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Climbing bean breeding for disease resistance and grain quality traits

Ana Elisabeth Portilla[1] | Victor Manuel Mayor-Duran[2] | Hector Fabio Buendia[1] | Matthew W. Blair[3] | Karen Cichy[4] | Bodo Raatz[1]

[1]Bean Program, Crops for Nutrition and Health, International Center for Tropical Agriculture (CIAT), Cali, Colombia
[2]Semillas Valle, Cali, Colombia
[3]Department of Agricultural and Environmental Sciences College of Agriculture Tennessee, State University Nashville, Nashville, TN, USA
[4]Sugarbeet and Bean Research, Agricultural Research Service, United States Department of Agriculture, East Lansing, Michigan, USA

Correspondence
Ana Elisabeth Portilla and Bodo Raatz, Bean Program, Crops for Nutrition and Health, International Center for Tropical Agriculture (CIAT), Cali, Colombia.
Email: a.e.portilla@cgiar.org; bodo.raatz@gmail.com

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Abstract

Common bean (*Phaseolus vulgaris*) is grown in two growth types, bush and climbing beans. The latter are preferred in several regions in East and Southern African as well as in Latin America (dominant in Rwanda and Colombia), due to higher yields and resilience compared with bush type. Common bean productivity is reduced by several pests and diseases between them. *Bean common mosaic virus* (BCMV), which is the most common and destructive poty-virus affecting bean production worldwide, and anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, can cause yield losses as high as 95% in susceptible cultivars. Further important traits in common bean are high micro mineral contents to alleviate malnutrition and grain quality traits such as the canning quality of bean varieties, which is important for farmers to access the processing market. In this study, new climbing bean populations were generated (coded ENF/CGA) to combine high seed iron (SdFe) and multiple diseases resistance. Double and triple crosses between parents with virus and anthracnose resistance, high SdFe, and good agronomic traits were employed. In trials in Darien and Popayan, lines were identified that combine BCMNV/BCMV and anthracnose resistance with seed yields above 4000 kg/ha. Phenotypic evaluations validated the usefulness of SNP markers tagging the genes *bc-3* and *I* for BCMN and *Co-3* for anthracnose as a selection tool for field resistance. These results show the genetic potential of the lines that are now being tested in target regions to be delivered to smallholder farmers.

KEYWORDS
anthracnose, BCMV, common bean (*Phaseolus vulgaris*), grain quality traits, marker-assisted selection

1 | INTRODUCTION

Common bean (*Phaseolus vulgaris L.*) is the most important grain legume for direct human consumption (Broughton et al., 2003; Câmara et al., 2013), a staple crop in many tropical regions in East and Southern African as well as in Latin America. It is grown especially by poor smallholder farmers and a valuable component of their diets due to its high nutritional value, rich in protein, micro minerals (iron and zinc), and vitamins (folate) (Messina, 1999). The global production of dry beans has reached 32 million tons per year (FAO, 2017).

Ever-increasing human population will aggravate land constraints, calling for more productive and sustainable crop systems. Climbing beans, also referred to as growth habit IV (Checa & Blair, 2012), are grown with trellis systems, stakes, or in intercropping systems where usually maize (*Zea mays* L.) stalks provide a structure to climb on (Isaacs et al., 2016). Climbing beans can have 2–3 times the yield as bush types, while requiring more manual labor, making them very suitable for usual living conditions of poor land limited smallholder farmers (Checa et al., 2006; Musoni et al., 2005). Climbing beans have spread in African countries over the last ∼30 years, becoming the dominant bean crop in Rwanda, and are now increasingly used in Kenya, Uganda, and surrounding countries (Graf et al., 1991; Musoni et al., 2005; Sperling & Muyaneza, 1995).

Investments in climbing bean research and dissemination efforts were shown to contribute significantly to improve household welfare (Katungi et al., 2018). One additional kilogram of climbing bean seed planted raised per capita consumption expenditure by 0.9% and decreased the likelihood of being poor by 0.6% (Katungi et al., 2018). Climbing beans, which have a much greater biomass and longer growth cycle that bush types, were reported to have the highest levels of symbiotic nitrogen fixation (SNF) found in bean present (Graham & Rosas, 1977; Manrique et al., 1993), and they can have a positive N balance (Beebe, 2012).

However, climbing beans have only found little attention of breeding programs; some known research centers such as CIAT (Colombia), Nicaragua, RAB (Rwanda), and ICTA in Guatemala (Tobar Piñón et al., 2021) have dedicated breeding programs. Yet new varieties have been developed and released. New breeding lines from the mid-altitude climber (MAC) group are generally adapted to lower altitudes, higher temperatures, and mature earlier than traditional climbing bean lines (Blair et al., 2007).

Bean production, including climbing beans, is reduced by several constraints, mainly abiotic stresses such as drought and low soil fertility and biotic constraints such as pests and diseases. Bean common mosaic virus (BCMV) is a seed-borne poty-virus with worldwide distribution that can cause substantial yield losses. The related bean common mosaic necrosis virus (BCMNV) is mainly found in Africa (Beebe, 2012). Resistance is genetically well defined and conditioned by a single dominant (*I*) and four recessive (*bc-u*, *bc-1*, *bc-2*, *bc-3*) genes. Introgression of *bc-3* is a common breeding strategy to provide BCMV/BCMNV resistance.

Anthracnose (Ant), caused by the fungus *Colletotrichum lindemuthianum* (Sacc. Et Magn.) Scrib, is one of the most economically damaging diseases affecting common bean production (Zuiderveen et al., 2016) and can cause yield losses as high as 95% in susceptible cultivars (Guzman et al., 1979). Lesions commonly appear on stems, hypocotyls pods, and leaves, and advanced infection in pods can cause abort (Padder et al., 2017). BCMV alike, Ant is a seed-borne pathogen that is particularly problematic for smallholder farmers in developing countries that do not access a formal seed market. Twenty-one major-effect resistance genes were reported; *Co-1 to Co-17*, *Co-u*, *Co-v*, *Co-x*, *Co-w*, *Co-y*, *Co-z*, *Co-Pa*, and *Co-AC* (Mungalu et al., 2020) conditioning resistance have been reported, probably a response to the wide variability of Ant races.

Seed quality traits are very important for marketability, consumer acceptance and human nutrition. Nutritional components that make common bean so valuable are mainly protein and micro minerals such as iron (Fe) and zinc (Zn) (Raatz, 2018). Micro nutrient malnutrition, also referred to by the term "hidden hunger" in human populations, is one of the greatest health challenges the world is facing. Currently, more than half of the world population is affected by micro nutrient malnutrition (Bouis, 1999, 2000; Nestel et al., 2006; WHO, 2000). Bean consumption patterns are often high in populations with iron deficiencies. For example, 27% of women within Latin America exhibit anemia due to iron deficiencies, and in sub-Saharan Africa, the situation is even worse with 40% of women suffering from iron deficiency. Hence, iron-rich beans could make a particularly important contribution to health in this region. Often the bean farmers are women, and even in areas in which male family members cultivate beans commercially, such as Uganda, women often tend their own plots of beans for home consumption. In addition, the consumption of iron-biofortified beans by women positively affects and has the potential to improve certain functional consequences of iron deficiencies such a physical and cognitive performance (Broughton et al., 2003; Haas et al., 2016; Luna et al., 2020; Murray-Kolb et al., 2017). Biofortification for higher Fe and Zn levels is an important component of the CIAT bean breeding program, which aims to release further improved varieties (HarvestPlus, 2012).

While beans are traditionally sold as dry seeds to consumers in Eastern and Southern Africa, the development of food processing infrastructure and demand for convenience foods is increasing the market potential for canned beans (Nakazi et al., 2019). Canning quality of bean genotypes is a complex trait that is determined by many factors, such as hydration characteristics of seed, cooking requirements, and consumer and processor preferences (Posa-Macallincag et al., 2002). The general appearance, degree of splitting, and hydration are routinely used by breeders to assess canned bean quality; genetic variability has been studied and is employed in breeding selections (Kelly & Cichy, 2012).

Traditional plant breeding is based on hybridization followed by selection of desirable trait combinations in subsequent generations. Disease resistance loci are introgressed into elite cultivars to develop superior cultivars. This process is time and resource consuming. The application of marker-assisted selection (MAS), for the detection of genes or genomic regions underlying a trait of interest, can aid phenotypic selection in breeding programs by reducing time and costs (Miklas et al., 2006).

This work aimed to identify new climbing bean breeding lines that combine anthracnose and BCMNV resistance, seed quality traits such as high seed iron content and canning quality, commercial grain types, and good agronomic performance. Furthermore, this work validated the usefulness of SNP markers tagging resistance genes for BCMNV and anthracnose.

2 | MATERIAL AND METHODS

2.1 | Plant material

The 235 ENF (by the Spanish word enfermedades referring to their disease resistance) or the 81 CGA (climbing beans selected for good agronomic performance) lines were codified in generations F7 or F8 based on agronomic evaluations in the field. They originate from single and triple crosses between bush and climbers parents with anthracnose and virus resistance. Some lines used like parents were G2333, Perry Marrow, MBC (mid-altitude BCMNV resistant climbing beans), BRB (bush bean resistant to black root), and BRC (climbing bean resistant to black root). High seed iron and good agronomic qualities targeting mid altitude production regions were extra attributes of these lines. They are all climbing bean lines with growth habit IV. From this panel, 80 genotypes were selected for yield trials of which are 67 ENF lines and 13 CGA lines. Twenty materials were used as checks in these trials, based on commercial grain qualities, good pod load, historical data, and diseases resistance. Among these are two MBC lines (mid-altitude BCMNV resistant climbing types), nine MAC lines (mid-altitude climbing beans), three gene bank accessions G5702 (super-nodulating), G2333 (landrace from Mexico with anthracnose resistance) and G19839 (bush, Peruvian germplasm accession), two non-nodulating genotypes, and four high-altitude climbing beans D Moreno, Bolivar, PO07A49, and PO07A50 (Table S1).

2.2 | Trial locations and experimental design

Two replicated field trials were carried out; several traits of these trials have been described by Barbosa et al. (2018). One field trial was conducted during the season from May to September of 2014 at the experimental station of the International Center for Tropical Agriculture (CIAT) in Darien, Colombia, located at 03°55′41.39″N latitude, 76°28′10.31″W longitude and an altitude of 1510 m above sea level (masl). A second field trial was carried out in Popayan, Colombia, during the season from April to August of 2015. This station is located at 2°25′39″N and 76°37′17″W with an altitude of 1750 masl. Basic characteristics of the field site were described previously by Barbosa et al. (2018), mainly biological, chemical soil analysis, and weather conditions. During the crop-growing season in field conditions, the total rainfall was 609 mm in Darien and 275 mm in Popayan.

Climbing beans normally are growing between habit 4A or 4B; these require of special structure to support them to stand and grow up. Trellis systems were made using local Poles of guadua (*Guadua angustifolia*) of 3 m large distributed in the field in squares of 5 m length × 5 m width. Wires in about 2.3 m height were spanned between "guadua" poles. Beans climbed on a string, about eight per row, hanging from the wire.

The experimental design at both locations Darien and Popayan consisted of a 10 × 10 alpha-lattice design with three replications. Experimental units consisted of two rows plots of 2.5 m length with row-to-row distance of 1.0 m with 25 seeds sown manually per row length.

2.3 | Phenotypic assessment

For yield determination, the grain was harvested from one row discarding the first and last plant, whereas the second row was used for physiological traits. Mean yields per hectare were corrected for 14% moisture in grain.

Phenological traits were recorded as:

Days to flowering (DF) number of days after planting until 50% of the plants had at least one open flower.

Days to physiological maturity (DPM): number of days after planting until 50% of plants had at least one pod losing its green pigmentation.

Seed weight (100SdW): weight of 100 seeds in grams.

Pod harvest index (PHI) (%): seed biomass dry weight at harvest/pod biomass dry weight at harvest × 100 as described by Polania et al. (2016).

Seed iron (SdFe) and seed zinc (SdZn) contents: measurement of iron in seed provided by Harvest plus laboratory (CIAT) evaluating ~5 g of ground seed quantified by X-ray fluorescence using an energy dispersive 162 X-ray fluorescence (EDXRF) instrument X-Supreme 8,000 (Oxford Instruments, UK).

2.4 | Canning quality evaluation

Canning quality was measured at USDA ARS Michigan using a single 90-g sample using a small-scale canning protocol (Hosfield et al., 1984). Due to capacity limitations, a subset of 72 bean lines was selected representing diverse types of grain, colors, sizes and shapes (Table S1) (Hosfield et al., 1984). One month after the beans were canned, visual appearance (APP) was evaluated by 21 trained panelists on a hedonic scale of 1 to 5, 1 being least desirable and 5 being most desirable. This scale takes into account whole bean integrity, uniformity of size, and brine color (Mendoza et al., 2017). Sample ratings were averages across all panelists. Texture (Tx) was measured with a standard shear-compression cell of a Texture Analyzer (Texture Technologies, MI). The Kramer Shear press uses a dynamic hydraulic system that reports the peak force needed for loss of total bean integrity. Hydration coefficient (HyC) was calculated as the weight of soaked beans (g) divided by the weight of dry beans equivalent to 90 g of solids. Hydration coefficient is an indicator of how well hydrated the beans were prior to thermal processing. Hydration coefficient in the range of 1.8 to 2.0 is ideal and an indication that the beans took up water sufficiently during soaking (Balasubramanian et al., 2000). The washed drained weight coefficient (WDC) was calculated as the weight of washed drained beans after canning divided by the soaked bean weight. This coefficient provides information on the water uptake during the canning process. Values between 1.4 and 1.6 are considered optimal. Values below 1.4 indicate insufficient water and above 1.6 indicate too much water in the final product (Hosfield et al., 1984).

2.5 | Disease evaluations

The panel of 316 lines was evaluated in Popayan (Colombia) during the season October 2012 to January 2013 for reaction to anthracnose. Disease severity was evaluated with the CIAT standard scale from 1 (no disease symptoms) to 9 (severe disease symptoms). The evaluation was carried out in leaves (Ant_L) and pods (Ant_Pd). The inoculation was carried out according to the greenhouse CIAT protocol (Castellanos et al., 2012) with some modifications including days after planting to first inoculation (21–23) and three total inoculations each 8 days. Mix of races using different pathogen isolates was included. Between them are as follows: Race 131 isolate CL597, Race 4 isolate CL598, Race 1033 isolate CL212, Race 133 isolate CL600, and Race 7 isolate CL601. All the isolates represent local pathotypes, relevant for Colombian production regions, and are covering all the spectrum of the Andean differential varieties.

The reaction to viral infection BCMNV was evaluated in the green house, aphid-proof conditions at CIAT. Plants were mechanically inoculated 20 days after sowing in plastic trays (53 × 27 × 7) containing sterilized soil. The inoculations were done with a sterile cheesecloth pad impregnated with a 1:10 homogenate of infected tissue ground in 10-mM potassium phosphate buffer (Morales, 1987). Virus strain used was BCMNV–NL3. The evaluations were made with the CIAT scale with three categories: no symptoms were observed in resistant plans (R), whereas mosaic symptoms were observed in susceptible plants (S). Furthermore, a hypersensitive necrotic reaction was observed in lines that carry the *I* gene alone (Bello et al., 2014).

2.6 | Genotyping

Molecular markers tagging *bc-3* resistance gene for BCMV were designed based on Sanger sequencing data; sequences were published by Hart and Griffiths (2013). SNP assays bc-3a and Bc-3b were established at genotyping outsourcing providers Intertek (www.intertek.com) and LGC genomics (www. lgcgroup.com), details in Table 1.

A SNP assays tagging the *I* gene were designed based on SNP ss715648456 reported by Bello et al. (2014) to provide resistance to BCMV. SNP assay ID at Intertek is BCMV_I_00453_M1, and at LGC the ID is sc00445ln245016_77514_C_T_217829459.

Ant markers were identified in the Andean Diversity Panel (ADP) and designed based on SNPs ss715646578, ss715640025, and ss715648452 published by Zuiderveen et al. (2016). A *Co-4* marker was designed using G2333 whole genome sequencing data; the SNP was selected identifying a unique SNP compared with a set of sequenced genotypes (Lobaton et al., 2018), in the estimated *Co-4* position based on Burt et al. (2015) and Oblessuc et al. (2015).

TABLE 1 Molecular markers used in this study

Common bean ID	Intertek ID	Alternative names	Chr	Position (ref 1.0)	Position (ref 2.1)	Trait	Gene	Resis. associated allele	SNP ref
bc-3a	snpPV0001	BCMNV_SNP_Chr06_27969872_T_A	6	27,969,872	27,204,533	BCMV	bc-3	A:A	Hart and Griffiths (2013)
Bc-3b	snpPV0002	BCMNV_SNP_Chr06_27969904_C_A	6	27,969,904	27,204,533	BCMV	bc-3	A:A	Hart and Griffiths (2013)
BCMV_I_00453_M1	snpPV0004	ss715648456, LGC: sc00445ln245016_77514_C_T_217829459	2	48,447,134	49,075,854	BCMV	I	T:T	Bello et al. (2014)
ANT_Co-u_ss715648452	snpPV0045	ss715648452	2	48,617,342	49,236,622	Ant	Co-u	G:G	Zuiderveen et al. (2016)
ANT_Co-1_ss715646578	snpPV0048	ss715646578	1	48,340,819	47,637,549	Ant	Co-1	A:A	Zuiderveen et al. (2016)
ANT_Co-3_ss715640025	snpPV0050	ss715640025	4	212,804	169,725	Ant	Co-3	G:G	Zuiderveen et al. (2016)
ANT_Co-4_08_CG_2329860	snpPV0069		8	2,329,860	2,262,272	Ant	Co-4	G:G	Lobaton et al. (2018), based on Burt et al. (2015) and Oblessuc et al. (2015)

For genotyping, two leaf discs were taken from individual plants at 25 days after planting using a paper disc puncher and placed in a 96-well storage plate (Thermo Scientific 1.2 ml). Plates with leaf samples were dried during 18 h at 55°C and sealed with film. Silica gel was added to keep samples dry and shipped to genotyping provider Intertek in Sweden (www.lgcgroup.com).

2.7 | Statistical analysis

All data were analyzed using the Plant Breeding Tools program (v 1.3) (IRRI, 2013). The adjusted means of the genotypes in each environment individually (Darien and Popayan) were obtained using mixed modeling with the MIXED procedure considering the effects of the replications and blocks within replications as random and genotypes as fixed. Correlations coefficients were calculated with XLSTAT (Addinsoft, 2020) from Excel package. Values marked with *, **, or *** are statistically significant at probability levels of 5%, 1% and 0.1%, respectively. The graphics to show the relationship between variables were made with R using the GGPLOT package (V. 3.3.0).

Marker effects of a specific marker on a specific trait were calculated as the average deviation from the population mean phenotype of the lines the carry a specific allele.

3 | RESULTS

3.1 | New climbing bean lines combine important agronomic traits

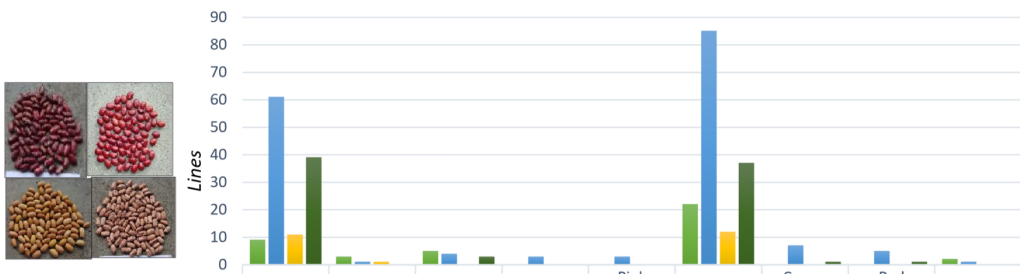
A group of climbing bean breeding lines was developed at the CIAT bean program based on simple and three-way crosses. Utilized parental lines were recent climbing bean breeding lines (coded MAC, MBC, LAS, BRC), BCMNV-resistant bush bean breeding lines (BRB), Colombian varieties Cargamanto Rojo/Blanco, Caballero, and Bolivar of the Andean genepool, and the Mesoamerican line G2333 known for its anthracnose (Ant) resistance.

A set of 316 ENF/CGA lines was coded based on visual selection of grain quality and agronomic traits in the field in F7 or F8 generation. The ENF/CGA population harbors several attributes such as good agronomic performance and diseases resistance (Ant and BCMV) (Figure 1). Red and white seed coat are most common followed by yellow, cream mottled, red mottled, cream, pink, pink mottled, and purple. Evaluations for BCMNV and Ant resulted in 41 lines with resistance to both diseases. Twenty-four lines proved resistant to only BCMNV and 170 materials to Ant, while 81 were generally susceptible.

3.2 | Phenotypic evaluations

Eighty most promising lines were chosen for yield trials, together with 20 elite climbing bean lines used as checks in Darien 2014 and Popayan 2015. Genotypic differences were found between the 100 genotypes for several traits measured in both locations (Table 2). Main agronomic data were previously reported by Barbosa et al. (2018). Seed yields were similar between Darien and Popayan, equally DF did not show major differences between locations; however, DPM was reached later in Darien where 100SdW was also higher. A more detailed analysis of grain quality traits was performed for the Darien trial. An evaluation of SdFe levels found a mean of 69.2 ppm, compared with standard check CAL96 at 52.8 ppm. In this trial, several lines with high SdFe levels above 80 ppm were identified with the best performing line containing 88.7 ppm. SdZn contents averaged 34.8 ppm with a maximum of 48.1 ppm.

Evaluation of canning quality traits showed many genotypes in the ENF/CGA population suitable for canning processing. For the most important variable, the visual APP score, a mean of 2.6 (scale from 5 [excellent appearance] to 1 [unacceptable]) with 13 lines >3.5 was recorded. HyC mean was 2.1 with 14 outstanding lines showing optimal values of 1.8–2 and the bean firmness evaluated as Tx ranged around a mean of 40.5. The water uptake during the canning process WDC had a mean of 1.2 with a group of seven samples between 1.4 and 1.6, which is considered an optimal coefficient.



	White	Cream	Yellow	Pink	Pink mottled	Red	Cream mottled	Red mottled	Purple
Antracnose & BCMV Resistant	9	3	5	0	0	22	0	0	2
Antracnose Resistant	61	1	4	3	3	85	7	5	1
BCMV Resistent	11	1	0	0	0	12	0	0	0
Susceptible	39	0	3	0	0	37	1	1	0

FIGURE 1 Combinations of grain colors and disease resistance in 316 ENF/CGA lines. Colors in each bar shows number of the lines per attribute and seed color

TABLE 2 Overview of phenotypic data evaluated in 100 climbing bean lines grown in Popayan and Darien. First five traits were previously reported by (Barbosa et al., 2018)

Trait	Location	Mean	Min	Max	%CV
Days to flowering (DF)	Darien	49.8	43	71.3	11.2
	Popayan	50.2	41.7	62.3	8.8
Days physiological maturity (DPM)	Darien	102.1	86.1	113.1	6.8
	Popayan	96.3	85	107.3	4.8
Pod harvest index (PHI), %	Darien	74.8	65.2	83.5	4.2
	Popayan	70.2	57.3	78.2	5.8
100 seed weight (100SW)	Darien	51.1	26.2	75.7	10
	Popayan	41.1	22.3	59	19.2
Seed yield (yield), kg ha^{-1}	Darien	3,593	1786	6,830	22.8
	Popayan	3,508	1715	6,674	24
Seed iron (SdFe), ppm	Darien	69.2	48.7	88.7	10.7
Seed zinc (SdZn), ppm	Darien	34.8	26.8	48.1	11.1
Hydration coefficient (HyC)	Darien	2.03	1.6	2.2	8.8
Texture (Tx), kg ha^{-1}	Darien	40.5	14	71	43.3
Washed drained coefficient (WDC)	Darien	1.2	1.0	1.7	13.8
Overall appearance (APP)	Darien	2.6	1.3	4.7	35

Note: Canning quality traits were evaluated in 72 lines.

Coefficient of variation (%CV) for yield exceeded 22%, which expectedly had among the highest variabilities. Most other traits had lower %CV values indicating good data quality and repeatability. Only for canning quality traits Tx and APP higher %CV were observed, indicating noisier data probably due to the smaller sub sample of the population that was evaluated (72 lines) and general variability in visual observations made by panels of testers (Mendoza et al., 2014).

Among the grain quality traits, a high correlation of 0.62 was found between SdFe and SdZn (Table 3). Yield in both locations was negatively correlated with SdZn and SdFe levels. PHI is positively associated with yield and accordingly also had negative correlations in both trials with SdZn as well as SdFe in Darien. SdFe was also negatively correlated with 100SdW in both trials. Good seed fill leading to high yield likely has a dilution effect on Fe and Zn in the seed, thereby causing the opposing associations.

Trait values for three canning quality traits (APP, Hyc, and Tx) were all significantly positively correlated with each other. WDC had a high negative association between HyC ($r = -0.97$) and Tx (-0.70) as well as with 100SW in both locations. So desirable appearance goes hand in hand with higher water uptake at the beginning of the process and higher pressure resistance. High water uptake during the canning process then appears predominantly in lines that did not soak well and is associated with breakage.

APP had a weak but positively significant correlation with yield in Darien. In line with this finding, three canning quality traits (APP, HyC, and Tx) were positively correlated with PHI and negatively with SdFe and SdZn. Inversely, WDC was correlated negatively with PHI and positively with SdFe, SdZn, and APP. Also both APP and Tx were positively related with all the maturity traits (except APP with DMP) and Tx and HyC (but not APP) with 100SdW. Hence, productive lines with well filled seed generally have good water absorption and—somewhat unexpected—superior structural strength and integrity after cooking.

3.3 | Best lines combine several important traits

The ENF/CGA lines are current elite breeding lines from which variety candidates will be selected to be tested for potential release and also for use in further germplasm development. Best lines will be identified for specific requirements.

Seed yield, which is often considered to be the most important trait, was strongly correlated between the two locations. Figure 2a shows available combinations between seed yield and disease response. Six ENF lines displayed Ant and BCMNV resistance and yields significantly above of the mean, while two of the top three yielding lines had Ant resistance. Early maturity is a trait valued by most producers as long as it is not accompanied by a yield penalty. In these trials, a weak positive correlation was observed between seed yield and DPM, yet lines were identified that combine earliness of <95 days with good productivity above 4.5 t/ha (Figure 2b). Several of these also show disease resistance.

Few lines combined high SdFe values >80 ppm with above average yield. Most of these also have high Zn content (Figure 2c). Lines with good APP scores >4 were identified that combine with above average yield. Most of these show some disease resistance (Figure 2d). In general, previously generated breeding lines that were included in the trials as checks (MAC, MBC lines) rate among the best in agronomic properties like yield and earliness, whereas for grain quality traits and disease resistance, the new ENF/CGA lines show the best performance.

Best lines for several trait combinations are shown in Table 4. ENF85, 112, 113 combine high seed yield with high SdFe levels and show Ant resistance (scores 1–3). ENF25, 27, and 31 have above average trait values for all the traits evaluated as well as resistance to both BCMV and Ant. High APP scores were noted in combination

TABLE 3 Phenotypic trait correlations in a climbing bean panels, evaluated in Popayan (P) and Darien (D)

Traits	100SW_D	100SW_P	DF_D	DF_P	DPM_D	DPM_P	SdFe_D	SdZn_D	PHI_D	PHI_P	Yield_D	Yield_P	HyC	Tx	APP	WDC
100SW_D	1															
100SW_P	0.81***	1														
DF_D	0.21	−0.1	1													
DF_P	0.03	−0.25*	0.80***	1												
DPM_D	0.06	−0.23*	0.64***	0.59***	1											
DPM_P	0.06	−0.25*	0.69***	0.77***	0.78***	1										
SdFe_D	−0.21*	−0.2*	0.05	0.06	0.08	0.04	1									
SdZn_D	−0.08	0.01	−0.1	−0.1	−0.1	−0.1	0.62***	1								
PHI_D	0.17	0.01	0.16	0.13	0.17	0.09	−0.2**	−0.3***	1							
PHI_P	−0.06	−0.1	0.13	0.09	0.04	0.05	−0.1	−0.2*	0.42***	1						
Yield_D	0.05	−0.02	0.12	0.12	0.21*	0.13	−0.18	−0.3**	0.23*	−0.02	1					
Yield_P	0.01	−0.05	0.06	0.09	0.20*	0.09	−0.14	−0.3**	0.14	−0.03	0.89***	1				
HyC	0.54***	0.45***	0.12	−0.3	−0.15	−0.24*	−0.37*	−0.32*	0.26*	0.14	0.09	0.03	1			
Tx	0.52***	0.18	0.51***	0.41***	0.31**	0.3*	−0.41***	−0.46*	0.27*	0.02	0.17	0.11	0.62**	1		
APP	0.2	0.07	0.4**	0.39***	0.05	0.08	−0.17	−0.39**	0.26*	0.14	0.01	−0.01	0.5**	0.54***	1	
WDC	−0.51***	−0.36**	−0.2	−0.06	0.07	0.16	0.4***	0.4**	−0.33*	−0.16	−0.15	−0.08	−0.97**	−0.7***	0.56***	1

Note: Abbreviations see Table 2.
*Significant at the 0.05 probability level.
**Significant at the 0.01 probability level.
***Significant at the 0.001 probability level.

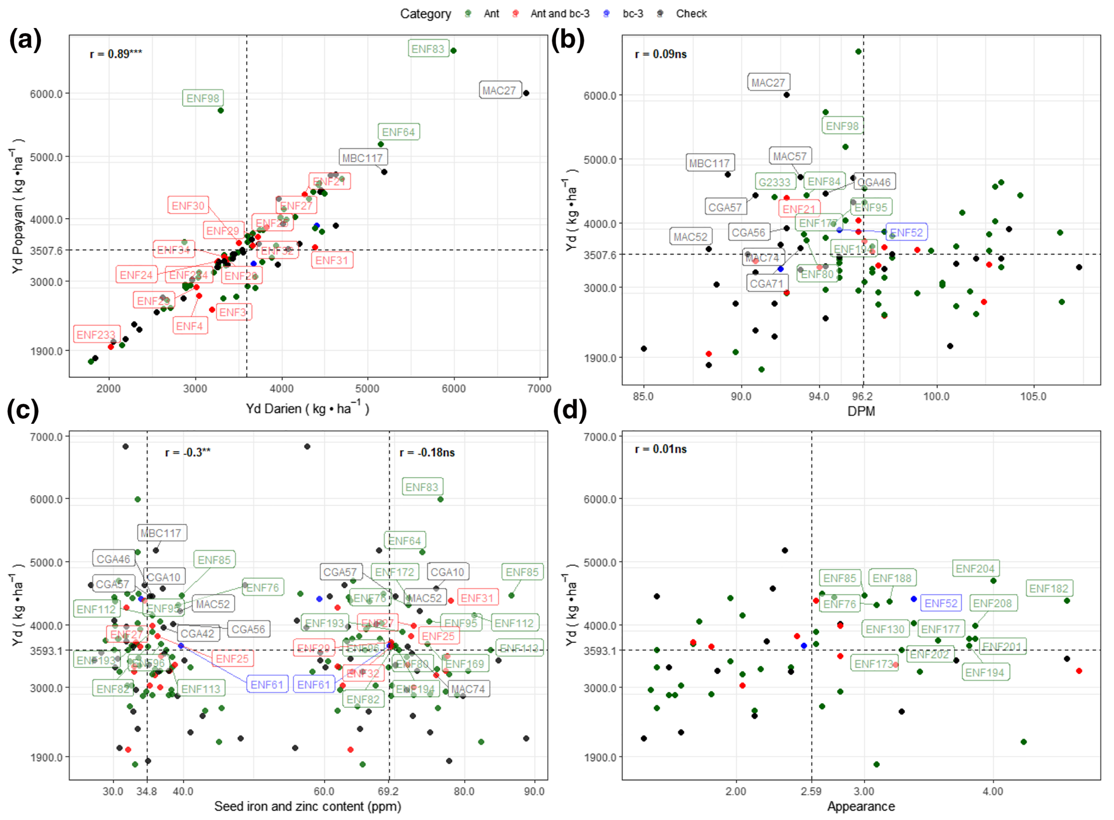


FIGURE 2 (a) Relationship between yield data at Darien and Popayan, (b) yield and DPM in Popayan, (c) yield and SdFe and SdZn in Darien, and (d) yield and APP in Darien, evaluated in 100 climbing bean lines. Dotted lines indicate the mean of each trait

TABLE 4 Superior lines that combine excellent properties in individual traits or trait combinations

Line	Yield_D	Yield_P	SdFe	SdZn	APP	BCMNV	Ant_L	SC
ENF24	3262	3326	77.3	38.0	4.7	R	3	Red
ENF25	3820	3857	72.3	36.3	2.5	R	2	Red
ENF27	**3984**	**4033**	**72.8**	**35.6**	**2.8**	**R**	**1**	Red
ENF30	**3499**	**3618**	**77.5**	**36.8**	**2.8**	**R**	**1**	Red
ENF31	4381	3548	77.9	34.4	2.6	R	3	Red
ENF85	4469	3789	86.6	39.7	3.0	S	3	White
ENF112	4154	4030	81.3	35.5	2.0	S	1	White
ENF113	3602	3630	83.7	37.5	1.4	S	2	White
ENF172	4425	4535	72.0	32.7	2.0	NA	2	Red
ENF204	4699	4633	64.1	30.7	4.0	S	1	Red
Population average	***3593***	***3507***	***69.2***	***34.9***	***2.5***			
LSD	***652.9***	***596.84***	***7.02***	***3.5***	-	-	-	

Note: Lines in bold are superior to the mean for all traits. Abbreviations see Table 2. BCNMV: R: resistance, S: susceptible, SC: seed color. Probability levels for LSD were calculated at 0.05.

with high SdFe in ENF24 and with high yields in ENF204 both showing Ant resistance. Commercial grain quality is among the most important traits for consumer acceptance. Highlighted lines of white or red seed color are acceptable in Colombia and other markets (Figure 3). From these lines, genotypes can be chosen for further breeding activities depending on the requirements of the product profiles.

3.4 | Validation of molecular markers for BCMV/BCMNV and Ant resistance

Molecular markers were evaluated following the phenotypic evaluations to validate their usefulness for marker assisted selection (MAS) in breeding. Phenotypic disease scores were compared with SNP genotyping data obtained from an outsourcing genotyping provider.

A molecular marker tagging the *I* gene was designed based on Bello et al. (2014) reported to provide resistance to BCMV. A marker tagging the *bc-3* gene based on Hart and Griffiths (2013) was reported to be associated with resistance to BCMV as well as Bean Common Mosaic Necrotic Virus (BCMNV), a variant that is commonly found in African be an production areas (Raatz et al., 2019).

Phenotypic data for BCMNV disease scores obtained in the greenhouse resulted in strong association (*r*: 0.88 ***) with the *bc-3* marker (Figure 4). Susceptible lines showed mosaic symptoms, whereas lines with the resistance associated allele of the *bc-3* or both *bc-3* and *I* gene markers had by large no symptoms. Lines with only the *I* gene (without *bc-3*) would be resistant to BCMV infection, but expectedly displayed a necrotic response to BCMNV, killing the plants at an early stage. The few lines where phenotype and genotype do not correspond may be due to phenotypic escapes or genotyping issues.

Ant markers tagging *Co-u*, *Co-1*, and *Co-3* resistance were designed based on Zuiderveen et al. (2016); a marker tagging *Co-4* locus from G2333 was designed using WGS data and positional information from Burt et al. (2015) and Oblessuc et al. (2015). These markers were chosen because they represent well-defined SNP markers for major Ant resistance genes. G2333 was used as a parent in the crosses; the presence of the other genes was not known. Ant markers for *Co-u*, *Co-1*, and *Co-3* genes all had a low significant effect with *p* values between 0.05 and 0.01, for both Ant evaluations in leaves and pods (Table 5). *Co-4* marker showed no significant effect. The *Co-u* marker allele associated with resistance according to (Zuiderveen et al., 2016) had a positive (increasing disease scores) effect on disease scores, associated with 0.85 higher Ant scores. That means this marker had the opposite effect compared with previous report. *Co-3* marker was actually reported to be associated with resistance to Ant race 109 with the G allele, while the opposing A allele of the same marker was associated with resistance to race 7. The data suggest a resistance response based on the GG allele. *Co-1* and *Co-3* makers showed moderate effects, reducing disease scores by 0.76 and 1.35 points, respectively. The lack of clear, strong phenotype genotype associations could be due to the specific interactions between the variable Ant pathotypes and resistance genes and may be affected by population structure which is not accounted for in such validations.

Taken together, the results show that BCMNV markers are good molecular breeding tools that precisely predict phenotype in complex genetic backgrounds of elite breeding lines. The usefulness of the Ant markers that that were investigated here is doubtful.

FIGURE 3 Representative grain color from the highlighted lines. ENF85 and ENF 25, respectively

4 | DISCUSSION

4.1 | Climbing bean breeding progress and goals

The ever-increasing global population will aggravate land constraints, calling for more productive and sustainable crop production systems. Climbing beans have 2–3 times the yield as bush types, while requiring more manual labor, thereby offer a solution for more productivity for land limited smallholder farming systems. In the Western highlands of Guatemala and the mid-highlands in Honduras, Nicaragua among others is still used the three sisters crop (maize-bean-squash) as an option of supplementary feeding (Zizumbo-Villarreal et al., 2012). Climbing beans are a minor crop compared with bush bean production, but over the last ∼30 years, they spread, becoming the dominant bean crop in Rwanda, and are now increasingly used in Kenya,

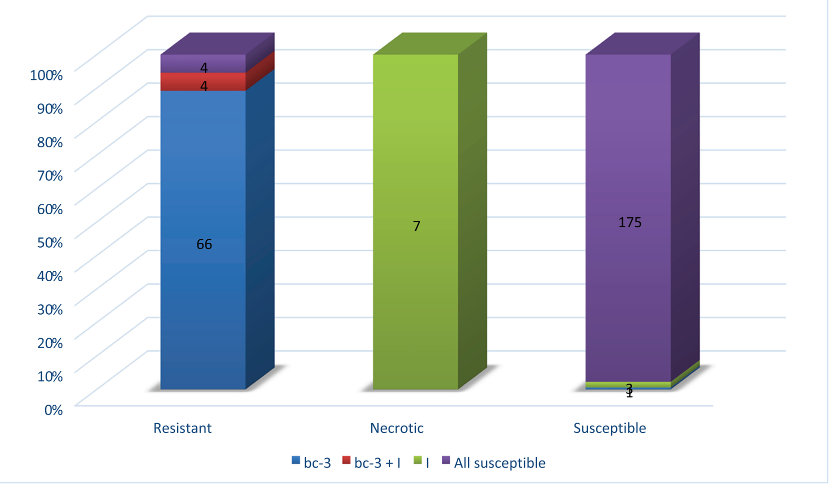


FIGURE 4 Marker validation for bc-3 and I gene markers associated with BCMNV response. Number of plants in each class shown in bars

TABLE 5 Validation of four molecular markers for anthracnose (Ant) resistance evaluated in leaves and pods

Marker	Expected resistance associated allele	Ant_L marker effect	Ant_L p-value	Ant_Pd p-value
Co-u_ss715648452	G:G	0.848	0.022	0.014
Co-1_ss715646578	A:A	−0.764	0.019	0.020
Co-3_ss715640025	G:G	−1.346	0.033	0.023
Co-4_08_CG_2329860	G:G	−0.608	0.561	0.589

Note: Marker effect shows the deviation from phenotypic means based on the marker allele, and *p* values indicate significance of the effect associated with the marker evaluated by *t* test.

Uganda, and surrounding countries (Graf et al., 1991; Sperling & Muyaneza, 1995). Climbing beans also dominate in Latin American Countries such as Colombia. Climbing beans are heavily promoted as having a great potential to improve situation of small holder farmers (Rwanda Agricultural Board [RAB], 2014). A recent study in Rwanda showed that climbing bean adoption increases household welfare (Katungi et al., 2018) and the bean variety MAC 44 that was developed at CIAT and released in 2010 has high adoption rates in Rwanda and surrounding countries. Hence, new highly productive climbing bean varieties can have a large impact in future in smallholder farming systems. Climbing beans for future impact need to be highly productive and combine traits that are required by producers targeting markets.

The challenges in climbing bean breeding are primarily the longer life cycle and larger plants, which means a generally more expensive breeding process. Internationally, only few institutes develop climbing beans germplasm; to our knowledge, only Rwanda, Nicaragua, Guatemala, and CIAT have sizable breeding programs. This poses a challenging task to keep climbing beans competitive and make their full potential available to producers. Resources need to be mobilized and used effectively to produce germplasm with the required trait combinations for the future.

4.2 | New lines will impact through several traits

Several traits need to be combined to produce germplasm products that will be adopted by farmers and create positive economic impact. In this project, selection focused on six classes of traits: production traits such as maturity, agronomic performance, and disease resistance and grain quality traits such as commercial grain type, nutritional content, and canning quality.

Yield is of course of major importance, which was highly correlated between locations here, indicating a good across location performance of these lines. Average yields of 3.5 t/ha were found with peaks calculated at over 6 t/ha. Reports on climbing bean trials are not numerous. Ramaekers et al. (2012) reported 2.2 t/ha in RIL parental line G2333 in the same Darien location; 0.5 to 3.4 t/ha were measured in Colombia at an altitude of 1000 masl (which is considered too low for optimal climbing bean production) (Francis et al., 1978); 5.1 to 6 t/ha were reported optimizing agronomy in trials in Turkey (Ece et al., 2007), and (Wortmann, 2001) reported average yields of 2.1 t/ha highland area of southwestern Uganda. Available data indicate that yields reported here are good; however, variability of conditions, agronomic practices, and germplasm impede a meaningful comparison. Clearly, climbing bean yields easily exceed those found in bush beans. Comparison with checks within trials are more relevant. The new ENF/CGA lines such as ENF27, ENF30, ENF85 etc are not necessarily superior in yield compared with recent breeding lines used as checks, as lines from groups MAC or MBC are among the top yielders. The new line's main quality is the combination of important traits such as diseases resistance (ANT, BCMV), SdFe, canning and grain type which has not been available so far.

Earliness is always among the traits desired by producers; however, the yield loss that is inseparably connected with very early maturity is not accepted. Several of the new breeding lines show a good balance of earlier maturity with high yield, which is a particular strength of a preceding group of breeding lines coded MAC. Classic highland varieties have much longer maturity; Bolivar and D Moreno mature around 103 days in Popayan, compared with high yielding new breeding lines of 90–95 days. Similar yields in less time are of large benefits for producers, reducing, for example, costly agrochemical applications against pathogens.

A major disadvantage of the MAC breeding lines is their susceptibility to BCMV/BCMNV, as reported in Malawi. BCMV/BCMNV is a major production constraint in many tropical regions in both Africa and the Americas. This is the first reported set of climbing bean lines in which several lines are resistant to BCMV, and these can be used either for variety release or for further breeding. Ant is the major fungal disease in moist higher altitude production areas; hence, Ant resistance is a valuable trait in most climbing bean production areas. Highly Ant resistant climbing beans were recently reported from Guatemala (Maldonado-Mota et al., 2021), which is the origin of the genotype G2333 used as a parent in this population.

Among seed traits, the grain color and shape are likely the most important, as high similarity to a commercial grain class is usually a prerequisite to market the produce. Market access is of prime importance for poverty reduction. Development in Ethiopia in recent decades, where bean yields have doubled within ~10 years (Lakew & Tadesse, 2018), shows that access to export markets can infuse investments into crop production systems that lead to the spread of better varieties and agronomic management resulting in poverty reduction. In this work, we present mainly white and red seed types. Solid red grain types are accepted in most regional markets in Eastern Africa; medium large whites are often considered to have a high export market potential.

Next to grain class, the processing quality is becoming increasingly important. Export markets are often directed at canning, and regionally sold products may be processed and marketed, for example, as pre-cooked beans. Here we evaluated the complex trait canning quality in climbing beans. Several lines had good scores for HyC above 1.8, Tx between 40 and 50, and a good overall appearance (APP > 4). These traits were well correlated with each other, as well as with other traits like seed weight, DF and PHI. A large, well-filled grain appears to take up a higher weight percentage of water and is at the same time mechanically stronger with less breakage and starch leakage after canning, which is not very intuitive. Efficient seed fill and possibly higher starch concentration seems to give bean a better structure during cooking.

Biofortification, the use of natural diversity to increase nutrients in crops, is used as a method to fight the biggest health problem this world is currently facing, the iron deficiency (Tulchinsky, 2010). The Harvest+ program has been funding biofortification breeding in bean leading to the release of new varieties in several countries (Bouis et al., 2011). In this work, we identified several lines with SdFe values above 80 ppm that meet biofortification criteria. They have higher levels of SdFe than the first wave of biofortified climbing bean materials such as MAC44 with ~58 ppm (data from previous trials in Darien). SdFe as well as SdZn are usually found in negative correlation with Yd, probably due to a dilution effect in high yielding lines (efficient seed fill with high carbohydrates translocation to grain) (Raatz, 2018). Several lines combine good Yd and SdFe, and should be tested for local adaptation for their usefulness as new biofortified varieties.

In summary, these new climbing bean lines show good trait combinations for several traits of importance for producers. They will be shared with partners to be tested as potential varieties or to be used for further breeding activities.

4.3 | Breeding strategies for climbing beans—what did we learn?

Breeding of climbing beans is more challenging that working with beans with bush growth habit, due to the longer life cycle, larger plants with higher plant to plant spacing and the requirement of a trellis or staking system. All this makes operations more expensive and time consuming, hence, efficient breeding practices need to be applied.

To generate the ENF/CGA lines both two- and three-way crosses were used, some of those as a backcrosses. All resulted in well performing lines, hence, no clear preference can be concluded. Twenty-three lines result from inter gene pool crosses with G2333, which is a well-known source for Ant resistance. Inter gene pool crosses are usually avoided in bush bean breeding, due to the difficulty to regain the desired growth habit and grain type, also a backcross is unlikely to yield a good proportion of acceptable grain types. Some successes were reported (Mayor-Duran & Raatz, 2016), but generally such a breeding strategy creates a lot of rejected waste. Climbing beans seem to be more receptive as good phenotypic performance was found in lines with presumably large inter gene pool introgressions.

Pod Harvest Index was found positively correlated with Yd in Darien, but does not represent such a strong yield indicator as in other reported studies (Assefa et al., 2013), which would have been very useful due to the larger costs of climbing bean yield trials. As a positive effect, PHI evaluated in Darien was significantly correlated with the three components of canning quality, the highest measured correlation was actually with the appearance score (APP). Notably, no correlation was observed between APP and 100SdW, so the linkage of PHI, yield, and APP is not primarily seed size effect, but connected to seed fill. This suggests that a seed that fills well has a different internal biochemical structure, an interesting indication that PHI may be affected by processes of carbon storage within the seed, rather than the loading of photoassimilates into the seed, as usually assumed.

The fact that seed fill and mechanical resilience during cooking (canning quality) is well correlated is good news for breeders. In contrast, the known consistent negative correlations between Fe and Zn levels and yield (Raatz, 2018) are aggravated here with even more negative correlations with PHI and canning quality traits. Biofortification breeding must overcome the confounding factor of carbohydrate accumulation which dilutes micro mineral levels. The breeder has to select those lines that really import and store more micro minerals in the seed, rather than those that have high Fe/Zn levels due to reduced carbon accumulation.

Here we validated molecular markers to support breeding with MAS. Marker validation has to be demonstrated in diverse breeding germplasm, as recently shown for ALS resistance (Gil et al., 2019). The marker for BCMV/BCMNV resistance reported to tag the *bc-3* gene (Hart & Griffiths, 2013) is very indicative of resistance. Also the *I* gene marker (Bello et al., 2014) is very predictive for the phenotype, in response to BCMNV strains the lines that only carry the *I* gene showed the expected hypersensitive response (plant death). We suggest to stack both *I* gene as well as *bc-3*, because BCMV strains were recently reported that overcome *bc-3* (Feng et al., 2016), while a combination of *bc-3* and *I* continues to provide resistance.

The validation of Ant markers was less successful; some correlations with the phenotype of moderate significance were found but are not fully convincing. We evaluated markers for four resistance genes, three of those (*Co-1*, *Co-3*, and *Co-u*) were published based on a study on a panel of Andean germplasm (just like most of the lines evaluated here) using a large number of SNPs. The *Co-4* marker was developed based whole genome sequencing data from genotype G2333 and appears to be highly specific. The right markers were used in the right germplasm; hence, a better result would have been expected. Bare any major errors, this would indicate a very specific interaction of resistance genes and those Ant races that were evaluated in Popayan.

5 | CONCLUSION

Taken together, the BCMV/BCMNV resistance markers work very well and have been implemented in mainstream breeding activities, whereas the evaluated Ant markers appear to be of limited utility for resistance breeding in Colombia. As regards the phenotypic analyses, breeding for canning and yield traits seems to work well based on the observed correlations. PHI looks like a suitable selection trait to support yield traits also in climbing bean. In contrast, combining high Fe with other traits continues to be challenging. These experiences together with the developed germplasm will strengthen the future breeding activities in climbing bean germplasm development.

ENF/CGA lines are available to other breeding programs for new crosses or testing with local checks. Seeds of these lines are available in the Bean Breeding Program at International Center for Tropical Agriculture-CIAT, Palmira, Colombia.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

M.B. lead the development and early selection stages of the population. B.R. advanced with the final experiments. A.P., H.B., and V.M. conducted the experiments in the field and provided the phenotypic data as part of the investigation. K.C. was part of post-harvest test (canning). A.P. and V.M. participated in the formal analysis related with data analysis, interpretation of phenotypic, and genotypic data. B.R. assisted in the experimental setup and data analysis. B.R. and A.P. drafted the manuscript. B.R. read and edited the manuscript. All authors read and approved the final manuscript.

ETHICS STATEMENT

This article does not contain any studies with human and animal subjects.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supporting information of this article.

ORCID

Ana Elisabeth Portilla https://orcid.org/0000-0002-8397-848X
Karen Cichy https://orcid.org/0000-0002-4311-0774

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