

## **‘San Blas’: a variety of Negro Opaco beans resistant to BCMV and BCMNV for Nayarit and The Bajío of Guanajuato**

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### **Abstract**

A new variety of beans is described, ‘San Blas’, whose main characteristics are its type of grain in high demand in Mexico and its resistance to the common bean mosaic virus (BCMV) and the mosaic virus and common bean necrosis (BCMNV), since it has the combination of genes *II + bc-3*. San Blas was derived from the cross between Jamapa Plus/XRAV-187-3. The final selection of the line that gave rise to San Blas was carried out by inoculation with the necrotic strain BCMNV NL-3, and genotyping with the molecular markers (MMs) SW13 and ENM, associated with genes *II* and *bc-3*, respectively. The San Blas plant has an indeterminate, semi-erect type II habit, with lilac-purple flowers, straw-colored pods at maturity and Negro Opaco grain, oval in shape and small in size similar to Jamapa. In the Bajío, its flowering and physiological maturity occur around 45 and 92 days after sowing (dds); while in Nayarit at 40 and 80 dds, respectively. After selection with MMs and inoculation, the average yield of San Blas in six different environments in Guanajuato was 1841 kg ha<sup>-1</sup>, 12% more than that of Negro 8025; while in three residual moisture environments in Nayarit it was 1 927 kg ha<sup>-1</sup>, surpassing the Jamapa and Coranay varieties by 50 and 36%, respectively.

**Keywords:** *Phaseolus vulgaris* L., residual moisture, virus resistance BCMV and BCMNV.

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The Negro Opaco bean of Mesoamerican race has a wide distribution in tropical areas of Mexico (FIRA, 2016). Its high demand in the country justifies its importation in large volumes and the need to have different varieties that promote the production of this type of grain. In Nayarit and other producing regions, due to ignorance of the various varieties that have developed within this type of bean, the Jamapa variety is still used, of which probably only the name remains, since the current version is very different from the original described by Cárdenas-Ramos and Velo (1964).

The viruses BCMV and BCMNV, cause the common mosaic diseases and the black root of the beans, these in addition to the virus of the golden yellow mosaic of the beans (BGYMV), which produces the disease of the golden yellow mosaic of the beans, significantly affect the production of the beans in Nayarit and other tropical areas of the country (Chiquito-Almanza *et al.*, 2014; Chiquito-Almanza *et al.*, 2017), while BCMV and BCMNV mainly affect cultivation in The Bajío region (Chiquito-Almanza *et al.*, 2017). The best strategy against these diseases is to use healthy seed of resistant varieties (Acosta-Gallegos *et al.*, 2016).

The dominant variety of black beans in the state of Nayarit is Jamapa (FIRA, 2016), which could be considered obsolete, however, its fame precedes it. In Guanajuato, the only Negro Opaco bean variety used on a small scale is Negro 8025. The Bajío, together with Nayarit, could contribute to the production of black beans to achieve self-sufficiency. For which it is necessary to develop high-yield varieties, tolerant to diseases and adapted to the conditions of both regions.

### Origin and process of obtaining

‘San Blas’ originated from the simple cross Jamapa Plus/XRAV-187-3 carried out in 2010 in The Bajío Experimental Field (CEBAJ) of the INIFAP (Table 1). The Jamapa Plus variety was used as a progenitor due to its wide adaptation to the humid tropics and The Bajío and because of its high commercial acceptance grain; the XRAV-187-3 line was used as a source of resistance to BCMV, BCMNV. Jamapa Plus was derived through mass selection of the Jamapa variety under pressure of a viral complex under field conditions (Comun. Pers. Ernesto Lopez Salinas CECOT-INIFAP, 2010).

**Table 1. Development process of the Negro Opaco bean variety ‘San Blas’.**

Year	Activity	Observations
2010 R and T	Cross Negro Jamapa Plus/XRAV-187-3	Crossing and advancement of F <sub>1</sub> in the greenhouse and of F <sub>2</sub> in the field at CEBAJ
2011 R	Sowing population F <sub>3</sub>	Selection of healthy plants in field conditions with high incidence of black root
2011 T	Planting resistant F <sub>4</sub> families	Furrow per family, and at the stage of physiological maturity, selection was made between and within furrows for health and pod load

Year	Activity	Observations
2012 R and T	Sowing F <sub>5</sub> and F <sub>6</sub>	Furrow per family, and in physiological maturity selection between and within furrows for health and pod load
2012/2013 and 2013 R	Sowing individual selections F <sub>6</sub>	Generation advance to get F <sub>7</sub> and F <sub>8</sub>
2013 T	Sowing uniform families F <sub>8</sub>	Preliminary yield trial sowing in Guanajuato, Veracruz and Chiapas
2013/2014 R	Sowing of F <sub>9</sub> lines	Preliminary trial planting in Nayarit (A-W)
2015/2016	F <sub>10</sub> line selection	Line clearance due to its resistance to inoculation with BCMNV NL-3, and the presence of MMs associated with genes <i>II</i> and <i>bc-3</i>
2016 T	Yield test	Sowing in Guanajuato in the spring-summer cycle (S-S) and in Nayarit (A-W)
2017/2018 R	Yield testing and validation in Nayarit	Sowing in Guanajuato (S-S) and Nayarit (A-W), validation in three locations

R= irrigation; T= temporary.

The XRAV-187-3 line was developed from the PR0003-124 Raven cross (Beaver *et al.*, 2014), Raven contains genes *II* and *bc-3* that confer broad spectrum resistance to BCMV and BCMNV (Kelly *et al.*, 1994). ‘San Blas’ was obtained by the genealogical method, carrying out two generations per year until its standardization and inclusion in yield tests.

In the F<sub>2</sub> and F<sub>4</sub> generations, under irrigation during the early season of the year, in CEBAJ healthy plants were selected under pressure of BCMV and BCMNV under natural field conditions, which was confirmed by reverse transcription and polymerase chain reaction (RT-PCR) following the protocol described by Chiquito-Almanza *et al.* (2017). Subsequently, individual selection was made in generations F<sub>5</sub> and F<sub>6</sub>.

In 2013, the F<sub>8</sub> lines were evaluated in Guanajuato under storm and natural presence of diseases such as rust, powdery mildew, common mosaic, common blight and halo blight. During the autumn-winter cycle (A-W) 2013/14 the trial was established in Nayarit, the line (Jamapa Plus/XRAV-187-3)-M-M-9-M-3, which gave rise to ‘San Blas’, showed resistance in the field to BCMV and BCMNV viruses. In 2015, together with other 70 bean genotypes, it was phenotyped by artificial inoculation with the BCMNV NL-3 strain at CEBAJ facilities under greenhouse containment conditions and genotyped with molecular markers (MMs) SW13 (Melotto *et al.*, 1996) and ENM (Naderpour *et al.*, 2010), associated with resistance genes *I* and *bc-3*, respectively, following the methodology described by Anaya-López *et al.* (2018). From 2016 to 2019, the line that gave rise to the ‘San Blas’ variety was evaluated in Guanajuato and Nayarit. Table 1 summarizes the development of the ‘San Blas’ variety.

### Agronomic characteristics of the San Blas variety

The San Blas plant is of indeterminate type II semi-erect habit, with lilac-purple flowers, medium-sized dark green leaves, straw-colored pods at maturity, and grain similar to Jamapa, Negro Opaco in color and small in size. In The Bajío, its flowering and physiological maturity occurs around 45 and 92 days after sowing, respectively (Tables 2 and 4), while in Nayarit at 40 and 80 dds, respectively (Table 4). The average yield of San Blas in six different environments in Guanajuato was 1841 kg ha<sup>-1</sup>, 12% more than Negro 8025; while in three different environments in Nayarit it was 1927 kg ha<sup>-1</sup>, 50 and 36% more than Jamapa and Coranay (Table 4).

In the 2013 storm cycle in CEBAJ, Celaya presented high pressure of common blight, halo blight, ash and rust. With the exception of the experimental line (Jamapa Plus/XRAV-187-3)-M-4-10 and the Jamapa Plus and Negro Guanajuato varieties, the San Blas variety had yield values slightly higher than the average of the other materials, in weight of 100 seeds, its value was similar to all materials and showed lower values of reaction to naturally occurring diseases (Table 2).

**Table 2. Comparison of agronomic characteristics and response to foliage diseases of ‘San Blas’ with seven Negro Opaco bean genotypes established in 2013 in Celaya, Guanajuato.**

Genotype	Yield	P100S	DF	MDZ	TC	TH	C	RY
(Jamapa Plus/XRAV-187-3)-M-4-10	2295	22.8	44	89	3	4	2	1
(Jamapa Plus/XRAV-187-3)-M-2-8	1507	23.3	47	93	4	4	2	2
San Blas	1941	24.6	44	92	2	3	2	1
Negro Comapa	1490	24.9	47	93	3	3	3	1
Verdin	1765	28.3	40	86	2	2	2	2
Jamapa Plus	2047	23.2	45	95	3	4	2	1
Negro Guanajuato	2052	23.6	48	95	3	3	1	1
Negro 8025	1928	23.1	46	93	4	3	2	2
Average	1831	24.4	46	91	3	3	2	2

Yield= yield (kg ha<sup>-1</sup>); P100S= weight of 100 seeds (g); TC= reaction to common blight; TH= reaction to halo blight; C= reaction to mildew; RY= reaction to rust. On a scale of 1 to 9 (Shoonhoven and Pastor-Corrales, 1987). DF= days to flowering; MDZ= days to maturity.

Regarding the incidence of viruses causing black root, Verdin, Jamapa, Negro Guanajuato and Negro 8025 showed a low percentage of infected plants, while San Blas and other sister lines were not affected. For common blight, the line (Jamapa Plus/XRAV-187-3)-M-2-8 and the Negro 8025 variety showed the highest incidence. For halo blight, the line (Jamapa Plus/XRAV-187-3)-M-4-10 and Jamapa Plus showed the highest incidence. For mildew only Negro Comapa showed a value above 3 and for rust only (Jamapa plu/XRAV-187-3)-M-4-10. In days of flowering and maturity, Verdin showed the least number, while Jamapa Plus and Negro Guanajuato were the latest.

In the evaluation of San Blas y Negro 8025 in Celaya, Guanajuato under three fertilization treatments during the S-S 2017 cycle in rainfed conditions plus supplementary irrigation to flowering, the highest average yield corresponded to San Blas (Table 3); this result was partly due to the characteristic of resistance to the BCMV and BCMNV viruses of San Blas, since it did not show incidence of common mosaic or black root under any fertilization treatment, while Negro 8025 showed incidence of black root through treatments (>10%). Across varieties, the average dose was higher in yield ( $p < 0.01$ ) and the sub-optimal and optimal doses showed a similar yield. Regarding the weight of 100 seeds, the two varieties were similar and were within the acceptable commercial range for beans of the small Negro Opaco type, similar to that of the Jamapa variety to which they belong (Table 3).

**Table 3. Yield of the variety ‘San Blas’ under rainfed conditions in Celaya, Guanajuato 2017, under three basic fertilization treatments plus foliar application of macro and microelements.**

Fertilization	Negro 8025*		San Blas		Average	
	Yield	P100S	Yield	P100S	Yield	P100S
Sub-optimal	2142	21.3	2476	22.2	2237 b	21.7 a
Half	2443	20.9	3041	21.3	2642 a	21.5 a
Optimal	2024	20.3	2694	22.7	2309 ab	21.2 a
Average	2137 b	20.9 b	2737 a	22.1 a		

\*= Negro Opaco bean control, Sub-optimal fertilization 20-20-0 (N-P-K); half fertilization= 40-40-0; optimal fertilization= 60-60-10; with 0, 1 and 2 complete foliar applications of macro and microelements in each dose, respectively; P100S= weight of 100 seeds (g); Yield= yield (kg ha<sup>-1</sup>). Values with different letters are different ( $p < 0.01$ ).

In rainfed and irrigated conditions in The Bajío, San Blas was occasionally superior to Negro 8025, both in seed weight and yield (Table 4). As for the days to start flowering, in general San Blas starts in less time than Negro 8025 and as the maturity is similar, San Blas has a longer reproductive period.

In 2017, under a storm plus an aid irrigation, the maximum yield of San Blas in the locality was observed, of 2 731 kg ha<sup>-1</sup>, while the lowest was obtained in the late 2018 sowing under temporary and under strong pressure by leafhopper (*Empoasca kraemeri*).

Regarding the weight of the seed, San Blas and Negro 8025 were similar. In an average of six different environments, San Blas exceeded the yield of Negro 8025 by 12% (Table 4). In residual humidity conditions in the A-W 2017/2018 cycle in the towns of Ruiz, Santiago Ixcuintla and San Blas, Nayarit; the average yield was 1 927 kg ha<sup>-1</sup>, 50 and 36% more than the Jamapa and Coranay varieties, respectively (Table 4). San Blas showed values of 1 in the reaction to the common mosaic and the black root on the Shoonhoven and Pastor-Corrales scale (1987) across all the test locations, while the control varieties showed values between 2 and 4 (Table 4), which confirmed the resistance to BCMV and BCMNV of San Blas.

**Table 4. Agronomic characteristics and resistance to common mosaic and the black root of ‘San Blas’ compared to ‘Negro 8025’ in a locality of The Bajío Guanajuatense, and with ‘Jamapa’ and ‘Coranay’ in three localities of Nayarit.**

Year	Variety	DF	DMZ	Yield	P100S	MC <sup>f</sup>	RN <sup>f</sup>
The Bajío, Guanajuato							
2016 T <sup>a</sup>	San Blas	50 a	92	1561 a	19.2 a	ND	ND
	Negro 8025	51 a	90	1569 a	19.3 a	ND	ND
2017 T <sup>a</sup>	San Blas	43 b	92	2731 a	22.1 a	1	1
	Negro 8025	47 a	91	2137 b	20.9 b	1	4
2018 R <sup>b</sup>	San Blas	42 b	97	1494 a	20.4 a	1	1
	Negro 8025	46 a	100	1525 a	20.9 a	1	3
2018 T <sup>c</sup>	San Blas	41 b	82 a	1242 a	25.8 a	1	1
	Negro 8025	47 a	82 a	1336 a	23.2 b	1	3
2019 R <sup>d</sup>	San Blas	40 b	96 b	2079 a	24.5 b	1	1
	Negro 8025	45 a	103 a	1217 b	26.7 a	1	3
Nayarit							
2017/2018 HR	Jamapa	48 b	101 b	963 c	18 ab	4 b	2 a
	Coranay	41 a	92 ab	1235 b	18 ab	3 b	1 a
	San Blas	40 a	80 a	1927 a	19 ab	1 a	1 a

DF= days to flowering; MDZ= days to maturity; Yield= yield (kg ha<sup>-1</sup>); P100S= weight of 100 seeds (g); MC= reaction to common mosaic; RN= black root reaction; ND= not determined; <sup>a</sup>= temporary low with supplementary irrigation in flowering; <sup>b</sup>= under temperatures above 32 °C in the reproductive stage; <sup>c</sup>= in storm under pressure by *Empoasca kraemeri*; <sup>d</sup>= with pre-sowing irrigation and two auxiliary irrigations; <sup>f</sup>= Scale from 1 to 9 (Shoohoven and Pastor-Corrales, 1987). Values with different literals are different ( $p < 0.01$ ).

## Conclusions

The ‘San Blas’ variety was resistant to BCMV and BCMNV in all the test locations. This is the first variety in Mexico that includes the combination of resistance genes *I* and *bc-3*. In The Bajío, the San Blas variety with an average yield of 1 841 kg ha<sup>-1</sup> was 12% higher than Negro 8025. In Nayarit the average yield was 1 927 kg ha<sup>-1</sup>, 50 and 36% higher than that of the varieties local Jamapa and Coranay. The first author can be contacted to obtain seed samples for research purposes or to be used as a parent by giving the corresponding credits to the National Institute of Forestry, Agricultural and Livestock Research.

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