Output 2: Improved, large-seeded, bean germplasm resistant to major biotic and abiotic stresses with greater nutritional and market value

Activity 2.1 Developing germplasm resistant to diseases

Highlights:

- Over 1000 populations of different generations F_3-F_6 in various market classes were selected during this reporting period.
- Several lines were selected from the large red kidney and sugar bean market classes nurseries, which combined more than one attribute such as high yield, resistance to diseases (ALS and FLS) and tolerance to low soil fertility. These will be available for distribution to NARIs partners in SABRN.
- Four advanced lines (HGA 24, HGA 25, HGA 26, and HGA 27) were identified that combined resistance to rust, common bacterial blight, anthracnose and the angular leaf spot.
- The recessive *bc-3* gene is being successfully incorporated in climbing bean genotypes to control the increasing incidence of bean common mosaic in the American highlands. This gene confers total resistance to the causal viruses (BCMV and BCMNV), and facilitates the selection of virus resistant common bean genotypes possessing seed colors that exhibit genetic linkage problems (e.g. cranberry and red-seeded types).
- Several common bean genotypes possessing resistance to Bean leaf crumple virus, a begomovirus transmitted by the whitefly *Bemisia tabaci*, have been identified in field screening plots planted in the Cauca Valley. The resistance sources identified are already present in many advanced common bean lines.
- Over 39 resistant and potential parental materials were evaluated under greenhouse conditions against nine isolates representing 6 *Pythium* species pathogenic to beans in eastern Africa. Thirty remained resistant.
- Over 500, F_2-derived F_5 lines and populations from simple, double and backcrosses were selected for *Pythium* and angular leaf spot resistance.
- Marker assisted selection for BCMV resistance continued this year in Andean bush and climbing beans with wide application of the SCAR markers ROC11 for the *bc*-3 gene and SW13 for the *I* gene. This work was expanded to collaboration with additional partners in the Andean region: CORPOICA, Universidad Nacional–Bogotá and PROMPEX-Peru.
- New heat tolerant, BCMV-resistant, red and cream mottled Andean climbing beans were produced. Peruvian varieties from the Alubia, Bayo, and Canario seed classes were also improved for BCMV resistance. Based on successes in marker assisted selection, a new project will begin next year for incorporating BCMV resistance into East African varieties with partners from Kenya, Rwanda and Uganda including ISAR, KARI, NARO, University of Nairobi and University of Rwanda.
- Marker assisted selection for anthracnose resistance genes was implemented in yellow, white and red seeded climbing beans using the SCAR markers SAS13 for the *Co-4*^{2} gene and SAB3 for the *Co-5* gene. The SAB3 marker was found to work well under a range of PCR conditions while SAS13 marker amplification was improved. The anthracnose resistance genes are being pyramided into lines with BCMV resistance and commercial

seed type. This project is important goal for breeding of Andean beans in both Africa and Latin America.

2.1.1 Breeding for specific bean market classes in Southern Africa Bean Research Network (SABRN)

Rationale: The bean breeding strategy for Africa, focusing on specific bean market classes, has been going on since 2001. In this strategy, national scientists within the network share responsibilities in generating multiple constraint crosses, for specific market classes where they have comparative advantages. Within SABRN the responsibilities for breeding programs are assigned by countries: program 1 Red Mottled (Malawi); program 2a Dark Red Kidney (Zimbabwe); program 2b Small Red (Tanzania); program 3 Brown Yellow and Tan (Zambia); program 4 Cream and Sugar (South Africa); program, 5a Small-white or Navy (South Africa); program 5b. Large White (South Africa); and program 6 Purples (Tanzania). Other countries that have interest in specific market classes collaborate in evaluation of fixed lines and cultivars, of the preferred types. The CIAT-SABRN regional breeding program, based in Malawi continued to provide backstopping support to the national scientists in 4 market classes: red mottled, brown/tan, dark red kidney and sugar. This section of the report covers activities of the regional breeding program in Malawi, capturing evaluation of segregating populations and nurseries in various market classes.

a) Segregating populations

Materials and Methods: The segregating populations (F4-6) evaluated here, were derived from crosses that were initiated a few years ago. The crosses were made for 4 different market classes (red mottled, brown/tan, dark red kidney and sugar), which are preferred by farmers and consumers in more than 2 countries in the SADC region. Good parents for the market classes and parental lines to serve as sources of resistance to various biotic and abiotic constraints were chosen. The parents for good biotic and abiotic attributes were: Mexico 54, AND 277 and AND 279 for angular leaf spot (ALS); VAX 5 and VAX 6 for common bacterial blight (CBB); RAO 55 and UBR (92) 25, for low P, low N and low pH complex and G52201 and Mlama 127 for bean stem maggot (BSM). The evaluation was done at Chitedze (1200 masl) and Bembeke (1650 masl).

Results and Discussion: The disease pressure for CBB at Chitedze (CTZ), was over-masked by widespread virus symptoms with similar appearance, making it difficult to screen for CBB resistance. However, the ALS pressure at Bembeke (BBK) was predominant, which made it easy for the program to screen for resistance to ALS at this site. The soils at Bembeke continued to provide a stressful environment, suitable to screen genotypes for adaptation to low soil fertility. A total of 1123 populations (Table 37) were selected, 856 at Chitedze and 267 at Bembeke. More seed of these populations has been multiplied using surface irrigation facilities at Bwanje in Malawi. These populations will be available for distribution to interested NARS partners within the SABRN network in the next season.

Table 37. Summary of segregating populations in various colors evaluated under fertility stress at Chitedze (CTZ) and Bembeke (BBK), Malawi, 2004.

Families Evaluated	Populations				
	Evaluated		Selected		
	CTZ	BBK	CTZ	BBK	Total
Normal soil fertility:					
F_3 Climbers	621		149		149
F_4 Khaki Bulk	25	57	7	13	20
F_4 Khaki Selections	57	24	19	4	23
F_4 Sugars Selections	454	454	50	117	167
F_4 Sugars Bulk	142		12		12
F_4 Red Kidney Selection	58	58	29	10	39
F_4 Red Kidney Bulk	33	31	4	7	11
F_4 General Selections	130	130	59	23	82
F_4 General Bulk	47	47	55	23	78
F_4 Red Mottled Selection	139	139	99	54	153
F_4 Red Mottled Bulk	85	84	25	16	41
F_5 Red Mottled	742		188		188
F_6 Red Mottled	521		160		160
Total	**3054**	**1024**	**856**	**267**	**1123**

b) Evaluation of bean germplasm developed for specific market classes for adaptation, yield and reaction to biotic and abiotic stresses in Malawi

In addition to evaluating the segregating populations for various attributes, the program evaluated fixed lines of various market classes for adaptation, yield and reaction to diseases at Chitedze and Bembeke, and for tolerance to low soil fertility stress at Bembeke.

Materials and Methods: Nurseries for specific market classes included: sugars (76 lines), red mottled (70 lines), red kidney (67 lines), carioca (50 lines) and small reds (44 lines). At Bembeke, a set of sugar and red kidney lines were also planted in a low soil fertility stress block. The soil fertility stress block has been used over years to identify potential bean lines, which can perform well under low P, low N and low pH complex conditions. The nurseries were planted during the second week of January, in single row plots without replication. Data were collected on yield and reaction to diseases in both sets of nurseries, under with and without low soil fertility stress conditions.

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

i. Large red kidney lines

Among the top 10 lines out of the 70 that were evaluated, all originated from CIAT derived segregating populations coded VTTT. These have gone through a series of selections in Malawi, such that the fixed lines are now being evaluated for yield, tolerance to low soil fertility and reaction to diseases. Some of the top-yielding lines like VTTT 917/6-1, VTTT 924/19-8-1, VTTT 928/9-1 and VTTT 918/15-4-4 have resistance to moderate resistance levels to ALS, (scores ranging from 3-6). These lines also have good grain yield levels ranging from 2900 to 3200 kg ha^{-1}, which is much more than the control RWR 1946, 1900 kg ha^{-1} (Table 38). The site mean is very low, 467 kg ha^{-1}, because some lines were very poorly adapted, such that their grain yields were very low, and sometimes 0.

Table 38. Yield performance and reaction to diseases of top 10 large red kidney lines evaluated at Bembeke, 2004.

	Disease Scores					Grain Yield
Identity	**ALS**	**CBB**	**ASC**	**ANT**	**FLS**	**(kg ha^{-1})**
VTTT 917/6-1	4	1	2	1	7	3219
VTTT 924/19-8-1	5	1	4	1	6	3108
VTTT 916/8-3	7	1	2	1	7	3108
VTTT 928/9-1	6	1	2	1	6	2914
VTTT 918/15-4-4	3	2	2	1	7	2914
VTTT 918/15-2	3	1	3	1	7	2886
VTTT 918/15-4-5	4	1	2	2	7	2886
VTTT 925/7-8-1	4	2	2	1	7	2775
VTTT 918/15-4-2	4	2	4	2	7	2775
VTTT 924/12-5-1	6	2	3	1	7	2775
RWR 1946 (control)	3	1	2	1	7	1915
Mean						**467**

A similar set of lines was planted in the low soil fertility block at Bembeke where a similar pattern of disease pressure was observed. However the grain yields were very low, almost 10% of those without soil fertility stress. The severe soil fertility pressure restricted the number of lines that were selected, because some of them had no yield data. Most of the selected lines had good levels of resistance to ALS, with scores ranging from 2-4 (Table 39). Among the 10 top lines selected under low soil fertility conditions, 2 of them (VTTT924/19/8-1 and VTTT18/15-2) were also among the top 10 lines selected in the block without soil fertility stress. This suggested that some lines combine good disease resistance, tolerance to low soil fertility and high grain yield potential.

Table 39. Yield performance and reaction to diseases of top 10 large red kidney lines evaluated under low soil fertility stress, Bembeke 2004.

	Disease Score					Grain Yield
Identity	**ALS**	**CBB**	**FLS**	**ASC**	**ANT**	**(kg ha^{-1})**
VTTT 923/7-2	4	1	4	2	1	333
VTTT 918/15-4	2	1	2	1	1	333
VTTT 916/8-3	3	1	4	1	1	333
VTTT 924/6-3-3	4	1	1	1	1	305
VTTT 925/7-8-1	4	2	5	2	2	278
VTTT 920/24-3	3	1	6	3	1	250
VTTT 924/19-8-1	3	1	4	1	1	250
VTTT 918/15-2	3	1	4	2	1	250
VTTT 915/14-2	4	1	5	2	1	250
VTTT 923/9-1	3	2	6	1	1	222
RWR 1946 (Control)	5	1	4	2	2	28
Mean						128

ii. Sugar lines

Among the 76 sugar lines that were evaluated at Bembeke, only VTTT lines emerged in the top 10 category (Table 40). They all had medium to susceptible rating for disease resistance for ALS and FLS. However, their yield performance was satisfactory, ranging from 2600 to 3000 kg ha^{-1}, which were well above the control cultivar SUG 131 (250 kg ha^{-1}), which was even below the site mean (374 kg ha^{-1}). The same set of lines was also planted in a low soil fertility block at Bembeke. Like the large red lines, the yield level under the low soil fertility conditions were also only 10% of the yields realized in the block that was not stressed (Table 41). Two of the lines, VTTT925/7-6 and VTTT924/15-2, were among the top 10 in both Tables 40 and 41, indicating again that some lines combined such attributes as disease resistance, tolerance to low soil fertility and high yield potential.

Table 40. Yield performance and reaction to diseases of top 10 sugar lines evaluated at Bembeke, 2004.

	Disease score					Grain Yield
Identity	**ALS**	**CBB**	**ASC**	**ANT**	**FLS**	**(kg ha^{-1})**
VTTT 923/10-6-2	6	2	3	2	6	3025
VTTT 924/15-2	5	2	3	1	7	2969
VTTT 924/19-3	6	1	2	1	7	2886
VTTT 925/7-6	6	1	3	1	5	2858
VTTT 924/10-7	7	1	2	1	7	2831
VTTT 923/10-2	5	2	2	1	6	2775
VTTT 924/2-4-2-1	7	2	5	1	7	2775
VTTT 925/7-5	7	1	2	1	7	2720
VTTT 925/9-3	6	1	2	1	6	2664
VTTT 924/12-5-3	6	1	4	1	7	2664
SUG 131 (control)	5	2	2	1	4	250
Mean						374

Table 41. Yield performance and reactions to diseases of sugar lines under low soil fertility stress at Bembeke, 2004.

	Disease Score					Grain Yield
Identity	**ALS**	**CBB**	**FLS**	**ASC**	**ANT**	**(kg ha^{-1})**
VTTT 925/7-6	2	1	3	1	1	333
VTTT 925/1-4	2	1	5	1	1	333
VTTT 925/1-2-1	3	1	4	2	1	222
VTTT 925/2-5-2-2	3	1	3	1	2	194
VTTT 925/2-7-1	2	1	4	2	1	194
VTTT 924/15-2	2	1	5	1	1	167
VTTT 924/2-4-1	2	1	4	2	1	139
VTTT 924/18-6	3	1	3	1	1	111
VTTT 925/4-3-3	4	1	3	1	1	111
VTTT 923/12-4	3	1	2	2	1	83
Sugar 131 (Control)						0
Mean						75

iii. Red mottled lines

The background of the lines that appeared among the top 10, within the red mottled market at Bembeke were varied, including such lines as RA 13170-5-1-3, BOA 1-5/34, RMA 21 and VTTT 925/6-4-1. Among the group, only RA 13170-5-1-3 showed a good level of resistance to ALS, the rest were mildly resistant to susceptible. Others like BOA 1-5/34 and VTTT 925/6-4-1 had good levels of resistance to FLS. The yield levels among the top 10 lines were reasonable, above 2000 kg ha-1, which were better than the control variety CAL 143, 1305 kg ha-1, (Table 42).

Table 42. Yield performance and reaction to diseases of top 10 red mottled lines at Bembeke, 2004.

	Disease Score					Grain Yield
Identity	**ALS**	**CBB**	**ASC**	**ANT**	**FLS**	**(kg ha^{-1})**
RA 13170-5-1-3	3	1	3	1	5	2831
BOA 1-5/34	6	2	2	1	1	2775
RMA 21	6	2	2	1	7	2609
VTTT 925/6-4-1	6	1	2	2	5	2553
RMA 12	7	2	2		5	2553
RMA 18	6	2	3	1	5	2553
AND 1064	6	2	2	1	2	2553
VTTT 915/7-3-2	5	2	2	1	7	2498
VTTT 916/14-4-3	5	1	2	1	6	2442
RMA 44	7	2	3	2	7	2387
CAL 143 (Control)	3	2	3	1	5	1305
Mean						368

Conclusion: The regional breeding program continued to make progress in creating and screening populations for multiple attributes, combining grain yield, preferred grain color and resistance to biotic and abiotic constraints. Over 1000 populations of different generations F_3-F_6 in various market classes were selected during this reporting period. In addition further progress has been achieved this season in selecting high yielding genotypes of various grain market classes that combine more than one attribute. For example, some genotypes in large red kidney and sugar bean market classes combined good grain yield, resistance to diseases (ALS and FLS) and tolerance to low soil fertility. More seed for all selected populations and fixed lines is under multiplication for further distribution to NARS partners.

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2.1.2 Identifying Andean genotypes with multiple disease resistance

Rationale: The increase in the importance and distribution of anthracnose and angular leaf spot of beans means that simultaneous incorporation of resistance genes against the two pathogens is a good breeding strategy. However, this is complicated by the high pathogenic variability found in both pathogens that makes the development and use of resistance to manage this disease difficult. Validating the usefulness and effectiveness of potential sources of resistance and breeding lines against multiple pathotypes of these pathogens and defining the deployment strategies of such varieties is a major activity in our program. With this in mind, we evaluated breeding lines developed through multiple crosses designed to pyramid resistance genes to anthracnose, common bacterial blight, angular leaf spot, and bean common mosaic virus (BCMV) for their effectiveness against several races of these pathogens under greenhouse conditions. These lines were developed to specifically target the Andean zone and taking into account the preferred grain types.

Materials and Methods: Forty genotypes were evaluated for resistance to anthracnose under greenhouse conditions. Thirty-three were advanced breeding lines (F_5-F_8) derived from multiple crosses involving 10 parents with resistance to a total of six factors (Table 43). These were inoculated with Andean and Mesoamerican pathotypes of *C. lindemuthianum, P. griseola*, one isolate of *Xanthomonas campestris* pv. *phaseoli*, and an Andean rust (*Puccinia appendiculatus*) isolate (Table 43). Plant handling, inoculum production, inoculations and disease evaluations were done as described previously (CIAT 2003).

Results and Discussion: Four lines, HGA 24, HGA 25, HGA 26, and HGA 27 were resistant to the four pathogens evaluated in this study (anthracnose, angular leaf spot, rust and common bacterial blight pathogens) (Table 43). The lines HGA4 and HGA 8 were resistant to three of the four pathogens (all races of anthracnose, anthracnose and rust used in this study). The majority of the lines were resistant to either one or two pathogens, and these lines can be deployed, depending on the distribution, prevalence and importance of the pathogens for which they lack resistance.

Table 43. Response of advanced lines to inoculation with anthracnose, angular leaf spot, rust and common bacterial blight pathogens.

	Colletotrichum lindemuthianum			*Phaeoisariopsis griseola*					
Genotype	**1**	**7**	**3481**	**1**	**15-0**	**31-0**	**7-55**	**Rust**	**CBB-123**
CHOCHO	1.0	9.0	1.0	1.0	8.0	8.0	4.1	1.0	5.3
CATRACHITA	1.0	1.0	9.0	1.0	1.0	1.0	8.0	1.0	5.7
G 5686	1.0	1.0	1.0	1.0	1.0	1.0	2.3	9.0	7.0
VAX 3	5.3	2.4	9.0	6.0	1.0	1.0	8.0	1.0	1.0
HGA 1	1.0	1.0	9.0	1.0	1.6	1.6	8.0	1.0	7.0
HGA 2	1.0	1.2	7.0	1.0	1.0	1.0	8.0	9.0	2.7
HGA 3	4.9	1.9	8.0	1.0	2.0	2.0	8.0	5.0	1.3
HGA 4	1.1	1.0	4.5	1.0	6.2	6.2	8.0	1.0	1.0
HGA 5	4.6	9.0	3.6	7.2	6.4	6.4	8.0	9.0	5.3
HGA 6	2.0	1.4	3.3	2.6	8.0	8.0	8.0	1.0	3.3
HGA 7	1.0	1.0	1.0	1.0	1.0	1.0	6.0	9.0	4.0
HGA 8	1.0	1.0	4.4	1.0	1.0	1.0	8.0	1.0	1.0
HGA 9	4.5	9.0	9.0	7.4	2.2	2.2	8.0	4.5	4.0
HGA 10	1.0	9.0	1.3	1.0	1.0	1.0	7.2	1.0	6.3
HGA 11		2.0	5.6	2.6	1.2	1.2	4.9	9.0	1.0
HGA 12	1.0	1.0	1.0	1.0	1.0	1.0	5.7	5.0	1.3
HGA 13	1.9	6.0	6.1	5.3	1.0	1.0	6.0	1.0	1.3
HGA 14	1.0	1.0	9.0	1.0	1.0	1.0	8.0	1.0	4.3
HGA 15	2.8	4.3	9.0	1.0	1.0	1.0	8.0	9.0	1.7
HGA 16	1.0	1.0	8.2	1.0	1.0	1.0	8.0	1.0	1.7
HGA 17	1.0	1.0	9.0	1.0	2.2	2.2	8.0	1.0	7.0
HGA 18	1.0	1.0	9.0	1.0	1.0	1.0	8.0	1.0	3.7
HGA 19	1.0	1.9	9.0	1.1	1.2	1.2	8.0	9.0	6.3
HGA 20	1.0	1.0	9.0	1.0	1.0	1.0	8.0	1.0	2.0
CALIMA	1.0	9.0	1.0	1.0	1.0	1.0	4.7	5.0	9.0
VAX 6	6.4	9.0	9.0	3.1	1.0	1.0	8.0	1.0	1.0
A 193	1.0	1.0	1.0	1.0	3.3	3.3	8.0	5.0	6.7
HGA 21	1.0	1.0	9.0	2.0	1.0	1.0	8.0	5.0	2.7
HGA 22	9.0	7.4	1.0	9.0	1.3	1.3	1.8	9.0	2.3
HGA 23	1.0	1.0	1.0	3.7	1.3	1.3	8.0	9.0	1.0
HGA 24	1.0	1.0	1.0	1.0	1.3	1.3	3.2	1.0	1.3
HGA 25	1.0	1.0	1.0	1.0	1.0	1.0	2.0	1.0	1.7
HGA 26	1.0	1.0	1.0	1.0	1.0	1.0	5.7	1.0	1.0
HGA 27	1.0	1.0	1.0	1.0	1.2	1.2	3.3	1.0	1.3
A 483	1.0	1.0	1.0	1.0	2.7	2.7	8.0	9.0	9.0
TALASH	9.0	9.0	9.0	9.0	8.0	8.0	8.0	8.0	9.0
WILKINSON 2			9.0	5.6			8.0		1.0
HGA 28	9.0	3.4	9.0	6.7	2.4	2.4	8.0	9.0	6.5
HGA 29	9.0	9.0	9.0	9.0	2.0	2.0	3.4	9.0	6.3
HGA 30	1.0	1.0	3.3	1.0	8.0	8.0	8.0	9.0	8.3
HGA 31	1.0	1.0	1.0	2.0	1.0	1.0	8.0	1.0	6.3
HGA 32	1.0	1.0	1.4	1.0	8.0	8.0	7.0	9.0	8.3
HGA 33	1.0	7.8	9.0	9.0	3.0	3.0	8.0	9.0	4.3
VAX 1	1.0	1.0	9.0	1.0	1.0	1.0	2.0	1.0	1.0
DICTA 17	1.0	1.0	9.0	1.0	1.8	1.8	8.0	1.0	7.7
MAR 1	2.7	1.0	9.0	2.9	1.0	1.0	2.0	1.0	3.0
NIC 159	9.0	1.0	9.0	3.1	1.0	1.0	2.0	1.0	7.7
ICA PIJAO	9.0	4.0	9.0	9.0	1.0	1.0	8.0	1.0	8.0
G 17198	1.0	2.7	1.0	1.0	5.0	5.0	8.0	9.0	6.7

Conclusion: The lines that combine resistance to all four pathogens in this study form a valuable group that can be deployed in those areas where the respective pathogens are important. In addition, these lines can serve as sources of resistance in breeding programs tasked with simultaneously developing resistance to several bean diseases. Some of the parents used in this study, e.g. VAX 1, has been shown to be adapted to low soil fertility conditions including aluminum toxicity, and has resistance to several root rot causing pathogens. These materials need to be tested for other pathogens such as several root rot causing pathogens, abiotic constraints (e.g. tolerance to aluminum and drought) and nutritional quality (iron and zinc).

References

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2.1.3 Incorporation of recessive resistance to BCMV/BCMNV

A SCAR marker, ROC11, and mechanical inoculation tests conducted at CIAT with a combination of mosaic- and necrosis-inducing strains of BCMV (NL4) and BCMNV (NL3) showed that the recessive *bc-3* gene can be transferred to common bean genotypes possessing seed colors known to be genetically linked to recessive traits conditioning susceptibility to these viruses in *Phaseolus vulgaris*. In a study conducted by Biol. Gloria Santana of CORPOICA, Rionegro, Antioquia, in collaboration with CIAT, the *bc-3* gene was incorporated into 14 Cargamanto lines. This highly commercial grain type had become increasingly affected by common mosaic in northwestern Colombia. This technology is also applicable to Africa, where the *bc-3* gene could be used to protect common bean cultivars against necrosis-inducing strains of BCMV and BCMNV.

The screening of common bean breeding materials for the presence of the *bc-3* gene has increased gradually in recent years, particularly in the case of climbing bean genotypes. This year, a total of 1,443 climbing lines for the Andean region and Africa were evaluated for their reaction to BCMNV and presence of the *bc-3* gene.

2.1.4 Search for sources of resistance to begomoviruses affecting common bean

Last year, we reported on the emergence of a new disease of common bean in the Cauca Valley of Colombia, caused by a new begomovirus transmitted by the whitefly *Bemisia tabaci*. Snap beans are severely attacked by whitefly-transmitted viruses because they are mostly climbing types not bred for the lowland regions affected by whitefly-borne viruses. Approximately 240 common bean genotypes consisting of advanced bush and climbing bean types, and potential sources of resistance, were planted and evaluated in the municipality of Pradera, Cauca Valley, Colombia. Two of three plantings (each having three replications), showed a high incidence of bean leaf crumple (Figure 26), and were used to score the selected genotypes for their reaction to the virus. Figure 27 shows the grouping and frequency distribution of the materials evaluated, based on their reaction to the virus expressed as an average score using a scale of 1 (symptomless) to 5 (severely affected) Table 44. As shown, only three bean genotypes: EMP

496, DICTA 113, and a breeding material (TLP35XG21212)F1 X ICTA Ligero, remained symptomless. Eight entries, mostly materials developed for their resistance to begomoviruses, had scores lower than 2. These materials included BAT 304, Tio Canela, DOR 390 and DOR476.

Figure 26. Bean leaf crumple disease in the Cauca Valley of Colombia.

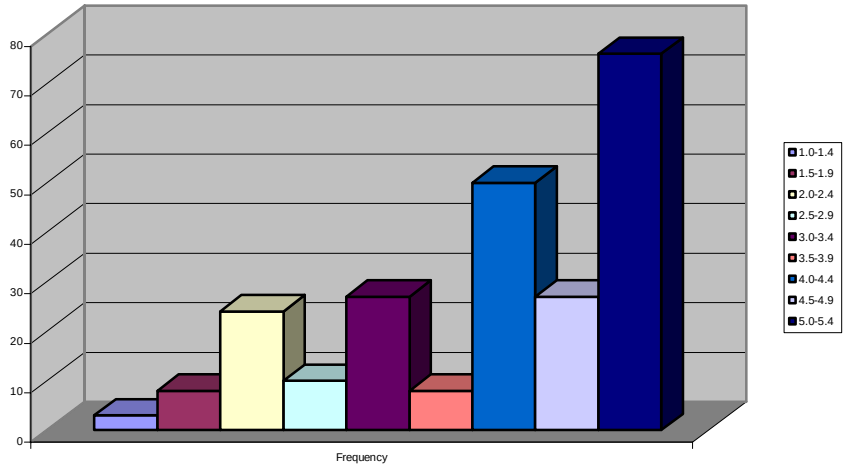

Figure 27. BLCrV score frequencies-La Tupia, September 2004

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
1	DOR 476		.	DOR 476		bgm1 + W12	1	1	1	**1.0**	1	2	2	**1.7**
2	DOR 482		.	DOR 482		bgm1 + W12	1	2	2	**1.7**	1	2	4	**2.3**
3	EAP 9504-30B		.	EAP 9504-30B		bgm1 + W12	1	1	1	**1.0**	1	2	3	**2.0**
4	EAP 9510-77		.	EAP 9510-77		bgm1 + W12	2	1	1	**1.3**	1	4	4	**3.0**
5	9653-16B-1		.	9653-16B-1		bgm1 + W12	1	1	1	**1.0**	2	2	3	**2.3**
6	9653-16B-3		.	9653-16B-3		bgm1 + W12	1	1	1	**1.0**	2	1	3	**2.0**
7	9824-47-1/F7		.	9824-47-1/F7		bgm1 + W12	1	2	1	**1.3**	3	4	3	**3.3**
8	9824-56-2/F7		.	9824-56-2/F7		bgm1 + W12	1	1	2	**1.3**	3	5	5	**4.3**
9	9825-46-1/F6		.	9825-46-1/F6		bgm1 + W12	1	2	1	**1.3**	2	4	3	**3.0**
10	RAB 609		.	RAB 609		bgm1 + W12	1	1	1	**1.0**	2	2	3	**2.3**
11	SAM 1		.	SAM 1		bgm1 + W12	1	4	2	**2.3**	4	5	5	**4.7**
12	TIO CANELA 75		.	TIO CANELA 75		bgm1 + W12	1	2	1	**1.3**	1	2	2	**1.7**
13	A 429		.	A 429		bgm1	1	1	1	**1.0**	1	2	3	**2.0**
14	DOR 714		.	DOR 714		bgm1	1	1	1	**1.0**	1	4	2	**2.3**
15	EMP 496		.	EMP 496		bgm1	2	1	1	**1.3**	1	1	2	**1.3**
16	FEB 212		.	FEB 212		bgm1	1	1	1	**1.0**	1	3	3	**2.3**
17	G 2402 [GARRAPATO]		.	G 2402 [GARRAPATO]		bgm1	1	4	3	**2.7**	4	5	5	**4.7**
18	ICTA LIGERO		.	ICTA LIGERO		bgm1	3	1	2	**2.0**	1	3	3	**2.3**
19	DOR 390		.	DOR 390		bgm1	1	2	1	**1.3**	1	1	3	**1.7**
20	RAB 608		.	RAB 608		bgm1	1	1	1	**1.0**	1	3	3	**2.3**
21	RAB 612		.	RAB 612		bgm1	1	2	1	**1.3**	1	4	4	**3.0**
22	RAB 619		.	RAB 619		bgm1	3	3	4	**3.3**	3	5	5	**4.3**
23	RAB 623		.	RAB 623		bgm1	4	3	3	**3.3**	3	4	5	**4.0**
24	RAB 630		.	RAB 630		bgm1	2	4	2	**2.7**	2	5	5	**4.0**
26	RJB 10		.	RJB 10		bgm1	1	1	1	**1.0**	2	2	3	**2.3**
27	ICA PIJAO		.	ICA PIJAO		W12	1	2	1	**1.3**	2	3	4	**3.0**
28	PORRILLO SINTETICO		.	PORRILLO SINTETICO		W12	1	1	1	**1.0**	1	4	3	**2.7**
29	BAT 304		.	BAT 304		W12	1	1	1	**1.0**	1	2	2	**1.7**
30	DOR 364		.	DOR 364		W12	1	1	1	**1.0**	1	3	4	**2.7**
31	DOR 582		.	DOR 582		W12	1	2	1	**1.3**	1	5	4	**3.3**
32	DICTA 113		.	DICTA 113		W12	1	1	1	**1.0**	1	1	2	**1.3**
33	EAP 9020-14		.	EAP 9020-14		W12	1	1	1	**1.0**	1	2	2	**1.7**
34	FEB 115		.	FEB 115		W12	1	1	1	**1.0**	3	5	5	**4.3**
35	G 17341		.	G 17341		W12	2	1	2	**1.7**	2	5	5	**4.0**
36	ICTA OSTUA		.	ICTA OSTUA		W12	1	1	1	**1.0**	1	2	4	**2.3**
37	MD 23-24		.	MD 23-24		W12	2	1	2	**1.7**	1	5	4	**3.3**
38	NEGRO COAXTLA 91		.	NEGRO COAXTLA 91		W12	1	1	2	**1.3**	1	3	4	**2.7**
39	RAVEN		.	RAVEN		W12	3	4	3	**3.3**	3	5	5	**4.3**
40	SAM 3		.	SAM 3		W12	3	2	3	**2.7**	2	4	3	**3.0**
41	TLP 35		.	TLP 35		W12	2	1	1	**1.3**	2	4	4	**3.3**
42	GMR 5		.	GMR 5		?	1	1	1	**1.0**	2	4	2	**2.7**
44	ASC 72 [Pv x Pc]		.	ASC 72 [Pv x Pc]		?	1	1	1	**1.0**	2	3	2	**2.3**
45	ASC 74 [Pv x Pc]		.	ASC 74 [Pv x Pc]		?	4	2	1	**2.3**	4	5	5	**4.7**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
46	ASC 75 [Pv x Pc]		.	ASC 75 [Pv x Pc]		?	1	2	2	**1.7**	5	5	5	**5.0**
47	G 35171 [P cocc]		.	G 35171 [P cocc]		?	1	1	1	**1.0**	4	2	4	**3.3**
48	G 35172 [P cocc]		.	G 35172 [P cocc]		?	1	2	2	**1.7**	2	2	2	**2.0**
49	G 35252 [P cocc]		.	G 35252 [P cocc]		?	1	1	1	**1.0**	2	3	3	**2.7**
50	MR	13937-15	BGMV 550	MD 23-24 x (RAB 655 x G 21212)F1/-MC-3P-MQ-MC-2C-MC-MC		W12	3	3	1	**2.3**	1	4	4	**3.0**
51	MR	13937-21	BGMV 550	MD 23-24 x (RAB 655 x G 21212)F1/-MC-7P-MQ-MC-9C-MC-MC		W12	2	1	2	**1.7**	3	4	5	**4.0**
52	MR	14000-2	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-1P-MQ-MC-15C-MC-MC		bgm1	3	2	4	**3.0**	5	4	5	**4.7**
53	MR	14000-2	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-1P-MQ-MC-21C-MC-MC		bgm1	2	3	4	**3.0**	5	5	5	**5.0**
54	MR	14000-2	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-10P-MQ-MC-1C-MC-MC		bgm1+W12	2	3	2	**2.3**	5	5	5	**5.0**
55	MR	14000-2	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-10P-MQ-MC-13C-MC-MC		bgm1	1	3	3	**2.3**	5	5	5	**5.0**
56	MR	14000-2	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-10P-MQ-MC-21C-MC-MC		bgm1+W12	1	2	3	**2.0**	5	5	5	**5.0**
57	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-3C-MC-MC		bgm1	1	1	1	**1.0**	1	3	2	**2.0**
58	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-4C-MC-MC		bgm1	1	2	1	**1.3**	1	2	3	**2.0**
59	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-6C-MC-MC		bgm1	2	2	1	**1.7**	1	2	2	**1.7**
60	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-8C-MC-MC		bgm1	1	1	1	**1.0**	1	5	4	**3.3**
61	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-15C-MC-MC		bgm1	1	1	1	**1.0**	3	3	2	**2.7**
62	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-17C-MC-MC		bgm1	1	1	1	**1.0**	3	4	3	**3.3**
63	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-20C-MC-MC		bgm1	1	2	1	**1.3**	3	4	3	**3.3**
64	MR	14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-25C-MC-MC		bgm1	1	1	1	**1.0**	3	3	3	**3.0**

Table 44. Sources of resistance to begomoviruses

Ent		Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
65	MR		14143-28	BGMV 451	(RAB 651 x TIO CANELA 75)F1 X (RAB 608 x SEA 15)F1/-MC-2P-MQ-MC-27C-MC-MC		bgm1	1	2	1	**1.3**	2	4	3	**3.0**
66	MR		14148-54	BGMV 453	(SEA 21 x RAB 623)F1 X 9653-16 B-1/-MC-3P-MQ-MC-13C-MC-MC		W12	1	2	2	**1.7**	4	5	5	**4.7**
68	MR		14148-80	BGMV 453	(SEA 21 x RAB 623)F1 X 9653-16 B-1/-MC-2P-MQ-MC-2C-MC-MC		bgm1+W12	1	2	1	**1.3**	3	4	5	**4.0**
69	MR		14148-80	BGMV 453	(SEA 21 x RAB 623)F1 X 9653-16 B-1/-MC-2P-MQ-MC-3C-MC-MC		W12	2	3	2	**2.3**	3	5	5	**4.3**
70	MR		14152-14	BGMV 561	(SEA 22 x (TLP 35 x G 21212)F1)F1 X EAP 9504-30 B/-MC-3P-MQ-MC-1C-MC-MC		W12	1	1	1	**1.0**	3	4	3	**3.3**
71	MR		14153-3	BGMV 562	(SEA 22 x (A 774 x G 21212)F1)F1X(RAB619xTIO CANELA 75)F1/-MC-2P-MQ-MC-4C-MC-MC		bgm1	1	2	1	**1.3**	5	5	5	**5.0**
72	MR		14153-3	BGMV 562	(SEA 22 x (A 774 x G 21212)F1)F1X(RAB619xTIO CANELA 75)F1/-MC-3P-MQ-MC-17C-MC-MC		W12	2	2	2	**2.0**	5	5	5	**5.0**
73	MR		14153-3	BGMV 562	(SEA 22 x (A 774 x G 21212)F1)F1X(RAB619xTIO CANELA 75)F1/-MC-3P-MQ-MC-21C-MC-MC		W12	3	3	3	**3.0**	5	5	5	**5.0**
74	MR		14198-13	BGMV 291	(RAB 618x(DOR 364x(DOR 364x(DOR 364xSAM 1)F1)F2)F3)F1 X ((RAB 655xG 21212)F1xSEA 21)F1/-MC-1P-MQ-MC-11C-MC-MC		W12	1	3	1	**1.7**	5	5	5	**5.0**
75	MR		14198-13	BGMV 291	(RAB 618x(DOR 364x(DOR 364x(DOR 364xSAM 1)F1)F2)F3)F1 X ((RAB 655xG 21212)F1xSEA 21)F1/-MC-1P-MQ-MC-23C-MC-MC		bgm1+W12	1	3	2	**2.0**	5	5	5	**5.0**
76	MR		14215-9	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-6P-MQ-MC-11C-MC-MC		W12	1	1	1	**1.0**	4	3	3	**3.3**
77	MR		14258-7	BGMV 351	(DICTA 122x(DICTA 122x(DICTA 122xSAM 1)F1)F3-1)F1 X (MD 23-24x(RAB 655xG 21212)F1)F1/-MC-9P-MQ-MC-4C-MC-MC		W12	2	2	2	**2.0**	3	3	4	**3.3**
78	MR		14258-7	BGMV 351	(DICTA 122x(DICTA 122x(DICTA 122xSAM 1)F1)F3-1)F1 X (MD 23-24x(RAB 655xG 21212)F1)F1/-MC-9P-MQ-MC-8C-MC-MC		W12	2	1	2	**1.7**	2	4	3	**3.0**
79	MR		14273-4	BGMV 366	(RAB 623 x DICTA 17)F1 X (RAB 630 x SEA 21)F1/-MC-3P-MQ-MC-6C-MC-MC		W12	3	2	3	**2.7**	5	5	5	**5.0**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
80	MR	14273-4	BGMV 366	(RAB 623 x DICTA 17)F1 X (RAB 630 x SEA 21)F1/-MC-3P-MQ-MC-11C-MC-MC		W12	2	4	4	**3.3**	5	5	5	**5.0**
81	MR	14292-63	BGMV 385	(DICTA 122x(DICTA 122x(DICTA 122xSAM 1)F1)F3-1)F1 X (RAB 651x(VAX 1xRAB 655)F1)F1/-MC-2P-MQ-MC-11C-MC-MC		W12?	1	2	2	**1.7**	4	4	4	**4.0**
82	MN	14059-8	BGMV 448	(FEB 192 x G 21212)F1 x ICTA LIGERO/-MC-4P-MQ-MC-4C-MC-MC		bgm1	1	2	1	**1.3**	5	5	4	**4.7**
83	MN	13934-63	BGMV 570	(FEB 192xG 21212)F1x(DOR500x(DOR390x(DOR390 xSAM1)F1)F2)F3/-MC-2P-MQ-MC-9C-MC-MC		W12	2	1	1	**1.3**	3	4	5	**4.0**
84	MN	13934-63	BGMV 570	(FEB 192xG21212)F1x(DOR500x(DOR390x(DOR390xSAM1)F1)F2)F3/-MC-2P-MQ-MC-15C-M-MC		W12	2	1	1	**1.3**	4	5	4	**4.3**
85	MN	13942-22	BGMV 575	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-1P-MQ-MC-1C-MC-MC		bgm1	1	1	1	**1.0**	2	2	2	**2.0**
86	MN	13942-22	BGMV 575	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-1P-MQ-MC-3C-MC-MC		bgm1	1	1	1	**1.0**	1	2	1	**1.3**
87	MN	13942-22	BGMV 575	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-1P-MQ-MC-5C-MC-MC		bgm1	1	1	1	**1.0**	1	2	3	**2.0**
88	MN	13942-22	BGMV 575	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-1P-MQ-MC-11C-MC-MC		bgm1	1	1	1	**1.0**	1	2	2	**1.7**
90	MN	13942-33	BGMV 575	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-7P-MQ-MC-6C-MC-MC		W12	2	2	1	**1.7**	5	5	5	**5.0**
91	MR	14144-11	BGMV 578	(SEA 18 x (FEB 192 x G 21212)F1)F1 X EAP 9020-14/-MC-1P-MQ-MC-2C-MC-MC		W12	1	1	1	**1.0**	4	4	5	**4.3**
92	MR	14144-11	BGMV 578	(SEA 18 x (FEB 192 x G 21212)F1)F1 X EAP 9020-14/-MC-1P-MQ-MC-8C-MC-MC		W12	2	2	1	**1.7**	4	4	4	**4.0**
93	MR	14144-15	BGMV 578	(SEA 18 x (FEB 192 x G 21212)F1)F1 X EAP 9020-14/-MC-1P-MQ-MC-1C-MC-MC		W12	1	1	1	**1.0**	4	4	3	**3.7**
94	MN	14154-10	BGMV 579	(RIB 68 x G 21212)F1 X ICTA LIGERO/-MC-4P-MQ-MC-11C-MC-MC		bgm1	1	1	2	**1.3**	4	4	3	**3.7**
95	MN	14154-31	BGMV 579	(RIB 68 x G 21212)F1 X ICTA LIGERO/-MC-9P-MQ-MC-4C-MC-MC		bgm1?	3	1	2	**2.0**	4	4	5	**4.3**
96	MN	14154-31	BGMV 579	(RIB 68 x G 21212)F1 X ICTA LIGERO/-MC-9P-MQ-MC-5C-MC-MC		bgm1	3	3	2	**2.7**	4	4	5	**4.3**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
97	MN	14154-31	BGMV 579	(RIB 68 x G 21212)F1 X ICTA LIGERO/-MC-9P-MQ-MC-9C-MC-MC		bgm1	2	3	2	**2.3**	5	4	5	**4.7**
98	MN	14154-31	BGMV 579	(RIB 68 x G 21212)F1 X ICTA LIGERO/-MC-9P-MQ-MC-14C-MC-MC		bgm1	2	2	3	**2.3**	5	5	5	**5.0**
99	MN	14154-36	BGMV 579	(RIB 68 x G 21212)F1 X ICTA LIGERO/-MC-12P-MQ-MC-2C-MC-MC		bgm1+W12?	1	1	1	**1.0**	4	2	3	**3.0**
100	MR	14194-19	BGMV 287	((A 774xG 21212)F1xRAB 609)F1x((DOR 500x(DOR 390x(DOR 390xSAM 1)F1)F2)F3xSEA 18)F1/-MC-3P-MQ-MC-6C-MC-MC		bgm1	1	3	2	**2.0**	4	3	4	**3.7**
101	MR	14212-4	BGMV 305	(SEA 15x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F3)F1 X (TIO CANELA 75x(FEB 192xG 21212)F1)F1/-MC-2P-MQ-MC-4C-MC-MC		W12?	1	2	2	**1.7**	4	4	4	**4.0**
102	MR	14212-12	BGMV 305	(SEA 15x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F3)F1 X (TIO CANELA 75x(FEB 192xG 21212)F1)F1/-MC-5P-MQ-MC-3C-MC-MC		bgm1	1	3	2	**2.0**	4	5	5	**4.7**
103	MR	14212-12	BGMV 305	(SEA 15x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F3)F1 X (TIO CANELA 75x(FEB 192xG 21212)F1)F1/-MC-5P-MQ-MC-4C-MC-MC		bgm1	2	1	1	**1.3**	5	5	5	**5.0**
104	MR	14212-12	BGMV 305	(SEA 15x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F3)F1 X (TIO CANELA 75x(FEB 192xG 21212)F1)F1/-MC-5P-MQ-MC-7C-MC-MC		bgm1	3	4	3	**3.3**	4	4	5	**4.3**
105	MR	14215-5	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-9P-MQ-MC-4C-MC-MC		bgm1[H]?	2	1	2	**1.7**	5	5	5	**5.0**
106	MN	13856-6	BGMV 252	LORE 24 x ((VAX 1xA 774)F1x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F1)F1/-MQ-3P-MQ-MC-2C-MC-MC		bgm1	2	2	2	**2.0**	4	4	5	**4.3**
107	MN	13856-35	BGMV 252	LORE 24 x ((VAX 1xA 774)F1x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F1)F1/-MQ-3P-MQ-MC-3C-MC-MC		bgm1	2	2	1	**1.7**	4	3	4	**3.7**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
108	MN	13862-44	BGMV 258	LORE 87 x ((VAX 1xIPA 7)F1x(G 18479x(G 18479x(G 18479xSAM 1)F1)F2)F1)F1/-MQ-1P-MQ-MC-2C-MC-MC		bgm1+W12	1	2	1	**1.3**	5	3	2	**3.3**
109	MR	14202-4	BGMV 295	(RAB 623 x MD 23-24)F1 X (SEA 15 x (RAB 655 x G 21212)F1)F1/-MC-3P-MQ-MC-9C-MC-MC		W12	3	4	3	**3.3**	5	5	5	**5.0**
110	MR	14202-4	BGMV 295	(RAB 623 x MD 23-24)F1 X (SEA 15 x (RAB 655 x G 21212)F1)F1/-MC-3P-MQ-MC-12C-MC-MC		W12	2	1	1	**1.3**	5	5	4	**4.7**
111	MR	14216-3	BGMV 309	(SEA 15x(DOR 364x(DOR 364x(DOR 364xSAM 1)F1)F2)F3)F1 X (RAB 651x(MD 23-24xG 21212)F1)F1/-MC-7P-MQ-MC-1C-MC-MC		bgm1+W12	2	2	1	**1.7**	5	5	5	**5.0**
112	MR	14216-3	BGMV 309	(SEA 15x(DOR 364x(DOR 364x(DOR 364xSAM 1)F1)F2)F3)F1 X (RAB 651x(MD 23-24xG 21212)F1)F1/-MC-7P-MQ-MC-9C-MC-MC		bgm1+W12	2	3	3	**2.7**	5	5	5	**5.0**
113	MR	14000-20	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-6P-MQ-MC-1C-MC-MC		bgm1	3	3	2	**2.7**	4	5	5	**4.7**
115	MR	14000-20	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-6P-MQ-MC-10C-MC-MC		bgm1	3	3	4	**3.3**	5	5	5	**5.0**
116	MR	14215-5	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-4P-MQ-MC-8C-MC-MC		bgm1	3	2	3	**2.7**	5	5	5	**5.0**
117	MR	14215-5	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-5P-MQ-MC-1C-MC-MC		bgm1	4	2	1	**2.3**	5	5	5	**5.0**
118	MR	14215-5	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-5P-MQ-MC-10C-MC-MC		bgm1+W12	4	2	3	**3.0**	5	5	5	**5.0**
119	MR	14215-6	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-4P-MQ-MC-12C-MC-MC		bgm1+W12	2	3	2	**2.3**	5	5	5	**5.0**
121	.	.	.	BAT 338 x G35252	F11	Interesp	1	1	1	**1.0**	4	4	5	**4.3**
122	.	.	.	BAT 338 x G35252	F10	Interesp	2	2	2	**2.0**	4	3	5	**4.0**
123	.	.	.	(G35649 x G 3807)x G35023	F7	Interesp	2	2	1	**1.7**	1	2	3	**2.0**
124	.	.	.	((G35876 x G 3807)x G35182)x A 114	F9	Interesp	1	1	1	**1.0**	3	3	3	**3.0**
126	.	.	.	BAT 338 x G35252	F11	Interesp	2	2	2	**2.0**	5	4	5	**4.7**
127	.	.	.	BAT 338 x G35252	F11	Interesp	3	3	4	**3.3**	5	4	4	**4.3**
129	.	.	.	BAT 338 x G35252	F11	Interesp	2	3	2	**2.3**	5	5	5	**5.0**
130	.	.	.	BAT 338 x G35252	F11	Interesp	1	2	1	**1.3**	1	2	3	**2.0**
132	.	.	.	BAT 338 x G35252	F11	Interesp	2	2	1	**1.7**	5	5	4	**4.7**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
133	.	.	.	BAT 338 x G35252	F10	Interesp	1	3	1	**1.7**	5	4	5	**4.7**
134	.	.	.	AND 107 x Piloy	F4	Interesp	2	3	4	**3.0**	5	5	4	**4.7**
135	.	.	.	BAT 338 x G35252	F11	Interesp	2	1	1	**1.3**	5	4	4	**4.3**
136	.	.	.	BAT 338 x G35252	F11	Interesp	3	3	4	**3.3**	5	5	5	**5.0**
137	.	.	.	BAT 338 x G35252	F10	Interesp	2	2	1	**1.7**	2	3	2	**2.3**
138	.	.	.	Pasto x G35122	F11	Interesp	3	3	2	**2.7**	4	4	4	**4.0**
139	.	.	.	BAT 338 x G35252	F11	Interesp	2	2	1	**1.7**	2	3	4	**3.0**
140	.	.	.	BAT 338 x G35252	F11	Interesp	3	2	4	**3.0**	4	5	4	**4.3**
141	.	.	.	Pasto x G35122	F10	Interesp	1	2	2	**1.7**	4	5	5	**4.7**
142	.	.	.	(ICA PIJAO X G 35171)F1 X ICA PIJAO)F1/-(NN)P-(NN)P(F8)-(NN)P-(NN)D	F10	Interesp	3	3	4	**3.3**	5	5	5	**5.0**
143	.	.	.	(ICA PIJAO X G 35171)F1 X ICA PIJAO)F1/-(NN)P-(NN)P(F8)-(NN)P-(NN)D	F10	Interesp	1	1	1	**1.0**	3	3	2	**2.7**
144	.	.	.	(ICA PIJAO X G 35172) X ICA PIJAO)F1/-4P-(NN)P(F8)-(NN)P-(NN)D	F10	Interesp	1	1	1	**1.0**	2	3	3	**2.7**
145	.	.	.	(ICA PIJAO X G 35172) X ICA PIJAO)F1/-19P-(NN)P(F8)-(NN)P-(NN)D	F10	Interesp	1	1	2	**1.3**	2	1	3	**2.0**
146	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	2	2	1	**1.7**	4	4	5	**4.3**
147	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	1	2	1	**1.3**	3	2	2	**2.3**
148	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	1	1	1	**1.0**	3	2	3	**2.7**
149	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	2	1	1	**1.3**	1	2	2	**1.7**
150	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	2	2	2	**2.0**	3	3	5	**3.7**
151	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	1	2	2	**1.7**	3	2	3	**2.7**
152	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	1	1	2	**1.3**	2	3	4	**3.0**
153	.	.	.	ICA PIJAO X (ICA PIJAO X G 35877)F1/-(NN)P-(NN)Q-(NN)P-(NN)D	F6	Interesp	1	2	1	**1.3**	2	2	2	**2.0**
154		.	.	RMC 2			3	2	2	**2.3**	5	5	5	**5.0**
155		.	.	RMC 3			2	2	2	**2.0**	5	5	5	**5.0**
156		.	.	RMC 4			2	3	4	**3.0**	5	5	5	**5.0**
157		.	.	RMC 5			2	2	1	**1.7**	5	5	5	**5.0**
158		.	.	RMC 6			2	3	4	**3.0**	5	5	5	**5.0**
159		.	.	RMC 7			1	4	1	**2.0**	5	5	5	**5.0**
160		.	.	RMC 9			1	2	1	**1.3**	5	4	4	**4.3**
161		.	.	RMC 10			4	3	3	**3.3**	5	5	5	**5.0**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
162		.	.	RMC 11			4	3	4	**3.7**	5	5	5	**5.0**
163		.	.	RMC 12			5	2	2	**3.0**	5	5	5	**5.0**
164		.	.	RMC 14			3	4	3	**3.3**	5	4	5	**4.7**
165		.	.	RMC 16			4	3	2	**3.0**	5	5	5	**5.0**
168		.	.	RMC 25			4	3	1	**2.7**	5	5	5	**5.0**
169		.	.	RMC 26			4	4	4	**4.0**	5	5	5	**5.0**
170		.	.	RMC 27			4	4	4	**4.0**	5	5	5	**5.0**
171		.	.	RMC 29			4	4	4	**4.0**	5	4	5	**4.7**
172		.	.	RMC 30			4	4	4	**4.0**	5	5	5	**5.0**
173		.	.	RMC 33			4	4	3	**3.7**	5	5	5	**5.0**
174		.	.	RMC 34			4	3	3	**3.3**	5	5	5	**5.0**
175		.	.	RMC 35			3	1	1	**1.7**	5	5	5	**5.0**
176	. 21598 -(M)W F2-(CM)W-CM(6)W- 7W-CM(8)W		.	UPR9745-138 X SEL 1446			4	4	4	**4.0**	5	4	5	**4.7**
178	. 21605 -(M)W F2-(CM)W-CM(30)W- 6W-CM(2)W		.	UPR9745-226 X SEL 1447			4	2	2	**2.7**	5	5	5	**5.0**
179	. 21605 -(M)W F2-(CM)W-CM(30)W- 9W-CM(6)W		.	UPR9745-226 X SEL 1447			3	3	1	**2.3**	4	5	4	**4.3**
181	. 21605 -(M)W F2-(CM)W-CM(30)W-12W-CM(8)W		.	UPR9745-226 X SEL 1447			2	2	3	**2.3**	5	5	5	**5.0**
183	. 21605 -(M)W F2-(CM)W-CM(30)W-16W-CM(5)W		.	UPR9745-226 X SEL 1447			2	2	2	**2.0**	5	4	5	**4.7**
184	. 21608 -(M)W F2-(CM)W-CM(25)W- 4W-CM(10)W		.	UPR9745-226 X S 31465			2	2	3	**2.3**	5	2	5	**4.0**
185	. 21609 -(M)W F2-(CM)W-CM(18)W-11W-CM(3)W		.	UPR9745-226 X CALIMA DAR			3	3	3	**3.0**	5	5	5	**5.0**
187	. 21609 -(M)W F2-(CM)W-CM(18)W-22W-CM(10)W		.	UPR9745-226 X CALIMA DAR			3	4	2	**3.0**	5	5	5	**5.0**
188	. 21609 -(M)W F2-(CM)W-CM(18)W-23W-CM(13)W		.	UPR9745-226 X CALIMA DAR			4	2	3	**3.0**	5	5	5	**5.0**
191	. 21597R-(M)W F2-(CM)W-CM(25)W-17W-CM(3)W		.	SEL 1445 X UPR9745-138			3	4	3	**3.3**	5	5	5	**5.0**
193	. 21607R-(M)W F2-(CM)W-CM(22)W- 1W-CM(6)W		.	SEL 1448 X UPR9745-226			2	2	3	**2.3**	5	5	5	**5.0**
195	. 21609R-(M)W F2-(CM)W-CM(50)W- 2W-CM(11)W		.	CALIMA DAR X UPR9745-226			3	3	4	**3.3**	5	5	5	**5.0**
196	. 21661 -(M)W F2-(CM)W-(CM)W-4W-CM(16)W		.	TIO CANELA X G 2333			1	1	1	**1.0**	4	4	3	**3.7**
197	. 21661 -(M)W F2-(CM)W-(CM)W-6W-CM(4)W		.	TIO CANELA X G 2333			2	1	1	**1.3**	4	4	5	**4.3**
198	. 21661 -(M)W F2-(CM)W-(CM)W-7W-CM(15)W		.	TIO CANELA X G 2333			1	2	1	**1.3**	4	3	3	**3.3**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
199	. 21661 -(M)W F2-(CM)W-(CM)W-15W-CM(15)W		.	TIO CANELA X G 2333			1	1	1	**1.0**	4	2	3	**3.0**
200	. 21662 -(M)W F2-(CM)W-(CM)W-2W-CM(10)W		.	G 685 X TIO CANELA			2	3	2	**2.3**	4	3	5	**4.0**
201	. 21662 -(M)W F2-(CM)W-(CM)W-3W-CM(15)W		.	G 685 X TIO CANELA			3	2	1	**2.0**	3	4	5	**4.0**
202	. 21662 -(M)W F2-(CM)W-(CM)W-4W-CM(7)W		.	G 685 X TIO CANELA			1	2	3	**2.0**	3	4	4	**3.7**
203	. 21662 -(M)W F2-(CM)W-(CM)W-6W-CM(17)W		.	G 685 X TIO CANELA			3	3	3	**3.0**	4	4	4	**4.0**
205	. 21662 -(M)W F2-(CM)W-(CM)W-13W-CM(24)W		.	TIO CANELA X G 685			1	2	1	**1.3**	3	3	3	**3.0**
206	. 21662 -(M)W F2-(CM)W-(CM)W-4W-CM(14)W		.	TIO CANELA X G 685			3	4	2	**3.0**	4	4	5	**4.3**
207	. 21662 -(M)W F2-(CM)W-(CM)W-5W-CM(2)W		.	TIO CANELA X G 685			1	1	1	**1.0**	4	5	4	**4.3**
208	. 21662 -(M)W F2-(CM)W-(CM)W-9W-CM(14)W		.	TIO CANELA X G 685			1	2	1	**1.3**	4	4	4	**4.0**
209	. 21662 -(M)W F2-(CM)W-(CM)W-14W-CM(8)W		.	TIO CANELA X G 685			3	3	3	**3.0**	4	5	5	**4.7**
210	. 21662 -(M)W F2-(CM)W-(CM)W-15W-CM(21)W		.	TIO CANELA X G 685			2	2	1	**1.7**	4	4	4	**4.0**
211	. 21662 -(M)W F2-(CM)W-(CM)W-15W-CM(21)W		.	TIO CANELA X G 685			3	2	3	**2.7**	4	4	5	**4.3**
212	. 21662 -(M)W F2-(CM)W-(CM)W-16W-CM(20)W		.	TIO CANELA X G 685			2	2	2	**2.0**	4	4	5	**4.3**
213	. 21661R-(M)W F2-(CM)W-(CM)W- 4W-CM(16)W		.	G 2333 X TIO CANELA			3	3	4	**3.3**	4	4	4	**4.0**
214	. 21661R-(M)W F2-(CM)W-(CM)W- 4W-CM(12)W		.	G 2333 X TIO CANELA			3	2	2	**2.3**	4	5	5	**4.7**
215	. 21661R-(M)W F2-(CM)W-(CM)W- 6W-CM(13)W		.	G 2333 X TIO CANELA			2	2	2	**2.0**	4	4	4	**4.0**
216	. 21661R-(M)W F2-(CM)W-(CM)W- 8W-CM(10)W		.	G 2333 X TIO CANELA			1	2	1	**1.3**	3	3	3	**3.0**
217	. 21661R-(M)W F2-(CM)W-(CM)W-12W-CM(12)W		.	G 2333 X TIO CANELA			3	3	2	**2.7**	4	4	5	**4.3**
218	. 21661R-(M)W F2-(CM)W-(CM)W-14W-CM(11)W		.	G 2333 X TIO CANELA			3	4	1	**2.7**	5	4	4	**4.3**
219	. 21661 -(M)W F2-(CM)W-(CM)W-7W-CM(11)W		.	TIO CANELA X G 2333			2	2	2	**2.0**	5	5	5	**5.0**
220	. 21662 -(M)W F2-(CM)W-(CM)W-6W-CM(5)W		.	G 685 X TIO CANELA			2	2	2	**2.0**	4	5	4	**4.3**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
221	. 21662 -(M)W F2-(CM)W-(CM)W-7W-CM(7)W		.	G 685 X TIO CANELA			1	1	1	**1.0**	4	4	3	**3.7**
222	. 21662 -(M)W F2-(CM)W-(CM)W-9W-CM(15)W		.	G 685 X TIO CANELA			2	3	3	**2.7**	5	5	5	**5.0**
223	. 21662 -(M)W F2-(CM)W-(CM)W-10W-CM(4)W		.	G 685 X TIO CANELA			1	2	1	**1.3**	3	2	4	**3.0**
224	. 21662 -(M)W F2-(CM)W-(CM)W-13W-CM(4)W		.	G 685 X TIO CANELA			1	2	1	**1.3**	3	3	4	**3.3**
225	. 21662 -(M)W F2-(CM)W-(CM)W-17W-CM(7)W		.	G 685 X TIO CANELA			2	1	1	**1.3**	4	5	5	**4.7**
226	. 21662 -(M)W F2-(CM)W-(CM)W-20W-CM(10)W		.	G 685 X TIO CANELA			2	2	2	**2.0**	5	5	4	**4.7**
227	. 21662 -(M)W F2-(CM)W-(CM)W-22W-CM(4)W		.	G 685 X TIO CANELA			2	1	1	**1.3**	3	4	3	**3.3**
228	. 21662 -(M)W F2-(CM)W-(CM)W-1W-CM(11)W		.	G 685 X TIO CANELA			1	1	1	**1.0**	3	2	5	**3.3**
229	. 21662 -(M)W F2-(CM)W-(CM)W-1W-CM(14)W		.	TIO CANELA X G 685			1	1	2	**1.3**	3	5	5	**4.3**
230	. 21661R-(M)W F2-(CM)W-(CM)W- 6W-CM(17)W		.	G 2333 X TIO CANELA			2	1	1	**1.3**	5	5	4	**4.7**
231	. 21661R-(M)W F2-(CM)W-(CM)W- 7W-CM(12)W		.	G 2333 X TIO CANELA			3	3	2	**2.7**	5	5	5	**5.0**
233				. G 685			2	3	2	**2.3**	4	5	5	**4.7**
235				RM - 35			1	-	-	**1.0**	5	5	5	**5.0**
236				G5746 (RGL - C)			3	3	3	**3.0**	5	5	5	**5.0**
237				Rojo Brasil			3	2	2	**2.3**	5	5	5	**5.0**
114*	MR	14000-20	BGMV 551	RAB 651 x (MD 23-24xG 21212)F1/-MC-6P-MQ-MC-4C-MC-MC		bgm1	4	4	4	**4.0**	-	-	-	
120*	MR	14215-9	BGMV 308	(SEA 15 x MD 23-24)F1 X (TIO CANELA 75 x G 21212)F1/-MC-8P-MQ-MC-1C-MC-MC		bgm1+W12	4	4	4	**4.0**	-	-	-	
125*	.	.	.	BAT 338 x G35252	F11	Interesp	5	4	5	**4.7**	-	-	-	
128*	.	.	.	AND 279 x G35337	F4	Interesp	3	4	4	**3.7**	-	-	-	
131*	.	.	.	BAT 338 x G35252	F10	Interesp	3	4	4	**3.7**	-	-	-	
166*		.	.	RMC 22			4	4	4	**4.0**	-	-	-	
167*		.	.	RMC 23			4	4	3	**3.7**	-	-	-	
177*	. 21602 -(M)W F2-(CM)W-CM(20)W-16W-CM(12)W		.	UPR9745-138 X CALIMA DAR			4	4	4	**4.0**	-	-	-	
180*	. 21605 -(M)W F2-(CM)W-CM(30)W-12W-CM(8)W		.	UPR9745-226 X SEL 1447			4	4	2	**3.3**	-	-	-	
182*	. 21605 -(M)W F2-(CM)W-CM(30)W-15W-CM(8)W		.	UPR9745-226 X SEL 1447			4	4	4	**4.0**	-	-	-	

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
186*	. 21609 -(M)W F2-(CM)W-CM(18)W-16W-CM(9)W		.	UPR9745-226 X CALIMA DAR			4	4	4	**4.0**	-	-	-	
189*	. 21609 -(M)W F2-(CM)W-CM(18)W-25W-CM(8)W		.	UPR9745-226 X CALIMA DAR			4	4	4	**4.0**	-	-	-	
190*	. 21597R-(M)W F2-(CM)W-CM(25)W- 2W-CM(4)W		.	SEL 1445 X UPR9745-138			4	4	2	**3.3**	-	-	-	
192*	. 21597R-(M)W F2-(CM)W-CM(25)W-20W-CM(5)W		.	SEL 1445 X UPR9745-138			3	4	4	**3.7**	-	-	-	
194*	. 21607R-(M)W F2-(CM)W-CM(22)W- 4W-CM(8)W		.	SEL 1448 X UPR9745-226			3	4	4	**3.7**	-	-	-	
204*	. 21662 -(M)W F2-(CM)W-(CM)W-8W-CM(11)W		.	G 685 X TIO CANELA			4	4	3	**3.7**	-	-	-	
232*			.	. SEL 1445			4	4	4	**4.0**	-	-	-	
234*				GN - 31			4	4	-	**4.0**	-	-	-	
238*				G - 76 (RED KLOUD)			4	4	3	**3.7**	-	-	-	
25*	RAB 651		.	RAB 651		bgm1	3	4	4	**3.7**	-	-	-	
43*	G 4090		.	G 4090 [ROJO DE SEDA]		Susceptible	4	4	3	**3.7**	-	-	-	
67*	MR	14148-72	BGMV 453	(SEA 21 x RAB 623)F1 X 9653-16 B-1/-MC-3P-MQ-MC-3C-MC-MC		W12	3	4	4	**3.7**	-	-	-	
89*	MN	13942-31	BGMV 575	(TLP 35xG 21212)F1 x ICTA LIGERO/-MC-1P-MQ-MC-4C-MC-MC		bgm1+W12	4	4	2	**3.3**	-	-	-	
10H	SB 14565-4	14		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-4T	F5		-	-	-		5	5	-	**5.0**
11H	SB 14565-4	13		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-3T	F5		-	-	-		5	5	5	**5.0**
12H	SB 14565-4	12		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-2T	F5		-	-	-		5	5	5	**5.0**
13H	SB 14565-4	11		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-1T	F5		-	-	-		5	5	5	**5.0**
14H	SB 14565-3	10		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-2T	F5		-	-	-		5	5	5	**5.0**
15H	SB 14565-3	9		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-1T	F5		-	-	-		5	5	5	**5.0**
16H	SB 14565-1	8		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-5P-4T	F5	bgm1+W12	-	-	-		5	5	5	**5.0**
17H	SB 14565-1	7	.	HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-5P-3T	F5	Susceptible	-	-	-		5	5	5	**5.0**
18H	SB 14565-1	6		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-5P-2T	F5		-	-	-		5	5	5	**5.0**
19H	SB 14565-1	5		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-5P-1T	F5		-	-	-		4	5	-	**4.5**
1H	SB 14565-7	23		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-2P-1P-2T	F5	Interesp	-	-	-		5	5	5	**5.0**

Table 44. Sources of resistance to begomoviruses

Ent	Code	Cross Number	#BGMV	Identification	Fn	BGYMV Res. genes	First trial (2003A) R1	R2	R3	Media	Second trial (2004A) R1	R2	R3	Media
20H	SB 14565-1	4		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-3P-1T	F5	Interesp	-	-	-		5	5	5	**5.0**
21H	SB 14565-1	3		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-1P-3T	F5	Interesp	-	-	-		5	5	5	**5.0**
22H	SB 14565-1	2		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-1P-2T	F5	W12	-	-	-		5	5	5	**5.0**
23H	SB 14565-1	1	.	HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-4P-1P-1T	F5	bgm1	-	-	-		4	5	5	**4.7**
2H	SB 14565-7	22		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-2P-1P-1T	F5		-	-	-		5	5	5	**5.0**
3H	SB 14565-4	21		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-3P-2P-2T	F5		-	-	-		5	5	5	**5.0**
4H	SB 14565-4	20		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-3P-2P-1T	F5		-	-	-		5	5	5	**5.0**
5H	SB 14565-4	19		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-2P-3T	F5		-	-	-		5	5	5	**5.0**
6H	SB 14565-4	18		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-2P-2T	F5		-	-	-		5	5	5	**5.0**
7H	SB 14565-4	17		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-2P-1T	F5		-	-	-		5	5	5	**5.0**
8H	SB 14565-4	16		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-6T	F5	bgm1+W12	-	-	-		5	5	5	**5.0**
9H	SB 14565-4	15		HAV 129 X (G 17723 x (G 685 x (ASC 73 x ICTA HUNAPU)F1)F1)F5/-1P-1P-5T	F5	bgm1	-	-	-		5	5	5	**5.0**

A screening of selected sources of begomovirus resistance was also conducted under glasshouse conditions to determine the efficiency of transmission of the virus by *B. tabaci*. Table 45 shows the average incidence of two inoculations of seven day old seedlings using eight whitefly adults per plant.

Table 45. Bean leaf crumple incidence in selected bean genotypes artificially inoculated using eight *B. tabaci* individuals per test plant.

Bean Genotype	Bean leaf crumple Incidence (%)
Topcrop	100
Red Kloud	100
Redlands Greenleaf-C	83.3
Garrapato	54.2
Great Northern 31	33.3
EAP-9510-77	16.7
Rojo Brasil	16.7
Red Mexican 35	4.5
ICTA Ligero	0
Porrillo Sintetico	0
BAT 304	0

The field and glasshouse inoculations of selected sources of resistance and the susceptible control (Topcrop), showed that the genetics of resistance to Bean leaf crumple is similar to that reported for other begomoviruses of common bean. Basically, the best common bean genotypes identified, are either virus-resistant genotypes or have virus-resistant parents of different racial origin. However, the best sources of virus resistance are found in the black-seeded genotypes (e.g. BAT 304, Porrillo Sintetico, and ICTA Ligero). Unfortunately, although the black-seeded genotypes have been widely used as sources of resistance to begomoviruses in bush types, the climbers and most snap bean varieties have not been previously bred for resistance to these emerging virus problems. This requires a concerted effort to improve snap beans for their resistance to begomoviruses.

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2.1.5 Resistance to different pathogenic species of *Pythium*

Rationale: In screenhouse evaluations of germplasm and potential sources of resistance against *Pythium* root rots, we have in the past used *P. aphanidermatum*. As a result we identified and confirmed a number of resistance sources. However, our recent pathogen characterization studies showed that over nine *Pythium* species (*Pythium ultimum*, *P. salpingophorum, P. irregulare, P. aphanidermatum, P. nodosum, P. spinosum, P. tulorosum, P chamaehyphon* and *P. pachycaule*) from east and central African countries infect beans, with *P. ultimum* being the most widespread species (found in 25-30% of samples collected). Besides, some of the genotypes observed to be

resistant in the screenhouse against *P. aphanidermatum* were susceptible under field conditions, while resistance in some varieties (RWR 221) varied with location (e.g. resistant in Rwanda but susceptible in Western Kenya). It is therefore vital to subject the resistant germplasm or potential parental materials against the different characterized *Pythium* species, so as to ensure that the resistance identified and used for introgression into commercial varieties is broad and durable.

Materials and Methods: Thirty-nine genotypes, previously screened and found to be resistant to *P. aphanidermatum* were evaluated against nine isolates representing six *Pythium* species (*Pythium ultimum*, *P. salpingophorum, P. spinosum*, *P. tulorosum, P chamaehyphon* and *P. pachycaule*) pathogenic to beans. Plants were grown in wooden trays containing soil artificially infested with each of the six species. Soil conditions favoring pathogen establishment and disease development (high soil moisture) were provided and plants were evaluated three weeks after germination. Seedlings were gently uprooted, washed in tap water, and severity of lesions on the root system scored using a CIAT severity rating scale of 1 (resistant) to 9 (susceptible). Twelve plants per entry were evaluated in two replications. CAL 96 and RWR 719 were used as susceptible and resistant checks respectively.

Results and Discussion: Thirty out of thirty-nine genotypes maintained their resistance against the different species of *Pythium* (Table 46), a demonstration of their potential value in genetic improvement of commercial varieties. Of interest and probably influenced by species was the susceptible reactions of DOR 708, VAX 2, DOR 622, RWR 1058 and RWR 1059 which were previously considered resistant (using *P. aphanidermatum*). However, there was no differential reaction of the bean genotypes to different *Pythium* species used in the current study, implying that the genes responsible for resistance might have a similar mode of action. Some of the small seeded varieties (Mex 54, 217/2, GLP 585 and GLP X 92) had intermediate reactions implying that they possibly have some levels of resistance but distinct from that in resistant varieties. This is consistent with observations under field conditions where some of these entries (e.g. GLP X92) are more tolerant to *Pythium* root rot than the susceptible ones (GLP 2). Mex 54, resistant to most races of angular leaf spot pathogen (*Phaeoisariopsis griseola*) found in Africa has been used in crosses to improve resistance against the disease. Its medium to susceptible reactions against *Pythium* species, demonstrates the need to pyramid ALS and *Pythium* root rot resistances. The large or medium seeded Andean varieties like CAL 96, GLP 2, GLP 24, RWR 1058, RWR 1092, and Urugezi were susceptible (reaction score of 9) and died 21 days after planting. Varietal improvement is targeting these materials some of which are important commercial varieties in east and central Africa.

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Table 46. Reaction of common bean genotypes to artificial inoculation with six *Pythium* species causing bean root rots. Kawanda, 2004

Entry	*Pythium* species / isolates								
	P. ultimum		*P. salpingophorum*		*P. sp.*	*P chamaehyphon*	*P. pachycaule*	*P. spinosum*	*P. torulosum*
	JIM 85A	JIM 7HI	JM 84	JM 65A	KAK 5B	VIH 2A	JIM 29A	JM70H1	JM 65A
MLB-49-89A	1	1	1	1	1	1	1	1	1
MLB-40-89A	1	1	1	1	1	1	1	1	1
MLB-48-89A	4	4	3	3	1	3	3	2	1
MLB-39-89A	1	1	1	1	1	1	1	1	1
MLB-17-89A	1	1	1	1	1	1	1	1	1
MLB-36-89A	1	1	1	1	1	1	1	1	1
MLB-22-88B	2	2	1	1	1	1	1	1	1
MLB-68-89A	1	1	1	1	1	1	1	1	1
RWR 719	1	1	1	1	1	1	1	1	1
RWR 1873	2	2	2	2	1	1	1	1	1
RWR 2075	2	2	2	2	1	1	1	1	1
RWR 1946	2	2	2	2	1	1	1	1	1
RWR 221	1	1	1	1	1	1	1	1	1
RWR 1092	1	1	2	2	2	2	1	1	1
RWR 1091	1	1	1	1	1	1	1	1	1
Ihumure	1	1	1	1	1	2	1	1	1
SCAM80-CM/15	1	1	1	1	1	1	1	1	1
SCAM80-CM/5	1	1	1	1	1	1	1	1	1
MCD 221	3	3	1	1	1	1	1	1	1
AND 1064	1	1	1	1	1	1	1	1	1
AND1055	1	1	1	1	1	1	1	1	1
AND 1062	1	1	1	1	1	1	1	1	1
MAM 38	3	3	2	3	1	1	1	1	1
UBR (95) 2	1	1	1	1	1	1	1	1	1
CIM 9314-1	1	1	1	1	1	1	1	1	1
CIM 9313-1	1	1	1	1	1	1	1	1	1
EC-DE-HAR	2	2	2	2	2	2	1	1	1
DFA 54	3	3	2	2	1	1	1	1	1
FEB 181	3	3	2	2	1	1	1	1	1
311/7	2	1	2	1	1	1	1	1	1
Mexico 54	6	6	6	7	7	7	7	8	7
217/2	5	5	7	7	7	7	7	7	7
GLP 585	7	7	7	7	7	7	7	7	7
GLP X 92	7	7	7	7	6	6	7	6	6
DOR 708	9	9	9	9	9	9	9	9	9
VAX 2	9	9	9	9	6	6	6	7	7
DOR 622	9	8	8	8	9	9	8	9	9
RWR 1059	9	9	9	9	9	9	6	9	9
RWR 1058	9	9	9	9	9	9	9	9	9
GLP 24	9	9	9	9	9	9	9	9	9
GLP2	9	9	9	9	9	9	9	9	9
URUGEZI	9	9	9	9	9	9	9	9	9
CAL 96	9	9	9	9	9	9	9	9	9

2.1.6 Evaluation and selection among segregating populations for resistance to *Pythium* root rot and angular leaf spot.

Rationale: In efforts to increase bean production through genetic improvement in Africa, and reduce yield losses caused by two major bean diseases; angular leaf spot (ALS) and bean root rots, several populations designed to transfer, combine and pyramid resistance against the diseases, into major commercial and adapted bush and climbing bean cultivars were generated. Some of the parents used (see Section 2.1.1 above) are resistant to several species of *Pythium* and important races of *Phaeoisariopsis griseola* in Africa. Selections from segregating populations and lines focused on seed types and resistance to *Pythium* root rot and ALS.

Materials and Methods: Several F_2 derived F_4 populations, lines and families were grown at Rubaya in southwest Uganda and at Kawanda. At Kawanda, populations were grown in the field and evaluated for ALS, common bacterial blight and bean common mosaic virus. At Rubaya, populations were evaluated for root rots and resistance to other foliar diseases. Some lines were evaluated in the screenhouse using artificial inoculation with *P. ultimum* as described under 2.1.1.

Evaluation for angular leaf spot resistance was done by artificially inoculating populations with a mixture of local Andean and Mesoamerican races. Three week-old seedlings were spray-inoculated with a spore suspension from 14 day-old cultures at a concentration of $2x10^4$ conidia ml^{-1}. Inoculated plants were incubated in a humid chamber for 4 days and thereafter in the open, inside the screen house. Assessment of disease reaction was done every third day over a three-week period using a CIAT severity scale of 1 (resistant) to 9 (susceptible).

Results and Discussion:

Selection of recombinant inbred lines (RILs) for improved resistance to *Pythium* root rot:

The F_4 single plants evaluated had been derived from two selection pathways of F_2 populations. The first set of F_2 population had been evaluated in soil artificially infested with *P. ultimum* in the screen house. F_2 resistant plants were selected and planted in single row progenies in the field at Kawanda. Resulting F_3 families were advanced to F_4 by single seed descent. The F_4 superior single plants were grown this year in Rubaya and Senge and selected for their resistance to root rots and for other agronomic traits. About 170 F_5 single plant progenies were selected (Table 47) and will be made available to partners in both ECBREN and SABRN countries for multi-ecology evaluations with farmer participation.

Another set of F_2, populations were screened in the field in Rubaya (a root rot hotspot) and advanced by selecting best individual plants to get F_3 families (pedigree selection). The best F_4 plants were selected from best rows within each family to produce F_5 families. These were evaluated in the screen house against *P. ultimum*. The best resistant entries ($\leq$ 3.9 in a CIAT scale of 1-9) and also representing a range of seed types (e.g. red mottle, red, black, beige, cream etc) are shown on Table 48. Seed multiplication is underway and the materials will be available for multi-location evaluation with farmer participation.

Table 47. F_5 recombinant inbred lines (RILs) derived by single seed descent from F_2 resistant progenies**.**

Pedigree	Lines			
	Evaluated			Selected
	F_3 at Senge 2003B	**F_4 at Senge 2004A**	**F_4 at Rubaya 2004A**	**F_5 at Rubaya 2004A**
GLP 2 x RWR 719	5	2	2	1
GLP2 x AND 1055	103	63	22	21
GLP2 x AND 1062	35	23	(under evaluation)	
GLP 585 x RWR 719	40	20	3	3
GLP 585 x MLB-49-89A	456	205	124	114
GLP 585 x SCAM 80-CM/15	7	3	3	1
GLP 585 x AND 1062	8	7	(under evaluation	
CAL 96 x MLB-49-89A	5	10	5	5
BCr_1GLP 585 x MLB-49-89A	30	20	36	21
BCr_1 CAL 96 x MLB-49-89A	11	10	5	4
Total	684	363	200	170

Table 48. F_5 lines resistant against *P. ultimum* (score of less then ≤ 3.9) derived from F_2 resistant progenies by pedigree method. Kawanda, screen house, 2004

Pedigree	Line codes	No. of lines with resistant disease severity scores[x]		
		1	**(1.1-2)**	**(2.1-3.9)**
GLP 2 xMLB-49-89A	RF RO2-12	4	2	8
GLP 2 x SCAM 80-CM/15	RF RO2-13	5	2	6
GLP 2 x AND 1055	RF RO2-14	3		6
GLP 2 x AND 1062	RF RO2-15	3		5
GLP 585 x RWR 719	RF RO2-21	2	1	
GLP 585 x SCAM 80-CM/15	RF RO2-23			1
GLP 585 x AND 1062	RF RO2-25	1	2	1
CAL 96 x MLB-49-89A	RF RO2-32			1
Total		**18**	**7**	**28**

[x] - Based on a CIAT scale of 1 to 9 where 1 is resistant and 9 susceptible

Development of backcross (BC) populations with *Pythium* root rot resistance

Parallel to the development of RILs, a backcrossing program initiated to transfer resistance into popular market class types grown in Kenya, Rwanda and Uganda has generated 20 backcross populations (Table 49). Two and five populations are at BC_5 and BC_4 respectively. The latter is being advanced to BC_5. The BC_5 will be available for heterogeneity test by partners in different countries for selecting lines of interest (resistance and farmer preferences) under different environments.

Table 49. Developed backcross (BCs) populations by 2004

Population codes	No. of populations	Generation
BCs_1 RFR O2-1-11	2	BC_5
BCs_1 RFR O2-1-12, 13, 15, 22, 24	5	BC_4
BCs_1 RFR O2-1-14, 21, 23, 25, 31, 32, 33, 34, 35, 40, 42, 43, 44-45.	13	BC_3
Total	20	**BC_{2-5}**

Selection from families combining ALS and *Pythium* root rot resistance

Out of 31 F_2 families (Table 50) designed to combine ALS and *Pythium* root rot resistant in different seed background and evaluated at Kabale for root rot and at Kawanda for angular leaf spot resistance, 205 F_5 families were selected by bulk breeding method. The families generated are heterogeneous for agronomic traits and seed types. They will be distributed to national program partners for further selection.

Table 50. F_5 Families combining resistance for bean root rot and angular leaf spot, Kawanda, 2004.

Cross	F_5 Families selected
F_1 (CAL 96 x RW 719) x F_1 (CAL 96 x MEX 54)	17
F_1 (CAL 96 x RWR 719) x F_1 (CAL 96 x BAT 332)	38
F_1(CAL 96 x MLB-49-89A) x F_1(CAL 96 x MEX 54)	16
F_1(CAL 96 x SCAM 80- CM/15) x F_1 (CAL 96 x BAT 32	68
F_1(CAL 96 x MLB-49-89A) x F_1(CAL 96 x BAT 332)	50
F_1(CAL 96 x SCAM 80CM/15) x F_1CAL 96 x MEX 54)	16
Total	**205**

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Collaborators: S. Beebe and M. Blair.

2.1.7 Use of the RAPD marker OPE -04 to select for angular leaf spot resistance gene contributed by Mex 54

Rationale: Marker-assisted selection (MAS) is considered a useful biotechnology tool in speeding and improving effectiveness of breeding and in pyramiding desired genes into commercial backgrounds. Mex 54, a good source of resistance to most races of *Phaeoisariopsis griseola* found in Africa, has been extensively used in simple and complex crosses to transfer resistance into commercial but susceptible varieties. Last year we showed that a RAPD primer OPE-04 is associated and segregates with a single resistant dominant gene in Mex 54 (detected when using race 63-39 of *P. griseola*) and therefore offers potential for use in selecting resistant progenies. The objective of this study was to develop and adapt a protocol for evaluating and selecting resistant progenies.

Materials and Methods: Segregating populations (F_1, F_2 and F_5) derived from simple and double crosses and several lines and varieties were inoculated with race 63-39 of *P. griseola* and evaluated as described under section 2.1.2. DNA was extracted from leaves of the same populations and lines according to Doyle and Doyle (1990), with some modification and by the alkaline method (Warner *et al* 2001). DNA samples were amplified by RAPD technique according to Williams *et al.* (1990) with a decamer primer OPE-04 (GTGACATGCC) (Sigma Genosys). Each amplification reaction of 25µl contained 1X PCR buffer,3mM of $MgCl_2$, 0.2mM of each dNTP, 1µM of primer, 1 unit of *Taq* DNA polymerase, 10ng of DNA and 0.1 mg of BSA (Bovine serum albumin). The amplification conditions used are as follows: DNA denaturation (94^oC for 15 seconds), primer annealing (35^oC for 30 seconds) and extension by *Taq* DNA polymerase (72^oC for 1 min). After 40 cycles, samples are subjected to a final extension of 7 minutes at 72^oC and finally kept at 4^oC. Amplification products were separated on a 2% agarose gel containing 5µg/ml ethidium bromide.

Results and Discussions: A modification of the DNA extraction method by Doyle and Doyle (1990) yielded more polymorphism than the alkaline method (Warner *et. al*. 2001) and we therefore opted to use the former to evaluate progenies and lines for the presence or absence of the marker associated with the resistance gene in Mex54. Primer OPE-04 amplified a 700bp DNA fragment that was present in individuals scored as resistant based on virulence data and absent in susceptible individuals. Results of some of the progenies/lines are shown on Figure 28 and clearly demonstrate the possibility of using the method and primer to select for the resistance gene associated with the RAPD primer. Efforts are underway to design protocols that will facilitate the efficient use of this method and to enhance effectiveness of selecting for ALS resistance. Similarly, collaborating arrangements and procedures are being developed to enable bean network partners in different countries to make use of these tools at selected laboratories.

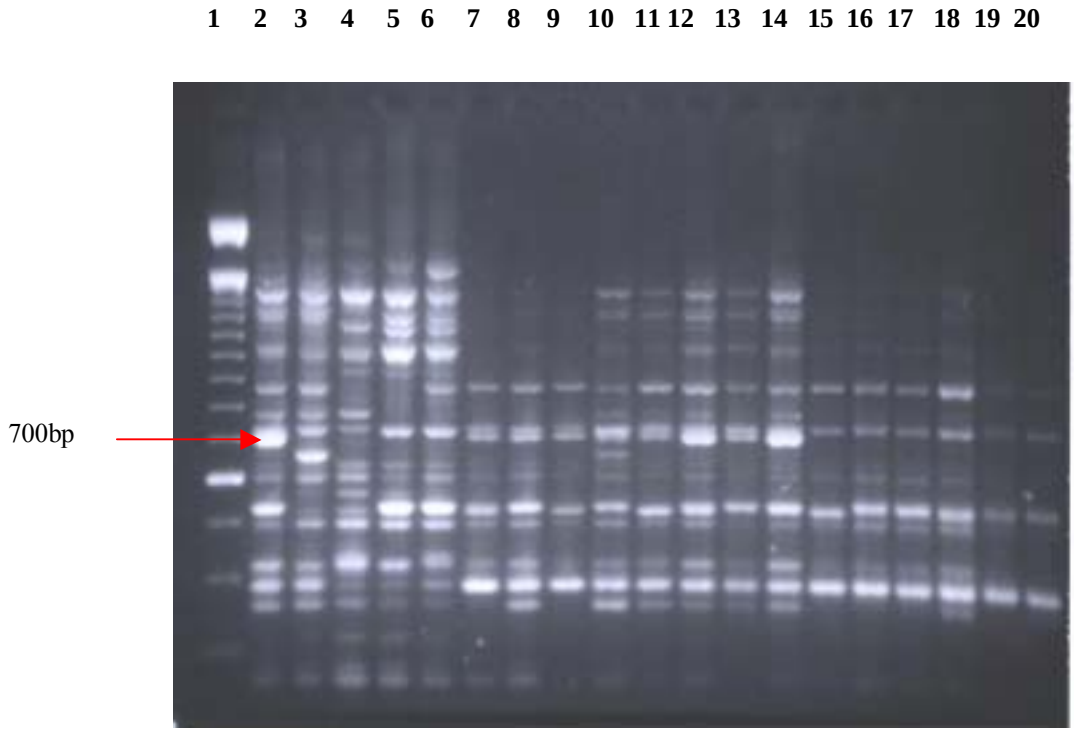

Figure 28. Identification of F_2 plants with a marker associated with ALS resistant gene in Mex 54 and detected by an OPE4, RAPD primer. Lane 1=100bp ladder, Lane 2 = Mex54; Lane 3 = GLP 585; Lane 4 = K132; Lane 5 = GLP2; Lane 6 = SCAM80-CM/15, Lane 7 - 14 = with the marker (resistant), Lane 15 - 19 = without marker (susceptible).

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2.1.8 Yield testing of BCMV resistant, heat tolerant Andean climbing beans developed with marker assisted selection and virus screening

Rationale: Climbing beans are grown in both intensive (trellised/staked monoculture) and extensive (inter-cropping with corn) farming systems. In either system the need to protect the crop from diseases is great, especially against seed borne or easily transmitted viral diseases such as bean common mosaic virus (BCMV or BCMNV). Bean common mosaic virus is found worldwide and is an aphid transmitted Potyvirus. A number of BCMNV resistance genes have been tagged including the *I* gene, *bc3, bc2* and *bc1*^{2}. The genes can be distinguished by inoculation with different viral isolates. BCMNV resistance is very important in Africa were necrotic strains are prevalent and has become a renewed priority for parts of Latin America where necrotic strains have been discovered. Very few climbing beans have been bred for resistance to bean common mosaic virus. Therefore it has been our goal to incorporate recessive resistance based on the *bc-3* gene into a series of climbing beans. One initial target of the breeding effort has been the MAC lines that we have recently developed and distributed to East Africa. These are mid-altitude climbing bean (MAC) lines, which are more heat tolerant, and higher yielding than many traditional climbing bean varieties and have great promise for the region. We have started this program with a series of backcrosses and simple crosses between MAC lines and sources of BCMV resistance. During the breeding program we used marker-assisted selection extensively based on the SCAR marker for *bc3* and combined this with virus screening to select resistant progeny. The objective of this report is to summarize yield testing of the most advanced climbing bean lines with BCMV resistance that are coming out of this breeding effort.

Materials and Methods: A total of 40 advanced $F_{5.7}$ lines were selected for yield testing. These represented the best selections from simple crosses between virus resistant bush bean parents and susceptible climbing bean parents which had been evaluated with marker-assisted selection using the SCAR marker for *bc3* resistance, ROC11, evaluated on the F_5 single plant selection and with virus inoculations performed on the $F_{5.6}$ progeny. MAS procedures and viral inoculations were as described in last year's annual report. Selections were also made based on agronomic performance, climbing bean architecture and large red mottled seed type. Parents involved in the crosses included a series of mid-altitude climbing (MAC) bean advanced lines (SEL1445, 1446, 1447, 1448 and 1449), as well as the local landrace Calima Voluble Darien (selected for its long grain and straight pods) crossed with three sources of *bc3* resistance, BRB29, BRB32 and BRB191. The trial was planted in Palmira in the 2003B season in a randomized complete block design with three repetitions. The genotypes were grown in 3m long single row plots with a trellis system. Agronomic management was as described previously in annual reports. Check varieties included three of the heat tolerant MAC lines (SEL1445, 1446 and 1449) as well as the heat susceptible genotype, Calima Voluble Darien, all of which had been used as parents.

Results and Discussion: BCMV resistance was predicted to occur in all of the advanced lines based on virus screening[1] (for those lines with BRB191 in their pedigrees) or based on marker assisted selection (for those lines with BRB29 or BRB32 in their pedigrees). Some lines still segregated for BCMV resistance as shown by either +/- signals for marker results or S/R, susceptible-resistant, segregation for virus inoculation results (Table 51). However, BCMV was prevalent in the border rows but not within the trial showing that the selections were indeed resistant to field pressure of the disease even in high temperatures that favor symptom development.

Heat tolerance was found in over half of the genotypes tested, as shown by their high yields (ranging from 1694 to 2746 kg ha^{-1}) which were comparable or surpassed the yields of the heat tolerant checks SEL1445, 1446 and 1449 (1371 to 2212 kg ha^{-1}) (Table 51). The heat susceptible genotype, Calima Voluble Darien, and the lines derived from this parent were the lowest yielding of the checks and of the lines, respectively. The two highest yielding lines, both from the cross BRB191 x SEL1447, produced 2746 and 2632 kg ha^{-1}, respectively, which were significantly higher than the yield for two of the heat tolerant checks, SEL1445 and SEL1449, based on LSD comparisons (P=0.05). Lines derived from crosses between BRB29 or BRB32 and SEL1448 or SEL1449 tended to perform less well than those between BRB191 and SEL1447 or SEL1445, indicating which may be the most favorable parents for further crosses. Days to flowering ranged from 31.5 to 46 while days to maturity ranged from 65.5 to 90 days, a range which surpassed that of the checks. This was as expected, since we selected both early maturing and late maturing climbing beans to see if there was an effect on yield due to growth cycle, which was not borne out by phenotypic correlations between days to flowering and productivity (r=-0.122 and –0.121, for plant and plot yields respectively) or days to maturity and productivity (r=0.058 and 0.032, respectively) all of which were not significant. Significant correlation was observed between days to flowering and days to maturity (r=0.877) and plant (g pl^{-1}) and plot (kg ha^{-1}) productivity (r=0.869).

Conclusions and Future Plans: We will begin to implement marker assisted selection for the I gene in these populations and compare BCMV resistance and yield capacity of the lines selected with this SCAR marker.

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[1] Viral inoculations using a necrotic strain of BCMNV to evaluate viral resistance or susceptibility reactions were evaluated after approximately 10 days, the plants were scored for necrotic hypersensitivity I gene resistance (N), susceptible mosaic symptoms (M) or immune resistance response indicating presence of the bc3 gene (0).

Table 51. Best yielding BCMNV resistant heat tolerant climbing bean F_7 advanced lines developed through marker assisted selection and progeny testing.

Entry no.	Pedigree	Cross no.	$F_{5.7}$ sel. no.	ROC11 SCAR	BCMV N	BCMV S	BCMV R	DF	DM	g/pl	Yield kg ha^{-1}
1	BRB 29 X SEL 1445	21614	5W	+	-	-	-	39.5	76	13.7	712.0
2	BRB 29 X SEL 1445	21614	8W	+	-	-	-	36	71.5	20.3	1699.5
3	BRB 29 X SEL 1445	21614	11W	+	-	-	-	36.5	73.5	21.3	1566.8
4	BRB 29 X SEL 1446	21615	5W	+	-	-	-	35.5	71.5	25.4	1853.6
5	BRB 29 X SEL 1449	21617	6W	+	-	-	-	39.5	79	21.2	1444.8
6	BRB 29 X SEL 1448	21618	4W	+	-	-	-	46	90	21.2	1697.1
7	BRB 29 X SEL 1448	21618	7W	+	-	-	-	38.5	74	14.9	1244.2
8	BRB 29 X CALIMA DAR	21620	3W	+	-	-	-	40.5	78	26.0	1224.3
9	BRB 29 X CALIMA DAR	21620	5W	+/-	-	-	-	37.5	77	13.1	997.2
10	BRB 29 X CALIMA DAR	21620	6W	+	-	-	-	38	74	24.0	1607.4
11	BRB 32 X SEL 1446	21622	11W	+	-	-	-	35	69	19.2	1463.1
12	BRB 32 X SEL 1446	21622	14W	+/-	-	-	-	37	71.5	27.4	1990.1
13	BRB 32 X SEL 1446	21622	17W	+/-	-	-	-	35	69.5	19.5	1481.3
14	BRB 32 X SEL 1449	21624	1W	+	-	-	-	32	65.5	18.2	1301.0
15	BRB 32 X SEL 1449	21624	4W	+/-	-	-	-	35.5	67	21.5	1740.7
16	BRB 32 X SEL 1449	21624	7W	+	-	-	-	39	73	24.0	1695.7
17	BRB 32 X SEL 1449	21624	10W	+	-	-	-	35	68	18.5	1519.4
18	BRB 32 X SEL 1449	21624	13W	+	-	-	-	39	74	24.7	1508.1
19	BRB 32 X SEL 1449	21624	16W	+/-	-	-	-	31.5	67	22.7	1636.7
20	BRB 32 X SEL 1449	21624	18W	+	-	-	-	35	68	20.3	1625.8
21	BRB 32 X SEL 1449	21624	19W	+/-	-	-	-	34	70	33.9	2131.0
22	BRB 32 X S 31465	21626	3W	+/-	-	-	-	38	71	23.4	1755.8
23	BRB 32 X CALIMA DAR	21627	4W	+	-	-	-	39	79.5	18.7	1036.4
24	BRB 32 X CALIMA DAR	21627	5W	+	-	-	-	38.5	75	14.7	1180.7
25	BRB 191 X SEL 1445	21628	2W	+/-	-	-	-	32.5	69	25.4	1878.9
26	BRB 191 X SEL 1445	21628	3W	NA	0	3	12	39	76	28.9	2195.9
27	BRB 191 X SEL 1445	21628	4W	NA	0	9	5	33.5	66.5	21.2	1645.6
28	BRB 191 X SEL 1445	21628	6W	NA	0	8	4	38.5	77.5	26.6	1533.4
29	BRB 191 X SEL 1445	21628	8W	NA	0	0	15	34.5	69	29.3	2129.8
30	BRB 191 X SEL 1445	21628	9W	NA	0	0	15	38.5	76.5	23.3	1560.5
31	BRB 191 X SEL 1445	21628	10W	NA	0	0	15	38	76.5	23.1	1758.7
32	BRB 191 X SEL 1445	21628	11W	NA	0	4	11	39.5	76	22.5	1709.4
33	BRB 191 X SEL 1449	21631	10W	NA	0	0	14	34.5	69.5	25.5	1526.3
34	BRB 191 X SEL 1447	21630	2W	NA	0	0	13	41.5	83.5	26.9	2157.8
35	BRB 191 X SEL 1447	21630	4W	NA	0	3	12	36	71	25.6	2131.2
36	BRB 191 X SEL 1447	21630	7W	NA	5	0	8	36.5	75.5	23.8	1999.1
37	BRB 191 X SEL 1447	21630	8W	NA	0	0	14	36.5	76.5	21.6	1694.7
38	BRB 191 X SEL 1447	21630	9W	NA	0	3	12	33	72.5	37.1	2175.2
39	BRB 191 X SEL 1447	21630	11W	NA	0	0	12	37.5	80	33.5	2631.9
40	BRB 191 X SEL 1447	21630	12W	NA	0	0	15	39	79.5	37.0	2746.5
41	SEL 1445	--	--	-	-	-	-	36.5	69	24.9	1918.8
42	CALIMA VOL DE DARIEN	--	--	-	-	-	-	39.5	77.5	12.2	1009.5
43	SEL 1446	--	--	-	-	-	-	38	75.5	28.7	2212.1
44	SEL 1449	--	--	-	-	-	-	37	72.5	19.1	1371.6
	LSD							4.7	4.1	27.3	752.8
	CV (%)							3.5	6.0	12.8	22.2

2.1.9 Adaptation and use of SCAR markers for marker assisted selection of two anthracnose resistance genes in Andean bean breeding

Rationale: Anthracnose, caused by the fungal pathogen *Colletotrichum lindemuthianum*, is a serious biotic constraint on common bean (*Phaseolus vulgaris*) in many areas of East Africa, and South America where Andean beans are grown. A set of differentials and mapping population have been used to identify over a dozen genes and QTLs affecting resistance/ susceptibility reactions of different races of the pathogen to different common bean genotypes. Co–evolution is known to have occurred between fungal pathotypes and the two common bean gene pools – such that Andean races attack Andean genotypes and Mesoamerican races attack Mesoamerican genotypes, while Andean genotypes resist Mesoamerican races and Mesoamerican genotypes resist Andean races. Therefore the best sources of resistance for breeding programs is often found in the complementary gene pool and genes for resistance must be introgressed through wide crosses and recurrent selection or backcrossing between Andean and Mesoamerican genotypes. In this case marker assisted selection (MAS) is a good option for rapidly breeding resistant varieties especially in the case of climbing beans which are longer season and more expensive to produce than bush beans. Our objective in this study was to introgress two important resistance genes against Andean races of anthracnose into Andean climbing bean breeding lines from their Mesoamerican sources. The principal source used for this work was G2333, a climbing bean landrace from Mexico that is known to have several target genes, namely *Co-4*^{2} and *Co-5* (Young et al., 1998).

Materials and Methods:

DNA extraction and Plant Material: Two extraction techniques were used: 1) Alkaline Extraction (a high-throughput "microprep", 96-well format method based on alkaline lysis of fresh leaf tissue disks) and 2) Miniprep (ammonium acetate based method using liquid N2 ground tissue from newly-emerging trifoliates). Both techniques have been described before. A total of 574 genotypes were extracted with the first method and 140 genotypes were extracted with the second method. The first group consisted in F_5 advanced lines from simple crosses and from backcross derived families that are part of the Andean bean breeding program, while the second group consisted of inbreeding lines and anthracnose differentials as controls. Alkaline extraction DNA after the neutralizing step was diluted 1:1, 1:5, 1:10, 1:20, 1:30 and 1:50 with sterile water to determine the optimum concentration for amplification. Resuspended miniprep DNA was diluted 1:10.

SCAR markers: A set of four SCAR markers were used in this study to target *Co-4*^{2} and *Co-5* resistance genes: SAS13 (Young et al., 1998), SH18 and SBB14 (Awale and Kelly, 2000) for *Co-4*^{2} and SAB3 (Vallejo and Kelly, 2000) for *Co-5*. PCR amplification amplification conditions were tested with a gradient cycler to find the annealing temperature that worked best with the diluted alkaline extraction DNA. PCR products were visualized on 30 well, 1.5% agarose gels run in 0.5X TBE buffer for 45 minutes at 220 volts, with up to two loadings per comb. Gels were photographed for scoring. The presence or absence of PCR products and the size of these were evaluated for each genotype to determine if the genotype was likely to contain the resistance allele or the susceptible allele.

Results and Discussion:

Genotyping: All four SCAR markers worked well with miniprep DNA diluted 1:10 and presented the expected size band (Table 52) in the correct source genotype (G2333) and other sources of the same gene or alternate allele of the same gene. In the case of SAS13 a band was amplified in the genotypes containing *Co-4*: G-2333, TO, PI, Widusa. This agrees with the results of Young et al. (1998) who found that the marker was not specific to G2333 and other *Co-4*[2] containing genotypes but also amplified a band in several genotypes that have the *Co-4* allele. Meanwhile in the case of the co-dominant marker SBB14 which was reported to only produce the expected band in G2333 and derived genotypes according to Awale and Kelly (2001) there was also amplification of the resistance-associated allele in the genotypes AB136, Cornell 47-292 and TO, in addition to G2333. The other specific marker from Awale and Kelly (2001), SH18, behaved as expected and amplified a band exclusively in G2333. The *Co-5* marker, SAB3 amplified in both G2333 and TU, as expected from the report of Vallejo and Kelly, (2000).

Table 52. Results of dilution series tests on SCAR marker amplification in the 12 Anthracnose differential genotypes.

Marker	Size bp	Gene	MP	Alkaline		
			Dil. 1:10	Dil. 1:5	Dil. 1:10	Dil. 1:20
SAB-3 (5.9cM)[2]	400	*Co-5* (dominant)	+	+ (Tu only)	+ (Tu, G2333)	+ (Tu, G2333)
SAS-13 (0cM)[3]	950	*Co-4, Co-4*[2] (dominant)	+	-	-	+ (G2333)
SBB-14 (5.89cM)[1]	1150-1050	*Co-4*[2] (co-dominant)	+	-	-	+/- (G2333,AB136)
SH-18 (4.2cM)[1]	1100	*Co-4*[2] (dominant)	+	-	-	-

Alkaline extraction DNA at 1:1 or 1:5 dilutions as template for PCR reactions, was not as reliable as miniprep DNA for SAS13, SBB14 or SH18 amplification but was for SAB3 amplification, which produced the expected bands for the genotypes G2333 and TU at this concentration (Table 52). Increasing the dilution to 1:20 was effective for the amplification of the correct band in G2333 by the markers SAS13 and SBB14 although band intensity was still low (Figure 29). The SAS13 marker which was reported by Young et al. (1998) to amplify in a number of genotypes containing *Co-4* and which did so when miniprep DNA was used, only produced a band for G2333. The SBB14 marker which was reported by Awale and Kelly (2001) to be co-dominant became a dominant marker when alkaline extraction DNA was used but still amplified the correct resistance-associated band in G2333 and AB136. Other dilutions were also tried, including 1:30 and 1:50, but only SAB-3 was observed to amplify the correct genotypes (G-2333 and TU) under all dilutions (data not shown). With alkaline extraction DNA, the most effective annealing temperature for SAS13 was 68ºC rather than the 72ºC as previously reported (Young et al., 1998).

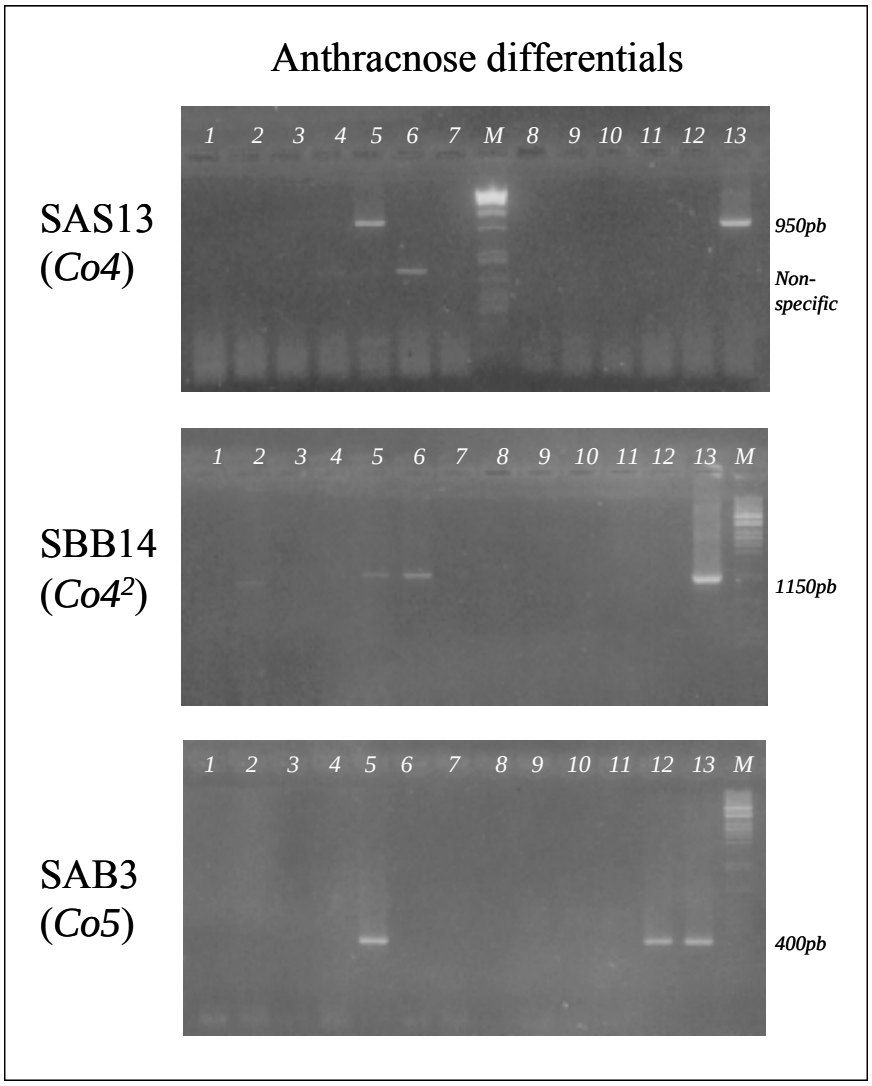

Figure 29. Amplification of anthracnose SCAR markers using alkaline extraction DNA for twelve differentials (lane 1. MDRK, 2. Perry Marrow; 3. Kaboon; 4. Michelite; 5. G-2333; 6. AB136; 7. Cornell 47-292; 8. Mexico 222; 9. PI207262; 10. Widusa; 11. TO; 12. TU) and one control (lane 13. 1:10 dilution of miniprep DNA for the source genotype, G2333).

Marker assisted selection: Of the 574 genotypes extracted a total of 132 had G2333 in their pedigree (the remaining individual DNAs were used for *bc3* marker assisted selection) and these were evaluated for whether they were positive or negative for the SAS13 (*Co-4*^{2}) or SAB3 (*Co-5*) markers (Table 53). Of this total, 41 genotypes were mid-altitude climbing bean selections in Palmira and 93 were mid-altitude/highland selections in Darién. Both groups of selections were made in the 2004A season. The SAB3 marker was run on the full set of genotypes while SAS13 was only run on the 41 genotypes from Palmira. A total of 21 selections were predicted to have the *Co-4*^{2} gene, while 35 were predicted to have the *Co-5* gene. The segregation ration was close to expected for the SAS13 marker (1:1) while the segregation of the SAB3 marker was skewed towards presence in the Palmira selection and towards absence in the Darién selections. This may indicate that SAS13 is in a region of the genome where the allele from G2333 has a neutral phenotypic effect on selection in both environments while SAB3 is in a region of the genome where the allele from G2333 has a positive effect in Palmira but a negative effect in Darién. This would need to be confirmed in larger populations as most of the crosses presented here produced from as little as 2 to at most 25 selections (average of 8.25 selections each). Interestingly mass selection to the $F_{5.6}$ generation was probably as effective as pedigree selection to the $F_{5.6}$ generation for selecting marker positive genotypes. Pedigree selection fixed the genes in an early generation and recombinants were fewer.

Table 53. Climbing bean advanced lines evaluated for the *Co-4*^{2} and *Co-5* SCAR markers in semester 2004A.

Pedigree	SAS13 Co-4^2		SAB3 Co-5		Total no. of lines
	+	-	+	-	
Palmira					
Pedigree Selection – $F_{2.3.5.6}$					
G2333 x SEL1447	0	2	0	2	2
G2333 x SEL1448	6	0	6	0	6
Mass Selection – $F_{5.6}$					
G2333 x BRB29	1	2	2	1	3
G2333 x BRB152	2	4	1	5	6
G2333 x BRB32	4	1	4	1	5
SEL1385 x G2333	1	1	2	0	2
G2333 x SEL1447	0	3	0	3	3
G2333 x BRB197	7	7	10	4	14
Subtotal	21	20	25	16	41
Darien					
BRB183 x (G12572 x G2333)	--	--	2	21	23
BRB183 x (G12621 x G2333)	--	--	1	24	25
(BRB29 x G 2333) x G23614	--	--	1	8	9
G2333 x Q'oscoporoto	--	--	1	5	6
Kori Inti x (Kori Inti x G2333)	--	--	5	10	15
(G12623 x BRB197) x (G12621 x G2333)	--	--	0	3	3
G20393 x G2333	--	--	0	9	9
Kori Inti x G2333	--	--	0	3	3
SubTotal			10	83	93
Total	**21**	**2**	**35**	**99**	**132**

The markers evaluated in this study were relatively easy to use since they are dominant and in coupling to the disease resistance gene. The marker was for the most part exclusive to the resistance gene source G2333 so that no false positives are expected. Marker assisted selection was beneficial because it allowed us to select for anthracnose resistance without using pathogen inoculation and in seasons where disease pressure would have been low due to environmental conditions. Marker assisted selection has other advantages over disease inoculation in allowing the harvest of clean seed and the evaluation for other characteristics which are masked by infection.

Future work: We plan to test the fidelity of the SCAR markers tested here by confirming resistance with inoculation of Andean races in Popayán. We also plan to make additional selections in another set of simple and triple cross populations and apply markers to earlier generation selection and/or gamete selection. We will attempt to increase the efficiency of the markers by testing multiplexing strategies both at the PCR and gel loading level. Furthermore, the single-resistance gene and multiple-resistance gene stocks created here will be distributed and used for further crosses.

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Vallejo, V., and J.D. Kelly. 2001. Development of a SCAR marker linked to Co-5 gene in common bean. Ann. Rept. Bean Improv. Coop. 44:121-122.

Young, R.A., M. Melotto, R.O., Nodari and J.D. Kelly. 1998. Marker assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar G2333. Theor. Appl. Genet. 96:87-94

Collaborators: MW Blair, LN Garzón, HF Buendia, (SB-2), R Chirwa (CIAT-Malawi), P. Kimani (CIAT– Kenya), G. Ligareto (Univ. Nacional – Bogota)

Progress towards achieving output milestones:

- Several stable and high yielding lines in various market classes (large red kidney, sugar, red mottled) showed to have multiple resistances to ALS and FLS at Chitedze and Bembeke in Malawi. A few others in large red kidney and sugar market types combined multiple disease resistance with tolerance to low soil fertility.

- Several advanced lines with resistance to 4 bean diseases were identified. These materials constitute an important set for use in breeding programs intended for multiple constraint improvement.

- The development of virus-resistant bean cultivars depends on the identification of sources of resistance. Bean Virology has been identifying suitable parental materials that possess resistance to the different begomoviruses that affect common bean in Latin America and elsewhere.

- Advanced lines and progenies resistant to *Pythium* root rot and others combining resistance to angular leaf spot and *Pythium* root rot were identified. They will be distributed to partners for multi-ecology evaluations and selection.

- High yielding mid-altitude climbers with *bc-3* resistance to BCMNV have been obtained.

- Markers for anthracnose resistance genes that are great utility for improving Andean beans have been adapted for use in MAS.

Activity 2.2 Developing germplasm resistant to insects

Highlights:

- Marker assisted selection of Arcelin-derived bruchid resistance was applied to advanced red mottled breeding lines and showed promise for substituting the serology-based assays presently in use for selection. These lines will be useful for Africa, the Andes and the Caribbean where *Zabrotes* weevils are prevalent in storage and cause severe losses.

- Tolerance to *Empoasca kraemerii* was confirmed in Andean bred lines.

2.2.1 Microsatellite-based marker assisted selection of Arcelin-derived bruchid resistance in Andean genotypes of common bean

Background: The Arcelin resistance gene is the most effective resistance factor for the most common storage pests of common bean, namely the Mexican bean weevil, *Zabrotes subfasciatus* (Boheman). We have tested a series of microsatellite markers that are linked to the Arcelin resistance gene and found two to be very effective at distinguishing resistant (of which there are seven variants) and susceptible alleles. Last year we tested these two markers using both miniprep and alkaline extraction derived DNA and found that miniprep DNA worked best and that Pv-ATCT001 (M68913) was the most closely linked marker. This year we improved the amplification conditions for alkaline extraction derived DNA so as to test for marker assisted selection in the field with a total of 261 genotypes segregating for Arc 1 resistance allele. The long-term objective of this work is to increase the efficiency of breeding for multiple constraint resistance and facilitate the pyramiding of bruchid resistance with other biotic and abiotic stress resistances. The conversion of the protein based selection of arcelin to a usable DNA marker obviates the need for arcelin-specific antibodies and protein electrophoresis and streamlines arcelin selection with the widespread use of other SCAR markers that we have also embarked on in our breeding program. In addition to its compatibility with other types of DNA based markers, the advantages of using the microsatellite over the time-consuming protein based selection was that it was amenable to high-throughput, rapid analysis.

Methodology:

Genetic materials and DNA extraction: A total of 261 advanced lines from two families of red mottled bush beans from the Andean bean breeding program were planted in Darién in the 2004A season. DNA was extracted by alkaline lysis from leaf disks harvested into 96 well plates that were packed on ice and processed at the CIAT marker lab. The alkaline extraction technique is a rapid, high-throughput "microprep" method based on alkaline lysis. The resulting DNA was diluted by 1:10 in sterile water before being used in PCR reactions.

Microsatellite markers: The microsatellite marker Pv-ATCT001 (M68913) was used for the marker assisted selection scheme. Microsatellite amplifications were conducted according to standard PCR protocols. Microsatellite amplifications were run at 1800 volts, 120 Watts and temperature of 45^{o}C for one to two hours on 4% polyacrylamide gels and silver-stained with a re-circulating tank system. Alleles were identified as reported in the 2002 annual report for the parental materials used in multiple crosses to generate the advanced lines tested this year.

Results and Discussion: The microsatellite Pv-ATCT001 (M68913) presented only two alleles in susceptible materials. This was an advantage for multiplexing. In addition, the marker presented a unique allele for the parent that provided Arcelin 1 (RAZ44) so was used as a diagnostic test for this widely used allele of the Arcelin gene. This marker produced single amplification products where the resistant allele of Arcelin 1 was associated with the 190 bp band while all susceptible alleles were associated with the 195 and/or 200 bp bands. The amplification of Pv-ATCT001 with alkaline extracted DNA (Figure 30) was comparable to the previous amplifications with miniprep DNA. As marker assisted screening proceeded, improvements were made in the experimental technique resulting in successful amplification of over 96% of the genotypes using Pv-ATCT001 (only 10 null alleles were registered among the 261 tested genotypes and these were not scored).

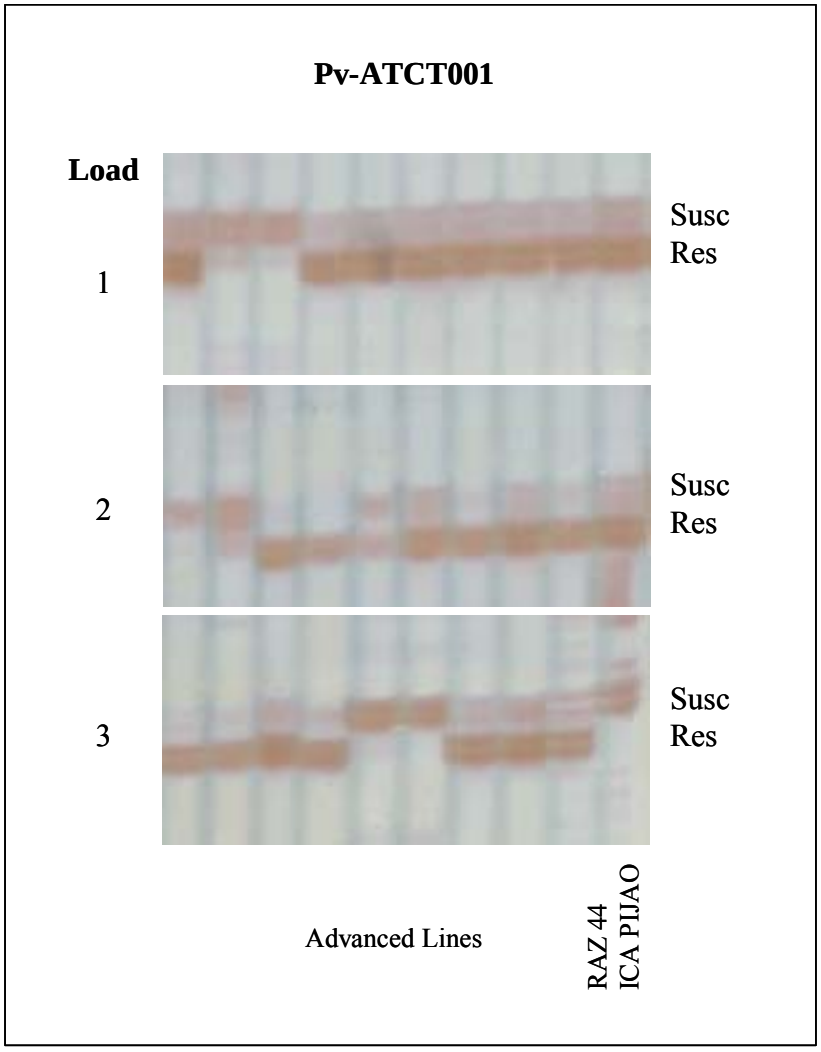

Figure 30. Examples of the marker assisted selection for the Arcelin gene in Andean red mottled bush bean lines. Control genotypes were RAZ44 (Arc1 positive) and ICA Pijao (Arc1 negative).

Marker assisted selection proved useful for screening the advanced lines derived from single plant selection in the F5 generation. Of the overall total of 261 advanced lines screened from the two cross combinations, a total of 58 positives were selected with 161 negatives and 32 heterozygotes (Table 54). In the case of the first cross A36 x (A36 x ((RAZ44 x ROYAL RED) x (CATRACHITA x WILK2))), the segregation ratio fit the expected 3:1 negative:positive ratio for the test crosses between the negative recurrent parent A36 and the positive Arcelin1 containing heterozygotes that had been selected by the Bean Entomology project in the F1 generation using the protein assay. These results show that the two assays can be combined effectively in a breeding program and the strength of the molecular assay in screening a large number of advanced lines.

Table 54. Results of microsatellite screening for the Arcelin resistance gene in two families of Andean red mottled bush bean lines.

Crosses	Positive	Negative	n. a.	Het	Overall
A36 x (A36 x ((RAZ44 x ROYAL RED) x (CATRACHITA x WILK2)))	42	161	10	32	245
A483 x ((MAR1 x RAZ50) x (PVA9576-34-1 x G 17340))	16	0	0	0	16
Grand Total	58	161	10	32	261

While it appeared that all the lines derived from the cross A483 x ((MAR1 x RAZ50) x (PVA9576-34-1 x G 17340)) were fixed, a substantial number of the lines derived from the cross A36 x (A36 x ((RAZ44 x ROYAL RED) x (CATRACHITA x WILK2))) were scored as heterozygous. The larger than expected number of heterozygotes may be a misinterpretation of a faint amplification product for the upper band (Figure 30). We will confirm whether these genotypes continue to segregate or whether the marker assay is sensitive to DNA quality or mixtures.

Future work:

- Improve the efficiency of the screening technique, adapting additional arcelin linked microsatellites to the alkaline extraction technique.
- Determine the level of linkage disequilibrium between the markers and the arcelin locus in breeding populations.
- Use the markers to select for greater recombination around the arcelin locus and break the linkage drag associated with this locus that has a negative affect on plant vigor of arcelin-derived lines.

Collaborators: M.W Blair, H.F. Buendia (SB-2) C. Cardona (IP-1)

2.2.2 Developing Andean lines tolerant to Empoasca

For details of breeding activities, please refer to section 2.2.1. As in 2003, studies were aimed at developing Andean type bean with improved tolerance to the leafhopper, *Empoasca kraemeri.* We will highlight results of the work trying to develop Andean type beans (crosses with PVA 773 and CAL 143) with improved tolerance to the leafhopper, *Empoasca kraemeri.* Lines selected for lower damage scores and higher reproductive adaptation scores in previous years performed relatively well under moderate levels of leafhopper infestation (4.3 nymphs per leaf, seasonal average) (Figure 31). Given that susceptibility to leafhopper is usually very high in large-seeded Andean beans, these results indicate that substantial progress has been made in incorporating resistance to leafhopper in these types of beans. Another set of lines derived from crosses between Saladin and selected EMP lines did not perform so well (Figure 32), possibly due to the inherent susceptibility of Pompadour-type beans. Nevertheless, eight lines that showed moderate tolerance were selected for further testing.

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

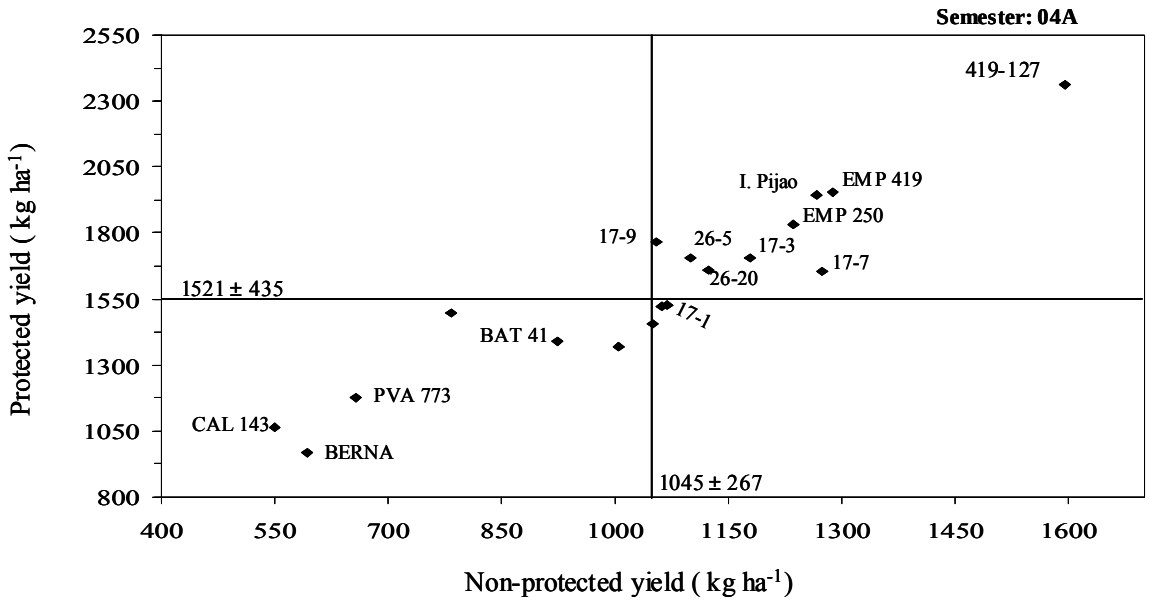

Figura 31. The relationship between protected and non-protected yield in selected Andean bean lines bred for tolerance to *Empoasca kraemeri*. PVA 773 and CAL 143 are susceptible parents. EMP 250 is the tolerant parent. BAT 41 and Pijao are susceptible and tolerant checks, respectively

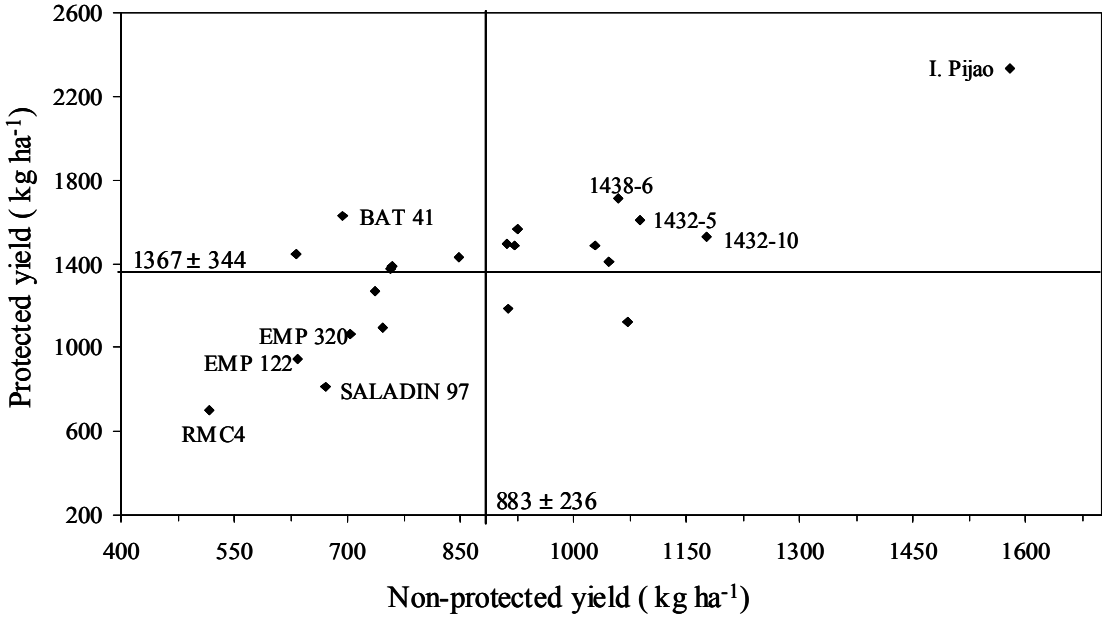

Figure 32. The relationship between protected and non-protected yield in selected Andean bean lines bred for tolerance to *Empoasca kraemeri*. Saladin 97 is a commercial variety in Dominican Republic.

Progress towards achieving output milestones:

- MAS for arcelin will permit integrating selection for bruchid resistance into other selection protocols for disease resistance genes, obviating the need for either antibody-based selection or protein analysis.

- Substantial progress has been made in incorporating resistance to leafhopper in Andean type beans.

Activity 2.3 Incorporating wider genetic diversity into beans

Highlights:

- Selections were made for low phosphorus tolerance in red and cream mottled Andean bush beans. Additionally, Colombian released varieties were compared for low phosphorus tolerance and yield index under low vs. high phosphorus conditions.

2.3.1 Improving Andean beans for tolerance to drought

Rationale: Andean beans are often planted in relatively more favorable environments compared to Mesoamerican beans, but in any case farmers are subject to variability in rainfall. Regions where Andean beans are preferred such as southern Africa are afflicted by frequent droughts. In previous years CIAT executed a project to improve Andean common bean for Iran, and the national program requested crosses for drought tolerance. In this report we present yield data of advanced lines derived from those crosses.

Materials and Methods: A small number of Andean genotypes (ICA Quimbaya, AND 125) had presented a relative degree of drought tolerance in previous years and were chosen as parents. Additionally, Durango type beans of the Mesoamerican gene pool (Pinto Villa, flor de junio Victoria) were identified for crossing. These have medium size seed and a type 3 growth habit that is similar to that used in Iran. These were crossed to Iranian cultivars including Talash, Khomein and CIAT line COS 16 which has *I* gene resistance to BCMV. Double crosses were selected over several years and lines were created from individual plant selections in F_3. Sixteen advanced lines were tested under the same conditions as described for the Mesoamerican germplasm in Output 1, activity 1.1.1.1. Checks in this trial included DOR 390, SEA 5, SEA 15, and ICA Quimbaya which is considered to be the best Andean genotype to date under drought stress in CIAT conditions.

Results and Discussion: The three best lines were all derived from the same cross, (Pinto Villa x Quimbaya) x (Talash x COS 16). Although the best line produced 22% more than Quimbaya, no line outyielded the Andean check Quimbaya by a statistically significant margin (Table 55). However, given the lack of genetic variability in Andean types, these lines should be studied further to determine if indeed they have an advantage. Several are segregating and may have genetic variability within families that can still be exploited.

Conclusion: Efforts to improving drought tolerance in Andean types have been very modest and progress slow. However, the present families may have a narrow advantage over available sources and will be purified for future evaluation.

Table 55. Andean lines with cream striped, cream or pink grain color derived from crosses for tolerance to drought.

Cross / Line	Yield as % checks[1]	Yield check
(Pinto Villa x Quimbaya) x (Talash x COS 16)		
SX 14337-MC-1C-MC	122	1057
SX 14337-MC-19C-MC	121	1057
SX 14337-MC-9C-MC	112	1057
(Flor de junio Victoria x CAL 125) x (Local de Khomein x COS 16)		
SX 14331-MC-20C-MC	111	1057
SX 14331-MC-22C-MC	103	1057
SX 14331-MC-35C-MC	102	1057

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

Contributors: S. Beebe, H. Terán, and M.A. Grajales

2.3.2 Inheritance of seed darkening / non-oxidizing seed coat trait in Andean bayo beans

Rationale: Seed coat darkening is thought to be due to oxidation of polyphenolics in the seed coat of common bean and affects consumer preference in common bean varieties especially those with cream-colored backgrounds, including both Mesoamerican types such as Pintos and Cariocas as well as Andean types such as Sugars and Cranberries (Cream Mottled classes). Oxidation is especially important in Bayo beans, found both among the Mesoamerican and Andean gene pools, and produced predominantly in Mexico and Peru. Among other Andeans, yellow beans especially Canarios, seem less affected by the problem, while in red beans darkening is masked by the already intense coloration of the grain. The seed coat of white beans does not generally darken. The objective of this study was to study the inheritance of seed coat darkening in a cross of two Peruvian Bayo varieties that were identified by INIA to differ in their rate of seed coat darkening.

Materials and Methods: The Peruvian cream seeded variety 'Bayo Mochica' (BM) with non-oxidizing seed coat character, was crossed with a similar Peruvian genotype, 'Bayo Florida' (BF) which does oxidize. Crosses were made in reciprocal directions. F_2 seed was harvested from individual F_1 plants to ensure that the cross was effective. F_2 plants were harvested individually and advanced by single seed descent until the $F_{4.5}$ generation. A total of 116 recombinant inbred lines (RILs) were developed from the BM x BF cross and 128 RILs from the reciprocal cross of BF x BM. Seed Darkening was evaluated by storing the $F_{3.4}$ seed for three months at room temperature and comparing to freshly harvested seed of the $F_{4.5}$ generation. A 1 to 5 color scale was used where 1 = non-oxidized, cream colored seed coat and 5 = very oxidized, brownish seed coat.

Results and Discussion: The inheritance of seed darkening appears to be quantitative as shown by the population distributions for BM x BF and BF x BM (Figure 33). However, the possibility of a major gene influencing the trait is suggested by the more binomial distribution of the BM x BF population. There were few overall differences between the reciprocal populations suggesting that maternal inheritance is not important for this trait.

Conclusions and Future Plans: We plan to use the recombinant inbred lines in QTL analysis of the trait if funding is available. This will also await the results of a parental survey to identify the level of genetic polymorphism between the parents of the cross. Once the inheritance of seed darkening is known in this cross it may be interesting to apply the results to other cream-colored seed classes.

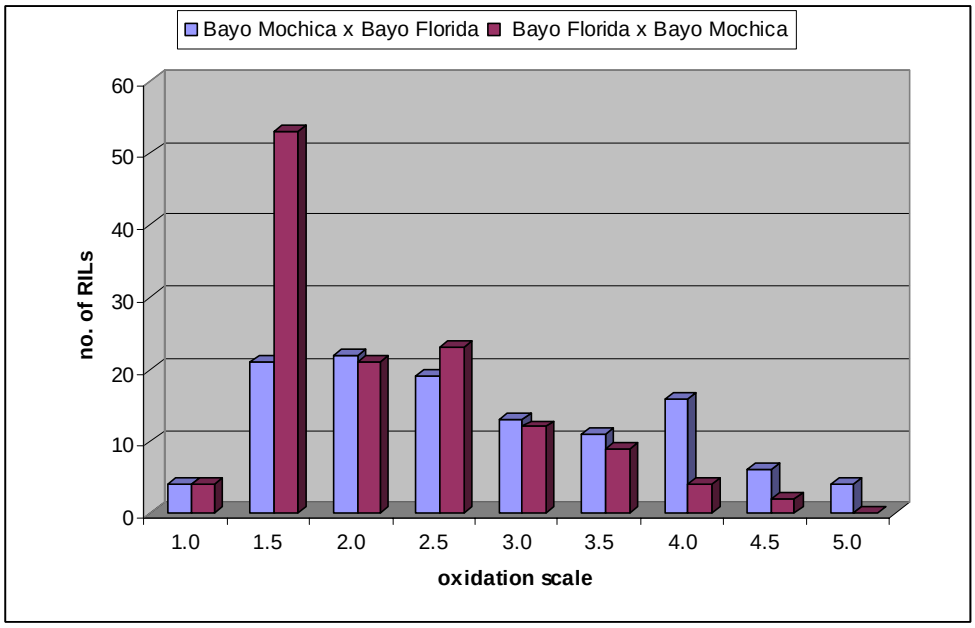

Figure 33. Recombinant inbred line (RIL) population distributions for seed darkening in two crosses of Peruvian Bayo beans.

Collaborators: M.W. Blair, A. Hincapie (IP-1), A. Valladolid (PROMPEX-Promenestras-Peru),

2.3.3 Yield testing of low phosphorus tolerant Andean bush beans

Rationale: Eighty-two percent of the soils in Latin America are deficient in phosphorus. Phosphorus deficiency is widespread in East Africa as well, possibly affecting over 50% of soils there. Studies at CIAT have identified several sources of tolerance to low phosphorus conditions. These genotypes can take up or use phosphorus more efficiently. Good tolerance to P-deficient environments has been observed in a liborino type, yellow-and-red seeded landrace from Peru, G19833, that is a parent of the CIAT mapping population (Blair et al., 2003). The mechanism of phosphorus deficiency tolerance in this genotype is thought to be better phosphorus use efficieincy through root hair exudation and other potential mechanisms (Liao et al., 2004). In the breeding programs at CIAT, one of our long-term objectives is to increase the capacity of new breeding lines to grow under low P conditions. In this research we analyzed advanced lines that were selected from crosses between G19833 and BCMV resistance sources from the BRB series.

Materials and Methods: A large number of advanced lines were developed from crosses between G19833 and four BRB lines (BRB156, BRB183, BRB211 and BRB217). Two trials were planted in Darien (Valle) during the rainy season in semester 2003B under low phosphorus (fertilization of 50 kg ha^{-1} TSP (7.5 kg P_2O_5)) conditions, one for red mottled selection and one for cream mottled selections, with totals of 112 and 93 genotypes, respectively. Each trial consisted in a randomized complete block with three replicates. Double row plots were 3 m long and were separated by a constant susceptible check genotype, DOR390, spaced between every plot. Additional controls within the trials were the breeding line checks BRB156, BRB183, BRB211, BRB217 and CAL149 for the red mottled trial and BRB211, BRB217 and SUG47 for the cream mottled trial. The genotypes, G4017 (low P tolerant Mesoamerican), G14665 (low P tolerant Andean) and G19833 (Parent 1) were also included as checks in each experiment.

Results and Discussion: Table 56 shows the top 20 lines selected for each experiment. The range in production was from 575 kg ha^{-1} and 645 kg ha^{-1} to close to 1000 kg ha^{-1} in the case of both experiments. The highest yielding line was 1036.7 kg ha^{-1} in the case of the cream mottled lines and 941.7 kg ha^{-1} in the case of the red mottled lines, significantly outyielding all the Andean checks including the parent G19833 in both experiments. Among the checks, the Mesoamerican genotype, G4017 was the most tolerant to the low P conditions, yielding an average of 1265 kg ha^{-1} across the two experiments. In the cream mottled lines SUG47 was the second highest yielding check, while CAL149 was the second highest yielding check among the red mottled lines. G19833 was the third highest yielding among the checks in the cream mottled lines but ranked lower among the checks of the red mottled line experiment. The average yield across both experiments for G19833 was 440 kg ha^{-1}.

These results indicate that breeding for low P tolerance in Andean beans is possible. Surprising results can occur with some evidence for transgressive segregation in the breeding process as shown by the higher yield of the progeny compared to the parents under low P conditions.

Table 56. Yield testing of low phosphorus tolerant Andean bush beans in Darién in semester 2003B.

Cream mottled					Red Mottled				
Entry no.	**pedigree**	**DF**	**g pl^{-1}**	**yield**	**Entry no.**	**pedigree**	**DF**	**g pl^{-1}**	**yield**
2	G19833 X BRB211	43.5	4.5	1036.7	12	G19833 X BRB211	43.5	5.5	941.7
78	G19833 X BRB156	41.0	4.3	946.3	94	G19833 X BRB156	38.5	4.7	905.0
9	G19833 X BRB211	43.5	5.6	822.7	78	G19833 X BRB183	44.5	4.8	814.0
13	G19833 X BRB211	41.5	4.1	813.7	18	G19833 X BRB211	44.0	3.7	795.0
93	G19833 X BRB183	43.5	4.4	811.3	4	G19833 X BRB211	40.0	3.9	780.3
11	G19833 X BRB211	44.5	5.3	803.7	3	G19833 X BRB211	40.5	3.4	714.0
58	G19833 X BRB217	41.5	3.7	767.0	106	G19833 X BRB156	37.0	4.1	705.0
4	G19833 X BRB211	38.5	3.9	759.7	77	G19833 X BRB183	44.0	4.0	669.7
60	G19833 X BRB217	42.0	4.3	731.0	17	G19833 X BRB211	44.5	4.5	668.0
39	G19833 X BRB217	39.5	3.1	720.3	8	G19833 X BRB211	41.5	5.3	663.3
3	G19833 X BRB211	41.0	3.5	715.0	10	G19833 X BRB211	43.5	3.1	660.3
61	G19833 X BRB217	42.5	3.1	693.3	101	G19833 X BRB156	38.5	3.9	653.0
23	G19833 X BRB211	45.0	3.9	691.3	75	G19833 X BRB183	44.0	3.0	616.0
81	G19833 X BRB156	41.5	3.1	689.0	1	G19833 X BRB211	40.0	3.3	598.3
1	G19833 X BRB211	41.5	3.7	680.0	112	G19833 X BRB156	44.5	2.9	593.0
17	G19833 X BRB211	41.0	3.3	669.7	9	G19833 X BRB211	43.5	3.3	583.7
24	G19833 X BRB211	43.0	2.9	665.0	15	G19833 X BRB211	44.0	2.7	577.7
15	G19833 X BRB211	39.5	3.9	660.7	16	G19833 X BRB211	44.0	2.6	576.3
62	G19833 X BRB183	44.5	4.1	654.7	30	G19833 X BRB217	37.5	2.9	576.3
71	G19833 X BRB183	41.5	3.8	645.0	90	G19833 X BRB156	41.0	2.9	575.0
97	G4017	45.0	8.3	1216.0	120	G4017	44.5	7.4	1314.3
99	SUG47	36.0	3.7	757.0	119	CAL149	44.0	6.0	1058.3
98	G19833	45.5	2.5	466.7	118	G14665	41.5	3.1	582.3
95	BRB211	38.0	2.5	394.7	116	BRB183	44.0	3.2	455.0
96	BRB217	36.0	2.1	351.3	117	G19833	44.5	2.4	412.7
100	G14665	42.0	1.9	339.7	115	BRB217	36.0	1.6	268.7
94	BRB156	34.5	1.4	316.7	114	BRB211	38.0	1.7	233.7
					113	BRB156	37.0	1.0	145.3
	LSD	3.7	3.2	316.9		LSD	2.3	1.8	350.7
	CV	4.5	55.6	32.4		CV	2.9	40.9	44.5

Future Plans: The lines developed in this project will be tested for anthracnose resistance and adaptation under low P soils in Popayán before being prepared for nurseries. The lines would be of interest in Eastern and Southern Africa given the high market value seed types: large red mottleds and sugars.

Collaborators: MW Blair, A. Hoyos

2.3.4 Yield testing of a collection of Colombian varieties under low and high phosphorus treatments

Background: Bean breeding in Colombia has produced a wide range of large-seeded Andean varieties for many different agroecological zones. The country is home to a wide range of traditional farmer varieties and landraces. The objective of this research was to test a representative number of improved and traditional Colombian varieties under low and high phosphorus treatments to get a more precise idea of their range of adaptation. This study was also used as a test case for biomass evaluations by kite aerial photography in collaboration with the Land Use/GIS unit.

Materials and Methods: A total of 40 bush bean genotypes were tested in a replicated yield trial in Darien in semesters 2003B and 2004A under two fertilization treatments: low phosphorus 50 kg ha^{-1} TSP (10 kg P) and high phosphorus 350 kg ha^{-1} TSP (70 kg P). Native soil P is 2 to 10 mg kg^{-1}. Experimental design consisted in three replications in randomized complete block with plots separated by DOR390 as check rows. Data is presented only from the 2004A semester trial since the 2003B semester trial was lost due to root rots that are a secondary problem of low phosphorus sites during very wet growing periods. During 2004A, Darién was a favorable site for all the genotypes given its location at 1500 masl and average temperature of 19°C and annual rainfall of 1,200 mm (500 during the cropping season). The genotypes included 22 large-seeded red mottled (Calima) or red (Radical) varieties released by ICA or CORPOICA over the past 30 years (DIACOL and ICA series), 11 landraces from Berruecos, Darién, Sevilla and Tenerife (Valle) as well as Ocaña and Zaragosa (N. Santander) and seven CIAT lines as controls (A36, AFR188, AFR612, AFR619, AFR735, AND279 and CAL96).

Results and Discussion:

The range in yields for the varieties went from a low of 309 kg ha^{-1} to a high of 1781 kg ha^{-1} in the low phosphorus treatment and from a low of 580 kg ha^{-1} to a high of 3164 kg ha^{-1} in the high phosphorus treatment (Table 57). The same variety was the lowest yielding in these two environments, namely the type I genotype DIACOL Nutibara, a variety poorly adapted given its origin in colder climates. The highest yielding variety in the high phosphorus treatment was the type II breeding line AFR619, while the highest yielding variety in the low phosphorus treatment was the type III landrace Palisero (Berruecos) which was also the second highest yielding in high phosphorus. As a result Palisero had a relatively high lowP/high P yield index (59.9%) which was similar to several of the landrace genotypes, including Blanquillo (73.8%), Morado Moteado (62.1%), Sangretoro (64.3% and 72.0%). The average index for the landraces (55.7%) was higher than for the improved released varieties (50.8%) showing that landraces tend to be better for low fertility conditions and pointing out a deficiency of some of the released varieties, except for ICA Tundama, a cold climate variety with a high yield index (72.0%) and reasonable yields under low P (1032 kg ha^{-1}) and high P (1433 kg ha^{-1}).

Table 57. Yield trial of Colombian released varieties and landraces in Darién, Valle in semester 2004A.

Entry no.		Low P				High P				LP/HP index
	Ident	DF	DM	g.pl	yield	DF	DM	g.pl	yield	
1	ICA Guali	36.0	81.3	3.6	774.8	36.3	79.7	6.1	1377.6	56.24
2	DIACOL Calima	38.3	78.7	2.2	515.9	37.0	81.3	5.2	1204.6	42.83
3	ICA Palmar. INIA 17	42.0	83.7	3.0	666.2	43.3	86.7	7.3	1502.3	44.34
4	DIACOL Nutibara	39.0	75.7	1.4	309.3	39.7	71.7	2.4	580.1	53.32
5	ICA Duva	37.3	84.3	3.5	802.5	37.3	82.0	8.5	1913.3	41.94
6	Sangretoro	42.3	84.7	3.7	885.2	39.7	79.0	6.6	1377.5	64.26
7	DIACOL Nima	41.7	86.7	3.6	759.0	40.3	85.0	8.3	1694.3	44.80
8	ICA Tone	38.3	80.3	2.4	450.0	38.7	77.3	4.4	943.8	47.68
9	ICA Cuna	39.7	82.0	2.4	585.3	38.3	82.3	5.5	1201.3	48.73
10	ICA Tundama	48.0	90.0	5.0	1032.4	48.0	90.0	9.1	1433.2	72.03
11	DIACOL Catio	42.7	85.7	2.6	592.9	40.3	85.3	7.9	1480.4	40.05
12	Frijolica P-1.1	43.0	87.0	3.5	849.0	41.3	79.3	3.9	852.4	99.60
13	AFR 619	45.7	92.3	5.5	1315.1	42.0	85.3	16.0	3164.4	41.56
14	ICA L-59 ARS-59	42.7	87.3	4.5	966.1	40.0	88.7	8.5	1821.5	53.04
15	ICA Bachue	35.7	82.0	3.1	675.4	38.0	80.7	5.6	1220.3	55.35
16	ICA Cerinza	42.7	84.7	2.2	560.6	39.3	78.0	6.2	1244.9	45.03
17	Radical Cerinza	36.7	83.7	3.6	816.2	37.3	84.0	8.2	1805.4	45.21
18	ICA Citara	35.7	79.3	3.3	798.1	36.3	82.7	6.8	1524.8	52.34
19	AND 279	40.3	86.0	5.7	1080.6	39.7	84.0	10.2	1741.9	62.04
20	ICA L-66	37.0	77.3	2.1	421.0	37.0	81.3	6.8	1529.1	27.53
21	ICA Caucaya	44.0	87.3	4.6	988.3	41.3	82.0	8.6	1720.5	57.44
22	ICA Cafetero	41.7	84.0	2.4	525.0	40.3	83.0	6.0	1365.7	38.44
23	ICA Quimbaya	38.0	84.0	2.5	577.7	37.0	83.3	5.9	1395.2	41.40
24	Chocho (Tenerife)	39.7	78.7	2.7	572.1	38.0	74.3	5.4	1211.6	47.22
25	ICA Guanenta	37.3	76.7	3.0	706.6	37.3	80.0	5.7	1280.3	55.19
26	Radical Froylan	39.7	85.0	2.7	633.7	38.5	88.0	8.6	1901.1	33.33
27	Cargabello (Darién)	41.0	80.0	2.7	663.6	40.0	80.7	6.4	1405.1	47.22
28	Rosado Zaragoza	35.7	75.0	2.7	558.5	36.0	74.3	3.9	923.1	60.50
29	Rosado Ocana	35.3	75.0	1.5	370.9	35.3	76.0	3.8	867.3	42.77
30	Radical (Restrepo)	40.3	85.3	5.0	1072.8	39.7	83.7	8.1	1927.0	55.67
31	Morado (Restrepo)	38.3	82.0	4.5	1082.3	37.3	82.3	7.2	1741.8	62.14
32	Sangretoro (Berruecos)	45.7	92.7	7.4	1658.4	43.0	90.3	11.2	2303.1	72.01
33	Palisero (Berruecos)	45.3	93.0	7.7	1781.6	44.7	92.3	13.8	2976.9	59.85
34	Blanquillo (Berruecos)	49.0	90.0	6.0	1466.2	48.3	90.0	10.8	1986.1	73.82
35	Mina (Berruecos)	38.3	83.3	3.4	773.5	37.5	80.0	12.3	2816.6	27.46
36	CAL 96	39.3	80.7	3.2	706.5	37.3	80.7	7.2	1490.1	47.41
37	AFR 188	40.3	91.3	6.1	1422.0	38.3	85.0	7.8	1660.4	85.64
38	AFR 735	37.7	82.7	5.3	1277.8	37.0	86.7	12.6	2302.5	55.50
39	A 36	47.0	95.0	4.1	850.0	46.0	90.0	9.4	2001.1	42.48
40	AFR 612	43.7	89.3	4.7	1147.0	41.3	90.0	13.8	2545.9	45.05
LSD		2.2	2.8	2.0	413.4	2.3	2.1	2.6	546.8	
CV		3.3	2.1	29.1	29.9	3.5	5.6	20.2	20.6	

The more recently-developed breeding lines tended to do better than the released varieties and had an average yield index of 54.2%. Two notable breeding lines in this respect were AFR188 (yield index of 85.6%) and AND279 (62.4%). As mentioned earlier, AFR619 was an interesting genotype due to its high responsiveness to P fertilization in the high P treatment but its yield index was relatively low (41.6%). Overall, the average yield of the breeding lines (2129.5 kg ha^{-1}) exceeded that of the landraces (1776.0 kg ha^{-1}) and the improved varieties (1446.3 kg ha^{-1}) under high P treatment. The same pattern was seen for breeding lines (1114.1 kg ha^{-1}), landraces (989.5 kg ha^{-1}) and released varieties (712.7 kg ha^{-1}) under low P treatment. The differences between groups may be due to the fact that the landraces included long season, small seeded higher yielding indeterminate genotypes while the released varieties included some with more specifc agro-ecological adaptation, especially those from colder climates. This was evident when comparing the days to flowering and maturity differences between the two groups (Table 57). Meanwhile the breeding lines included genotypes that were locally selected at the experimental site which might explain their greater adaptation. All together, the results are interesting in pointing out the yield stability of traditional landraces, the site-specificity of some of the released varieties and the improvements made in low P adaptation in breeding lines when compared to released varieties. Darién was a good environment to test the genotypes because most genotypes were well adapted there and the native low P soils were uniform and easy to manage for both the low P and high P treatments. Many of the landraces adapted quite well even though they were from very different environments originally. This might reflect a wider stability of some landraces over some of the improved germplasm. Apart from yield, the effect of low P treatment was notable in delaying flowering and days to maturity by an average of 1.8 and 2.2 days respectively overall and in some cases by as much as 3.7 days for flowering date and 7.7 days for maturity date. Interestingly this occurred most notably in some but not all of the varieties with better performance under low P stress (eg. AFR188, AFR619 and Sangretoro) suggesting that delayed phenology may be a mechanism for low P stress tolerance in Andean beans, although significant correlations were observed between many of the traits.

Conclusions and Future Work: We plan to test the same genotypes in Popayán for adaptation to biotic and abiotic stresses. Collaborators at CORPOICA have planted the same genotype in Rionegro, Antioquia so we will compared data from that location. We have also analyzed the nutritional quality in terms of micronutrients possessed by these varieties when grown in Palmira, Darién, as well as by FIDAR in Nariño as part of our biofortification work. In collaboration with the land use project of CIAT we have used this experiment for low altitude photographic comparisons of biomass accumulation, data for which will analyze in the coming months. In collaboration with CORPOICA, FIDAR and GIS we would like to make a compendium of information about these varieties that could be useful for producers, consumers and plant breeders.

Collaborators: MW Blair, A. Hoyos (IP-1), T. Oberthur, V Gonzales and H.Usma (Land Use Project).

Progress towards achieving output milestones:

- Initial studies on abiotic stress resistance in Andean types suggests that significant genetic variability exists that should be exploited, and that recent bred selections may have a slight advantage over earlier released varieties.

Activity 2.4 Developing more nutritious large seeded bean varieties

Highlights:

- The high iron NUA (Andean nutrition) breeding lines developed last year are being tested in on-farm trials with an NGO in Nariño and Valle departments, Colombia. Mineral analysis has confirmed the high iron status of four sister lines that are all well-adapted red mottled derivatives of CAL96. Low iron sister lines have also been identified and are also well adapted. Notably, one of the high iron sister lines, NUA56, has average iron content of 93 mg kg^{-1} across three sites and up to 112 mg kg^{-1} in one site, one of the highest seed iron values found to date. Additional Andean red seeded high iron varieties have been identified. From this analysis we have identified potential genotypes to include in nutrition nurseries and hybridizations.
- At CIAT, we are analyzing genotype x environment interaction in trials with recombinant inbred lines and finding good correlations across sites for iron content.
- Mineral analysis is also being used to identify the highest iron content in Cerinza-derived red and red mottled advanced breeding lines (BIF series) and among all Colombian released varieties.
- A crossing block for nutritional improvement of bush and climbing beans from Africa and Latin America has generated a total of 120 combinations using high iron landraces and the breeding lines being developed above.
- QTL mapping of nutritional traits has become an important way to increase our understanding of how to breed common bean for better mineral content as part of the HarvestPlus Challenge Program. We are using a mix of traditional breeding, biochemistry, genetics and genomics to dissect nutritional quality traits.
- We are also studying the inheritance of soluble and insoluble tannins, which are implicated as anti-nutrients in mineral absorption. We have conducted a QTL analysis of five segregating populations of common bean. We have also analyzed a population for the seed darkening/non-oxidizing seed coat trait.
- Collaboration between CIAT and USDA-Houston is centering on a basic mechanism for mineral uptake in legumes using common bean as a model for the tropical legumes, namely iron reductase. Initital results suggest a relationship between a QTL for seed iron and one for iron reductase.

2.4.1 Multi-location testing of high iron NUA (Andean-nutrition) lines in Nariño

Introduction: Iron deficiency anemia and other micronutrient deficiencies affect large number of people worldwide and in Colombia. 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 that these traits are likely

to be inherited quantitatively. We have started a breeding program to introgress the high iron trait into new red mottled breeding lines using the high iron climbing bean genotype G14519. A single backcross to the recurrent parents CAL 96 and CAL 143 was used to develop BC_1F_1 families that were selected amongst and within to develop BC_1F_3 and BC_1F_4 derived breeding lines that were high in seed iron accumulation. In this study we tested the best of these new NUA (Andean nutrition) lines in three locations in southern Colombia in on farm trials in collaboration with farmers and a local non-governmental organization.

Materials and Methods: A total of seven advanced NUA breeding lines selected for high and low iron content were tested at three sites in the Department of Nariño during the 2003B season. The high iron selections were NUA 30, NUA 35, NUA 45 and NUA 56, while the low iron selections were NUA 4, NUA 43 and NUA 50. The check varieties included four large, red mottled genotypes: AFR612, CAL96, CAL143 and Diacol Calima; and four large, red-seeded genotypes: Chocho, Quimbaya, Sangretoro and Radical Cerinza. The total number of genotypes in the experiment was 15. Three replicated trials were conducted in the municipalities of Consacá, Sandoná and Yacuanquer all at elevations between 1500 and 2000 masl, where the genotypes were evaluated for growth habit, resistance to diseases (anthracnose, ascochyta, web blight, rust and powdery mildew were prevalent) and consumer acceptability during the 2003B season. All experiments consisted in randomized complete block designs with three replicates each. Seed mineral content was evaluated at CIAT by grinding 4 g of grain from each separately harvested replicate plot into a fine powder using a modified Retsch mill with aluminum chambers and grinding balls and analyzing the resulting powder with a wet digestion method and atomic absorption spectrophotometry in the Analytical Services Laboratory of CIAT for both iron and zinc concentration measured in mg kg^{-1}.

Results and Discussion: In the analysis of variance, significant differences were observed between locations and varieties for iron, however for zinc content variety effects were significant but location effects were not (Table 58). In addition, variety x location effects were less significant for iron ($P<0.05$) than for zinc ($P<0.0001$). This may have to do with soil status for zinc as reflected in Table 59 for Consacá which were lower than those for Yacuanquer and Sandoná (data not shown). Replication x Location effects were not significant for iron but were significant for zinc, therefore field replication was still useful especially for the more variable factor, zinc. Coefficients of variation were low for the complete experiment (5.6% for iron and 4.5% for zinc).

Table 58. Analysis of variance for iron and zinc concentration in 15 varieties tested over three locations (Consacá, Sandoná and Yacuanquer) in the department of Nariño, Colombia in 2003B.

Source	DF	SS	MS	F	P
Iron Analysis					
LOC	2	4663.5	2331.77	132.04	0.0000
TRAT	14	9035.6	645.40	36.55	0.0000
LOC*REP	3	19.3	6.45	0.36	0.7786
LOC*TRAT	28	1060.1	37.86	2.14	0.0123
Error	42	741.7	17.66		
Total	89	15520.3			
Zinc Analysis					
LOC	2	1.89	0.9467	0.46	0.6327
TRAT	14	1354.65	96.7604	47.30	0.0000
LOC*REP	3	31.67	10.5576	5.16	0.0040
LOC*TRAT	28	340.39	12.1569	5.94	0.0000
Error	42	85.92	2.0458		
Total	89	1814.53			

Table 59. Analysis of soils from Consacá in the department of Nariño, Colombia in 2003B.

Sample No.	Site	N-Total (mg/kg)	P-BrayII (mg/kg)	K (cmol/kg)	B (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	pH (Un)
S-151-1	Consacá	4614.76	8.77	1.15	0.96	6.12	2.17	5.26
2		3003.72	4.13	0.53	0.82	7.01	1.95	5.49
3		2731.86	2.48	0.28	0.41	12.17	1.51	5.38
4		2779.25	4.93	1.08	0.79	11.65	1.86	5.51

For the variety contrasts, the highest iron lines were the advanced breeding lines from CIAT in all three environments and on average (Table 60). NUA 56, NUA 45 and NUA 35 were notable in having average iron contents close to 90 mg kg^{-1} and one location, Yacuanquer presented iron contents above 100 mg kg^{-1}. These values surpassed those of all of the agronomic check varieties including Quimbaya and Chocho, two red seeded genotypes that were relatively high in iron in the previous experiment (Section I). Zinc concentrations were correlated with iron concentration. Notably, Quimbaya was at the higher end of the range of zinc content. Among the three locations, average iron content was higher in Yacuanquer (85 mg kg^{-1}) than Sandona (71 mg kg^{-1}) or Consaca (69 mg kg^{-1}). In contrast average zinc content was similar in all three environments (average 32 mg kg^{-1}).

Table 60. Mean seed iron and zinc concentration in mg kg^{-1} of 15 bean varieties among and within three locations in Nariño (Consaca, Sandoná and Yacuanquer) in 2003B.

YACUANQUER			
	Iron		Zinc
NUA 56	112	NUA 56	43
NUA 45	102	AFR 612	41
NUA 35	102	Quimbaya	38
Chocho	91	Chocho	36
Quimbaya	90	NUA 45	35
Radical Cerinza	90	Radical Cerinza	34
AFR 612	86	NUA 35	33
NUA 50	85	NUA 30	32
NUA 4	80	NUA 50	27
NUA 30	79	CAL 143	27
CAL 143	77	Diacol Calima	27
CAL 96	75	Sangretoro	27
Diacol Calima	75	NUA 43	26
Sangretoro	67	CAL 96	24
NUA 43	65	NUA 4	23

SANDONA			
	Iron		Zinc
NUA 35	83	NUA 56	36
NUA 56	82	Radical Cerinza	36
NUA 45	79	Quimbaya	35
NUA 30	77	NUA 45	35
Chocho	74	Chocho	35
Quimbaya	74	NUA 35	33
Radical Cerinza	74	NUA 30	33
CAL 143	73	AFR 612	32
AFR 612	70	Diacol Calima	32
NUA 50	69	Sangretoro	29
NUA 4	69	CAL 143	29
CAL 96	65	NUA 4	29
Diacol Calima	65	NUA 43	28
NUA 43	53	NUA 50	28
Sangretoro	52	CAL 96	27

CONSACA			
	Iron		Zinc
NUA 56	86	Quimbaya	38
NUA 45	84	Radical Cerinza	36
NUA 35	81	NUA 45	36
Quimbaya	79	NUA 56	35
NUA 4	73	Chocho	33
Radical Cerinza	70	NUA 35	32
NUA 30	68	AFR 612	32
AFR 612	67	Diacol Calima	32
Diacol Calima	66	NUA 30	32
CAL 143	66	NUA 4	31
NUA 50	66	NUA 50	29
CAL 96	65	Sangretoro	29
Chocho	64	CAL 96	28
NUA 43	53	CAL 143	27
Sangretoro	51	NUA 43	9

MEAN			
	Iron		Zinc
NUA 56	93	NUA 56	38
NUA 35	89	Quimbaya	37
NUA 45	88	NUA 45	36
Quimbaya	81	Radical Cerinza	35
Radical Cerinza	77	AFR 612	35
Chocho	76	Chocho	34
Sangretoro	75	NUA 35	33
NUA 30	75	NUA 30	32
AFR 612	75	Diacol Calima	30
NUA 4	74	Sangretoro	28
NUA 50	73	NUA 50	28
CAL 143	72	NUA 4	28
Diacol Calima	69	NUA 43	27
CAL 96	69	CAL 143	27
NUA 43	57	CAL 96	27

Future Plans and Conclusions: The genotypes described here will be analyzed for mineral content by ICP (Inductive coupling plasma) analysis to validate the results obtained with the wet digestion method. We will be comparing these genotypes to other advanced lines from the Andean breeding as they are produced and have introduced them as parents into crosses with other Andean genotypes with favorable agronomic characteristics.

Collaborators: M.W. Blair, C. Astudillo, S. Beebe (IP1 Project, CIAT), J. Restrepo, P. Ojeda, LC Bravo (FIDAR), O. Mosquera (CIAT – Analytical Services)

2.4.2 Genotype x Environment studies of mineral accumulation in advanced lines and released varieties from Colombia

Introduction: We are continuing to evaluate advanced lines and released varieties for their mineral content. One group of genotypes derived from Cerinza are of special interest given that this Colombian variety has favorable commercial characteristics and average iron content between 75 and 80 mg kg^{-1}. Given variability between sites detected in previous studies we are replicating these trials at two to three locations each. To date a total of two trials were prepared:

1) A total of 64 advanced lines (from the BIF series) were selected from an advanced backcross CAL 96 x (RAD-CERINZA x (G 24390 x RAD-CERINZA))) breeding population with a high iron parent (Radical Cerinza) in the hope of obtaining a higher iron segregant with red or red mottled seed type derived from the Andean ALS-resistant line CAL96. Of these 26 were large-seeded red lines and 37 were large seeded red mottled lines. The full set of genotypes was tested for agronomic adaptation in Nariño during the 2004A growing season. The trial was prepared for two locations as a randomized complete block design with 3 repetitions each and 4 row plots.

2) A total of 39 Colombian varieties were prepared as a nursery for one site in Nariño (Consacá) and for one site in Valle (Darién). The experiments were designed as randomized complete block design with three replicates of four-row plots for each genotype. These genotypes represent all the bush bean varieties released by ICA and CORPOICA over the past thirty years along with standard check varieties and a few standard landraces. The majority of the varieties are red mottled 'Calima' or 'Nima' classes followed by large reds 'Radicales' or 'Duva' types.

Methodology: Atomic absorption mass spectrophotometry was practiced to obtain information on mineral content. The lines from trial I had average amounts of iron and zinc (Figure 34) except for two lines which surpassed 70 mg kg^{-1} (no. 8, a red seeded line and no. 61, a red mottled line). The iron and zinc content of the trial II genotypes is being evaluated.

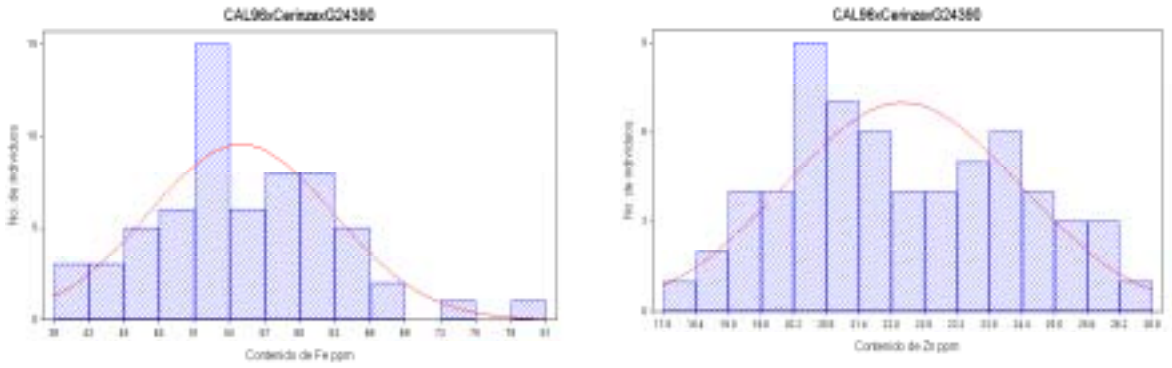

Figure 34. Distribution of iron and zinc content in advanced BIF lines derived from advanced backcross breeding with the moderate iron parent, Radical Cerinza.

Collaborators: M.W. Blair, A. Hoyos, C. Astudillo (IP-1), O. Mosquera (Analytical Services), J. Restrepo, D. Villada, P. Ojeda, LC Bravo (FIDAR).

2.4.3 Hybridizations of Andean genotypes for high micronutrient content

Introduction: As part of the HarvestPlus Challenge program we are developing new populations of Andean bush and climbing beans using three proven sources of high iron and high zinc genes: G14159 (Mesoamerican genotype from the USA with brown-colored high protein seed and average iron content of over 80 mg kg^{-1}); G21242 (Andean genotype from Colombia with cream mottled seed and average iron content close to 90 mg kg^{-1}); G23824E (Andean genotype from Peru with average iron content of 100 mg kg^{-1}).

Methodology: A total of 84 and 36 simple crosses were made for bush and climbing beans, respectively, using the 3 high iron landraces (G14519, G21242 and G23823E), 2 high iron backcross inbred lines (BID29 and BID115) as well as 13 selections from large-red high iron populations (SEL1457 to 1469) that were crossed to 12 Andean bush bean (AFR298, AFR612, CAL143, CAL144, CAL96, Canadian Wonder, Dore Kirundo, Kablanketi, PVA773, Radical Cerinza, Radical San Gil and SUG131) and 6 Andean climbing bean (AND497, Bolivar, CAB19, Caballero, Cargamanto and ICA Viboral) varieties from Africa and Latin America as recurrent parents (Table 61).

Table 61. Simple crosses made in the 2004A season for nutritional quality in Andean beans.

1) Bush beans

AFR298 x G23823E	Dore Kirundo x G23823E	BID 29 x CAL144
AFR298 x BID 29	G23823E x Kablanketi	BID 29 x G23823E
AFR298 x BID115	Kablanketi x G23823E	BID 29 x Kablanketi
AFR612 x G23823E	PVA733 x BID 29	BID 29 x PVA773
AFR612 x BID 29	PVA773 x G23823E	BID 29 x SUG131
AFR612 x BID115	Radical Cerinza x G23823E	BID115 x AFR298
CAL143 x G23823E	Radical Cerinza x BID115	BID115 x Canadian Wonder
CAL144 x G23823E	Radical San Gil x G23823E	BID115 x G23823E
CAL144 x BID 29	Radical San Gil x BID115	BID115 x Kablanketi
CAL96 x G23823E	SUG131 x G23823E	BID115 x PVA773
Canadian Wonder x G23823E	SUG131 x BID115	BID115 x Radical San Gil
Canadian Wonder x BID115	BID 29 x AFR298	BID115 x SUG131

2) Climbing beans

AFR298 x G14519	G14519 x SEL1461	G23823E x G14519
AFR298 x G21242	G14519 x SEL1462	G23823E x ICA Viboral
AFR612 x G14519	G14519 x SEL1463	G23823E x SEL1457
AFR612 x G21242	G14519 x SEL1464	G23823E x SEL1458
AND497 x G14519	G14519 x SEL1469	G23823E x SEL1460
AND497 x G23823E	G14519 x SEL1467	G23823E x surco 6517
Bolivar x G14519	G14519 x SEL1468	G23823E x SEL1462
CAB19 x G23823E	G21078 x G23823E	G23823E x SEL1464
CAL144 x G14519	G2124 x SEL1463	G23823E x SEL1469
CAL144 x G21242	G21242 x AND497	G23823E x SEL1465
Canadian Wonder x G14519	G21242 x Bolivar	G23823E x SEL1466
Dore Kirundo x G21242	G21242 x CAB19	G23823E x SEL1467
G12242 x SEL1458	G21242 x Caballero	G23823E x SEL1468

Table 61. cont'd

G14519 x AND497	G21242 x G23823E	G23823E x SEL1461
G14519 x CAB19	G21242 x SEL1457	G4825 x G23823E
G14519 x Caballero	G21242 x SEL1458	ICA Viboral x G21242
G14519 x Canadian Wonder	G21242 x SEL1460	Kablanketi x G14519
G14519 x Cargamanto	G21242 x SEL1461	Kablanketi x G21242
G14519 x Dore Kirundo	G21242 x SEL1462	PVA773 x G14519
G14519 x ICA Viboral	G21242 x SEL1464	PVA773 x G21242
G14519 x Kablanketi	G21242 x SEL1465	Radical Cerinza x G14519
G14519 x SUG131	G21242 x SEL1466	Radical Cerinza x G21242
G14519 x SUG31	G21242 x SEL1466	Radical San Gil x G14519
G14519 x BID 29	G21242 x SEL1467	Radical San Gil x G21242
G14519 x BID115	G21242 x SEL1468	SUG131 x G14519
G14519 x SEL1457	G23823E x Bolivar	SUG131 x G21242
G14519 x SEL1458	G23823E x Caballero	BID 29 x G2124
G14519 x SEL1460	G23823E x Cargamanto	SEL1463 x G23823E

Of these, several are released varieties in their respective countries: CAL144 in Bolivia; AFR298 (ICA Quimbaya), AFR612 (unofficial release – widely adopted), Cargamanto, ICA Viboral, Radical Cerinza and Radical San Gil in Colombia; Dore Kirundo in Congo; SUG131 and CAL143 in Malawi; Caballero in Peru; AND497 and CAB19 in Rwanda; Canadian Wonder and Kablanketi in Tanzania; and CAL96 in Uganda (K132). Three standard types for improvement among the red mottled bush bean are CAL96, CAL143 and PVA773 which has been released in Bolivia (Rojo Oriental), Colombia (as ICA Caucaya), Ecuador (INIAP Yunguilla) and Mozambique.

Results and Discussion: F_1 seed has been obtained and selection of populations is pending.

Future Work:

All three high iron genotypes are indeterminate climbing or semi-climbing beans therefore for those crosses with bush bean parents we are planning to backcross the F1 plants so as to be assured of good bush bean architecture in the segregants. For the climbing bean crosses we plan to make selections within simple crosses and backcrosses. We will also plan to make a range of double and triple cross populations for the climbing beans. For bush beans, we plan to involve a larger number of Andean genotypes from Eastern and Southern Africa as well as from Latin America.

Collaborators: MW Blair, A. Hincapie (SB-2), P. Kimani, R. Chirwa, S. Beebe (IP-1)

2.4.4 Analysis of iron reductase as a mechanism for enhanced iron uptake and mineral seed content in common beans: QTL analysis and candidate gene cloning

Background: Nutritional genomics is being used as part of the HarvestPlus Challenge Program to discover the basic mechanisms for mineral uptake and accumulation. As part of this program CIAT is collaborating with the Grusak lab at the USDA-Baylor College of Medicine to determine the genes in common beans that determine iron uptake and utilization. As part of the overall genomics approach, information from other well studied species such *Medicago truncatula*, peas and soybeans, as well as other model species such as *Arabidopsis thaliana* that have extensive genetic and molecular resources are being used for gene discovery and functional analysis. The underlying concepts of this work are to take advantage of metabolic unity among plants to characterize gene function and to apply bioinformatics and molecular cloning approaches to identify potential orthologous genes. As a first example of this approach the Grusak lab is trying to dissect the importance of iron reductase in the accumulation of iron in beans by assaying iron reductase activity in roots and by cloning an ortholog of the gene from common bean based on similarity to the same gene already isolated from *Medicago truncatula* and from *Pisum sativum*. Iron reductase is a member of the protein superfamily of flavocytochromes and functions to convert iron from an unavailable form (ferric, Fe^{3+}) to an available form (ferrous, Fe^{2+}) that can be readily absorbed by plants. The iron reductase protein (FRO) is located in roots and straddles the root cell membrane where it is active for iron reduction. The gene for iron reductase was first isolated from Arabidopsis and Pisum (Pea). Both species are fairly efficient at extracting iron from the soil and serve as models for enzyme activity. Other candidate genes include a family of zinc transporters.

Methodology:

Plant Material: The experiments have been carried out on two populations of recombinant inbred lines that are being used to genetically map seed micronutrient content QTL: DOR364 x G19833 and G21242 x G21078. The first population is from an Andean x Mesoamerican inter-gene pool cross and the second is from an Andean x Andean intra-gene pool cross.

Mineral Analysis: Seed mineral accumulation data has been obtained for both populations using atomic absorption (AA) spectrophotometry at CIAT and/or inductive coupling plasma (ICP) analysis at the University of Adelaide in Australia. Samples consisted in ground whole bean seeds that were processed in a Retsch Mill in the case of the AA samples and in a coffee grinder in the case of the ICP samples.

Library screening: the Grusak lab selected conserved primers for RT-PCR based on the Pisum iron reductase gene (PsFRO1) and used them to amplify common bean reductase candidates which were then used to screen filters from a leaf cDNA library made in the CUGI-CIAT collaboration.

Reductase Assay: In the Grusak lab, seeds are germinated for 3-4 days then planted in a hydroponic system for 12 days of growth in various levels of iron concentration (eg. 2, 5 , 10 and 20 uM Fe). Iron reductase assay is conducted at the end of this period by removing the entire root systems of four plants and staining for reduced iron measured as umol Fe reduced g FW^{-1} hr^{-1}.

Results and Discussion:

Phenotyping: The distribution of iron content in seeds of the DOR364 x G19833 population RILS is shown in Figure 35. Iron content ranged from 39 to 84 mg kg^{-1} and zinc content ranged from 17 to 37 mg kg^{-1}. Correlations between iron and zinc were significant (r=0.612). Results of the G21242 x G21078 screening are described separately in this annual report.

Library Screening: The Grusak lab has been screening a common bean leaf cDNA library which we made at CIAT and has found partial sequences with homology to iron reductase using the Medicago reductase clone and sequences with similarity to Aquaporins using the Zinc transporter family. They will begin next year to screen a set of two leaf cDNA libraries, where the mRNAs for iron reductase and zinc transporters are more likely to be expressed.

Reductase assays: The iron reductase assay is producing interesting differences between the parents of both mapping populations for their ability to reduce iron at two different iron regimes. These differences are evident more at low Fe concentration than at high iron concentration and seem to be somewhat correlated with the seed iron status of the parents.

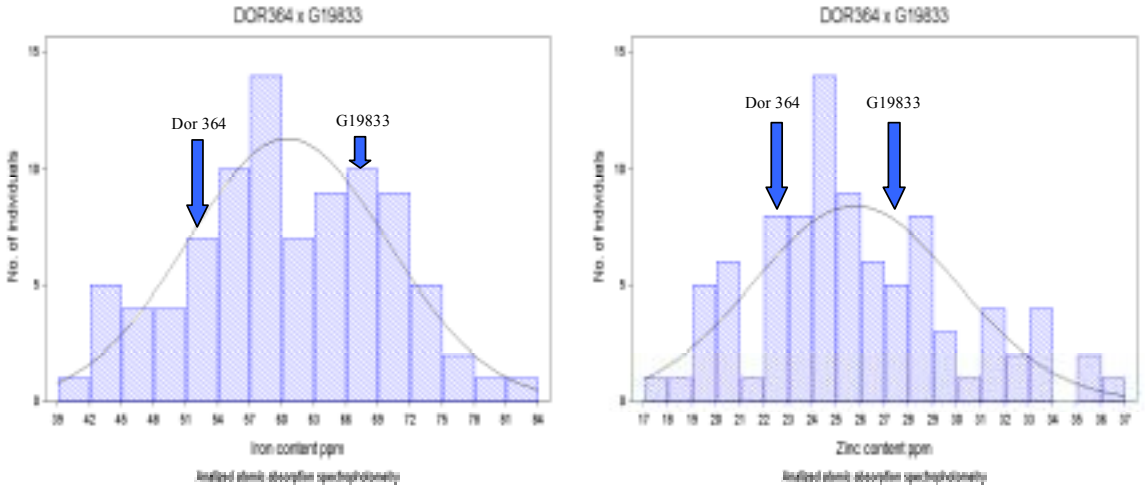

Figure 35. Distribution of seed iron and zinc content among RILs of the DOR364 x G19833 population as determined by atomic absorption spectrophotometry.

The bimodal nature of the population distribution for DOR364 x G19833 recombinant inbred lines (Figure 36) suggests that inheritance of iron reductase activity may be due to one or a few genes. QTL analysis confirms that there is one major QTL under iron sufficiency and this QTL is different from the one under iron deficiency. Therefore it can be postulated that iron reductase is represented by several differentially expressed genes, whereby: one iron reductase gene is expressed in iron deficiency (1 uM) and is found on Chromosome b02; another iron reductase gene is expressed in iron sufficiency (15 uM) and is found on Chromosome b11.

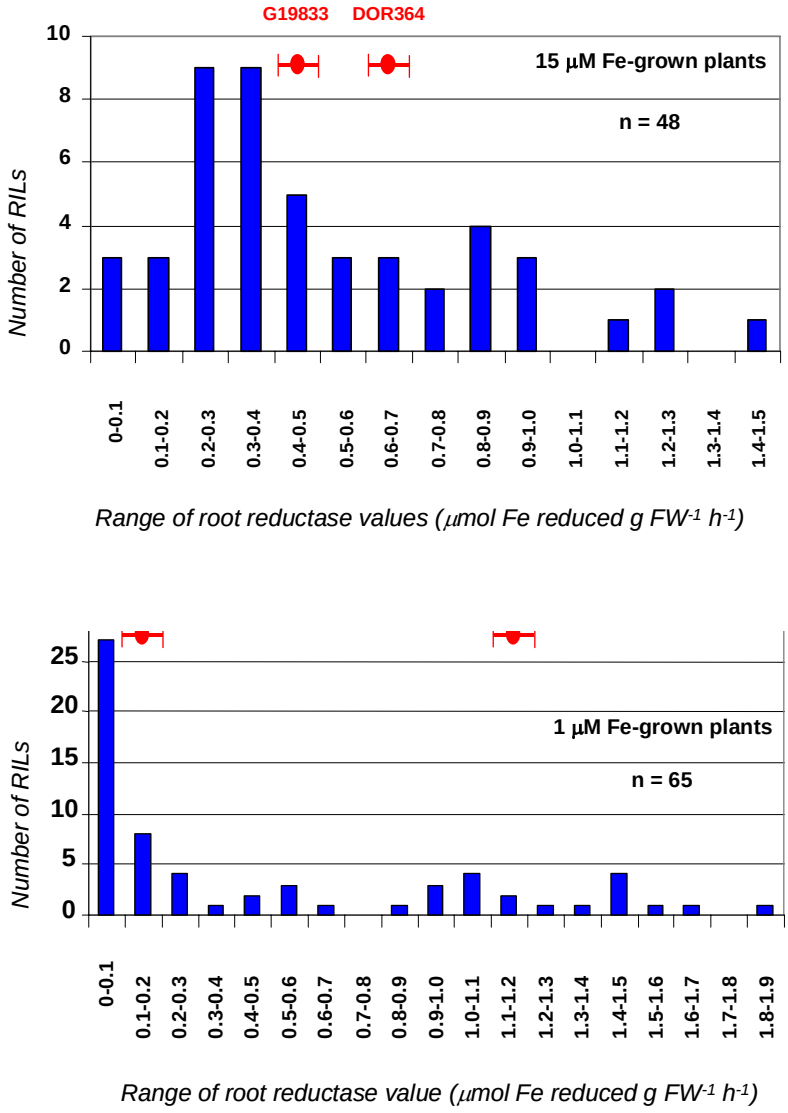

Figure 36. Distribution of iron reductase values among RILs of the DOR364 x G19833 population.

To understand the relationship of iron reductase to seed iron accumulation we analyzed the ICP results that we had obtained on seed of the recombinant inbred lines in a preliminary QTL analysis and identified two QTLs controlling seed iron accumulation in this population – one very important QTL on Chromosome b11 and another important QTL on Chromosome b08.

Although the correlation of iron reductase activity with seed iron accumulation was not significant in this populations at either 1 uM (r=0.044) or at 15 uM (-0.163), it was interesting to see that the iron reductase activity QTL on chromosome 11 was located in the same place as the seed iron content QTL. This did not hold true for the other seed iron QTL on chromosome 8.

Future Steps:

- Evaluate Fe reductase activity in a greater number of parents of other populations and a range of Fe concentrations for each parent.
- Perform QTL analysis with the G21242 x G21078 population and an improved genetic map that we have constructed.
- Identify any other potential QTL for iron accumulation and for Fe reductase activity at other genomic locations based on other populations.
- Develop a DNA marker for Fe reductase activity either based on QTL mapping or cloning and mapping of orthologs of the Fe reductase gene.

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2.4.5 Field evaluation of an Andean population developed to study seed mineral accumulation

Rationale: Legumes provide essential micronutrients that are found only in low amounts in the cereals or root crops. 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 that these traits are likely to be inherited quantitatively. The objective of our most recent studies has been to tag some of the quantitative trait loci (QTLs) controlling seed mineral content in several recombinant inbred line (RIL) populations of common bean. In this study we analyzed the results of a single RIL population derived from an Andean x Andean cross between G21242, a Colombian cream mottled climbing bean with 'C' Phaseolin and G21078, an Argentinean cream seeded climbing bean with 'T' Phaseolin, that was grown in multiple locations and in a replicated trial.

Methodology:

Plant materials: The population was analyzed over two seasons, Popayán 1998B and Darién 2003A with a total of 100 RILs planted in Popayán and a subset of 83 of the same RILs planted in Darién. A lattice design was used for the first trial (with 3 repetitions) and a randomized complete block design was used for the second trial (with 2 repetitions). Both experiments were planted with trellis supports since the population is predominantly made up of climbing bean genotypes and agronomic management consisted in recommended practices. In both seasons, plots were bulk harvested and grain was combined across repetitions before sub-sampling for mineral analysis.

Mineral Analysis: Two methods of mineral analysis were implemented. The harvest from Popayán was analyzed first with Inductive Coupling Plasma (ICP) analysis at the University of Adelaide and second with Atomic Absorption (AA) Spectrophotometry at the CIAT analytical services lab. The harvest from Darién was only analyzed with the second technique. Sample preparation for the ICP technique involved grinding 10 g of seed in a coffee mill, while for the AA technique 5 g of seed was ground in aluminum chambers using a Retsch mill and aluminum grinding balls. Replicate sampling with two repetitions each was done for the AA mineral analysis but not for the ICP analysis due to cost considerations. As the ICP analysis was presented in the 2002 Annual report, here we concentrate on the results of the AA analysis.

Results and Discussion: Iron and zinc content in the RILs presented a continuous distribution, suggesting that mineral content behaved as a quantitative trait (Figure 37). Iron content ranged from 27.3 to 96.2 mg kg^{-1} (average 55.3 mg kg^{-1}) in Darién, and from 22.5 to 91.3 mg kg^{-1} (average 49.2 mg kg^{-1}) in Popayán. Zinc content ranged from 14.5 to 37.7 mg kg^{-1} (average 26.5 mg kg^{-1}) in Darién and from 21.0 to 59.6 mg kg^{-1} (average 31.4 mg kg^{-1}) in Popayán.

The parents of the population were significantly different and tended to be on the edges of the population distribution (Figure 37). G21242, the high mineral parent, was always higher in mineral content than G21078, the low mineral parent. In the case of iron concentration, G21078 tended to have values similar to the means of the population while G21242 was closer to the upper extreme of the population distribution, while in the case of zinc concentration the parents were more intermediate but still contrasting. Given this, transgressive segregation for low iron and for both high and low zinc was evident in the population.

Previous results with ICP analysis showed a similar trend for the population distribution as with AA spectrophotometry. However G21078 had been lower in iron concentration in the ICP analysis (36.6 mg kg^{-1}) than in the AA analysis, even though G21242 had been similar (89.3 mg kg^{-1}). For zinc, ICP values were higher than those found with AA (49 mg kg^{-1} for G21242 and 28.5 mg kg^{-1} for G21078) but the population distribution was similar. AA spectro-photometry provided a savings in reagent costs and required smaller amounts of ground samples so was the preferred method. Reliability of the AA spectrophotometric method was high with coefficients of variation averaging 6.8% for iron and 5.6% for zinc in the analysis of variance conducted for each location.

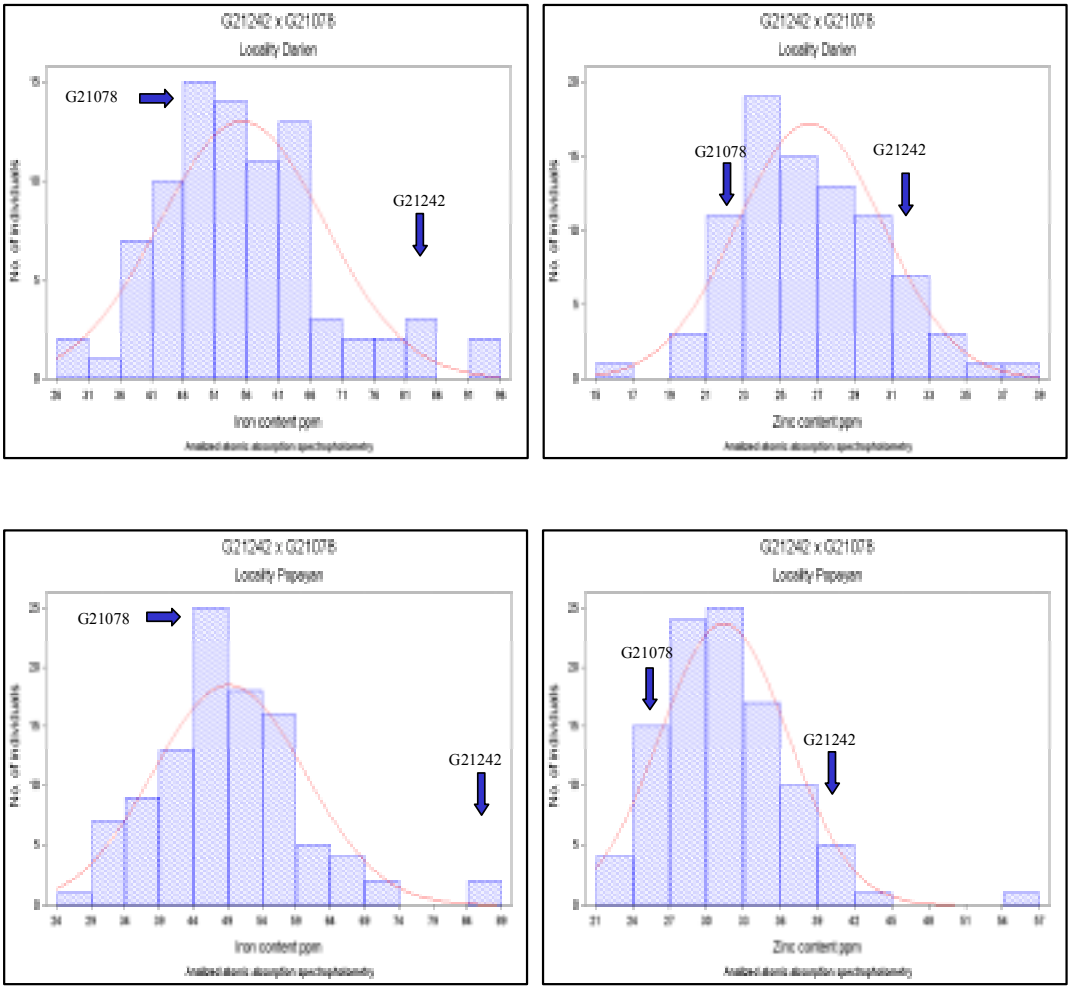

Figure 37. Population distribution for seed iron and zinc content in Andean recombinant inbred lines from the cross of G21242 x G21078 tested over two locations in Darién and Popayán, Colombia.

Genotype x environment interaction was measured for the AA results for seed iron and zinc content in a combined analysis over the two locations, Darién and Popayán (Table 62). Location, treatment and location x treatment effects were all significant at P=0.0000 level, showing that G x E was important for both minerals, confirming the difference in the distribution and parent means seen in the histograms. It was notable that location effects were stronger for zinc than for iron however location x treatment effects were similar for the two minerals. Despite the significant G x E effects, highly significant correlations were also observed between locations for both iron (r=0.665 and r=0.715) and for zinc content (r=0.439 and r=0.450) irregardless of the mineral detection method. Indeed, correlations were even higher between the AA and ICP results for both iron (r=0.849) and zinc (r=0.860) for the harvest from Popayán

(Table 63). Correlations were also high between iron and zinc concentration in both Darién (r=0.301) and Popayán (r=0.653 for AA and r=0.651 for ICP).

Table 62. Analysis of variance for seed iron and zinc content in the G21242 x G21078 population tested over two locations (Popayán 1998b and Darien 2003a).

Source	DF	SS	MS	F	P
Iron Analysis					
LOC	1	2213.7	2213.66	202.45	0.0000
TRT	77	39118.1	508.03	46.46	0.0000
LOC*TRT	77	7621	98	9.05	0.0000
Error	154	1683.9	10.93		
Total	311	51091			
Zinc Analysis					
LOC	1	2128.61	2128.61	798.32	0.0000
TRT	77	4637.78	60.23	22.59	0.0000
LOC*TRT	77	1757.97	22.83	8.56	0.0000
Error	154	410.62	2.67		
Total	311	8935.09			

Table 63. Correlations between locations and mineral detection methods for seed iron and seed zinc content and between minerals within each location and method in an Andean recombinant inbred line population grown in Darién and Popayán and analyzed with Inductive coupling plasma (ICP) and atomic absorption spectrophotometry (AA).

Location	Mineral detection method	Iron vs. Iron			Zinc vs. Zinc			Iron vs. zinc
		1	2	3	1	2	3	
1. DARIEN	AA	1.0			1.0			0.301
2. POPAYAN	AA	0.665	1.0		0.439	1.0		0.653
3. POPAYAN	ICP	0.715	0.849	1.0	0.450	0.860	1.0	0.651

Future Plans:

- Complete the QTL analysis with this population and a more complete genetic map for the G21242 x G21078 RILs.
- Determine correlations with other minerals analyzed in the ICP study, which include Mn, Ca, Mg, K, P and S, and with iron reductase activity.
- Compare agronomic response, heritability and QTLs across populations.
- Characterize the population for other nutrition related traits including, tannin content and sulfur containing amino acids (SAA).

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2.4.6 Evaluation of five common bean mapping populations for tannin content

Rationale: Part of the effort to increase the nutritional quality of common bean has concentrated on increasing iron bioavailability, where bioavailability is the proportion of the consumed nutrient that is digested, absorbed and utilized by human beings. Bioavailability is determined by both food composition and the nutrient status of the consumer as well as a mix of promoters (such as sulfur amino acids: methionine and cysteine, vitamin A or C) and anti-nutritional factors (including fiber, lectins, phytates, polyphenolics and tannins, as well as Calcium and Manganese). Among the anti-nutrients, tannins are important because of their ability to interact with proteins and to chelate minerals which results in reductions in protein digestibility and mineral bioavailability. Tannins are derived from phenolic compounds and contribute to the coloring found in the seed coats of common beans (*P. vulgaris*) and their relatives. They can be divided into hydrolizable / soluble tannins (derived from Gallic acid) and condensed tannins / proanthocyanidins (derived from polymerized flavonoids), which are measurable by different techniques. Some of the tannin fractions and flavonoid precursors have been suggested to have a positive effect on health through anti-oxidant activity. Therefore it is important to have a greater understanding about the inheritance of total tannin content and its component fractions. Given this we began a program to identify genetic variability for condensed tannins in seed coats of common bean mapping populations. In the previous year we reported the results of this analysis in the DOR364 x G19833 population and in this study we applied the extraction techniques developed over the last two years to the analysis of four additional populations of recombinant inbred lines. Our ultimate objective is to identify the QTLs associated with tannin accumulation in common bean.

Materials and Methods:

Plant Material: A total of 500 lines from five populations (averaging 100 RILs each) were analyzed in this study. The populations represented both inter-gene pool crosses (DOR364 x G19833, G19839 x G2333, BAT93 x JALOEEP558) and intra-gene pool crosses (Andean G21242 x G21078 and Mesoamerican G14519 x G4825). Each population was grown at a single location and seed was freshly harvested for analysis.

Seed Coat preparation: Seed coats were peeled from common bean seed and ground into a fine powder to use in the analysis. An n-heptane treatment was used facilitate seed peeling which consisted in 12 hours immersion in n-heptane after which the seed was dried and peeled by hand. Different amounts of ground seed coat were used for the parents (15 g) than for the individual recombinant inbred lines (10 mg). This was done to obtain enough purified tannin from the parents to construct the concentration calibration curves used in the analysis of the progeny. Three replicates were used per seed coat sample for the analysis.

Tannin Extraction: Total condensed tannin extraction and analysis of soluble and insoluble condensed tannis were as reported last year. Colorimetric tannin analysis was realized with a Butanol-HCl method which allows total condensed tannins to be measured. A butanol-water (5%) mix was used as a blank.

Tannin Purification and Establishment of the Calibration Curve: Seed coat of the parents of each population was extracted for tannins and purified with Sephadex LH-20. A dilution series of different tannin concentrations was evaluated in the colorimetric assay described above. The calibration curve was then established by plotting average absorbance against tannin concentration for the two parents of each population.

Results and Discussion: Condensed tannins were successfully purified from the parents of each population to use for the determination of the calibration curves for absorbance vs. concentration and to use in estimating the amount of tannins in the progeny. When the progeny and parents were evaluated, a range of seed coat tannin concentrations (expressed in percent) were observed for the four populations (Figure 38). Tannin measurements were consistent between repetitions showing that the Butanol-HCl method used for analysis is technically sound.

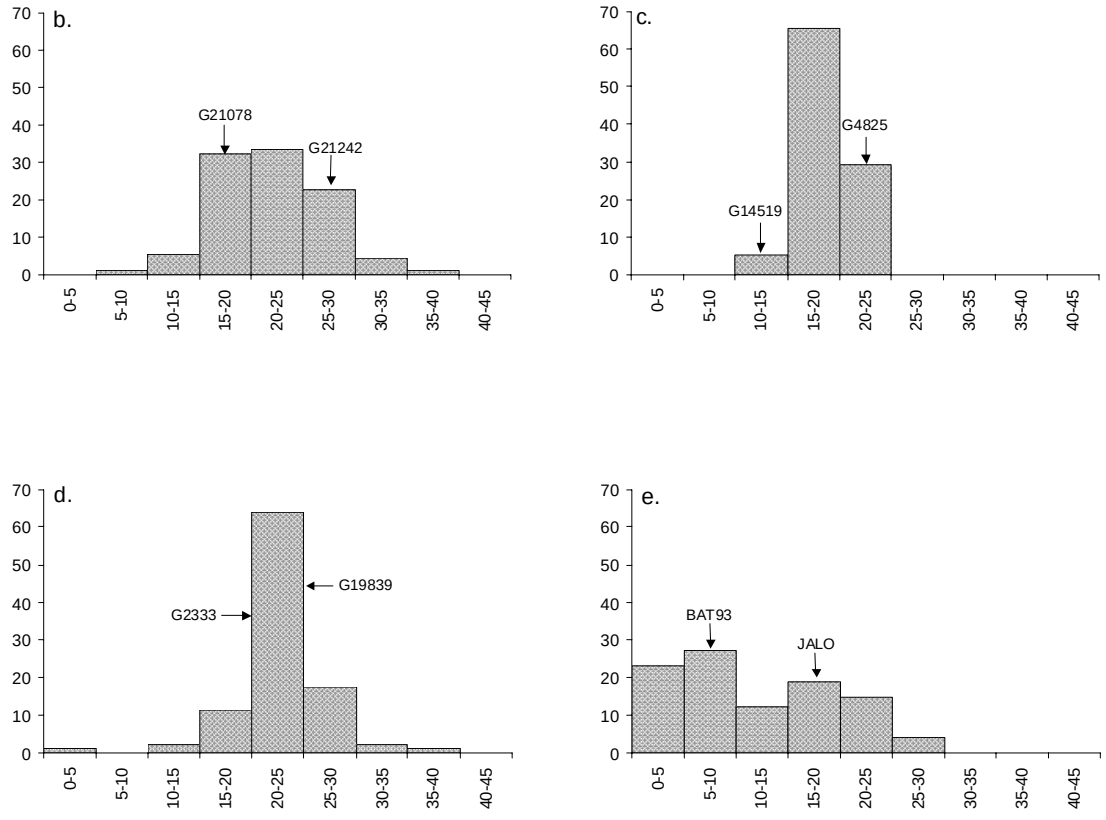

Figure 38. Population distribution for total condensed tannins (as percentage of total tannins) in four populations of common bean: a. G21242 x G21078, b. G14519 x G4825, c. G2333 x G19839, d. BAT93 x JALO EEP558. The Y-axis is the per cent of individuals in each phenotypic class.

Average soluble, insoluble and total condensed tannins were found to vary significantly between populations (Table 64). The DOR364 x G19833 population had the highest soluble condensed tannins and since soluble condensed tannins make up the bulk of total condensed tannins this population was also highest in this category. G2333 x G19839 and G21242 x G21078 has lower amongst of soluble tannin but had the highest amounts of insoluble tannins. The other two populations, especially BAT93 x Jalo EEP558 were low in both soluble and insoluble condensed tannins. The normal distribution found in total condensed tannin concentration, and both soluble and insoluble condensed tannins, in most of the populations suggests that the traits are inherited quantitatively (Figure 38). However given that the BAT93 x Jalo population was distributed binomially, there is the possibility that inheritance of tannin content is more qualitative. Correlations between the amount of soluble and insoluble tannins (Table 65) was low in the intra-gene pool populations but was significant for the inter-gene pool populations (ranging from r=0.20 to r =0.55). This may indicate that the inheritance of soluble and insoluble tannin content is independent in the first set of populations but linked in the other populations.

Table 64. Average condensed tannin content in five populations of common bean recombinant inbred lines.

Population[1]	Soluble	Insoluble	Total
DOR364 x G19833	26.45 A[(2)]	2.88 B	29.38 A
G2333 x G19839	19.31 B	3.41 A	22.74 B
G21242 x G21078	18.34 B	3.52 A	21.88 B
G14519 x G4825	15.88 C	2.98 B	18.88 C
BAT93 x JALO	9.49 D	2.36 C	11.87 D

[1] Population differences significant at P<1%; [2] Means separation by Ryan Einot Gabriel Welch test

Table 65. Correlation between soluble and insoluble condensed tannin content and total tannin content for each of five recombinant inbred line populations.

Population	Correlation		
	Ins vs. Sol	Ins. vs Tot	Sol vs. Tot
DOR364 x G19833	0.20	0.39	0.98
G21242 x G21078	0.05	0.21	0.99
G14519 x G4825	-0.04	0.18	0.97
G2333 x G19839	0.44	0.58	0.99
BAT93 x JALO	0.55	0.69	0.99

The search for QTLs in the DOR364 x G19833 population identified two QTLs for total tannin content on linkage groups b03 and b10 (Figure 39). The QTL on linkage group b10 was also associated with QTLs for both traits independently while the QTL on linkage group b03 was only significant for total tannin content. These QTLs map to the same locations as the seed coat color genes "Z" (b03) and "J" (b10) on the classical genetic map of common beans showing an association between seed color and tannin content which will be investigated further.

Conclusions and future plans: We plan to complete the QTL analysis with the additional populations to determine the genes involved in tannin content in common bean seed coats and use this information to devise a strategy for reducing specific fractions of tannins with the hope of increasing bioavailability of iron in beans. We also plan to evaluate the flavonoid components of the tannin fraction using HPLC analysis and determine if there are correlations between tannin content and iron/zinc accumulation in the seed.

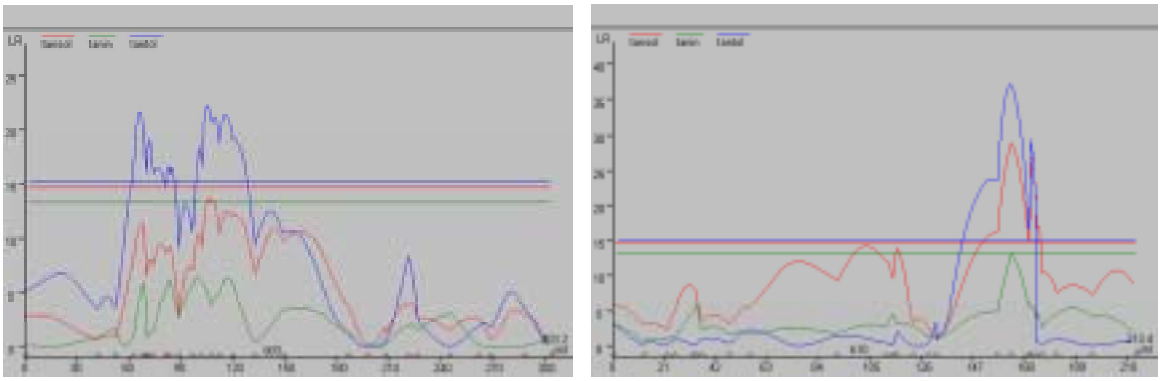

Figure 39. Quantitative trait loci (QTLs) for condensed tannin content detected with composite interval mapping analysis in the DOR364 x G19833 mapping population of common bean.

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Collaborators: P. Avila, C. Lazcano (IP-3), S. Beebe (IP-1), P. Gepts (UC-Davis).

Progress towards achieving output milestones:

- One high iron sister line, NUA56, has average iron content of 93 mg kg^{-1} across three sites. Additional Andean red seeded high iron varieties have been identified.

- Good correlations across sites for iron content confirm the potential of identifying a stable genetic component of improved iron content of beans.

- A total of 120 combinations of high iron landraces and breeding lines crossed to elite breeding lines with commercial grain and agronomic characters have been developed.

- QTL mapping of nutritional traits has revealed QTL for iron concentration, tannin concentration, and iron reductase.