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BRIEF REPORT

Soybean chlorotic spot virus, a novel begomovirus infecting soybean in Brazil

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Abstract A novel soybean-infecting begomovirus from Brazil was identified in Jaíba, in the state of Minas Gerais, and molecularly characterized. By using rolling-circle amplification-based cloning of viral DNAs, three DNA-A variants and a cognate DNA-B were isolated from infected samples. The DNA variants share more than 98 % sequence identity but have less than 89 % identity to other reported begomovirus, the limit for demarcation of new species. In a phylogenetic analysis, both DNA-A and DNA-B clustered with other Brazilian begomoviruses. Infectious cloned DNA-A and DNA-B components induced distinct symptoms in *Solanaceae* and *Fabaceae* species by biolistic inoculation. In soybean, the virus induced mild symptoms, i.e., chlorotic spots on the leaves, from which the name soybean chlorotic spot virus (SoCSV) was proposed. The most severe symptoms were displayed by common beans, which exhibited leaf distortion, blistering, interveinal chlorosis, mosaic and golden mosaic. The possibility that SoCSV may become a threat to bean production in Brazil is discussed.

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The genus *Begomovirus* (family *Geminiviridae*) includes viruses with mono- or bipartite ssDNA genomes that are transmitted to dicotyledonous plants by the whitefly *Bemisia tabaci* [10, 16]. Typically, the native begomoviruses from the Americas have two genomic components, DNA-A and DNA-B. DNA-A encodes the proteins that are required for replication (Rep and REn), the transcriptional activation of viral genes (TrAP), encapsidation (CP) and the suppression of RNAi defense functions (TrAP and AC4) [16, 19, 20]. The genes on DNA-B are required for the intra-(NSP) and intercellular (MP) movement of viral DNA-A and DNA-B [16]. NSP also functions as a virulence factor to suppress innate plant defenses [9, 17].

Collectively, the begomoviruses infect a large variety of relevant crops and cause major agricultural losses worldwide. In Brazil, the tomato- and bean-infecting begomoviruses severely impact crop productivity and are widely distributed throughout Brazil [4]. In contrast, soybean-infecting begomoviruses have been reported only sporadically, and these viruses have not been associated with yield losses [7, 12]. However, with the recent report of soybean-infecting begomoviruses causing moderate to severe losses in the neighboring country Argentina [15], there is concern that begomovirus infection in soybean may increase in Brazil. This potential threat to the sustainable agriculture of soybean in Brazil has intensified the surveys for begomovirus infection in the major soybean-producing areas. In this report, we describe the identification of a new soybean-infecting begomovirus, designated soybean chlorotic spot virus (SoCSV), which, nevertheless, was found in symptomatic (chlorotic spots) leaves from two soybean plants

(samples 9253 and 9254) grown in a tomato field in Jaíba, in the state of Minas Gerais, Brazil. They were spontaneously growing plants, probably from a previous cultivation.

Total DNA extracts from leaves of both infected soybean plants were subjected to full-length viral genome amplification using phage ϕ29 DNA polymerase, as described [11]. The 2.6-kb amplified fragments were cloned into pBluescript II KS + (Stratagene) and sequenced. The resulting clone, pUFV1588, harbors a full-length DNA-B, whereas the clones pUFV1587, pUFV1871 and pUFV1872 are genetic variants of DNA-A. Among them, the DNA-A variants were 98 % identical and displayed a DNA-A genomic organization typical of bipartite begomoviruses, with five ORFs (Rep, TrAP, REn, CP and AC4). The DNA-B plasmid, pUFV1588, harbored genes homologous to NSP and MP. The common region of SoCSV is highly conserved between the DNA-B and DNA-A variants pUFV1587 (94 % sequence identity, SI), pUFV1872 (93 % SI) and pUFV1873 (90 % SI) over a region of 172 nucleotides (Fig. S1). For both viral components, the intergenic region separating the divergent transcription units contained functional elements representing the geminivirus origin of replication, such as the conserved 30-bp stem-loop structure (Fig. S1) and the conserved TAATATTAC nonanucleotide sequence (Fig. S1), which contains the nicking site for initiation of virion-sense DNA replication [10]. As cognate DNA-A and DNA-B components, in addition to having a highly conserved common region, pUFV1587 (DNA-A) and pUFV1588 (DNA-B) share identical directly repeated iterons (GGTGA) separated by three nucleotides (ATT) upstream of the Rep gene TATA box, which is the putative Rep-binding site for the origin-specific recognition of New World begomoviruses [2, 3, 8, 13]. The putative binding site for Rep on SoCSV resembles the repeated iterons of other leguminous crop- and weed-infecting begomoviruses found in Brazil, such as bean golden mosaic virus (BGMV; GGTGY), Macroptilium yellow spot virus (MaYSV; GGTGT), Macroptilium yellow vein virus (MaYVV; GGTGY) and Macroptilium yellow net virus (MaYNV; GGTGN). The presence of the iteron consensus sequence GGTGA/Y in the common region of legume-infecting begomoviruses for Rep-specific recognition of replication origins may hold the potential for driving genetic reassortment in multiple infections.

Among begomoviruses, the SoCSV DNA-A nucleotide sequence was most closely related to that of MaYSV (85 % SI), whereas the DNA-B shared the most sequence conservation with BGMV (67 % SI). This level of nucleotide sequence conservation (DNA-A less than 89 % identity to known begomoviruses) is considered sufficiently dissimilar to define a distinct virus [6]. Therefore, the soybean isolate was assigned as a member of a new species of begomovirus, designated "Soybean chlorotic spot virus" (SoCSV; GenBank accession numbers JX122965 for DNA-A and JX122966 for DNA-B), based on the symptomatology of the natural host from which the virus was obtained.

Phylogenetic analysis based on the nucleotide sequence of the SoCSV DNA-A and DNA-B and other begomoviruses from the Americas confirmed a geographical-origin-based relatedness of the novel viral components (Fig. 1). The DNA-A was inserted into a cluster of Brazilian begomoviruses under a sub-branch of leguminous-host-infecting viruses, which included BGMV, two leguminous-weed-infecting viruses (MaYSV and Macroptilium yellow vein virus, MaYVV) that were recently detected in Northeastern Brazil [18], and Blainvillea yellow spot virus (BIYSV), which was recently identified in Southeastern Brazil. The DNA-B also grouped with mostly Brazilian viruses, forming a highly stable sub-branch (100 % bootstrap support) with BGMV and cleome leaf crumple virus (ClLCrV), which was recently identified in Northeastern Brazil [14].

To determine the experimental host range properties of this novel begomovirus, we constructed infectious DNA-A and DNA-B recombinant plasmids harboring partial tandem repeats of the A and B components of SoCSV. The plasmids pUFV1587 and pUFV1588, which contain single copies of DNA-A and DNA-B, respectively, were used to subclone fragments that included the SoCSV-A or B common region and flanking sequences into pBluescript II KS +. The resulting subclone pUFV1589 contains the 0.5-kb SacI/XbaI fragment (0.2-mer) from pUFV1587, whereas pUFV1590 harbors the 0.8-kb SacI/EcoRV fragment (0.3-mer) from pUFV1588. Partial tandem copies of SoCSV DNA-A and DNA-B were constructed by inserting the SacI fragment (1-mer) from pUFV1587 into SacI-digested pUFV1589 and the Sac I fragment (1-mer) from pUFV1588 into SacI-digested pUFV1590. The resulting plasmids pSCS1.2A (pUFV1591) and pSCS1.3B (pUFV1592) contained 1.2 copies of SoCSV DNA-A and 1.3 copies of DNA-B, respectively, and had duplicated common regions.

The clones pSCS1.2A and pSCS1.3B were used for the biolistic inoculation of plants from the families *Solanaceae* (*Nicotiana rustica, N. benthamiana, N. clevelandii, N. debneyi, N. glutinosa*, *N. tabacum*, *Solanum lycopersicum* 'Santa Clara' and 'Rutgers', *Capsicum annuum* 'Casca Dura Ikeda'), *Fabaceae* (Glycine max 'Conquista' and "Msoy7908 RR", *Phaseolus vulgaris* 'Pérola' and 'Ouro Negro') and *Chenopodiaceae* (*Chenopodium quinoa*). For the biolistic delivery of viral DNAs, DNA-coated tungsten particles were spread onto macrocarrier discs (24 mm and 15.2 mm, Bio-Rad), and inoculations of five-week-old seedlings (*Solanaceae* and *Chenopodiaceae* species) or emerging radicles (*Fabaceae* species) were performed using a non-commercially constructed PDS-1000/He-like

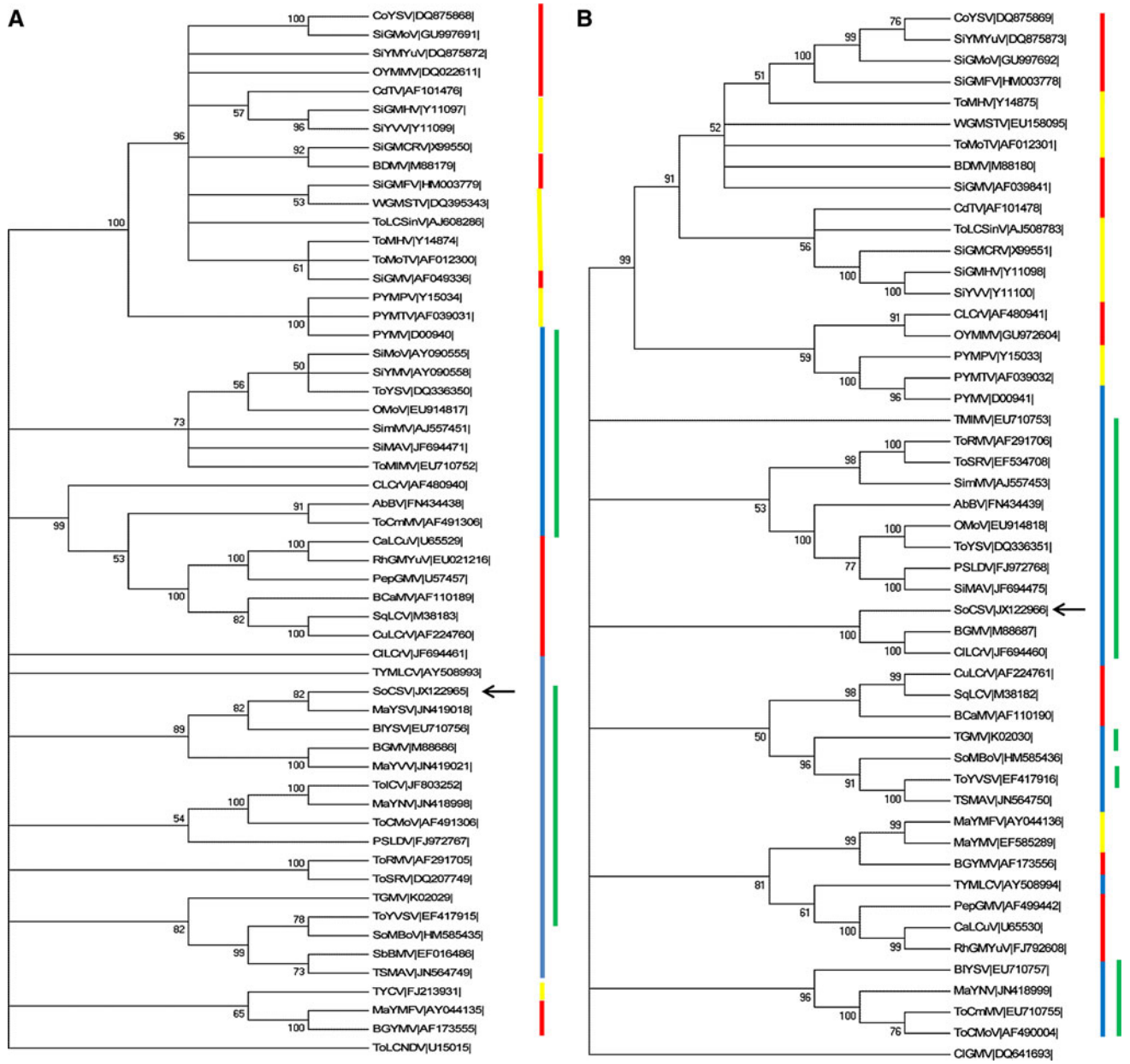

Fig. 1 Phylogenetic tree based on the alignment of the complete genome, DNA-A (**a**) and DNA-B (**b**) of SoCSV and selected begomoviruses from the Americas and the Old World. The trees were constructed by the neighbor-joining method using Mega 5.0 with 100,000 bootstrap replicates. The numbers shown at the nodes indicate the percentage bootstrap scores. SoCSV DNA-A and DNA-B are indicated by arrows. The DNA-A tree was rooted using tomato leaf curl New Delhi virus *(*ToLCNDV, U15015) as an outgroup. For the DNA-B tree, Clerodendrum golden mosaic virus *(*ClGMV, DQ641693) was used as an outgroup. Red bars delimit virus found in North America; yellow bars, Central America; blue bars, South America; and green bars, Brazil. Virus acronyms and GenBank accession numbers are shown in Table S1

accelerator at 900 psi. In each experiment, seven plants of each species were inoculated with 2 μg of tandemly repeated DNA-A plus DNA-B per plant and grown in a greenhouse under natural conditions of light. Inoculated plants were sampled between 14 and 21 days post-inoculation (DPI). Total DNA was extracted from the systemically infected leaves (young), and viral DNA was detected by PCR with the SoCSV DNA-A-specific primers SoCSVNcoI-R (GGAGCCATGGGCTCCTCCGTTTC) and SoCSV-F (CAATAATAATGCGTTTTCCG). The results of the host-range test are displayed in Table 1.

SoCSV infected all of the tested species of the family *Solanaceae* except for *S. lycopersicum* 'Santa Clara' and 'Rutgers'. It also infected all of the tested species of the

Table 1 Host range of SoCSV infectious clones

Plant species	Plants infected/inoculated (no. of experiments)	Symptoms
Capsicum annuum	7/7 (1)	Mild interveinal chlorosis
Chenopodium quinoa	0/7 (1)	–
Glycine max 'Conquista'	7/7 (3)	Chlorotic spots
Glycine max 'Msoy7908 RR'	7/7 (1)	Chlorotic spots
Nicotiana benthamiana	7/7 (1)	Interveinal chlorosis
N. clevelandii	5/7 (1)	–
N. debneyi	7/7 (1)	Interveinal chlorosis
N. glutinosa	7/7 (1)	–
N. tabacum	6/7 (1)	–
N. rustica	6/7 (1)	Interveinal chlorosis
Solanum lycopersicum 'Rutgers'	0/7 (2)	–
Solanum lycopersicum 'Santa Clara'	0/7 (2)	–
Phaseolus vulgaris 'Ouro Negro'	6/7 (3)	Leaf distortion, blistering, interveinal chlorosis, mosaic, golden mosaic
Phaseolus vulgaris 'Pérola'	5/7 (3)	Leaf distortion, blistering, interveinal chlorosis, mosaic, golden mosaic

family *Fabaceae*. Although we detected viral DNA accumulation in newly emerging, systemic leaves of *N. clevelandii, N. tabacum* and *N. glutinosa* at 21 DPI, no visible symptoms were observed in these permissive hosts (data not shown). The infected *C. annuum, N. benthamiana, N. debneyi, N. Rustica* (Fig. S2), *G. max* "Conquista" and *G. max* "Msoy 7908 RR" plants displayed mild symptoms (Fig. 2a and b, described in Table 1). The most-severe symptoms were observed in *P. vulgaris* 'Pérola' and *P. vulgaris* 'Ouro Negro', which exhibited leaf distortion, blistering, interveinal chlorosis, mosaic and golden mosaic (Fig. 2c, d, e and f). Therefore, under our experimental conditions and based on symptomatology, SoCSV seems to be more pathogenic to the experimental host bean than to soybean.

The discrepancy in the severity of SoCSV-induced symptoms in soybean and common bean resembles that seen with BGMV infection, which causes severe symptoms in *P. vulgaris* and represents a serious constraint on bean production in Brazil but produces attenuated symptoms in *G. max* and does not limit soybean productivity [7]. However, in spite of the apparent higher pathogenicity of SoCSV to beans and unlike BGMV, the virus has not been found infecting beans, in the field. Particularly, in Brazil, only BGMV has been found in common beans. In fact, a survey conducted in the major bean-producing regions of seven different states in Brazil revealed the dominance of BGMV in the collected samples [5]. This scenario is not likely to have changed since then; a recent circomics study revealed that only BGMV and intra-species genetic variants (99.7 % SI) were found in infected bean samples collected in Northeastern Brazil [21], a region in which distinct begomoviruses infecting leguminous weeds were identified [18]. The prevalence of BGMV in bean samples from a region in which the virus co-exists with other leguminous weed-infecting begomoviruses may indicate that the low diversity of bean-infecting begomoviruses is due to the absence of resistant bean cultivars that could drive the selective pressure towards the emergence of more-pathogenic species. If this is the case, an increase in the genetic variability of bean-infecting begomoviruses is expected to occur with the introduction of transgenic beans that are immune to BGMV to Brazil [1]. The identification of the novel soybean-infecting begomovirus SoCSV, which apparently can adapt to beans, supports the prediction that opportunistic infection of pre-existent pathogenic begomoviruses may become a threat to BGMV-resistant transgenic beans.

The current investigation adds a new virus to the growing list of soybean-infecting begomoviruses found in Brazil [7, 12]. All of these viruses were identified in a relatively small sample of infected soybean leaves, suggesting that, in contrast to beans, soybeans may host a large diversity of begomoviruses. Therefore, the hypothesis that soybean can host multiple infections, which can then drive the emergence of more viruses that are pathogenic and virulent, with the capacity to infect both beans and soybeans, cannot be discarded in the Brazilian agricultural scenario. The recent release of transgenic bean lines that are immune to BGMV for field cultivation in Brazil may accelerate this evolutionary process.

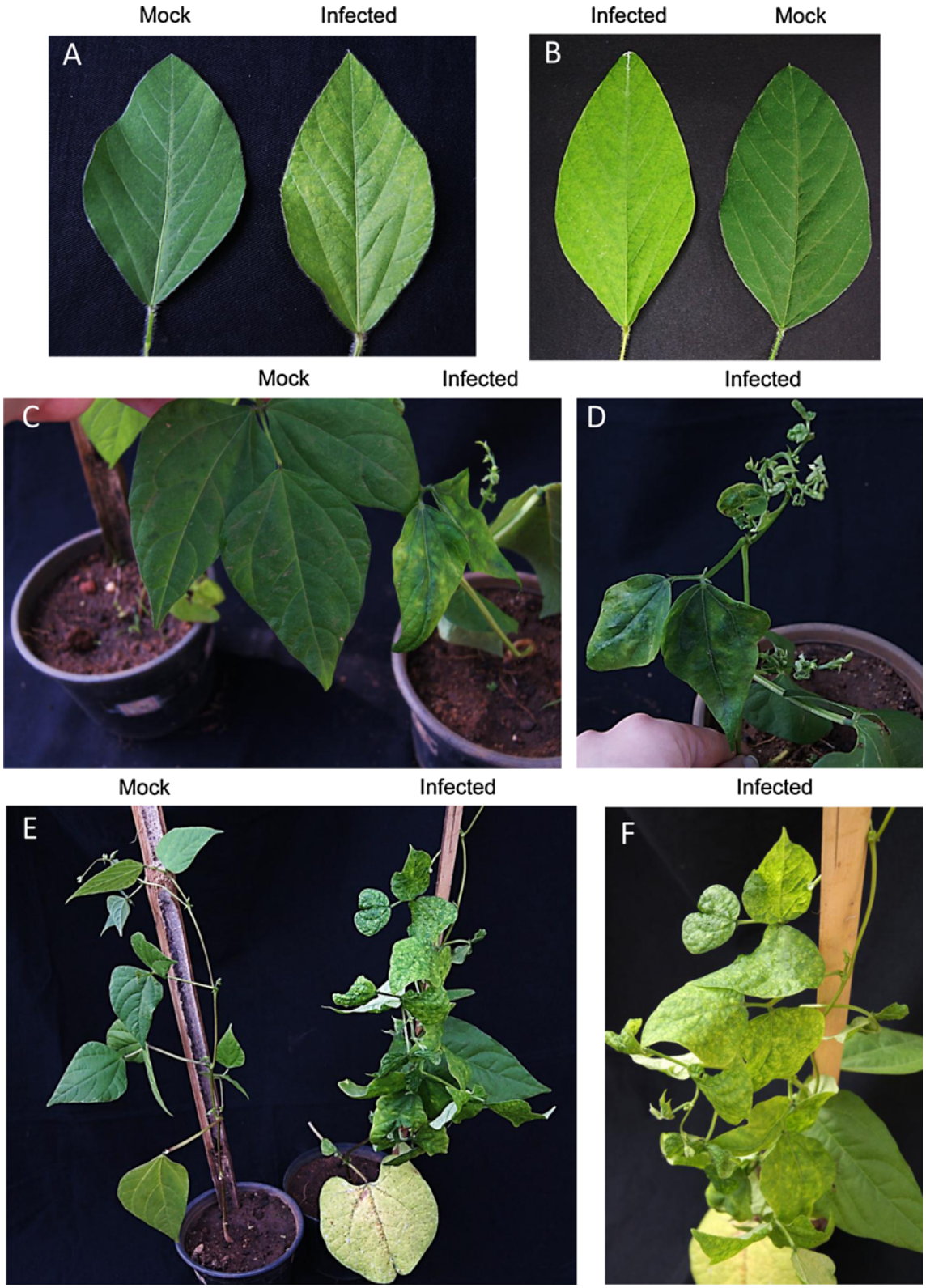

Fig. 2 Symptoms induced by biolistic inoculation of infectious SoCSV DNA-A and DNA-B into leguminous crops. Photographs were taken 35–41 days after biolistic inoculation of soybean or bean cotyledons with tandemly repeated copies of SoCSV DNA-A and DNA-B. **a** and **b** show the symptoms observed on newly emerging leaves of *Glycine max* Conquista (**a**) and *G. max* Msoy 7908 RR (**b**). The symptoms displayed by *Phaseolus vulgaris* 'Pérola' are shown in **c** and **d**, and the symptoms caused in *P. vulgaris* 'Ouro Negro' are shown in **e** and **f**

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