Agronomic evaluation of biofortified beans in Antioquia producers' farms



Evaluación agronómica de frijoles bio-fortificados en fincas de agricultores de Antioquia

doi: 10.15446/rfnam.v73n2.75588

Álvaro Tamayo-Vélez^{1*}, Gloria E. Santana-Fonseca², Mathew W. Blair³ and Carolina Ortiz-Muñoz¹

ABSTRACT

Keywords:

Human diet Iron Micronutrients *Phaseolus vulgaris* L. Zinc The objective of this research was to evaluate genotypes of iron- and zinc-enriched common beans during breeding in producers' farms. Yield, disease reaction, and commercial grain characteristics were evaluated to achieve this objective. In three locations of Antioquia (Rionegro, Jardín, and Betulia), seven bush beans and eight climbing bean genotypes were planted. A randomized complete block design with four replications was used in each location. There were significant differences between the bush and climbing bean genotypes that were evaluated. The highest yields, in all locations, were for the biofortified bean NUA 45 and the control variety Uribe Rosado, followed by the CAL 96 and AFR 612 genotypes. For the climbing beans, the highest yields were found in the G2333 genotypes, being this treatment equal to the MAC 27, a bean that is adapted to mid-climate and altitudes. The MAC 27 material is presented as a promising variety because of its high yields and tolerance to diseases, mainly anthracnose.

RESUMEN

Palabras clave: Dieta Humana Hierro Micronutrientes *Phaseolus vulgaris* L. Zinc El objetivo de esta investigación fue evaluar genotipos de fríjol común enriquecidos con hierro y zinc en fincas de productores. Para alcanzar este propósito se evaluó el rendimiento, la reacción a enfermedades y características comerciales del grano. En tres localidades (Rionegro, Jardín y Betulia) de Antioquia se sembraron siete genotipos arbustivos y ocho genotipos volubles de fríjol. Se utilizó el diseño de bloques completos al azar con cuatro repeticiones en cada localidad. Se presentaron diferencias significativas, entre los materiales arbustivos y volubles evaluados. Los mayores rendimientos en todas las localidades se presentaron para el material bio-fortificado NUA 45 y el control Uribe Rosado, le sigue en su orden los genotipos CAL 96 y AFR 612. Para los materiales volubles los mayores rendimientos se presentaron para el material G2333, este tratamiento fue igual al genotipo MAC 27, un frijol adaptado a clima y altitud medio. Se presenta el material MAC 27, como promisorio por sus altos rendimientos y tolerancia a enfermedades, principalmente a la antracnosis.

¹ Corporación Colombiana de Investigación Agropecuaria, AGROSAVIA. C.I. La Selva. Km 7 vía Rionegro – Las Palmas, sector Llanogrande. Rionegro, Antioquia, Colombia.

* Corresponding author: <atamayo@agrosavia.co>

² Facultad de Ciencias Agropecuarias. Universidad de Caldas. Cra. 25 No. 70-06, CP 170004. Manizales, Caldas, Colombia.

³ Department of Agricultural and Environmental Sciences. Tennessee State University. 3500 John A Merritt Blvd. Nashville TN, United States of America.

he common bean (*Phaseolus vulgaris* L.) is the third most important legume for human consumption worldwide (Broughton *et al.*, 2003; Saltzman *et al.*, 2013). Most of the protein consumed by poor populations comes from plant sources, which are rich in protein (Beebe, 2012), and beans play an important role in the human diet. Although they are much less important than cereals as a source of calories, beans often provide a significant percentage of carbohydrates. Like other legumes, they are also a key source of minerals, especially iron and zinc (Carvalho and Vasconcelos, 2013, FAO *et al.*, 2015).

Bean is one of the most traditional crops of Colombian agriculture because it is part of the staple food of Colombian families; it is also a source of income for peasant families. It is important for food consumption due to its nutritional content of vegetable origin and an alternative for the rural population with scarce economic resources. Also, it is important to take into account what is recommended in the nutrition system of the World Health Organization (WHO) for the design of public policies, which considers the recommended daily allowance (Recommended Dietary Allowance, RDA) of such nutrients (Simpson *et al.*, 2011; Tofiño *et al.*, 2015). Likewise, it has been detected that beans have iron and zinc contents that can supply the daily requirements of these minerals (McClean *et al.*, 2011).

Biofortification seeks to improve the nutrient density of primary food crops through conventional plant breeding, agronomic management, or genetic engineering (Blair et al., 2008; Blair, 2013; Thavarajah et al., 2009). Currently, the carotenoids as sources of provitamin A, iron, and zinc are important due to the high prevalence of deficiencies of these micronutrients in children under five years of age and women on reproductive age in developing areas of Africa, Asia and Latin America (Saltzman et al., 2013; Nestel et al., 2013). Therefore, increasing the concentration of bioavailable micronutrients (Ariza-Nieto et al., 2007) in edible crops (biofortification) has become a promising strategy in modern agriculture, which allows more nutritious foods to be accessed by more people and with the use of fewer resources (Nestel et al., 2006; Bouis and Welch, 2010; Blair et al., 2013; Vaz-Tostes et al., 2016).

As part of a biofortification program, new lines of Andean beans with high iron and zinc contents have been developed by the Andean plant breeding program of the Andean II, group led by Dr. Blair at CIAT (International Center for Tropical Agriculture). They are an alternative to solve the problem of public health in Colombia caused by a lack of micronutrients, which presents some degree of undernourishment, malnutrition, and anemia. These varieties of beans are developed to be grown in the Colombian Andean Region; they contain 60% more iron (82 ppm) and 50% more zinc (43 ppm), than the traditional varieties that have an average of 50 ppm and 28 ppm, respectively (Beebe, 2012; Brigide *et al.*, 2014).

For this reason, the objective of this research was the evaluation of bush or climbing bean genotypes enriched with iron and zinc and adapted to production areas of mid-climate and altitudes (NUA or MAC lines) in farms of producers. Participatory evaluation methodologies were applied, and trials were carried out in three locations with seven and eight, bush and climbing beans respectively, using a local control variety (Uribe Rosado) and one resistant to anthracnose (G2333).

MATERIALS AND METHODS Plant material

In three locations (Rionegro, Jardín, and Betulia), the NUA 30, NUA 35, NUA 45, NUA 56, CAL 96, and AFR 612 bush genotypes were planted, coming from the CIAT Agrosalud nursery and Dr. Blair's breeding program for Andean Nutrition (NUA). These genotypes were compared with the regional variety Uribe Rosado, which acts as a control. All NUA lines come from the backcrossing CAL 96×CAL 96×G14519), according to Blair *et al.* (2010). AFR 612 was a genotype bred during the '90s for Africa, and CAL 96 is an improved bred Calima type for mid- climates.

The varieties of climbing beans planted were also from Dr. Blair's program for mid-climate and altitude adaptation (MAC): MAC 9, MAC13, MAC 27, MAC 31, MAC 33, MAC 52, MAC 54. The variety of G2333, accession of the germplasm bank of Mexican origin, was used as a control.

A randomized complete block design with four replications was used for each group of beans (bush and climbing separately). The planting distance for bushy beans was 0.20 m between plants and 0.80 m between rows in plots of 5 m in length, and for climbing beans was 0.20 m between plants and 1.0 m between rows. For each row, 25 seeds

were sown for a total of 100 plants per replication of each genotype. The climbing beans were supported with 2 m long bamboo canes tied together with polyethylene threads, while the bush beans were planted without any type of support.

Trials in Rionegro

For the experiment in the municipality of Rionegro the plantations were held in the Research Center/Experimental Station 'La Selva' with soils of Andisol type (TyPic Melanaquand/Medial Isothermal), a flatland with high contents of organic matter, high in calcium, potassium, phosphorus, and medium to low magnesium. Regarding the minor elements, medium to high contents was present (Table 1). According to the soil analysis, it is a land of medium to high fertility.

Trials in the municipality of Jardin

The farm's soil of a bean grower in this municipality has low organic matter content, very low in phosphorus, low in potassium, high in calcium and medium in magnesium; but very high in iron content and low in zinc; in general it is a soil of low fertility (Table 1).

Table 1. Soil chemical characteristics of the municipalities of Rionegro, Jardín and Betulia.

Site	рН	OM	AI	Са	Mg	Κ	Р	Fe	Cu	Mn	Zn	В	
		%		cmol _c kg ⁻¹				mg kg ⁻¹					
Rionegro	5.4	20.2	-	8.1	1.58	1.83	80.4	229	6.7	6.5	14.3	0.20	
Jardín	5.0	3.6	-	6.6	3.10	0.15	2.0	167	1.7	30	1.9	0.08	
Betulia	4.8	5.8	2.4	5.6	2.30	0.09	2.0	201	5.5	160	2.9	nd	

pH in water (1:1); OM: Organic Matter Walkley and Black; AI: KCI (1 M); Ca, Mg, K, and Na: Ammonium acetate 1 M; P: Bray II; S: Calcium Phosphate 0.008 M; Fe, Mn, Cu, and Zn: Olsen-EDTA; B: Hot water.

Trials in the municipality of Betulia

The soil studied in this municipality of Antioquia has average contents of organic matter, low in phosphorus, potassium, and medium in calcium and magnesium; very high in iron and manganese content and low in zinc; in general, it is a soil of low fertility (Table 1).

RESULTS AND DISCUSSION Bush Bean Yields

In Table 2, it can be observed the production of the bred lines of biofortified bush beans. There were highly significant differences for the variables: pods per plant, beans per pod, and significant differences for the variables weight per row and total yield in kg ha⁻¹.

Location of Rionegro. The highest yield was obtained with the NUA 45 material, which was statistically equal to the Uribe rosado, CAL 96, and AFR 612 materials. The last two had the same performance statistically as the NUA 35 and NUA 30 materials. These materials exceeded the SCR3 genotype (1.45 t ha⁻¹) evaluated by Tofiño-Rivera *et al.* (2016); similarly, the genotype AFR 612 produced proper levels of yield and similar to those reported by Astudillo and Blair (2008). NUA 56 had a low yield, only reaching 1,390 kg ha⁻¹ compared to 2,260 kg ha⁻¹ of NUA

45 and approximately 2,000 kg ha⁻¹ of AFR 612, CAL 96, and NUA 35. Regarding the number of pods per plant, none of the materials presented significant differences. For the variable number of grains per plant, the NUA 56 material was statistically superior to the others.

Location of Jardín. In this locality, the genotypes behaved similarly to those of Rionegro. In the lines of bush beans, there were significant differences in the three measured characteristics among the different materials evaluated. The highest yields were for the material NUA 45 and the control variety Uribe Rosado followed in order by the genotypes CAL 96 and AFR 612 (Table 2). These results are similar to those obtained by Tofiño *et al.* (2011) with biofortified materials for the Caribbean region. The performance of this location was low.

Regarding the number of pods per plant, the NUA 45 material was statistically different from the NUA 56. The others had a similar behavior among them. However, for the number of grains per pod, there were no significant differences between the materials.

Location of Betulia. There were significant differences between the different bush materials evaluated. The highest

yields were for the Uribe Rosado material and the AFR 612 material, followed in order by the CAL 96, NUA 45, and NUA 35 genotypes (Table 2); however, the performance is statistically similar among them but different from the NUA 56 and NUA 30 materials. During

the evaluation, it occurred a period of intense rainfall, but the yields were medium. Maybe this environmental factor affected yields. For the number of grains per pod, the materials NUA 56 and NUA 30 were statistically lower than the others.

Table 2. Production of biofortified Bush bean lines in three locations of Antioquia.

Genotypes	Rion	egro (C.I. La	Selva)	J	ardín (in fai	Betulia (in farm)		
	Pods per plant	Grains per pod	Dry weight (kg ha ⁻¹)	Pods per plant	Grains per pod	Dry weight (kg ha ⁻¹)	Grains per pod	Dry weight (kg ha⁻¹)
NUA 56 NUA 30	27 a 25 a	5.75 a 4.00 c	1,390 c 1,719 bc	47 c 72 abc	13 a 18 a	760.4 bc 739.6 bc	5.38 c 5.89 c	796 c 870 c
NUA 45	26 a	4.25 c	2,260 a	93 a	18 a	1,072.9 a	9.25 ab	1,370 ab
NUA 35	22 a	4.00 c	1,560 bc	87 ab	20 a	653.3 c	8.25 ab	1,222 ab
Uribe Rosado	30 a	5.00 b	1,967 ab	82 ab	18 a	1,052.1 a	1.20 a	1,778 a
CAL 96	27 a	4.00 c	2,046 ab	57 bc	19 a	968.8 abc	9.38 ab	1,396 ab
AFR 612	24 a	4.50 bc	2,060 ab	77 abc	15 a	885.4 abc	1.05 a	1,556 a
SD	1.88	0.31	258.08	12.78	1.88	135.69	2.77	275.43

Means with the same letter among the column do not differ statistically, Tukey's ($P \le 0.05$).

The high yield of NUA 45 distinguishes in the studied area compared to other evaluations in Valle del Cauca (Colombia), where NUA 35 has been preferred for its precocity and high yields. Despite being an undetermined bush bean, NUA 56 has a low yield in both areas of Antioquia and Valle del Cauca.

Nutritive Quality of Biofortified Bush Beans

The overall mean for the locations was 58.6 mg kg⁻¹

for iron concentration and 34.1 mg kg⁻¹ for zinc concentration. With the ANOVA, significant differences were identified between the genotypes for both iron and zinc (Table 3). The mean comparison of each genotype showed that NUA 35 had a higher concentration of iron (71.05 mg kg⁻¹) and zinc (40.40 mg kg⁻¹), followed by CAL 96 with 60.0 mg kg⁻¹ and 32.1 mg kg⁻¹ of iron and zinc, respectively. The correlation between iron and zinc was highly significant, with a *P*<0.0123.

Table 3. Iron and zinc content for the seven genotypes evaluated in the locations of Jardin, Betulia, and Rionegro in the department of Antioquia.

Genotypes		Iron (mg kg ⁻¹)			Zinc (mg kg ⁻¹)	
	Jardín	Betulia	Rionegro	Jardín	Betulia	Rionegro
AFR612	57.59 a	52.57 b	57.06 b	31.10 a	31.86 ab	35.80 ab
CAL 96	49.96 a	51.36 b	60.06 b	25.60 a	26.15 c	32.05 bc
NUA35	55.91 a	66.77 a	71.05 a	27.72 a	33.60 a	40.40 a
NUA45	59.18 a	48.41 b	52.38 b	27.42 a	24.60 c	29.69 c
NUA56	59.40 a	54.10 b	59.40 b	26.93 a	29.73 abc	31.85 bc
Uribe Rosado	54.52 a	49.03 b	54.99 b	28.87 a	27.59 abc	34.77 bc
NUA30	-	-	55.11 b	-	-	34.28 c

Means with the same letter among the column do not differ statistically, Tukey's ($P \le 0.05$).

It was determined that Rionegro had the highest mean among the three evaluated locations, and it was the place where the NUA 35 genotype obtained the highest concentration of iron and zinc. These results were similar to those reported by Tofiño *et al.* (2016) in genotypes for the Colombian Caribbean Region; it is necessary to clarify that the soils of the moderate and cold climate in Antioquia have high contents of Fe, different from the soils of the Caribbean Region, where previous studies were conducted with Mesoamerican biofortified beans but not Andean biofortified beans as studied in Antioquia. The bioavailability of some of the NUA lines is high (Ariza Nieto *et al.*, 2007).

Climbing Bean Yields

In Table 4, it is observed the production of the climbing beans. There were highly significant differences for the variables pods per plant, grains per pod and dry weight in kg ha⁻¹ of grain.

Location of Rionegro. In this location, the material G2333 presented the highest number of grains per pod (8.25) and is statistically different from the others. The highest yields were for the climbing bean material G2333. This treatment was equal to the genotype MAC 27 and similar to the results of Blair *et al.* (2007). The other materials had similar performance. All these climbing genotypes had high yields (2 to 5 t ha⁻¹), agreeing with

what was exposed by Sida-Arreola *et al.* (2015) in which biofortified crops must be of high yield and profitable for the farmer.

Location of Jardín. Concerning the number of grains per pod, the MAC 27 (4.56) and G2333 (5.31) presented similar behavior, being statistically superior to others. For the climbing beans, significant differences were found for the materials G2333 and MAC 27, which obtained high yields between 3.8 and 4.4 t ha⁻¹ (Table 3). The other materials were equal in yield.

Location of Betulia. In the evaluation of the climbing beans, significant differences were observed between the treatments; yields fluctuated between 2,900 and 1,660 kg ha⁻¹. The highest production was for the material G2333, which was statistically different from the other materials.

Materials G2333 and MAC 27 obtained the highest production of grains per pod (2.95 and 2.41), respectively, being statistically different from the other materials. In this variable, the material with the lowest number of grains per pod (1.25) was MAC 52. For the dry weight, the same trend was observed, being statistically the best material the G2333 material with 3,933 kg ha⁻¹ and that of lower response MAC 52 with 1,660 kg ha⁻¹.

Table 4. Production of climbing bean lines adapted to mid-climate and altitude (MAC) in three locations of Antioquia.

	Rioneg	ro (La Selva)	Ja	rdín	Betulia		
Genotypes	Grains per pod	Dry weight (kg ha ⁻¹)	Grains per pod	Dry weight (kg ha ⁻¹)	Grains per pod	Dry weight (kg ha⁻¹)	
MAC 31	6.00 bc	3,142 b	2.250 b	1,875 b	1.575 cd	2,100 cd	
MAC 9	4.50 e	3,227 ab	3.125 b	2,064 b	1.588 cd	2,117 cd	
MAC 52	5.00 de	3,092 b	2.562 b	2,135 b	1.250 f	1,660 f	
MAC 33	5.00 de	3,127 b	2.812 b	2,343 b	1.650 cd	2,200 cd	
MAC 27	5.00 de	4,056 a	4.562 a	3,802 a	2.413 b	3,217 b	
G2333	8.25 a	4,073 a	5.312 a	4,427 a	2.950 a	3,933 a	
MAC 13	6.00 bc	3,121 b	3.312 b	2,760 b	1.913 c	2,550 c	
MAC 54	5.50 cd	3,054 b	3.062 b	2,552 b	1.814 cd	2,400 cd	
MAC 4	6.25 b	2,875 b	3.062 b	2,552 b	1.950 c	2,600 c	
SD	0.80	336.47	0.71	626.44	0.36	483.75	

* Means with the same letter among the columns do not differ statistically, Tukey's ($P \le 0.05$).

CONCLUSIONS

For biofortified bush beans, the highest yields were for NUA 45, surpassing CAL 96 and AFR 612, two nonbiofortified beans. The yields of these were similar to the local control variety Uribe Rosado in Jardín but not in Betulia, Antioquia. For climbing beans, the highest yields in all locations of the municipalities of Rionegro, Jardín, and Betulia were for the climbing bean material G2333. This material is equal to the MAC 27 genotype. The genotype that had the lowest yields was the material MAC 52. While the majority of MAC lines were of spotted red color, MAC 27 has a full red color that could compete with the high-priced varieties in category "Bola Roja," although they are more elongated in the form of a seed. The materials NUA 45 and MAC 27 are shown as promising beans and potential varieties for their high yields.

ACKNOWLEDGMENTS

The authors thank the farmers of Rionegro, Jardín, and Betulia, for their participation in the evaluation of the bean materials. We appreciate the careful work of Agobardo Hoyos, Alcides Hincapié, Fredy Monserrate, Guillermo Ortiz (RIP), Iván Gómez and above all, Yercil Viera in the development of the NUA and MAC lines through crosses in Darien or Palmira, Valle del Cauca, field trials and selections made in the second of these sites. It is also recognized the great support of Ing./Dr. Jose Restrepo, in the NGO, called FIDAR for believing in the usefulness of biofortified bean genotypes for the Colombian countryside and his help through multiple years in selecting in a participatory way with farmers the bush and climbing lines that were part of this study. The authors also thank the funding institutions for this research: Colombian Agricultural Research Corporation (Agrosavia), the United States Department of Agriculture (USDA), the 1890s Evans Allen Program, and the International Center for Tropical Agriculture (CIAT), Agrosalud Program.

REFERENCES

Ariza-Nieto M, Blair MW, Welch RM, and Glahn RP. 2007. Screening of iron bioavailability patterns in eight beans (*Phaseolus vulgaris* L.) genotypes using the Caco-2 cell in vitro model. Journal of Agricultural and Food Chemistry 55(19): 7950-7956. doi: 10.1021/jf070023y

Astudillo C y Blair MW. 2008. Contenido de hierro y cinc en la semilla y su respuesta al nivel de fertilización con fósforo en 40 variedades de fríjol colombianas. Agronomía Colombiana 26(3): 471-476. Beebe S. 2012. Common Bean Breeding in the Tropics. pp. 357–426. In: Janick J (ed.). Plant Breeding Reviews (Vol. 36). John Wiley & Sons, Hoboken. 535 p.

Blair MW, Hoyos A, Cajiao C and Kornegay J. 2007. Registration of two mid-altitude climbing beans with yellow grain color, MAC56 and MAC57. Journal of Plant Registration 1(2): 143-144. doi: 10.3198/ jpr2006.09.0571crg

Blair MW, Monserrate F, Astudillo C, Hoyos A, Vieira Y and Hincapié A. 2008. Bean with improved micronutrient concentration that have a positive impact of human health. Annual Report Bean Program, CIAT. 302 p.

Blair MW, Monserrate F, Beebe SE, Restrepo J and Ortubé J. 2010. Registration of high mineral common bean germplasm lines NUA35 and NUA56 from the red mottled seed class. Journal of Plant Registration 4(1):55-59. doi: 10.3198/jpr2008.09.0562crg

Blair MW. 2013. Mineral Biofortification Strategies for Staples: The Example of Common Bean. Journal of Agricultural and Food Chemistry 61(35): 8287-8294. doi: 10.1021/jf400774y

Blair MW, Izquierdo P, Astudillo C and Grusak MA. 2013. A legume biofortification quandary: variability and genetic control of seed coat micronutrient accumulation in common beans. Frontiers in Plant Science 4: 275. doi: 10.3389/fpls.2013.00275

Bouis HE and Welch RM. 2010. Biofortification. A sustainable agricultural strategy for reducing micronutrient malnutrition in the global South. Crop Science 50(51): S20–S32. doi: 10.2135/ cropsci2009.09.0531

Brigide P, Canniatt-Brazaca S and Silva MO. 2014. Nutritional characteristics of biofortified common beans. Food Science and Technology. 34(3):493-500. doi: 10.1590/1678-457x.6245

Broughton WJ, Hernandez G, Blair MW, Beebe S, Gepts P and Vanderleyden J. 2003. Beans (*Phaseolus* spp.) – model food legumes. Plant and soil 252: 55-128. doi: 10.1023/A:1024146710611

Carvalho SMP and Vasconcelos MW. 2013. Producing more with less: Strategies and novel technologies for plant-based food biofortification. Food Research International 54(1): 961–971. doi: 10.1016/j.foodres.2012.12.021

FAO, FIDA y PMA. 2015. El estado de la inseguridad alimentaria en el mundo 2015. Cumplimiento de los objetivos internacionales para 2015 en relación con el hambre: balance de los desiguales progresos. FAO, Roma.

McClean PE, Burridge J, Beebe S, Rao IM and Porch TG. 2011. Crop improvement in the era of climate change: an integrated, multidisciplinary approach for common bean (*Phaseolus vulgaris*). Functional Plant Biology 38(12): 927-933. doi: 10.1071/FP11102

Nestel P, Bouis HE, Meenakshi JV and Pfeiffer W. 2006. Biofortification of staple food crops. The Journal Nutrition 136(4): 1064–1067. doi: 10.1093/jn/136.4.1064

Saltzman A, Birol E, Bouis HE, Boy E, De Mouea FF, Islam Y and Pfeiffer WH. 2013. Biofortification: progress toward a more nourishing future. Global Food Security 2(1): 9–17. doi: 10.1016/j.gfs.2012.12.003

Sida-Arreola JP, Sánchez E, Ávila-Quezada GD, Acosta-Muñíz CH and Zamudio-Flores PB. 2015. Biofortificación con micronutrientes en cultivos agrícolas y su impacto en la nutrición y salud humana. Tecnociencia Chihuahua 9(2): 67-74.

Simpson JL, Bailey LB, Pietrzik K, Shane, B and Holzgreve W. 2011. Micronutrients and women of reproductive potential: required dietary intake and consequences of dietary deficiency or excess. Part II - vitamin D, vitamin A, iron, zinc, iodine, essential fatty acids. The Journal of Maternal Fetal Neonatal Medicine 24(1):1-24. doi: 10.3109/14767051003678226

Thavarajah D, Thavarajah P, Sarker A and Vandenberg A. 2009. Lentils (*Lens culinaris* Medikus Subspecies *culinaris*): A Whole Food for Increased Iron and Zinc Intake. *Journal of Agricultural and Food Chemistry 57*(12): 5413-5419. doi: 10.1021/jf900786e

Tofiño A, Tofiño R, Cabal D, Melo A, Camarillo W y Pachón H. 2011. Evaluación agronómica y sensorial de fríjol (*Phaseolus vulgaris* L.) mejorado nutricionalmente en el norte del departamento del Cesar, Colombia. Perspectiva en Nutrición Humana 13(2):161-177.

Tofiño A, Melo A, Ruidiaz Y and Lissbrant S. 2015. Evaluation of the potential dietary impact of the implementation of nutritionally improved crops in rural areas of the department of Cesar (Colombia). Agronomía Colombiana 33(3): 383-390. doi: 10.15446/agron.colomb. v33n3.51984

Tofiño-Rivera AP, Pastrana-Vargas IJ, Melo-Ríos AE, Beebe S y Tofiño-Rivera R. 2016. Rendimiento, estabilidad fenotípica y contenido de micronutrientes de genotipos de fríjol biofortificado en el Caribe seco colombiano. Corpoica Ciencia y Tecnología Agropecuaria 17(3): 309-329. doi: 10.21930/rcta.vol17_num3_art:511

Vaz-Tostes MG, Verediano TA, de Mejia EG and Costa NMB. 2016. Evaluation of iron and zinc bioavailability of beans targeted for biofortification using *in vitro* and *in vivo* models and their effect on the nutritional status of preschool children. Journal of the Science of Food and Agriculture 96(4): 1326–1332. doi: 10.1002/jsfa.7226