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ANNOTATED SEQUENCE RECORD

Complete genome sequence of bean leaf crumple virus, a novel begomovirus infecting common bean in Colombia

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Abstract A copy of the complete genome of a novel bipartite begomovirus infecting common bean (*Phaseolus vulgaris* L.) in Colombia was obtained by rolling-circle amplification (RCA), cloned, and sequenced. The virus is associated with leaf crumple symptoms and significant yield losses in Andean and Mesoamerican beans. Such symptoms have been reported increasingly in Colombia since at least 2002, and we detected the virus in leaf material collected since 2008. Sequence analysis showed that the virus is a member of a distinct species, sharing 81% and 76% nucleotide (nt) sequence identity (in DNA-A and DNA-B, respectively) to other begomoviruses infecting common bean in the Americas. The data obtained support the taxonomic status of this virus (putatively named 'bean leaf crumple virus', BLCrV) as a member of a novel species in the genus *Begomovirus*.

Begomoviruses (family *Geminiviridae*) are emergent pathogens of crops whose dissemination has been favoured by the increasing populations of their whitefly vectors [1]. In the Americas, a series of whitefly-transmitted begomoviruses have been reported to affect bean crops, and most have been associated to leaf mosaic symptoms and important yield reductions [1, 2]. In Colombia, distinct symptoms of leaf crumpling have been reported in fields of dry and snap bean varieties since 2002 [3]. Since then, there has been a drastic decrease (60-70%) in the cultivated area in localities where the symptoms have been reported [3, 4]. The most recent outbreak was recorded during December 2015 in Rozo, Department of 'Valle del Cauca' (Colombia), with a prevalence of 40% in fields of 'Blue Lake' beans, a popular but extremely susceptible commercial variety (Fig. 1A). The putative begomovirus causing the disease was originally named "virus del arrugamiento foliar" (Spanish for 'leaf crumple virus') [3], but the complete sequence was not available until now.

Thirty-seven leaf samples from affected fields in the departments of Valle del Cauca (municipalities of Rozo, Palmira and Pradera) and Quindío (municipality of Quimbaya) were collected, and DNA was extracted from 20 mg of silica-dried leaves or 100 mg of fresh leaves using cetyltrimethylammonium bromide (CTAB). Samples from fields surveyed between 2008 and 2014 in the same region were also included in this study. PCR detection of begomovirus was carried out using generic primers targeting the DNA-A and the DNA-B components [5]. Twenty-six samples tested positive with at least one of these primer sets, and 22 PCR fragments were sent for sequencing (Macrogen). A copy of the complete genome of BLCrV was obtained by rolling-circle amplification (RCA) using phi29 polymerase (New England Biolabs, USA) as reported previously [6]. RCA fragments of ~2.5-2.9 kb obtained by digestion with EcoRI were cloned and sequenced (Macrogen, Korea). The genome organization obtained was that of a typical bipartite begomovirus genome, including a common region (CR) of 195 nt (93.7% identity) containing the characteristic iteron, TATA box and invariant nonanucleotide sequences (Fig. 1B). DNA-A (GenBank accession number KX857725, 2598 nt) has one virus-sense open reading frame (ORF) encoding the capsid protein (AV1)

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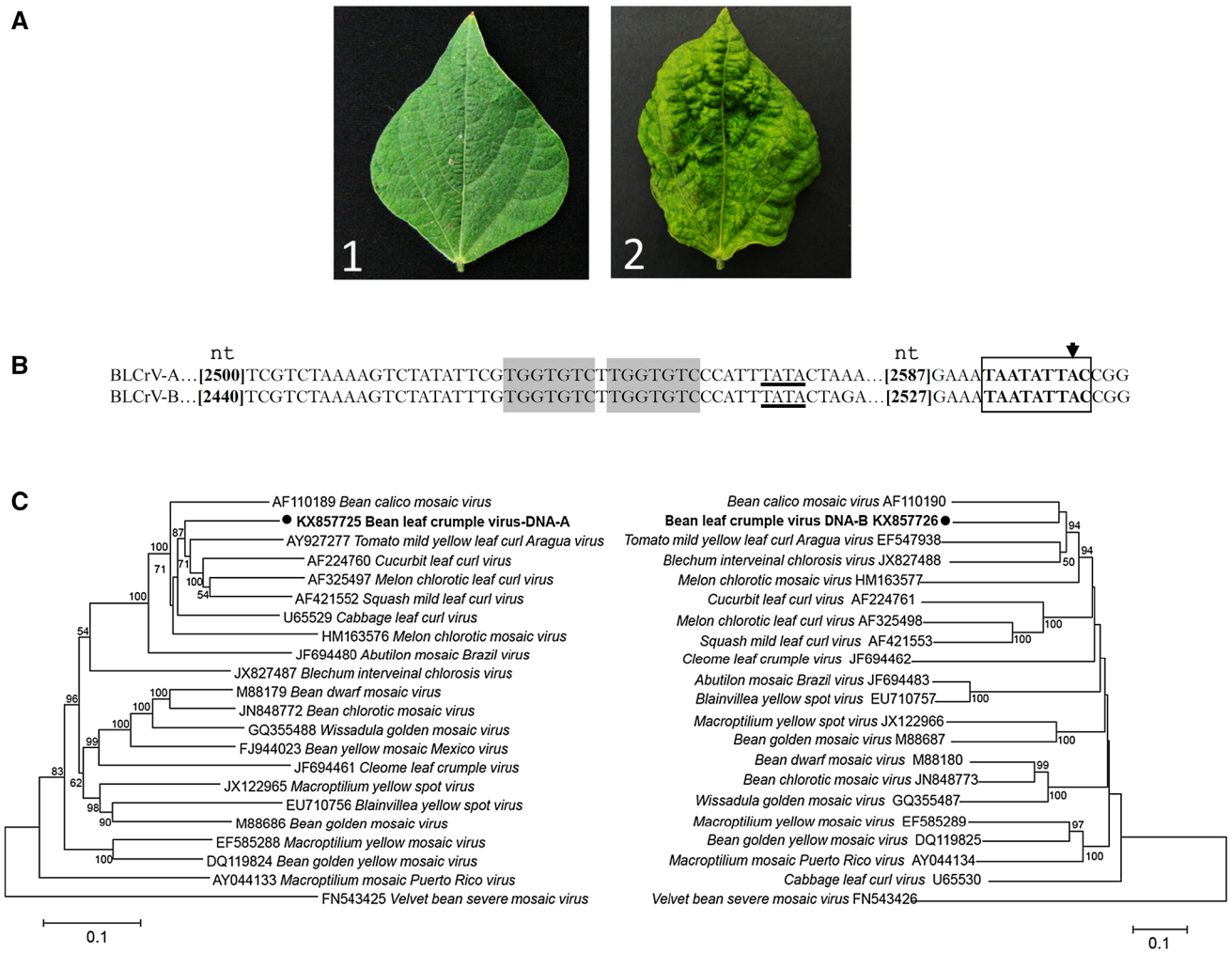

Fig. 1 A) Uninfected (1) and BLCrV-infected (2) bean leaves of variety Blue Lake (sample 13, isolate HA) showing characteristic leaf crumple symptoms observed in the municipality of Rozo (see Table 1). B) A segment of the common region containing the iteron sequences (grey boxes), TATA boxes (underlined) and the invariant nonanucleotide sequence at the origin of replication (white box). An additional iteron sequence (TGGTGTC) is found at position 21, but only in DNA-A. The black arrow indicates the position of nucleotide 1, and the numbers flanking the sequences indicate their position in the genome. nt, nucleotide. C) Phylogenetic relationship of BLCrV (black circle) to other New World bipartite begomoviruses reported in the Americas. The tree was constructed using full genome sequences and the neighbor-joining algorithm in MEGA 5 [8]. A sequence of velvet bean severe mosaic virus was used to root the tree. Bootstrap values above 50% are shown (1000 replicates)

and four ORFs on the complementary-sense strand, encoding the replication-associated protein (Rep/AC1), a transcriptional activator protein (TrAP/AC2), a replication enhancer protein (REn/AC3) and the AC4 protein (AC4). DNA-B (GenBank accession KX857726, 2538 nt) has one virus-sense ORF encoding the nuclear shuttle protein (BV1) and a complementary-sense ORF (BC1). Sequence analysis using the MUSCLE option in SDT v1.2, following current recommendations for begomovirus classification [7], indicated that BLCrV should be regarded as a member of a new species of the genus *Begomovirus*. DNA-A has the highest nt sequence identity (84.17%) to tomato mild yellow leaf curl Aragua virus (73.17% for DNA-B). Phylogenetic analysis using MEGA v5 [8] showed that BLCrV is more closely related to begomoviruses associated with leaf curling symptoms than to leaf-mosaic-associated begomoviruses (Fig. 1C). Sequence comparisons of a portion of the AC1 gene with the corresponding region from viruses obtained from samples collected in previous years confirmed the presence of BLCrV in Colombia since at least 2008 in all symptomatic plants (nt sequence identity >98%). Interestingly, leaf mosaic symptoms were observed in some affected plants that were positive for BLCrV but not for other begomoviruses. (Table 1).

In the bean-breeding program at CIAT, plants have been bred successfully for virus resistance for decades, mainly focusing on BGMV and BGYMV in Mesoamerican bean gene pools [9]. On the other hand, in the Andean bean-

Table 1 Samples evaluated in this study. DNA-A and DNA-B PCR products were obtained using generic primers [5], and sequences were confirmed for the samples indicated by an asterisk (*). Samples from symptomless plants are shown in bold. Samples 4 (F199) and 13 (HA) were selected for RCA. Only sample 13 (HA) was fully sequenced. ND, not determined

Samples	Sample name	Fresh (F)/Dried (D)	Municipality	Department	DNA-A	DNA-B	Collection date
1	LA*	D	Rozo	Valle del Cauca	(+)	(+)	2008
2	PD*	D	Pradera	Valle del Cauca	(+)	ND	2008
3	PV*	D	Quimbaya	Quindio	(+)	(+)	2012
4	F199*	F	Palmira	Valle del Cauca	ND	(+)	2014
5	1_1-2	F	Palmira	Valle del Cauca	(-)	(-)	2015
6	1_2-2*	F	Palmira	Valle del Cauca	(+)	(+)	2015
7	1_5-2	F	Palmira	Valle del Cauca	(-)	(-)	2015
8	1_6-2	F	Palmira	Valle del Cauca	(+)	(-)	2015
9	**1_7-1**	F	Palmira	Valle del Cauca	(-)	(-)	2015
10	**1_11-1**	F	Palmira	Valle del Cauca	(-)	(-)	2015
11	1_12-1	F	Palmira	Valle del Cauca	(-)	(-)	2015
12	FA*	F	Popayan	Cauca	(+)	(+)	2015
13	HA*	F	Rozo	Valle del Cauca	(+)	(+)	2015
14	E1AM*	D	Palmira	Valle del Cauca	(+)	(+)	2016
15	E2AM*	D	Palmira	Valle del Cauca	(+)	(+)	2016
16	E3AM*	D	Palmira	Valle del Cauca	(+)	(+)	2016
17	EAAM*	D	Palmira	Valle del Cauca	(+)	(+)	2016
18	E5A*	D	Palmira	Valle del Cauca	(+)	(+)	2016
19	A6M*	D	Palmira	Valle del Cauca	(+)	(+)	2016
20	A7AM*	D	Palmira	Valle del Cauca	(+)	(+)	2016
21	A8M*	D	Palmira	Valle del Cauca	(+)	(+)	2016
22	A9M*	D	Palmira	Valle del Cauca	(+)	(+)	2016
23	**S1**	D	Palmira	Valle del Cauca	(-)	(-)	2016
24	**S2**	D	Palmira	Valle del Cauca	(-)	(-)	2016
25	**S3**	D	Palmira	Valle del Cauca	(-)	(-)	2016
26	**S4**	D	Palmira	Valle del Cauca	(-)	(-)	2016
27	E1	D	Palmira	Valle del Cauca	(-)	(-)	2016
28	E2 *	D	Palmira	Valle del Cauca	(+)	(+)	2016
29	E3	D	Palmira	Valle del Cauca	(-)	(+)	2016
30	AYM*	D	Palmira	Valle del Cauca	(+)	(+)	2016
31	SN1*	D	Palmira	Valle del Cauca	(+)	(+)	2016
32	SN2*	D	Palmira	Valle del Cauca	(+)	(+)	2016
33	SN3	D	Palmira	Valle del Cauca	(-)	(-)	2016
34	SN4*	D	Palmira	Valle del Cauca	(+)	(+)	2016
35	SN5*	D	Palmira	Valle del Cauca	(+)	(+)	2016
36	SN6*	D	Palmira	Valle del Cauca	(+)	(+)	2016
37	SN7	D	Palmira	Valle del Cauca	(-)	(+)	2016

breeding program, due to its product focus, plants are not bred for geminivirus resistance. Our observations on recent local outbreaks indicate that breeding lines of the Mesoamerican gene pool show some resistance to BLCrV, while genotypes of the Andean gene pool (representing most of the varieties grown in Colombia) are significantly more susceptible (not shown). Climate change is expected to aggravate this as whitefly populations colonize higher-altitude areas [1, 2]. These results underscore the need for continued virus diagnostics and surveillance in order to formulate proper management strategies. Introgression of begomovirus resistance sources into Andean bean breeding lines is under way.

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Compliance with ethical standards

The authors declare no conflict of interest. This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Navas-Castillo Fiallo-Olivé E, Sánchez-Campos S (2011) Emerging virus diseases transmitted by whiteflies. Annu Rev Phytopathol 49:219–248
2. Morales FJ (2010) Distribution and dissemination of begomoviruses in Latin America and the Caribbean. In: Stansly PA, Naranjo SE (eds) Bemisia bionomics and management of global pest. Springer, Berlin, pp 283–318
3. Morales FJ, Martinez AK, Velasco AC (2002) Nuevos brotes de begomovirus en Colombia. Fitopatol Colomb 26:75–79
4. Departmental Agricultural Evaluations, Valle del Cauca, Colombia (2016) http://www.valledelcauca.gov.co/agricultura/publicaciones.php?id=33384. Accessed Oct 2016
5. Rojas MR (1993) Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. Plant Dis 77:340–347
6. Cuellar WJ, Galvez M, Fuentes S, Tugume J, Kreuze J (2014) Synergistic interactions of begomoviruses with sweet potato chlorotic stunt virus (genus *Crinivirus*) in sweet potato (*Ipomoea batatas* L.). Mol Plant Pathol 77:523–534
7. Brown JK, Zerbini FM, Navas-Castillo J, Moriones E, Ramos-Sobrinho R, Silva JC, Fiallo-Olivé E, Briddon RW, Hernández-Zepeda C, Idris A, Malathi VG, Martin DP, Rivera-Bustamante R, Ueda S, Varsani A (2015) Revision of Begomovirus taxonomy based on pairwise sequence comparisons. Arch Virol 160:1593–1619
8. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739
9. Blair MW, Morales FJ (2008) Geminivirus resistance breeding in common bean. CAB reviews: perspectives in agriculture, veterinary science, nutrition and natural resources 89:1–14