na	na	na
PVA 773	2.5	1169.3	7.5	5.0	8.0	2228.0	3472.0
PVA 800A	2.5	660.0	5.5	4.0	6.5	2732.0	3696.0
PVA 1441	na	1128.0	na	na	na	na	na
SEQ 1039	1.5	1500.9	7.5	5.0	5.0	3832.0	3325.3
Montcalm	na	na	8.0	7.0	9.0	736.0	1984.0
G 4494	na	na	7.5	5.5	7.5	1716.0	2594.7
Average of lines	2.1	1586.0	5.8	4.3	5.6	2646.1	2869.9

a. Scores on a scale of 1-9, where 1 = resistant and 9 = susceptible.
b. I = inoculated and U = uninoculated.

Results and Discussion: Good levels of resistance were found in many of the advanced lines in both experiments. Tables 24 and 25 show the number of gametes and selection made per cross and the cross's average yield with and without inoculation. Disease pressure and hence average ALS scores were lower in the 2000B season than in the 2001A season. In the first trial, ALS scores for the lines ranged from 1 to 5.5 and none of the checks were severely infected; while in the second trial ALS scores for the lines ranged from 2 to 8.5 and there was good differentiation of the susceptible checks. The epidemic may have been lighter in the first trial because of the preponderance of resistant material that kept the epidemic at bay, or because of weather conditions that were unfavorable for disease development. In the second season, the ALS inoculation took hold early and good differences could be observed at 40 DAP and again at 48 DAP (although ratings were lower then), and finally at 68 DAP, when the disease began to affect pod filling. The three separate ALS readings were highly correlated amongst each other (r = 0.738 to 0.843). Yield under disease pressure was highly correlated with ALS disease rating (r = -0.524 to -0.700) showing that the disease had strong effects on the yield potential of the lines. Average yield and ALS resistance were higher for the resistant checks such as AFR 612, AFR 735, AND 277, and CAL 143, than for the susceptible checks, such as the breeding lines, A 36 and A 193, or the varieties Calima (G 4494), Montcalm (this variety is often used as a standard for ALS susceptibility), and ICA Caucaya (PVA 773). Surprisingly, CAL 96 was even more

susceptible than some of these checks, although it has been reported to have some resistance at other locations. Yields in the disease inoculation trials were somewhat correlated with yield potential in the un-inoculated trials (r = 0.258), although less so than with ALS susceptibility or resistance.

Conclusions and Future Plans: Good levels of resistance to the prevalent ALS isolates existed in the red-mottled seed class parents and progeny we tested at Darién. Most of the lines were derived from bulk selections made over several generations at Darién from multiple crosses that included various sources of ALS resistance. Single plant selections were first made in 2000A from a group of 834 Andean (red-mottled, cream-mottled, large red, and yellow -seeded) F_5 bulks (CIAT 2001, p. 77-78). The average level of resistance found in the advanced lines from any given pedigree was correlated with (a) whether the final parent was ALS resistant, and (b) how many parents in the multiple cross pedigree were ALS resistant. The most outstanding pedigrees included the parents AND 277, AND 279, Cornell 49-242, G 5686, and MAR 3, which are all well-known sources of ALS resistance. The genotypes CAL 143 and PVA 800A also appeared to be proportioning resistance to some of the crosses. For example, an especially promising cross was AND 279 x ((MAM 38 x CAL 143) x (PVA 800A x AND 277)), which produced 93 selections from 12 gametes over the generations of selection leading up to this experiment. Among the other resistant checks tested in this trial, AFR 612 was promising. The popularity of AFR 612 in Cauca may partly be because of its good ALS resistance. The breakdown of CAL 96 confirms the variability in isolates. Several other parents have also been successful in other parts of the world where ALS is a problem, so we are hopeful that the material developed here will be useful in other regions. POA 12 and PVA 1441 will need to be tested because they are being included in new crosses. A farmer-led participatory research committee in Restrepo, Valle will evaluate the advanced lines from this trial to help us determine if ALS resistance is site-specific and different there than at Darién. The pathogen's diversity seems to require the deployment and rapid rotation of varieties with multiple genes for resistance. To understand how to control this variable pathogen in Andean beans, we need to know how ALS resistance genes are inherited and whether resistance against Andean isolates in Colombia hold up against Andean, Mesoamerican, and Afro-Andean isolates elsewhere.

References:

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2.1.3 Marker-assisted selection of BGYMV-resistant, red-mottled beans

Rationale: BGMV is the most serious viral disease of beans in lowland tropical Latin America. It is caused by a bipartite geminivirus that is transmitted by the whitefly, *Bemisia tabaci*. Red-mottled beans are one of the few Andean types that are grown in low- and mid-altitude areas of the region where the vector and virus are present. The virus is a well-established endemic problem in red-mottled beans grown in the Caribbean (Haiti and the Dominican Republic) and has the potential of being a serious threat to some production areas in South America (Colombia, Ecuador, and especially lowland Bolivia). Other diseases that often occur when BGMV is present

are CBB, rust, and ALS. The whitefly vector often occurs in conjunction with heavy infestations of leafhoppers. Therefore, it is important to pyramid genes for resistance to all these pests and pathogens together with BGMV resistance into the same genotypes. The objective of this work was to deploy BGMV resistance genes into new, red-mottled genotypes through MAS of advanced lines in later generations. Because BGMV cannot be field-screened at CIAT and the virus is difficult to inoculate artificially, MAS is a good substitute for phenotypic selection. The objective of this research was to implement MAS for two BGMV resistance markers in two stages of the breeding process: (1) to eliminate gamete or segregants in early generation material and save space and resources dedicated to population development; and (2) to screen advanced lines that were selected for multiple traits and confirm the presence of BGMV resistance genes before including these lines in nurseries destined for the Caribbean region.

Materials and Methods: Marker-assisted selection for BGMV resistance in red-mottled and large red beans was conducted in early and advanced generations (F_1, F_2, and F_{6-7}) derived from either simple, triple, or multiple crosses. Two molecular markers were used to select for BGMV resistance in red-mottled beans. The SCAR marker, DOR 21, is closely linked to the major recessive resistance gene, *bgm-1*, while a second SCAR marker, W012, is associated with a QTL for BGMV resistance found on chromosome B04. A third SCAR marker, SU 91, was used to select for a major QTL for CBB resistance in a subset of the segregating lines. DNA was collected with a hole-puncher from field-grown plants and extracted with an alkaline lysis procedure (CIAT biotechnology protocols). In the case of early F_1 or F_2 generation material, plants were individually tagged and analyzed, while for F_6 or F_7 generation derived material, a bulk of four plants per row was analyzed. We did not analyze any plants presenting the dwarf-lethal phenotype that is common among crosses between Andean and Mesoamerican gene pools, especially in early generations. The early generation material was grown in short rows in an F_1-F_2 nursery, while the F_6-F_7 advanced lines were grown in a preliminary yield test, with three repetitions and 3-m, individual-row plots. Six red-mottled checks, A 36, AFR 699, AFR 735, AND 1090, G 4494, and PVA 773, were used in the yield trials.

Results and Discussion:

F_1 multiple crosses, 2000B: 381 individual plants derived from 52 triple crosses were tested for the *bgm-1* marker, of which 70 plants from 31 cross combinations were positive. Plants with no amplification of the DOR 21 marker or the alternate allele of this SCAR were considered to be negative. Table 27 shows the number of gametes selected for each cross that had positive plants. Positive plants were identified from the crosses with all of the commercial parents used (G 18264, Saladin 97, BRB 191, COS 16, POA 12, and CAL 143).

Although we expected 50% of the plants to be positive, only 18% were observed to have the marker for the *bgm-1* recessive allele. The lower than expected prevalence of positive plants could have been because of the mixed nature of the BGMV resistance sources used in the crosses. Although RMC 4, RMC 6, and RMC 10 are stable lines that have been screened in the Vivero Caribeño de grano Andino (VICARIBE) nursery, the other four sources, SEL 1428, SEL 1432, SEL 1438, and SEL 1439, are earlier generation bulks that were positive for the *bgm-1* marker, but may still have been segregating. Gamete selection was applied to the F_1 plants from these triple crosses based on the marker results, resulting in a reduction in 83% of the individual selections that would be made if all of the plants were saved for phenotypic evaluation. A second

selection criterion was seed color. Ideally, segregants needed to have bright red colors, but seed size was less important because medium and even small seed is accepted in the Caribbean if the grain is the proper shade of red mottled. The selected F_1 plants were advanced at Palmira 2001A as F_2 families and grown for mass selection based on plant architecture and grain color. The progeny of these families will be advanced to homozygosity, selected in the F_5 generation, when they will be screened again to make sure they contain the arcelin and *bgm-1* genes.

Table 27. Number of gametes positive for *bgm-1* from triple crosses with Andean parents.

Cross no.	***Pedigree***	No. gametes *bgm-1* positive Palmira 2000A
DR 21856	G 18264 X (EMP 122 X RMC 4)	2
DR 21857	G 18264 X (EMP 122 X SEL 1428)	1
DR 21858	G 18264 X (EMP 122 X RMC 6)	1
DR 21861	G 18264 X (EMP 122 X SEL 1438)	1
DR 21862	G 18264 X (EMP 122 X SEL 1439)	1
DR 21863	G 18264 X (EMP 320 X RMC 4)	3
DR 21868	Saladin 97 X (EMP 122 X SEL 1428)	2
DR 21869	Saladin 97 X (EMP 122 X RMC 6)	2
DR 21871	Saladin 97 X (EMP 122 X RMC 10)	2
DR 21873	Saladin 97 X (EMP 122 X SEL 1439)	2
DR 21875	Saladin 97 X (EMP 320 X SEL 1432)	2
DR 21880	BR 191 X (EMP 122 X SEL 1438)	1
DR 21881	G 18264 X (EMP 122 X SEL 1439)	2
DR 21883	G 18264 X (EMP 320 X SEL 1439)	1
DR 21884	COS 16 X (EMP 122 X RMC 4)	4
DR 21885	COS 16 X (EMP 122 X SEL 1428)	1
DR 21886	COS 16 X (EMP 122 X RMC 6)	3
DR 21887	COS 16 X (EMP 122 X SEL 1432)	3
DR 21888	COS 16 X (EMP 122 X RMC 10)	2
DR 21889	COS 16 X (EMP 122 X SEL 1438)	5
DR 21890	POA 12 X (EMP 122 X SEL 1428)	7
DR 21891	POA 12 X (EMP 122 X RMC 6)	8
DR 21893	POA 12 X (EMP 122 X RMC 10)	1
DR 21894	POA 12 X (EMP 122 X SEL 1438)	3
DR 21895	POA 12 X (EMP 122 X SEL 1439)	2
DR 21900	CAL 143 X (EMP 122 X RMC 4)	6
DR 21901	CAL 143 X (EMP 122 X SEL 1428)	2
DR 21902	CAL 143 X (EMP 122 X RMC 6)	2
DR 21903	CAL 143 X (EMP 122 X SEL 1432)	4
DR 21906	CAL 143 X (EMP 122 X SEL 1439)	1
DR 21907	CAL 143 X (EMP 320 X RMC 4)	1
	Total	78

F_1 triple crosses, 2001A: In a second round of MAS in the subsequent F_1 nursery grown at Palmira 2001A, 605 individual plants derived from 98 triple crosses were tested for the *bgm-1* marker. Of these, 304 plants were positive for the marker. The segregation of 50% positive plants agrees with the expected ratio of 1:1 homozygous to heterozygous plants for the F_1 of triple crosses and proves the fidelity of this molecular screening and validates the normal segregation

of the *bgm-1* gene. Table 28 shows the BGMV sources that were used in these crosses and the BCMV and *Empoasca* (EMP) resistance parents with which they were pyramided.

Table 28. Resistance sources, final parents, and number of combinations in triple crosses planted at Palmira, Colombia, 2001A.

Resistance sources	Final parents	Number of combinations
Bean golden mosaic virus sources:	Large red	
DOR 476	AFR 298	8
DOR 482	BRB 151	2
UPR 9745-138	Velasco Largo	6
UPR9 745-226	SEQ 1033	10
	BRB 181	8
Bean common mosaic virus sources:	BRB 189	6
BRB 181		
BRB 32	Cream mottled	
BRB 151	COS 16	3
BRB 181	BRB 203	7
BRB 189		
BRB 190	Red mottled	
BRB 203	BRB 32	10
COS 16	BRB 190	5
	CAL 143	7
Empoasca sources:	EMP 122	5
EMP 122	EMP 320	4
EMP 250		
EMP 320	Purple mottled	
EMP 364	G 18264	8
EMP 496	Saladin 97	9
	Total	98

The BGMV-resistant parents included the small, red-seeded Mesoamerican lines, DOR 476 and DOR 482, and two small-seeded, red-mottled lines from the University of Puerto Rico, with resistance derived from DOR 482. Among the *Empoasca* parents were three red-mottled lines (EMP 122, EMP 320, and EMP 364) and two Carioca lines (EMP 250 and EMP 496). The advantages of the latter two lines were that EMP 496 was positive for the *bgm-1* marker and the Carioca genotypes combined well with the red-mottled parents for seed color. Gamete selection was applied to the F_1 plants from these triple crosses based on the marker results, resulting in a savings of 50% of the individual selections that would be made if the plants had been saved for phenotypic evaluation.

F_6-$_7$ advanced lines: 82 advanced red-mottled lines containing the *bgm-1* marker were yield-tested at Palmira in 2001A. Table 29 shows the number of F_6 and F_7 lines and their pedigrees. Resistance to BGMV was incorporated into these advanced lines through a mixture of gamete and pedigree selection. The 82 lines were originally selected from a group of 174 single plant selections made in the F_5 and F_6 generations that were screened for the marker. The cross RG 12668 (PVA 800A x AND 277) x (PVA 773 x ((EMP 385 x A 483) x (NW 63 x A 429))), provided 64 lines out of the total. Two crosses also provided both F_6 lines that were yield-tested

and additional F_7 lines that were positive for the marker, but were not tested, namely A 193 x ((VAX 3 x EMP 366) x (Tío Canela x PVA 800A)); and CAL 143 x ((Tío Canela x PVA 800A) x (VAX 3 x A 483)). In these crosses, A 429 and Tío Canela were the sources of BGMV resistance.

Table 29. Pedigree of *bgm-1* positive, red-mottled advanced lines used in yield trial at Palmira, Colombia in 2001A.

Cross	Pedigree	Generation	Gamete (no.)	Selection (no.)
RX 21222	A193 X ((VAX3 X EMP366) X (Tío Canela X PVA 800A))	F_6	na	1
RX 21224	CAL143 X ((Tío Canela X PVA 800A) X (VAX 3 X A 483))	F_6	na	6
NM 12803	PVA773 X ((ICA Pijao X Calima) X (Montcalm X CAL 143))	F_7	2	3
NM 12805	PVA773 X ((PVA 800 A X DOR 482) X (Belmineb RMR-3 X Montcalm))	F_7	1	8
RG 12668	(PVA 800 A X AND 277) X (PVA773 X ((EMP385 X A483) X (NW63 X A 429)))	F_7	4	64
		Total	7	82

Additional populations segregating for the bgm-1 marker: A set of F_2 populations from simple crosses and a backcross population involving the Pompadour variety, PC50 or G 18264, were also screened for the *bgm-1* marker. A variety of BGMV resistance sources were used in the simple crosses, while SAM 1, a line derived from the cross DOR 476 x SEL 1309, was the donor parent for the backcrosses. Twenty-one individual plants were selected in the BC_1F_1 generation and advanced two generations by pedigree selection. Eight $BC_2F_{2:3}$ progeny per family were grown in the greenhouse for MAS. DNA was extracted from a balanced bulk of plant tissues from each family member and genotyped. Several families were phenotypically very similar to PC50 and contained the marker. These families have been advanced in the field to select the best individual plants in a later generation.

Conclusions and Future Plans: Here we show the application of MAS to (1) select segregating F_1 plants from triple and multiple crosses in early generations; and (2) determine which advanced $F_{6:7}$ lines carry the gene of interest. The advanced lines will be included in a regional testing program for the Caribbean coordinated by the University of Puerto Rico. As a service for this regional effort, we have been testing entries from the national programs of the Caribbean, for the presence of the *bgm-1* marker in new breeding lines. A substantial proportion of new lines has the resistance gene and has been field selected under BGYMV pressure. This confirms the value of the marker in selecting for resistance. Here at CIAT, we have started to pyramid BGMV resistance with *Empoasca*, CBB, and BCMV resistance sources. *Empoasca* is an ongoing problem of red-mottled beans in the Caribbean, where pest control is not practiced widely. Common bacterial blight sources are available, but are almost universally Mesoamerican. We have started to use the SU 91 marker for CBB resistance, but find that it works well only for crosses derived from VAX 6. Bean common mosaic virus resistance will become a renewed priority for the Caribbean region: Necrotic strains of BCMNV have been discovered in the San Juan de la Maguana region of the Dominican Republic and in the Port-au-Prince region of Haiti and threaten bean production in the Caribbean. Therefore, it is important to incorporate recessive resistance based on the *bc-3* gene into varieties for the region. Once we have incorporated the *bgm-1* gene widely into red-mottled germplasm and combined it with *Empoasca* and BCMNV resistance, we plan to use the W012 SCAR more intensively in MAS to accumulate an additional

QTL for BGMV resistance into these lines. We also plan to pyramid arcelin-based resistance to bruchids (see Activity 2.2) into the red-mottled lines. Although the emphasis has been on bush beans, we also hope to add BGMV resistance to red-mottled climbing beans that might be grown in the Caribbean in the near future.

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2.1.4 Crosses for anthracnose and BCMV-resistant climbing and bush type popping beans (ñuñas)

Rationale: Nuñas, or popping beans, are a special class of common bean whose seed will burst open and expand to double their size when toasted, fried, or microwaved. They are a traditional crop of the Andean highlands of South America. Almost all varieties of ñuñas are climbing beans, which have a good yield potential, but require a long growing season. As a result of the extended time that ñuñas are in the field, they are exposed to a range of diseases, such as anthracnose, *Ascochyta*, halo blight, and rust. The objective of this research is to improve popping beans for disease resistance, adaptation to wider range of environments, and early maturity. We also hope to create bush-type popping beans.

Materials and Methods: Nine popping beans (six climbing and three bush types) were selected from 58 genotypes from Peru, the CIAT germplasm bank, and the core collection for their popping ability in hot oil and in the microwave (CIAT 2001, p. 83-86). These were crossed to non-popping beans that were sources of BCMV and anthracnose resistance, early maturity, and bush bean architecture. The anthracnose resistance source was G 2333 and the three bush type non-popping beans were AFR 612, CAL 96, and CAL 143. We gave extra weight to crosses with popping beans that had type III (bush) growth habit because we wanted to develop bush-type ñuñas. The bush-type popping beans were G 11785, a small, round, white-seeded genotype; G 23604, a large-seeded, black-speckled genotype; and G 23614, a brown and white mottled genotype. Among the climbing bean popping beans there were red- (G 12572), brown- (G 12621 and G 12623), and gray- (G 23691) seeded genotypes. Most of the ñuña parents were round seeded, while the non-popping beans were oblong (e.g.. G 2333 or CAL 143) to elongated (e.g., CAL 96). The F_1s of simple crosses between bush-type popping bean and non-popping beans were double crossed amongst each other and backcrossed to the non-popping bean parent.

Results and Discussion: Tables 30, 31, and 32 indicate the number of selections that were made in the simple, double, and backcrosses with type III ñuñas. All the selections were of bush-type growth habit (types I and II), attractive seed colors, and good to fair architecture. Among the simple cross progeny, most of the individual F_2 (71%), F_3 (66%), and F_4 (68%) selections were derived from G 23614, reflecting its status as the best type III ñuña parent in terms of seed type and combining ability. G 23614 is a brown and white "vaquita" type, which in crosses with red-mottled parents, AFR 612, CAL 96, and CAL 143, produced a wide range of segregants from yellow-seeded Canario types all the way to red and white Soldier type beans. G 11785, a pure white type III ñuña, in combination with the same parents, produced the next largest proportion of F_2 (21%), F_3 (24%), and F_4 (24%) selections. Many of the segregants were large, rounded, white-seeded genotypes and would be readily acceptable as either Bolon Blancos or ñuñas.
G 23604, another type III ñuña, but with small wild-type seed and unappetizing colors, produced few selections among its progeny. The simple crosses between bush beans and bush ñuñas were

also backcrossed to the red-mottled, non-popping bean and to their bush ñuña parents. The backcrosses with the red-mottled parents were bulk harvested in the F_3 generation. Thirty-three F_4 bulks are being grown in 2001B and will be tested for their ability to pop. Meanwhile the BC_1F_1 plants of the backcross to the type III ñuña parent were surprisingly vigorous and needed to be staked at Darién during the previous season. Therefore, despite the inclusion of the bush beans in their pedigree, these crosses will be used to develop short-season climbing ñuñas rather than bush-type ñuñas. The F_1 plants have been harvested individually, the best seed types selected and the $F_{1:2}$ families planted at Darién in 2001B as climbing beans.

Table 30. Selections made from simple crosses between ñuñas and non-popping beans.

Population code	Cross	F_2 individual selections	F_3 individual selections	F_4 individual selections
	Darién 1999B	Darién 2000B	Darién 2001A	Darién 2001B
NA 21718	AF 612 X G 11785	2	10	9
NA 21719	AFR 612 X G 23614	4	19	33
NA 21720	AFR 612 X G 23604	1	7	9
NA 21721	CAL 96 X G 11785	2	8	16
NA 21722	CAL 96 X G 23614	12	41	105
NA 21723	CAL 143 X G 11785	4	28	60
NA 21724	CAL 143 X G 23614	12	68	103
NA 21725	CAL 143 X G 23604	2	13	19
	Total	39	194	354

Table 31. Selections made from double crosses between ñuñas and non-popping beans.

Population code	Cross	F_1 bulks		F_2 individual selections	F_3 individual selections
	Darién 2000A	Darién 2000B		Darién 2001A	Darién 2001B
NA 21729	(CA 143 X G 11785) X (AFR 612 X G 11785)			5	12
NA 21730	(CAL 143 X G 23614) X (CAL 143 X G 11785)			3	4
NA 21731	(CAL 143 X G 11785) X (CAL 143 X G 23614)			1	3
NA 21732	(CAL 143 X G 23614) X (AFR 612 X G 11785)			2	2
NA 21733	(CAL 143 X G 11785) X (CAL 96 X G 11785)			1	1
NA 21734	(CAL 96 X G 23614) X (CAL 143 X G 11785)			4	5
NA 21735	(CAL 96 X G 23614) X (CAL 143 X G 23614)			1	2
NA 21736	(CAL 96 X G 11785) X (CAL 143 X G 11785)			3	3
NA 21737	(CAL 96 X G 11785) X (AFR 612 X G 11785)			3	17
NA 21738	(CAL 96 X G 11785) X (CAL 96 X G 23614)			5	11
NA 21737R	(AFR 612 X G 11785) X (CAL 96 X G 11785)			2	10
			Total	30	70

Table 32. Selections made between ñuñas and non-popping beans from backcross to bush bean (F_2).

Population code	Cross	F_1 bulks	F_2 individual selections	F_3 bulks	F_4 bulks
	Darién 1999B	Darién 2000A	Darién 2000B	Darién 2001A	Darién 2001B
NA 21726	CAL 96 X (CAL 96 X G 11785)		5	5	4
NA 21727	CAL 96 X (CAL 143 X G 11785)		6	6	5
NA 21728	CAL 96 X (CAL 96 X G 23614)		10	10	9
NA 21739	AFR 612 X (CAL 143 X G 11785)		2	2	1
NA 21740	AFR 612 X (CAL 96 X G 11785)		1	1	0
NA 21741	AFR 612 X (AFR 612 X G 11785)		1	1	0
NA 21742	AFR 612 X (AFR 612 X G 23614)		1	1	1
NA 21743	CAL 143 X (CAL 143 X G 23614)		10	10	10
NA 21726	CAL 96 X (CAL 96 X G 11785)		3	3	3
		Total	39	39	33

Conclusions and Future Plans: The popping ability of the selections will be tested in the coming year. We will also be studying the inheritance of popping ability to help expedite the transfer of the trait from ñuñas into bush types. Other combinations of ñuñas and anthracnose or BCMV resistance are also being made. Triple and simple crosses are being made to incorporate anthracnose and BCMV resistance simultaneously into climbing popping beans. A wider range of ñuñas are being used, including the popular varieties Ñuña Limona, Ñuña Pava, and Ñuña Poroto Blanco, and the accessions G 12582, G 12621, G 12623, G 23603, G 23691, and G 23767. The BCMV-resistance genes, *bc-3* and *I*, are being introgressed from BCMV-resistant breeding lines such as BRB 29, BRB 32, BRB 151, BRB 156, BRB 183, BRB 197, BRB 203, BRB 204, BRB 211, and BRB 217. A wider range of anthracnose resistance sources including all the differential genotypes (AB136, MDRK, Perry Marrow, Kaboon, To, Widusa, and G 2333) is being used in crosses with ñuñas. In crosses, we have also used G 19833, a well-studied source that provides high levels of resistance to certain isolates, and G 2337, another climbing bean, as well as G 2333, which is from Guatemala. Triple crosses between the ñuñas and the F_1 of the simple cross between the anthracnose- and BCMV-resistant genotypes, will be made this season. We also plan to make crosses with Gloriabamba, a Mesoamerican climbing bean variety released in Peru that has good anthracnose resistance. Selections will be made directly from the simple and triple crosses, and in some cases backcrossing will be employed to incorporate the resistance genes into ñuñas. With these crosses we hope to create new varieties of ñuñas that will increase the availability of this product. We hope that by combining between growth habits, seed colors, levels of resistance, and popping ability we will come up with new ñuñas for a variety of agricultural systems. Increased supply of ñuñas might stimulate exports and marketing of popping beans, especially from Peru. This project will work with the Peruvian Institute for Grain Legumes (IPEL), the National University of Cajamarca, and other partners in Peru (Instituto Nacional de Investigación Agraria [INIA], Universidad Nacional Agraria "La Molina" [UNALM]) to develop, test, and promote the new popping beans.

Reference:

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2.1.5 Identifying genotypes with resistance to anthracnose

Rationale: Race-specific resistance has been extensively used to control specific races of *C. lindemuthianum* in different parts of the world. However, because of the incredible pathotype diversity displayed by *Colletotrichum lindemuthianum*, the resistance that is effective against some races in one region is not necessarily effective in another or different region. As a result, no resistance genes are effective against all known races of this pathogen. For example, some of the well-known resistance genes effective in Europe (e.g., *Co-2*, *Co-3*, *Co-4*, and *Co-5*) are not effective in Colombia, Brazil, Costa Rica, and Mexico. The variety G 2333, which has three resistant genes (*Co-4*^{2}, *Co-5*, and *C0-7*), although resistant to most known anthracnose races, is susceptible to races 3481, 3545, 3977, and 3933 from Costa Rica, Mexico, and Argentina. The breakdown of the resistance in G 2333, once thought to be effective against all races, calls for a diversification of resistance genes by looking at other *Phaseolus* gene pools. We have previously found the secondary gene pool (*P. polyanthus* and *P. coccineus*) to be highly resistant to many *C. lindemuthianum* races including races that infect G 2333. In addition, the secondary gene pool has been known to be a source of interesting agronomic traits, among which is resistance to biotic (ALS, *Ascochyta*, BGMV) and abiotic (drought) stresses. We therefore screened 75 interspecific lines derived from simple and complex crosses involving *P. vulgaris* and *P. polyanthus* or *P. coccineus*. These materials were previously found to have high levels of resistance to ALS. We hypothesize that durable resistance to ALS and anthracnose can be found in the secondary gene pool. We therefore sought to identify lines that combined ALS and anthracnose resistance to be used as parents to simultaneously introgress, into commercial type beans, resistance to these two diseases.

Materials and Methods: 75 interspecific lines resulting from simple and complex crosses of *P. polyanthus*, *P. coccineus*, and *P. vulgaris* (Table 33), which had been found to have high levels of resistance to ALS under field and greenhouse conditions, were screened using three *C. lindemuthianum* races (6, 15, and 3481). Ten seeds of each of the genotypes were planted in flats and grown under greenhouse conditions for 10 days until seedlings had reached the full, expanded primary leaf stage. Seedlings of each accession were sprayed with spore suspension (1×10^4 spores mL^{-1}) until runoff on the stem and both surfaces of the cotyledons. After inoculation, plants were maintained in high humidity (>95%) at about 22 ºC, with a 12 h light/dark cycle for 8 days. Disease was rated 10 days after inoculation using a CIAT 1 to 9 severity scale (van Schoonhoven and Pastor-Corrales, 1987). Plants with no visible disease symptoms or with only a few, very small, lesions mostly on primary leaf veins were recorded as resistant (scale 1 to 3). Plants with numerous small or enlarged lesions, or with sunken cankers on both the lower surfaces of leaves and the seedling stem were rated as susceptible (scale 7 to 9). Plants that had a rating of (3.1 – 6.9) were scored as intermediate.

Results and Discussion: Of the 75 interspecific lines, 49 were resistant to the most aggressive race 3481, whereas 69 were resistant to races 6 and 15 (Table 33). In total, 22 lines were susceptible to race 3481, while two lines were susceptible to race 15 and one line was susceptible to race 6. The differences in the number of lines that were resistant and susceptible between the races used signify the complex nature of this pathogen. The low frequency of resistance found (49 lines) compared to the total resistance found in the secondary gene pool core collection (CIAT, 2001) could have resulted from prior selection of interspecific lines for other traits.

Table 33. Reaction[a] of interspecific lines derived from simple and complex crosses of primary and secondary common bean gene pools to inoculation with three races of *Colletotrichum lindemuthianum* under greenhouse conditions.

Cross[b]	Family	Type of cross[c]	CL56 Col	CL172-ECU	CL77 CRI
BAT 338 x G 35252 (5)	F_8	Pv x Pc	R	R	R
BAT 338 x G 35252	F_8	Pv x Pc	R	I	S
BAT 338 x G 35252	F_8	Pv x Pc	I	I	S
BAT 338 x G 35252	F_8	Pv x Pc	I	I	R
BAT 338 x G 35252	F_8	Pv x Pc	R	R	S
BAT 338 x G 35252 (3)	F_9	Pv x Pc	R	R	R
BAT 338 x G 35252	F_9	Pv x Pc	S	S	S
BAT 338 x G 35252 (4)	F_9	Pv x Pc	R	R	S
BAT 338 x G 35252	F_9	Pv x Pc	R	R	I
BAT 338 x G 35252	F_9	Pv x Pc	I	S	I
(G 35649 x G 3807) x G 35023 (2)	F_5	(Pcw x Pv) x Pc	R	R	R
((G35876 x G 3807) x G 35182) x A 114 (10)	F_7	((Pcw x Pv) x Pp	R	R	R
((G 35876 x G 3807) x G 35182) x S 31003 (12)	F_7	((Pcw x Pv)x Pp	R	R	R
AND 279 x G 35337	F_2	Pv x Pp	R	R	R
PVA 1426 x G 35180	F_3	Pv x Pp	R	R	R
BAT 1253 x G 35325	F_3	Pv x Pp	R	R	R
((G 35876 x G 3807) x G 35325) x VRA 81043 (2)	F_3	((Pcw x Pv) x Pp	R	R	R
((S 13811 x G 677) x G 35023) x BAC 24	F_4	((Pcp x Pv) x Pc	R	R	S
(G 35649 x G 3807) x BAC 24	F_4	(Pcw x Pv) x Pv	R	R	S
(G 35649 x G 3807) x BAC 24 (2)	F_4	(Pcw x Pv)x Pv	R	R	R
(G 35649 x L 32) x BAC 24	F_6	(Pcw x Pv) x Pv	R	R	S
ZAV 83102 x G 35182	F_3	Pv X Pp	R	R	R
AND 84 x Piloy	F_2	Pv x Pp	R	R	R
E 1056 x G 35060	F_4	Pv x Pp	R	R	R
AND 107 x Piloy	F_2	Pv x Pp	R	R	R
Mortiño x X 7 (2)	F_9	Pv x Pp	R	R	R
((G 35876 x S 30985) x G 35182) x G 21715	F_2	((Pcw x Pv) x Pp	R	R	I
((G 35876 x S 30985) x G 21715) x G 35336	F_2	((Pcw x Pv) x Pv	R	R	R
((G 35876 x G 3807) x BAT 1276)x G 35182	F_4	((Pcw x Pv) x Pv	R	R	R
(ICA Pijao x G 35171) F_1 x ICA Pijao)	F_8	(Pv x Pc) x Pv	R	R	R
(ICA Pijao x G 35171) F_1 x ICA Pijao)	F_8	(Pv x Pc) x Pv	R	R	S
(ICA Pijao x G 35172) x ICA Pijao)	F_8	(Pv x Pc) x Pv	R	R	S
(ICA Pijao x G 35172) x ICA Pijao)	F_8	(Pv x Pc) x Pv	R	R	I
ICA Pijao x (ICA Pijao x G 35877) (6)	F_5	Pv x (Pv x Pc)	R	R	S
ICA Pijao x (ICA Pijao x G 35877) (2)	F_5	Pv x (Pv x Pc)	I	I	S
ICA Pijao x (ICA Pijao x G 35877)	F_5	Pv x (Pv x Pc)	I	R	S

a. R = resistant, I = intermediate, and S = susceptible.
b. Genotypes used to make the crosses. The number in parentheses represents the total number of lines from that cross with the specified reaction.
c. Pv = *Phaseolus vulgaris*, Pc = *P. coccineous*, and Pp = *P. polyanthus*.

Because these materials were in advanced generations (F_5 to F_9), it is possible anthracnose resistance genes might have been lost through several generations of selection for *P. vulgaris* phenotype, BGMV, and/or *Ascochyta* resistance to the exclusion of introgressed segments containing anthracnose resistance genes.

Conclusions: These materials constitute an interesting set of potential sources of durable anthracnose resistance. The high levels of anthracnose resistance found in these materials provide evidence for the genetic potential of the *Phaseolus* secondary gene pool as a source of durable anthracnose resistance in a breeding program. In addition, this resistance is present in inter-specific lines, which means that breeders can take advantage of these materials to shorten the amount of time required to transfer this resistance to commercial types of beans. These materials also combine resistance to most ALS races known, including the most aggressive races identified to date. Therefore, use of these materials will allow for simultaneous introgression of ALS and anthracnose resistance. However, central to successful use of the resistance in these materials is a knowledge and understanding of the nature and inheritance of this resistance.

References:

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

van Schoonhoven, A.; Pastor-Corrales, M.A. 1987. Standard system for the evaluation of bean germplasm. CIAT, Cali, CO. 53 p.

Contributors: G. Mahuku, C. Jara

Progress towards achieving output milestones:

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

- Advanced red-mottled breeding lines were identified with CBB and these were yield-tested in inoculated trials in preparation for their distribution to national programs.
- Advance red-mottled and large red-seeded lines were identified with ALS resistance.
- BGMV resistance genes were incorporated into red-mottled seed type populations and advanced breeding lines by marker-assisted selection.
- Early generation selections were made for seed type in populations segregating for BGMV resistance genes and resulting lines will be tested for other characteristics including multiple constraint resistance.
- New crosses have been made to create popping beans (ñuña) with anthracnose and BCMV resistance. Both bush-type and climbing plant types were selected in F_2, F_3, and F_4 generations.
- Several inter-specific lines that combine ALS and anthracnose resistance were identified. These lines are currently being screened for their reaction to other pathogens to identify lines with multiple constraint resistance.

- Stable and durable resistance to anthracnose and ALS is likely to be found from the secondary gene pool.

Activity 2.2 Developing germplasm resistant to insects

Highlight:

- New crosses were made to incorporate *Empoasca* resistance into red-mottled beans for the Caribbean and other regions.

2.2.1 Pyramiding *Empoasca* resistance in red-mottled beans

Rationale: Leafhoppers are one of the most serious pests of Andean beans grown in low- to mid-elevation areas. Andean beans have traditionally had less *Empoasca* resistance than Mesoamerican beans. The purpose of this research was to transfer the *Empoasca* resistance found in some Mesoamerican genotypes into red-mottled Andean beans.

Materials and Methods: Several sets of populations have been prepared since last year for *Empoasca* tolerance screening; these include:

(1) 41 red-mottled selections from four simple crosses between *Empoasca*-resistant lines and susceptible commercial types that were advanced to the F_4 generation.
(2) 440 triple-cross F_2 families from plantings at Palmira in 2000B and 267 in 2001A.
(3) 505 triple-cross F_1 plants, 460 with one resistant parent and 45 with two resistant parents.

The resistant parents used included the red-mottled genotypes, EMP 122, EMP 277, EMP 320, EMP 322, and EMP 364; and the Carioca genotypes, EMP 250 and EMP 496. When Carioca EMP lines were used in triple crosses, they consisted in only one quarter of the pedigree. The susceptible parents included large-seeded commercial types for South America (Colombia, Ecuador, and Bolivia), and small-seeded types for the Caribbean (Haiti and the Dominican Republic).

Results and Discussion: The simple crosses with EMP 250 and EMP 496 in their pedigree have produced the progeny with the best levels of resistance; however, the progeny tends to be smaller seeded than the progeny from triple crosses with two Andean parents. In the triple crosses, the red-mottled and Carioca seed types combined well to produce progeny with good red-mottled seed types (Table 34). Some of the progeny of the triple crosses had better seed quality than the current red-mottled EMP lines. Some of the triple crosses, especially with the Mesoamerican lines, DOR 482, EMP 250, and EMP 496, and the red-mottled breeding lines, EMP 122, EMP 320, and EMP 364, produced dwarf lethal segregants in combination with Andean parents.

Table 34. Selection of red-mottled bean types from simple and triple crosses for *Empoasca* resistance.

Cross	Pedigree	F_2 selection	F_3 selection
ER 21260	EMP 496 x PVA 773	3	7
ER 21261	EMP 496 x CAL 143	5	7
ER 21264	EMP 250 x G 18264	1	2
ER 21263	EMP 250 x PVA 773	20	25
DR 21456	UPR 9945-38 x (EMP 496 x PVA 773)	3	9

Conclusions and Future Plans: Because pyramiding appears to be necessary to achieve high levels of resistance to *Empoasca*, we are following a strategy of using more than one EMP parent per cross. In our latest series of triple crosses, we included either one of two parents from the EMP series in about 10% of the crosses for red-mottled beans. The F_2 families will be divided into two groups based on screening with the *bgm-1* marker—those that are positive will be advanced an additional generation before *Empoasca* screening, those that are negative will be screened in the F_2 generation. In additional crosses we are targeting *Empoasca* resistance to genotypes that will need this insect resistance the most—in areas such as the Caribbean, where beans are produced in lowland conditions, infestation is high, and small-scale farmers cannot afford chemical control. With this in mind we have initiated a series of simples crosses between three Haitian landraces, X029-43, 47, and 52, and EMP 250 and EMP 496. Although resistance has not been found in local materials and advanced lines coming from outside the *Empoasca* breeding project before, 227 Andean entries will be tested for *Empoasca* resistance, including 43 IBN entries (CIAT, 2001), 77 VICARIBE entries (including RMC, RMA, DRK, and UPR lines (see this Annual report), 16 Haitian accessions, and 55 Dominican accessions.

Reference:
CIAT. 2001. IP-1 Annual Report 2000. CIAT, Cali, Colombia. (Working document no. ?)

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

Progress towards achieving output milestones:

- Triple cross families selected from red-mottled breeding populations developed for *Empoasca* resistance.

Activity 2.3 Incorporating wider genetic diversity into beans

Highlights:

- Preliminary germplasm and advanced breeding line nurseries for climbing beans were tested at two locations to evaluate yield and yield components.

- Cream-mottled advanced lines were developed from multiple crosses.

- Higher iron beans were bred from advanced backcrosses with a wild accession as donor parent, and from recurrent backcrosses with a high-iron mapping population parent as donor parent.

2.3.1 Development of mid-elevation, commercial-type, Andean climbing beans

Rationale: The most outstanding characteristic of climbing beans is their high yield potential compared to more commonly grown bush beans. Climbing beans have been an important component of traditional societies in Central America and the Andes for centuries. More recently, climbing beans have become important in certain areas of Africa. The principal limitation to the expansion of climbing bean technology into new areas has been the lack of new varieties. Most currently available climbing beans come from high-altitude areas of Central and South America and do not grow well in lower elevations or hotter climates. An urgent need exists for climbing bean varieties that are adapted to lower elevations (800 to 1800 m) and resistant to the diseases encountered there. Currently, there are also very few climbing bean varieties with the red-mottled or red kidney seed types that are preferred in many areas of the Caribbean, Africa, and South America. Therefore, an additional challenge for breeders is to develop climbing bean varieties that produce grain with the proper color and size. The objective of this research is to hybridize, develop, and test mid-altitude adapted, commercial-type, Andean climbing beans.

Materials and Methods: In 2001A and 2000B seasons, we collected data on two yield trials of (1) 55 accessions from Rwanda, Mexico, and the core collection; and (2) 55 advanced Andean breeding lines from CIAT. Both experiments were planted at Darién (1450 m) and Palmira (1000 m) during the rainy seasons with randomized complete block designs and two repetitions each. Experiments at Palmira were protected from insect damage (primarily *Empoasca*, *Epinotia*, Thrips, and mites), while the experiments at Darién had two preventative fungicide treatments at planting and again at flowering. In all experiments, G 85 and G 333 were used as visual checks, every 20 rows at Palmira, and every 25 rows at Darién. The climbing beans were all planted at a low density of 10 plants per meter of linear row. Plots consisted of a single 2-m row with 1.2 m between rows. The vines were supported on bamboo and wire trellises approximately 2.0 m above the ground. Data collected included yield per plant, pods per plant, grain per plant, 100 seed weight, days to flowering, days to maturity, and harvest index based on stem and pod weight. Agronomic adaptation and climbing ability were evaluated on a 1 to 9 scale (where 1 = good and 9 = poor). The scale for climbing ability is an expanded scale compared to the accepted values for growth habit scale, which go from I to IV. Plant height, raceme length, number of pods per raceme, pod length, number of vines per guide, and internode length at a height of one meter above the ground were evaluated for two plants per row and averaged to produce plot values. Analysis of variance was conducted for repetition, genotype, location, and location x genotype effects (Table 35).

Results and Discussion: The 55 germplasm accessions evaluated this year were a set of selections for climbing beans with Andean type commercial seed colors (red mottled, red, yellow and cream mottled, and white) made from 335 accessions from Mexico (92 genotypes), Rwanda (182 genotypes), and the core collection (61 genotypes). Many of the accessions from the core collection adapted to the Palmira site were almost entirely Mesoamerican genotypes and therefore were related to the accessions tested from Mexico. The Rwandan accessions were

highly diverse in term of seed color and their climbing ability and probably represent natural hybridizations between climbing and bush beans from both Andean and Mesoamerican gene pools. The 55 single plant selection evaluated this year were derived in the $F_{5:7}$ generation at Palmira 1999B from 50 bulk selections of Andean climber x Andean bush populations. The F_7 lines were tested in 2000A to confirm their adaptation to mid-elevation regions and increase seed. Of the selections, 27 were red mottled, 13 were large red, and 32 were cream mottled, all with average seed sizes around 50 g / 100 seed. Fifty-five of the lines with the best seed types were seed increased at Palmira in 2000B so that the yield trial could be conducted at both Darién and Palmira in 2001A. Yield, yield components, and agronomic adaptation were higher on the average at Darién then at Palmira for both groups of genotypes. Genotype x location effects was significant for all the traits, except plant height in the germplasm trial and pods per plant and internode length in the advance line trial (Table 35). The check varieties, G 85 and G 333 and G 337, performed much better at Darién than at Palmira, indicating their lack of heat tolerance and adaptation to lower elevations. Palmira was a warmer and less hospitable location for climbing beans, and therefore a good site for selecting heat tolerance in them. Meanwhile, Darién was an ideal mid-elevation site where a wide range of germplasm performed well. High-elevation Andean climbers (ICA Viboral, Calima Darién, etc) yielded almost nothing at Palmira and even suffered from poor adaptation at a mid-elevation site such as Darién. These results show the importance of selecting parental germplasm in the appropriate environment.

Table 35. Significance[a] (*F* statistic) of location, genotype, and genotype x location effects for the trials grown at Palmira and Darién, Colombia, in 2001A.

Dependent variable	Data collected[b]								
	DF	100s	P/P	Y/P	AA	CA	PH	DM	IL
55 accessions from Rwanda, Mexico, and the core collection:									
Rep (loc)	2	4.77 *	0.83 ns	0.01 ns	2.1 ns	2.43 ns	2.14 ns	8.78 ***	2.16 ns
Location	1	648.24 ***	494.41 ***	559.54 ***	521.98 ***	182.52 ***	124.9 ***	3.69 ns	16.0 ***
Genotype	54	29.38 ***	3.48 ***	2.75 ***	4.68 ***	23.35 ***	4.66 ***	5.5 ***	8.04 ***
G x L	51	7.03 ***	2.47 ***	3.21 ***	5.24 ***	1.81 **	1.29 ns	1.5 *	2.49 ***
55 advanced Andean breeding lines and three checks (G 685, G 2333, G 2337):									
Rep(loc)	2	0.1 ns	0.7 ns	1.13 ns	0.25 ns	0.93 ns	0.64 ns	0.09 ns	0.24 ns
Location	1	443.59 ***	192.85 ***	469.14 ***	745.87 ***	86.98 ***	264.35 ***	5.41 *	36.49 ***
Treatment	57	11.57 ***	1.58 *	2.42 ***	8.79 ***	1.78 **	2.33 ***	3.17 ***	2.65 ***
Loc. treat	57	1.96 **	1.33 ns	0.04 ***	5.9 ***	1.18 ns	1.5 *	1.62 *	1.15 ns

a. Significance at $P = <0.001$ (***), 0.01 (**), 0.05 (*), or not significant (ns).

b. DF = days to flowering, 100s = 100-seed weight, P/P = pods per plant, Y/P = yield per plant (kg ha^{-1}), AA = agronomic adaptation, CA = climbing ability, PH = plant height, DM = days to maturity, and IL = internode length.

In the trait analysis, some characteristics were highly correlated (Table 36). Climbing ability was a good measure of plant height and internode length. These two traits can be said to be components of climbing ability along with lateral branching or vinyness. Climbing ability was evaluated visually on a whole-plot basis and was a rapid and accurate substitute for quantitative phenotypic measurements that are time consuming and must be taken on a per-plant basis. Yield per plant, yield components (such as pods per plant), and the visual evaluation of agronomic adaptation were correlated with climbing ability in the germplasm study at Darién, but not always

at Palmira. In the advanced breeding lines there was less variability for climbing ability than in the germplasm trial so differences in yield were not correlated with this factor, but rather with agronomic adaptation. The non-significant or negative correlations between yield and climbing ability at Palmira were because of the greater heat stress encountered by climbing beans at this site than at Darién. Even the Mesoamerican climbing bean check varieties, G 685, G 2333, and G 2337, had problems of adaptation in the hot season that occurred at Palmira. Many of the advanced Andean breeding lines outperformed these checks at Palmira, indicating a higher level of heat tolerance of these standard varieties that are grown throughout climbing bean areas of Africa.

Table 36. Correlation values (*r*) between traits of beans grown at Palmira and Darién, Colombia in 2001A.

Characteristic	Location	100s	P/P	Y/P	AA	CA	PH	DM	IL
55 accessions from Rwanda, Mexico and the core collection:									
100s weight (100s)	Palmira	1.000	-0.006	0.081	-0.275	0.356	-0.321	-0.342	-0.185
	Darién	1.000	-0.222	-0.141	0.085	0.075	-0.066	-0.197	-0.017
Pods per plant (P/P)	Palmira		1.000	0.161	-0.550	0.167	-0.124	-0.142	0.009
	Darién		1.000	0.690	-0.486	-0.347	0.170	0.273	0.252
Yield per plant (Y/P)	Palmira			1.000	-0.576	0.088	-0.076	-0.110	-0.074
	Darién			1.000	-0.585	-0.539	0.296	0.475	0.448
Agronomic adaptation	Palmira				1.000	-0.260	0.165	0.344	0.183
(AA)	Darién				1.000	0.687	-0.343	-0.320	-0.522
Climbing ability (CA)	Palmira					1.000	-0.845	-0.580	-0.710
	Darién					1.000	-0.596	-0.626	-0.772
Plant height (PH)	Palmira						1.000	0.441	0.649
	Darién						1.000	0.237	0.722
Days to maturity (DM)	Palmira							1.000	0.301
	Darién							1.000	0.398
Internode length (IL)	Palmira								1.000
	Darién								1.000
Correlation between sites		0.281	0.204	-0.092	-0.058	0.803	0.397	0.527	0.534
55 advanced Andean breeding lines and 3 checks (G 685, G 2333, G 2337):									
100s weight (100s)	Palmira	1.000	0.053	0.178	-0.190	-0.074	-0.048	-0.185	0.033
	Darién	1.000	0.250	0.406	-0.642	0.266	0.167	-0.341	0.090
Pods per plant (P/P)	Palmira		1.000	0.626	-0.241	-0.252	0.112	-0.104	0.015
	Darién		1.000	0.871	-0.483	0.007	0.165	-0.260	0.059
Yield per plant (Y/P)	Palmira			1.000	-0.405	-0.157	0.252	-0.190	0.157
	Darién			1.000	-0.622	0.010	0.189	-0.337	0.127
Agronomic adaptation	Palmira				1.000	0.151	-0.334	0.396	-0.327
(AA)	Darién				1.000	-0.141	-0.187	0.609	-0.220
Climbing ability (CA)	Palmira					1.000	-0.352	-0.094	-0.327
	Darién					1.000	-0.076	-0.136	-0.220
Plant height (PH)	Palmira						1.000	-0.194	0.700
	Darién						1.000	0.043	0.107
Days to maturity (DM)	Palmira							1.000	-0.227
	Darién							1.000	0.006
Internode length (IL)	Palmira								1.000
	Darién								1.000
Correlation between sites		0.617	0.108	0.072	0.265	0.212	0.169	0.341	0.289

Some of the yield advantage of climbing beans over bush beans was because of later maturity. Days to maturity were correlated with climbing ability (and its components). Most of the climbing beans in both environments matured in 100 to 120 days from planting. For both experiments, significant correlations across sites were seen between a genotype's 100-seed weight, climbing ability, plant height, days to maturity, and internode length at Darién and Palmira, showing that these traits have medium to high heritability. Yield, pods per plant, and agronomic adaptation were not correlated between sites, indicating that these traits, as expected, have lower heritability. For yielding ability we will be conducting parallel selection at both locations to see the influence of specific versus general adaptation in climbing beans.

Conclusions and Future Plans: These yield trials have given us a new appreciation of the multiple characteristics that make up a good climbing bean variety. We have seen significant correlations between traits associated with climbing ability. To address this, we developed several scales for agronomic adaptation and climbing ability that will be useful for the selection of breeding lines without time-consuming phenotypic measurements of plant height, internode length, etc. The sensitivity of climbing beans to genotype x environment interaction will have to be factored into our breeding program for climbing beans. We are dealing with the issue of specific adaptation by using a parallel selection system at both the Palmira and Darién sites. We have initiated the following series of crosses, selections, and nurseries to develop and test climbing beans with mid-altitude adaptation:

***Triple crosses*:** So far we have selected climbing bean lines from 15 simples crosses between Mesoamerican climbers and Andean bush beans and from 24 triple crosses between Andean bush x (Mesoamerican climber x Andean bush beans). These have been selected for seed size and adaptation to growing conditions at Palmira. The growing conditions have been difficult because of warmer than usual seasons and compacted soils in the climbing bean lots, which are planted twice (without rotation) to avoid the expense of re-establishing the trellises every season. Therefore, selection criteria have been strict and only 10%-20% of the original families have moved forward each season.

New F_1 crosses: We are hybridizing the best climbing bean accessions from Mexico, Rwanda, and the core collection with single plant selections from Andean populations with tolerance to higher temperatures. We are also beginning to pyramid in the resistance and tolerance genes to ALS, BCMV, and low fertility that will be necessary for successful production of climbing beans at mid-elevation production areas of South America and east Africa. Crosses with BGMV-resistance sources and the best climbing bean parents have been made with the hope of developing climbing bean varieties for the Caribbean.

***International nursery*:** New lines developed from these crosses will be included in an international climbing bean nursery to test the best advanced lines and accessions at a range of elevations. We hope to test early generation climbing bean populations in participatory plant breeding projects and on-farm trials.

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2.3.2 Cranberry advanced breeding lines developed from multiple parent crosses

Rationale: Cream-mottled beans are popular in many areas of the world, including southern Africa, southern Europe, and the Middle East. The class includes both Cranberry and Sugar beans. In Africa, cream-mottled beans are grown in Malawi, Zambia, Zimbabwe, and particularly in South Africa (CIAT, 1998). In South America, Cranberry beans are grown in Chile, Bolivia, and Peru and a special type of cream-mottled bean, known as Cargamanto, is grown in Colombia. These beans are larger than the other cream-mottled seed types and are renowned for their flavor and cooking characteristics. Cream-mottled beans often command a good price in the market. The objective of this study was to yield-test cream-mottled breeding lines from multiple parent crosses to select the best lines for distribution to partners in national programs.

Materials and Methods: The trial was designed as a randomized complete block with three replications. The plots consisted of single 2-m hill plots. The crop was fertilized with 100 kg ha^{-1} of superphosphate at planting and a foliar application of recommended zinc-boron chelate micronutrient. Weed control and other cultural practices were as recommended. A protectant fungicide was used once to control ALS and other fungal diseases such as *Ascochyta* blight. The following check varieties were used: COS 16, BRB 151, BRB 203, FOT 28, FOT 54, SEQ 1027, SEQ 1040, SUG 46, SUG 47, SUG 130, and SUG 137.

Results and Discussion: 49 advanced cream-mottled breeding lines derived from six multiple parent crosses were grown in a replicated yield trial at Darién in 2000B. Table 37 shows the pedigree of each cross and the number of F_1 plants (referred to as gametes) selected in the first filial generation after the last cross to the final parent. Single plant selections were made in the F_2 and F_3 generations at Santander de Quilichao. Generations were advanced by bulk selections until the F_8 when single plant selections were made at Darién. A large number of superior genotypes were identified among the F_8 selections, many of which surpassed the cream-mottled check varieties grown in the experiment. Most importantly, many of the new genotypes yielded as well as the best check varieties and had the commercial seed color.

Table 37. Origin of advanced cream-mottled lines tested at Darién in 2000B for yielding ability.

Cross	Number of selections	Gametes (no.)	Selection (no.)
CT 11972	Cardinal x ((A 483 x Taylor) x (Cardinal x G 17341))	1	7
CT 11974	Cardinal x Taylor/	2	13
CT 11978	Taylor x TIF 1/	1	7
CT 11981	(COS 16 x G 17341) x (WAF 151 x Taylor)	1	8
CT 12477	Cardinal x (PVA 773 x ((A 483 x MAR 1) x (A 483 x G 17341))	1	7
CT 12252	(Cardinal x Araucano 85) x ((COS 16 x G 17341) x (WAF 151 x Taylor))	1	7

Conclusions and Future Plans: The selections made during this season will be tested for the presence of several diagnostic molecular markers for BCMV resistance. These results will be confirmed with viral inoculation if needed. The bush bean lines will be offered to collaborators in South America (Bolivia and Peru) and eastern Africa (Malawi, South Africa, and Zimbabwe) where this grain type is preferred or has potential for export. The lines may also be of interest for Iran. The new lines compare favorably to the Cranberry lines developed at CIAT that served as checks. One of them, COS 16, has been released as a variety in Bolivia. The Bolivian national

program along with its partners in farmer and producer associations hope to find additional Cranberry type beans that will be acceptable in the export market. We hope that some of the new Cranberry lines may provide high yield and good grain quality for Latin American and African producers and consumers of the cream-mottled seed class.

Reference:
CIAT. 1998. Bean project annual report 1997. CIAT, Cali, CO. (Working doc. no. 177)

Contributor: M.W. Blair

2.3.3 Breeding for higher iron and zinc content in populations of common bean

Introduction: Iron deficiency anemia and other micronutrient deficiencies affect over 3.5 billion people and, because of a generalized decrease in the quality of poor people's diets, has actually increased over recent decades, even in developed countries and even in areas where food is not limiting. Legumes are a good source of iron and other essential micronutrients that are found only in low amounts in the cereals or root crops. Unlike many cereals that are polished before eating, resulting in significant loss of nutrients, beans and other grain legumes are usually consumed whole, thus conserving their nutritional content. An ongoing project has shown that bean seeds are variable in the amount of minerals (iron, zinc, and other elements), vitamins, and sulfur amino acids that they contain, and shown that these traits are likely to be inherited quantitatively. These results suggest that is will be possible to breed for better micronutrient content in common bean. The objective of this work was to use advanced and recurrent backcrossing to increase the mineral content in four varieties of Andean bush and Mesoamerican climbing beans. We also lay the groundwork for mainstream breeding for micronutrient content in medium- to large-seeded, red and red-mottled beans that will be useful in Africa, the Caribbean, and South America.

Materials and Methods: Two different breeding procedures were used for the micronutrient breeding program—advanced backcrossing and recurrent backcrossing. In the advanced backcross program, a wild accession from Mexico (G 10022) was used as the source of high mineral content for three populations. The recurrent parents for these populations were the Andean variety Cerinza and two Mesoamerican varieties, DOR 390 or Tacana, and Pinto Villa. The Cerinza advanced backcross population was grown at Palmira in 2000A. For the recurrent backcrossing program, the source of high mineral content was a Mesoamerican cultivar (G 14519) that has been used to create a RIL population that was studied in a QTL analysis of mineral content (see SB-02 Annual Report). The recurrent parents for this program were the Andean bush bean, CAL 96, and the Mesoamerican climbing beans, G 685 and G 2333. The CAL 96 backcrosses were grown at Darién in 2001A, while the G 685 and G 2333 backcrosses were grown at Palmira in 2001A. The experiment site may influence the amount of iron uptake. The progeny of these crosses were analyzed by inductive coupling plasma (ICP) analysis that determines the content of 16 minerals including iron, zinc, and other macro and micronutrients (Mn, B, Cu, Mo, Co, Ni, Ca, Mg, Na, K, P, S, Al, and Cd). As part of this study, a set of 791 genotypes (237 Rwandan climbing beans, 97 Mexican and Andean climbing beans, 95 Colombian and Ecuadorian varieties, and 362 CIAT parents) was also analyzed for mineral content. During the course of that analysis we found that the Andean variety, Cerinza, which had been used in some of the advanced backcross populations, also contained high amounts of iron.

Cerinza is a large red-seeded, "Radical" type commercial variety from Colombia. Having found this, we decided to yield-test a series of multiple parent crosses that were made between the BC_1F_1 hybrids of Cerinza x (Cerinza x wild accession, G 24390), and the varieties CAL 96, ICA Quimbaya, and PC 50. The best-yielding lines from these crosses will be analyzed for mineral content.

Results and Discussion: Iron and zinc content in the advanced backcross lines was normally distributed (for iron, kurtosis 0.10 and skewness 0.418; for zinc, kurtosis 0.91 and skewness 0.507), indicating that micronutrient accumulation is probably a quantitative trait (Figure 40). The range in mineral content was 55 to 103 ppm for iron and 26 to 43 ppm for zinc. For both minerals, the recurrent parent, Cerinza, had average mineral content that was similar to that for the population as a whole. Twenty six lines had significantly more iron than the recurrent parent, Cerinza, and 24 lines had significantly more zinc than Cerinza; therefore it appears that transgressive segregation plays an important role in the accumulation of genes or QTLs for higher mineral content. The correlation between iron and zinc content in the lines was significant (r = 0.373), indicating that the linked genes or the same genes may be involved pleitropically in controlling the accumulation of both minerals. If the same QTLs contribute simultaneously to both iron and zinc content, it may be easy to select for these traits jointly. The advanced backcross method had been shown previously to be a useful method for incorporating wild germplasm into cultivar breeding programs. Although wild beans have been used before to transfer resistance to diseases and insects, as in the noted case of the Arcelin gene, and are being used to transfer yield traits, this is the first study to breed higher nutritional quality from them.

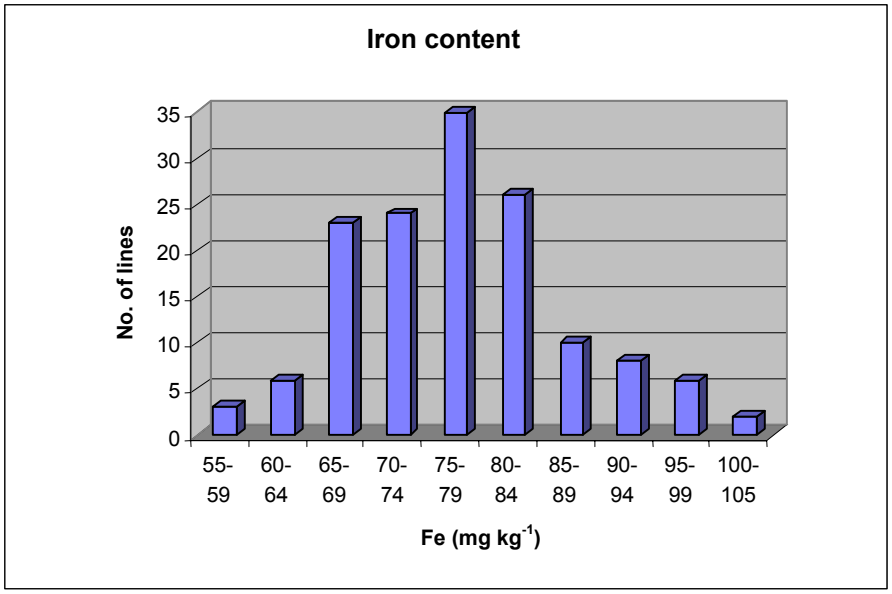

Figure 40. Population distribution of iron and zinc content in the advanced backcross populations from the Cerinza x (Cerinza x (Cerinza x G 10022)) crosses.

The recurrent backcross lines from CAL 96 have been analyzed and produced lines that were substantially higher (up to 119 ppm Fe, 36 ppm Zn) than the recurrent parent (88 ppm Fe, 25 ppm Zn), CAL 96 (Table 38). As for the advanced backcross lines, the correlation between iron and

zinc content in the recurrent backcross lines was highly significant (r = 0.614). The average content of the lines was similar to that found in the recurrent parent. Lower levels of iron and zinc were also found in some of the progeny. These results also suggest that iron and zinc content are inherited as a quantitative trait. The number of lines with higher iron than the recurrent parent in this small sample of progeny indicates that two backcrosses can be used for the incorporation of better nutritional quality. We will be analyzing the recurrent backcross lines from the two climbing beans, G 685 and G 2333, for mineral content in the next few months.

Table 38. Iron and zinc content of lines from the CAL 96 backcross.

Line[a]	Fe (mg kg^{-1})	Zn (mg kg^{-1})
NH 21566- 1F_2-CM(21) W	84	26
NH 21566- 2F_2-CM(7) W	61	23
NH 21566- 3F_2-CM(13) W	73	27
NH 21566- 4F_2-CM(6) W	98	35
NH 21566- 5F_2-CM(17) W	78	27
NH 21566- 6F_2-CM(8) W	81	30
NH 21566- 7F_2-CM(7) W	119	30
NH 21566- 8F_2-CM(23) W	87	33
NH 21566- 9F_2-CM(20) W	81	27
NH 21566-10F_2-CM(11) W	81	26
NH 21566-11F_2-CM(7) W	84	34
NH 21567- 1F_2-CM(18) W	75	28
NH 21567- 2F_2-CM(12) W	87	30
NH 21567- 3F_2-CM(15) W	100	28
NH 21567- 4F_2-CM(18) W	70	29
NH 21567- 5F_2-CM(16) W	107	31
NH 21567- 6F_2-CM(15) W	65	25
NH 21567- 7F_2-CM(23) W	106	36
Average of lines	85.3	29.2
s	15.33	3.57
CAL 96	88	25

a. All of pedigree CAL 96 x (CAL 96 x G 14519).

The modified multiple parent crosses produced lines that were commercially acceptable. This was probably because of the high proportion of cultivated germplasm in the cross. Although the wild accession was part of the pedigree, this was basically a good x good cross of two Andean varieties. The proportion of wild genome in these crosses is low. Therefore segregation for mineral content was probably because of the differences between the Cerinza and the low mineral Andean parents except where it is influenced by a gene or set of genes from the wild accession.

Future Plans: With this work we are close to producing advanced breeding lines in some classes with higher iron content. The advanced backcross method is especially promising for creating high iron lines that are commercially undistinguishable from their recurrent parent. Advanced

backcross populations from three other wild donor parents, G 24390 (from Mexico), G 24404 (Colombia), and G 23585 (Peru), may also be of interest for micronutrient screening if any of these accessions are found to contribute genes for higher mineral content. The good nick between the Cerinza x commercial parents crosses was also promising because many of the derived lines had very good grain quality and adaptation to multiple zones of production. With the prospect of creating almost finished varieties with high iron content in the near future, we are talking with nongovernmental organizations (NGOs) in Colombia, Guatemala, and Haiti about preliminary testing of the health benefits from consuming these bean varieties. In these pilot projects, we envision an integrated approach whereby producers and consumers are linked in a program to supply and use a nutritionally improved product. The program hopes to target small-scale farmers who need a market for higher value beans, and consumers who need extra iron in their diets, especially children and women from communities where poverty has led to problems of anemia.

The regular creation of high mineral varieties will require more efficient phenotypic screening procedures and MAS. In a parallel project we are examining the QTLs that control micronutrient accumulation in beans. We hope to focus on certain parts of the genome to determine if desirable alleles for higher mineral content are located at the same loci in the different populations developed specifically for this purpose. Future analysis will also include the detection of QTLs for the amount of sulfur containing amino acids (SAA), as well as for the amount of the other minerals analyzed in the ICP study. The QTL analysis will help us consider the possibility of genetically increasing SAA (methionine or cysteine) or Lysine as uptake promoters of iron, or decreasing anti-nutritional factor such as tannins or lectins that reduce the uptake of iron. We also plan to integrate the information about the map locations of QTLs for micronutrients with those for other agronomic traits that we have been studying, so that we can select for the best recombinants from crosses between high micronutrient lines and the best varieties.

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Progress towards achieving output milestones:

- A number of potential parents for climbing bean crosses were identified in a preliminary germplasm and advanced breeding line nursery at two locations.
- Understanding was increased of yield and yield components in climbing beans and the importance of genotype x environment interactions.
- Crosses are being made to incorporate disease resistance genes into climbing beans.
- Yield testing of cream-mottled advanced breeding lines developed from multiple crosses was completed at one location.
- Higher iron containing families were identified in advanced and recurrent backcross populations.
- Advanced lines will be selected from the high-iron families based on agronomic adaptation.