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Detection of Virus Damaging the Dry Bean Crop in Nayarit, Mexico

Elizabeth Chiquito-Almanza[1], Jorge A. Acosta-Gallegos[2], Nadia C. García-Álvarez[3], Wilmer Cuellar[4], Talina O. Martínez-Martínez[2], José Luis Anaya-López[2,*]

[1] Instituto Tecnológico de Celaya, Biochemistry Department, México.

[2] INIFAP-Campo Experimental Bajío, Biotechnology Unit, México.

[3] INIFAP-Campo Experimental Santiago Ixcuintla, Bean and Chickpea Program, México.

[4] International Center for Tropical Agriculture, Virology Laboratory, Colombia.

Abstract: In the state of Nayarit, Mexico, the common bean crop is severely damaged by viral diseases. The aim was to identify the viral species that damage the bean crop in Nayarit, a knowledge needed to generate resistant cultivars for this state. During the fall-winter cycle 2013/14, forty-two samples of foliar tissue from plants displaying symptoms were gathered from fifteen commercial rainfed bean fields in the main producing areas of Nayarit. Total DNA was extracted from each sample to detect by PCR, DNA viruses and total RNA to detect via RT-PCR, RNA viruses. We use the primers that identify the eight most prevalent viruses in Latin America. In 45 and 52 % of the samples we detected BCMV and BCMNV, respectively. The genus *Begomovirus* was detected in 46 % out of 35 samples. In regard to samples showing single infection, 45 % were either infected by BCMV, BCMNV or a *Begomovirus*; in 43 % mixed infections were detected, 14 % of those were BCMV and BCMNV, 10 % BCMV plus a *Begomovirus*, 14 % BCMNV plus a *Begomovirus*, and 5 % were a mixture of the three viruses. Undetected viruses were CMV, AMV, CPMMY, BYMV, PStV and PeMoV. *Potyviruses* BCMV and BCMNV were the predominant viruses in the dry beans of Nayarit followed by the genus *Begomovirus*. Studies are being conducted to identify the *Begomovirus* species collected.

Key words: *Phaseolus vulgaris*, viral diseases, infections.

INTRODUCTION

The bean crop is an important occupational and source of economic resources for the population and its grain is the main and only source of proteins for the poorest sectors that represent 45.5 % of the Mexican population[1]. More than 50 % of the national area planted with dry beans occurs in the states of Chihuahua, Durango and Zacatecas under rainfall conditions. In 2011 those states produced 28 % of the national dry bean volume. However, from 2009 to 2013 the national production[2] diminished 21 % mainly due to the effects of the climatic change that in addition to drought stress is contributing towards the surging of new insect pests and diseases that are affecting the bean crop even in regions with high moisture availability. This situation has prompted the bean crop to be moved to new areas with other particular problems, such as the state of Nayarit in the central Pacific region of Mexico.

In 2012 Lepe-Soltero *et al.* [3] conducted surveys in the main bean producing areas of Mexico with the aim of defining the distribution of the *Bean common mosaic virus* (BCMV) and *Bean common mosaic necrotic virus* (BCMNV), two of the main viral diseases that cause significant dry bean losses around the world. In that study the BCMV was detected in almost all regions sampled, whereas BCMNV was preferentially found in the lowland tropical regions[3], and although samples were taken on the basis of characteristics viral symptoms for those viruses, more than 50 % of the samples were negative, a fact that suggests that other viruses are damaging the dry bean crop in those regions. In addition, the high incidence of BCMNV found in the state of Nayarit suggests that infected grain is being used as seed for planting[3].

The BCMV and BCMNV are seed transmitted and in the field by aphids; its incidence is related to cropping susceptible cultivars, prevalent climatic conditions and interstate and international movement of infected seed. The infected seed is the most effective vehicle for the propagation of these viruses. For above reasons, the use of highly infected areas with BCMV and BCMNV, such as the state of Nayarit, for seed increases of susceptible cultivars adapted to other regions, represents a high risk of distributing infected seed towards other states. For example, during the fall-winter season of 2013/14, 4000 tons of seed of the BCMNV susceptible cultivar Pinto Saltillo (it carries the *II* gene that gives resistance towards BCMV, Sanchez *et al*., 2004) was produced in Nayarit and distributed in the state of Durango[4].

The most economical way of preventing damage by these viral diseases is to grow cultivars developed with durable resistance throughout the pyramiding of resistance genes. This process can be facilitated with the aid of molecular markers (MAS) in combination with direct confrontation against these *Potyvirus*. However, we do not know which strains of BCMV and BCMNV are present in Nayarit or if there are other viruses affecting dry bean production in this state. In general, the knowledge of the viruses that attack the bean crop in each region of the country is needed in order to develop cultivars with specific or broad resistance against viruses for each region, as well as the establishment of norms to regulate dry bean seed movement across regions and states. The development of efficient molecular markers for MAS is essential in order to anticipate the distribution and development of more aggressive viral strains due to the possible recombination between the viruses that co-infect the dry bean crop. The aim was to identify the viral species that damage the bean crop in Nayarit, Mexico. In the near future, this information will be useful for the development of virus resistant cultivars for this state.

METHODS

During the fall-winter growing season of 2013/2014, forty-two samples of foliar tissue from plants displaying viral symptoms were collected in 15 rainfed commercial bean fields in nine municipalities of Nayarit **(Table 1)**. From each plant three or four young trifoliate were collected, preferably from 0.6 to 2 cm in length, immediately they were placed between two sheets of silk (CHE Scientific) and introduced in plastic ziploc bags with 30 g of silica gel (Golden Bell). Plastic bags were kept in a hermetic plastic container. After two days the original silica gel was replaced with 10 g of new silica and kept in a hermetic container at -80 °C until its use for the extraction of total RNA or DNA.

Table 1: Incidence of BCMV, BCMNV and *Begomovirus* in dry bean fields grown in Nayarit, Mexico. Fall-winter cycle of 2013/14.

Location	Number of infected samples		BCMV plus BCMNV	*Begomovirus* plus			ND¶
	Single	Mixed*		BCMV	BCMNV	BCMV and BCMNV	
Santiago Ixcuintla	6	6 (33 %)	0	0	5	1	0
Ixtlán del Río	1	0	0	0	0	0	1
Compostela	2	5 (28 %)	0	4	0	1	0
Ruíz	3	3 (17 %)	3	0	0	0	1
Rosamorada	1	1 (5.5 %)	1	0	0	0	1
Tuxpan	2	1 (5.5 %)	0	0	1	0	0
Aguamilpa	2	1 (5.5 %)	1	0	0	0	0
Acaponeta	1	0	0	0	0	0	0
Tecuala	0	1 (5.5 %)	1	0	0	0	2
% Total	45	43	14	10	14	5	12

***: Between parenthesis is the percentage of mixed infections per site. ¶: No detected.**

For the detection of DNA viruses, total DNA was extracted by the CTAB method[5]. For the detection of RNA viruses by RT-PCR, RNA was extracted using Trizol (Invitrogen) following the instructions of the maker. For the study, primers of eight of the most commonly virus associated to dry beans in Latin America were selected: *Potyvirus*, *Begomovirus*, *Cucumovirus*, *Alfamovirus* and *Carlavirus*[6]. PCR and RT-PCR amplifications were done with specific primers and conditions reported for each virus: *Bean yellow mosaic virus* (BYMV)[7], *Peanut stripe virus* (PStV)[8], *Peanut mottle virus* (PeMoV)[8], *Cucumber mosaic virus* (CMV)[9], *Alfalfa mosaic virus* (AMV)[10], and *Cowpea mild mottle virus* (CPMMV)[11]. *Potyvirus* BCMV and BCMNV were detected in a multiplex reaction with the primers described by Rodríguez *et al.*[12] (RU: 5'GCAACAACCAGCGAGAATACTGA3', D1BCMV: 5'AAATGTGGTACAATGCTGTGAAGG3' and D1BCMNV: 5´GAGGTGTATGAATCCGTGTCAACA3'), whereas the genus *Begomovirus* was detected with the degenerated primers reported by Rojas *et al.*[13]. Amplifications were done in a thermo cycler Thermal T-100 from BioRad. Amplified products were verified in agar gels at 1 % with TAE 1X and were compared with the amplicon size reported in the corresponding paper.

RESULTS

Single and mixed infections of the *Potyvirus* BCMV, BCMNV and from the genus *Begomovirus* were detected. In 45 and 52 % of the samples, BCMV and BCMNV were detected, respectively. The genus *Begomovirus* was detected by degenerated primers in 46 % out of 35 analyzed samples. Same samples were further analyzed by PCR using specific primers against the *Begomovirus* more frequently associated with dry bean, such as the *Bean golden mosaic virus* (BGMV), *Bean dwarf mosaic virus* (BDMV), *Bean calico mosaic virus* (BCaMV) and *Bean golden yellow mosaic virus* (BGYMV).

In regard to those samples with single infections, 45 % were infected with BCMV, BCMNV or some virus from the genus *Begomovirus*. Forty-three percent displayed mixed infections, of these 14 % corresponded to the mixture of BCMV and BCMNV, 10 % to BCMV and a *Begomovirus*, 14 % to BCMNV and a *Begomovirus*, and 5 % to the mixture of BCMV, BCMNV and a *Begomovirus* **(Table 1**). Virus CMV, AMV, CPMMY, and the *Potyvirus* BYMV, PStV and PeMoV were undetected.

The municipalities where larger number of mixed infections were observed are Santiago Ixcuintla with 33 %, Compostela 28 %, Ruiz 17 %, Rosamorada, Aguamilpa and Tecuala with 5.5 % each **(Table 1)**. The incidence of mixed infections of BCMV, BCMNV and/or a *Begomovirus* was of 43 %. Cultivars with single infections of BCMV, BCMNV or some *Begomovirus* were Coranay (Mesoamerican), Mayocoba (Andean), Peruano (Andean), Negro Jamapa (Mesoamerican), Azufrado (Nueva Granada), and more black seeded cultivars non-identyfied, probably from the Mesoamerican gene pool **(Table 2).**

The lack of BCMV in cultivars Coranay and Costenay suggests that these cultivars must carry a *bc* gene that gives them resistance to this viral species. This observation is supported by the fact that both cultivars presented BCMNV along with mosaic and curly symptoms, without the presence of necrotic lesions, indicating that the resistance to the BCMV in not mediated by the *II* gene. For example, differential cultivars from group 4: Sanilac, Michelite 62 and Red Mexican 34, with the recessive *bc2* gene are resistant to strains NL1, NL7, FLA, NL6 and NL4 of BCMV, but develop mosaic symptoms when inoculated with strains NL3, NL5 and NL8 of BCMNV[14]. This finding emphasizes the importance of knowing the specific strains of BCMV and BCMNV that exist in the state of Nayarit.

In cultivars Mayocoba (Andean) and Pinto Saltillo (Durango) there were mixed infections of BCMV, BCMNV and a *Begomovirus*; whereas in Mayocoba, Peruano, Negro Jamapa and Pinto Saltillo, BCMV and a *Begomovirus* were found. The mixed infection of BCMV and BCMNV were identified in cultivars Peruano and Azufrado **(Table 2)**. The occurrence of mosaic symptoms along with the detection of BCMV and BCMNV in cultivars Mayocoba, Peruano, Negro Jamapa, Pinto Saltillo and Azufrado without the development of necrotic reactions, suggested the lack of *II* gene and the action of a *bc* gene. In all cultivars tested, except Coranay, a *Begomovirus* associated with the symptoms of *Bean golden yellow mosaic virus* was detected, which suggest that all these cultivars lack resistance to this viral species. In 17 %, 33 % and 9 % of the plants from cultivars Peruano, Negro Jamapa and Azufrado, respectively, none of these last viruses were detected **(Table 2).**

Point mutations and genetic exchange are the main sources of genetic variation in plant viral populations[15]. In many DNA and RNA viruses these exchanges are realized by recombination[16-18]. For that reason, the presence of mixed infections of BCMV, BCMNV and *Begomovirus* detected in the dry bean samples from Nayarit, Mexico, is an important element that must be taken into consideration to cope with viral diseases

in the region, since them can lead to viral recombination, producing phenotypic changes that can promote the adaptation of the virus, modify its virulence and/or the range of hosts[19, 20].

Table 2: Incidence of virus detected in distinct dry bean cultivars collected in Nayarit state

Cultivar	Race	% Incidence of							ND	TS
		BCMV	BCMNV	Bego	BCMV plus BCMNV	Bego plus BCMV	Bego plus BCMNV	Bego plus BCMV and BCMNV		
Coranay	M	-	100	-	-	-	-	-	-	4
Costenay	M	-	-	-	-	-	100	-	-	4
Mayocoba	NG	17	-	17	-	50	-	17	-	6
Peruano	NG	33	-	-	17	33	-	-	17	6
Negro Jamapa	M	-	17	17	-	17	17	-	33	6
Pinto Saltillo	DGO	-	-	-	-	50	-	50	-	2
Azufrado	NG	36	27	9	18	-	-	-	9	11
Negro†	M	-	-	50	-	-	50	-	-	2

†: Non-identified cultivar. In the Race column M: Mesoamerican, NG: Nueva Granada, DGO: Durango[21]. The acronyms Bego: indicated infection caused by *Begomovirus*, ND: No detected and TS: Total of samples.

Mixed infections of *Begomovirus* and *Potyvirus* has been detected in other crops, such as cassava (*Manihot esculenta* Crantz)[5] and bell pepper (*Capsicum annuum* L.)[22]. In dry beans viral recombination has been reported between the pathotypes BCMV US-5 and BCMNV NL-8[23], being the recombinant of higher virulence as had occurred with BCMNV NL3-K that provokes earlier symptoms and more severe than NL3. The analysis of the sequence of the putative P1 protein suggested that the strain BCMV NL3-K is a recombinant between the strain BCMNV NL3-D and BCMV RU-1[24]. Another case is that of the strain BCMV RU-1M, recently isolated and identified, that in contrast with the known strains of BCMV, is capable of inducing necrosis at temperatures below 30 ºC in bean plants carrying the dominant gene *I* and the recessive gene *bc-1*. The genomic confronting of this new strain with the genome from other strains suggests that the strain RU1M is a recombinant between strain BCMV RU1-OR and an unknown *Potyvirus*[25].

The recombination among *Begomovirus* is also a common phenomenon and is the driving force in the evolution of new species and viral strains[26, 27]; in tomato the recombination among strains from the *Begomovirus tomato yellow leaf curl sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV) has been studied in mixed infections[28], while in *capsicum* an enhanced combined effect of the *Begomovirus Pepper huasteco yellow vein virus* (PHYVV) and *Pepper golden mosaic virus* (PepGMV) has been demonstrated[29].

From the above, the high incidence of mixed infections of BCMV and BCMNV in the bean production systems of Nayarit suggest that in this state there is a high potential risk for the evolution of new strains of *Potyvirus* and *Begomovirus* by recombination. Therefore, the detailed evaluation of the diversity and adaptive capacity of these virus will help to improve the efficacy of the practices of control, and for adjusting the integrated management of the crop and to take decisions on the bean areas where those

practices must be strictly implemented year after year, as well as to indicate in which areas new bean cultivars developed with broad resistance, must be introduced.

CONCLUSIONS

The *Potyvirus* BCMV and BCMNV were the more frequently detected in the bean crop grown in state of Nayarit during the fall-winter cycle, followed by the genus *Begomovirus*. Further studies are required to identify the *Begomovirus* species that are prevalent in this state. An option might be to use more robust molecular techniques such as Small RNA Deep Sequencing (SRDS) that do not requires previous knowledge on the presumptive viral species infecting the plant.

Cultivars of all seed types being grown in the state displayed the presence of *Potyvirus* and *Begomovirus*, therefore, the development of cultivars at least resistant to BCMV and BCMNV of the main seed types grown in the state are needed, while the prevalent *Begomovirus* species are identified.

In this study, virus CMV, AMV, CPMMY, and the *Potyvirus* BYMV, PStV and PeMoV were undetected.

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*** Corresponding Author: José Luis Anaya-López**
INIFAP-Campo Experimental Bajío, Biotechnology Unit, México.
Email: anaya.jose@inifap.gob.mx; jose.luis.al@hotmail.com