Phytopathology Research

RESEARCH

Open Access

Epidemiological evaluation and identification of the insect vector of soybean stay-green associated virus

Ruixiang Cheng[1,2†], Rong Yan[1,2†], Ruoxin Mei[1,2], Yaodi Wang[1,2], Wei Niu[1,2], Hao Ai[1,2], Sijing Qiao[1,2], Mengjia Xu[1,2], Wei Yu[3], Wenwu Ye[1,2], Yuanchao Wang[1,2*], Xiaorong Tao[1,2*], Xueping Zhou[4*] and Yi Xu[1,2*]

Abstract

In recent years, the emergence of soybean stay-green syndrome (SGS), also referred to as 'zhengqing', in the Huang-Huai-Hai region of China has resulted in significant yield losses. SGS is a phenomenon characterized by the delayed senescence of soybean, resulting in stay-green leaves, flat pods, and stunted seed development at harvest. We previously identified a distinct geminivirus, named soybean stay-green associated geminivirus (SoSGV), as the causative agent of SGS by fulfilling Koch's postulates. To further understand the epidemiology of SoSGV, in this study, we collected 368 stay-green samples from 17 regions in 8 provinces including the Huang-Huai-Hai region and surrounding areas. The results showed that 228 samples tested positive for SoSGV (61.96%), and 96.93% of these positive samples showed severe pod deflation. Our epidemiological assessment reveals that SGS caused by the SoSGV is prevalent in the fields, and it is undergoing geographical expansion and genetic differentiation. Additionally, we determined other natural hosts grown in the Huang-Huai-Hai region. By capturing insects in the field and conducting laboratory vector transmission tests, we confirmed that the common brown leafhopper (*Orosius orientalis*) is the transmission vector of SoSGV. With a better understanding of the transmission and epidemiology of SoSGV, we can develop more effective strategies for managing and mitigating its impact on soybean yields.

Keywords Soybean stay-green syndrome, Soybean stay-green associated virus, Epidemiology, Transmission vector, Leafhopper

†Ruixiang Cheng and Rong Yan contributed equally to this article.

*Correspondence:
Yuanchao Wang
wangyc@njau.edu.cn
Xiaorong Tao
taoxiaorong@njau.edu.cn
Xueping Zhou
zzhou@zju.edu.cn
Yi Xu
xuyiqdpd@njau.edu.cn
[1] Department of Plant Pathology, Nanjing Agricultural University, Nanjing 210095, China
[2] Key Laboratory of Soybean Disease and Pest Control (Ministry of Agriculture and Rural Affairs), Nanjing Agricultural University, Nanjing 210095, China
[3] Fuyang Academy of Agricultural Sciences, Fuyang, 236000, China
[4] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China

Background

Soybean is a highly valuable legume crop worldwide, providing 25% of the global edible oil supply and two-thirds of the global concentrated protein for livestock feed (https://www.fas.usda.gov/data/oilseeds-world-markets-and-trade). However, the emergence of soybean stay-green syndrome (SGS), also known as 'zhengqing', in the Huang-Huai-Hai region of China in recent years has caused significant yield losses, with some areas experiencing a complete loss of seed yield (Xu et al. 2019; Cheng et al. 2022; Wang et al. 2022). SGS is known as delayed senescence at harvest, which is characterized by the retention of green leaves, poor pod production, and stunted seed development at harvest (Xu et al. 2019; Cheng et al. 2022; Li et al. 2022; Wang et al. 2022). Recently, we discovered for the first time that a new geminivirus named soybean stay-green associated geminivirus (SoSGV) is the pathogen of soybean stay-green syndrome by fulfilling Koch's postulates (Cheng et al. 2022). The SoSGV infection alone can cause the typical soybean stay-green symptoms, including delayed leaf senescence (stay-green), increased numbers of abnormal seeds, and many flat pods. This novel distinct virus is a monopartite single-stranded DNA virus, which is most likely formed by intergeneric recombination of geminiviruses (Cheng et al. 2022).

In our previous study, we discovered that SoSGV not only infects soybean, but also various experimental hosts, such as *Nicotiana benthamiana*, *N. tabacum*, *N. glutinosa*, and *Datura stramonium*. However, the natural hosts of SoSGV, particularly crops grown in the same area as soybean in the Huang-Huai-Hai region, were unknown. Additionally, we have previously investigated the transmission mode of SoSGV and provided compelling evidence that it cannot be transmitted through mechanical inoculation or seeds. Although the virus could enter the seed coat of soybean, it was unable to penetrate the embryo and cotyledon, indicating that seed transmission of SoSGV is unlikely. We further confirmed that the predominant *Bemisia tabaci* Mediterranean (Q biotype) species in the Huang-Huai-Hai region does not act as a vector for the transmission of SoSGV. Based on the finding that the CP structure of SoSGV was highly similar to that of CP from *Mastrevirus*, we proposed that SoSGV might be transmitted by leafhoppers (Cheng et al. 2022).

Otherwise, the mechanisms behind the formation of soybean stay-green syndrome are not well understood, but it is believed to be caused by the disruption of the interaction between source activity and sink capacity due to external factors (Ayre 2011; Zhang et al. 2016; Kumar et al. 2019). Previous studies have shown pod removal and seed injury, as well as feeding by bean bugs *Riptortus pedestris* (Fabricius) (Hemiptera: Alydidae) and red-banded stink bugs *Piezodorus guildinii* (Westwood) (Hemiptera: Pentatomidae), can cause delayed senescence, probably by impairing the sink capacity (Zhang et al. 2016; Wei et al. 2023). To gain a better understanding of the epidemiology of SoSGV and identify the main factors contributing to SGS in the Huang-Huai-Hai region, we conducted a study in 2022 to collect and test stay-green soybean plants at harvest from 17 different regions spanning 8 provinces. Our epidemiological assessment of SoSGV reveals it is undergoing geographical expansion and genetic differentiation. We also identified the natural hosts and transmission vector of SoSGV. With a better understanding of the transmission and epidemiology of SoSGV and its, we can develop more effective strategies for managing and mitigating its impact on soybean yields. Our study provides valuable information for guiding agricultural production and integrated control measures for SoSGV, which can help reduce yield losses and ensure sustainable soybean production in China.

Results

SoSGV is widely distributed in soybean fields and continues to expand its range to other areas

To advance our understanding of the epidemiology of SoSGV, we conducted a study from September to November 2022 in which we collected and tested 368 soybean plants that remained green at harvest from 17 distinct geographic regions across 8 provinces in China for the presence of the virus (Fig. 1a). The results of the study revealed that SoSGV was present in 61.96% (228 out of 368) of the stay-green soybean samples analyzed (Table 1). Specifically, 100% of the samples collected from Xuzhou in Jiangsu Province, Zhoukou in Henan Province, Xinxiang in Henan Province, Jieshou in Anhui

(See figure on next page.)

Fig. 1 Distribution and symptoms of SoSGV. **a** Geographic map highlighting all regions with confirmed incidences of SoSGV (in orange), as well as regions with new emerging cases reported in the current study (in purple). Red dots represent sites where SoSGV was detected, while white dots indicate no SoSGV was found in the samples. The right panel shows the field symptoms commonly associated with SoSGV infection, including the typical stay-green syndrome. Two regions displaying these symptoms are shown. **b** Symptom classification of collected stay-green soybean samples. Class I (I) refers to samples with obvious flat pod symptoms and a normal number of pods, while Class II (II) indicates samples with non-flattened pods and a significantly reduced number of pods. Class III (III) denotes samples with non-flattened pods and a normal number of pods

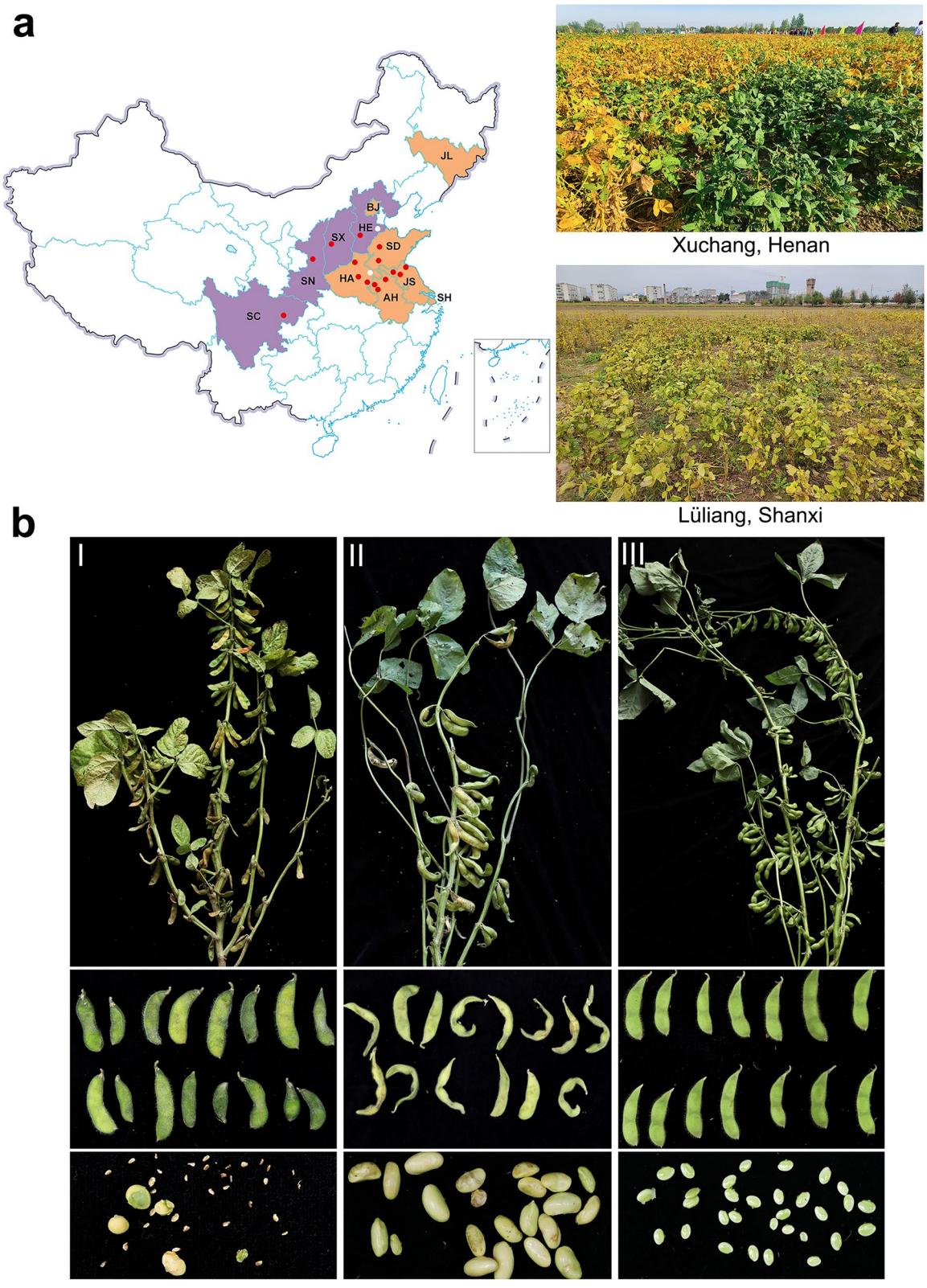

Fig. 1 (See legend on previous page.)

Table 1 PCR detection results of soybean samples and classification of diseased soybean types

Sampling location	Number of samples	Incidence rate of SoSGV (%)	Symptom type
Jiangsu			
Xuzhou	57	100	I
Suining	18	100	I
Lianyungang	21	4.76	I&III
Henan			
Zhoukou	30	100	I
Xinxiang	13	100	I
Shangqiu	8	0	III
Xuchang	7	85.71	I
Anhui			
Jieshou	15	100	I
Suzhou	14	57.14	I&II
Fuyang	50	98.00	I
Shandong			
Jining	28	3.57	I&II&III
Jinan	7	57.14	I&III
Shanxi			
Lüliang	15	100	I
Shaanxi			
Yanan	23	4.35	I&III
Hebei			
Shijiazhuang	29	31.03	I&II
Cangzhou	15	0	II&III
Sichuan			
Nancong	18	5.56	I&II&III

Province, and Lüliang in Shanxi Province tested positive for SoSGV. The incidence of SoSGV in samples from Xuchang in Henan Province, Suzhou and Fuyang in Anhui Province, and Jinan in Shandong Province was also notably high, with more than 50% of the samples testing positive. In contrast, the incidence of SoSGV in samples from Lianyungang in Jiangsu Province, Jining in Shandong Province, Yan'an in Shaanxi Province, and Nanchong in Sichuan Province was found to be below 10%. No SoSGV was detected in samples collected from Shangqiu in Henan Province or Cangzhou in Hebei Province. The statistical analysis results are presented in Table 1.

Furthermore, the study revealed that the symptoms of SGS exhibited variability among the stay-green soybean samples tested (Fig. 1b). Based on the number of pods and the degree of pod filling observed in the samples, the symptoms were classified into three types as depicted in Fig. 1b: Type I, characterized by evident flat pod and normal pod number; Type II, featuring no pod collapse but a reduced number of pods; and Type III, displaying no flat pod and a normal pod number. Of the 368 samples collected, 227 samples displayed symptoms characteristic of Type I, while 82 samples exhibited symptoms of Type II and 59 samples of Type III. In terms of the virus-carrying rate, 97.36% of the Type I samples were tested positive for SoSGV, with a 6.1% virus-carrying rate in Type II and a 3.39% rate in Type III (Table 1). These results indicate that infection with SoSGV resulted in abnormal filling and flattening of soybean pods, which is consistent with the previously defined symptoms of SGS. However, samples exhibiting symptoms of Type II and Type III did not exhibit flattening of pods (Fig. 1b). Importantly, our large-scale investigation demonstrates that SoSGV infection of soybean does not always exhibit the characteristic virus symptoms of leaf shrinking and plant dwarfing. As shown in Additional file 1: Figure S1, two soybean cultivars, 'Shidou' and an unidentified cultivar collected from the field, exhibited conspicuous flat pod and delayed senescence symptoms, yet did not show any signs of viral symptoms, such as leaf shrinking or plant dwarfing. Additionally, the proportion of Type I, Type II, and Type III samples varied across different locations (Table 1), potentially due to environmental and other factors. In addition to its previously identified presence in the Huang-Huai-Hai Region, the spread of SoSGV has now been discovered in numerous other soybean producing regions, including Hebei, Shaanxi, Shanxi, and Sichuan provinces, based on the results of our large-scale survey (Table 1 and Fig. 1a).

Phylogenetic analysis of SoSGV in stay-green soybean samples

To better understand the spread and evolution of SoSGV, a phylogenetic analysis was conducted on soybean plant samples collected from various regions. Specifically, 44 samples of 228 stay-green soybeans from 15 sites in 8 provinces were selected for SoSGV full-length cloning and sequencing. The genomic sequences of the SoSGV isolates were compared, revealing 92.08–99.9% nt identity among them (Additional file 2). Subsequent phylogenetic analysis of the complete genomes of 44 SoSGV isolates demonstrated that the Lüliang and Shijiazhuang isolates form a separate, coherent, and bootstrap-supported clade (Fig. 2). The genome sequences of the Lüliang and Shijiazhuang isolates vary significantly with those from other regions (Additional files 2 and 3), placing them in a distinct clade. In addition to geographical expansion, our epidemiological assessment of SoSGV in China suggests that it is undergoing genetic differentiation.

Determination of the natural host range of SoSGV

In our previous study, we discovered that SoSGV can infect not only soybean, but also other experimental hosts such as *N. benthamiana*, *N. tabacum*, *N. glutinosa*,

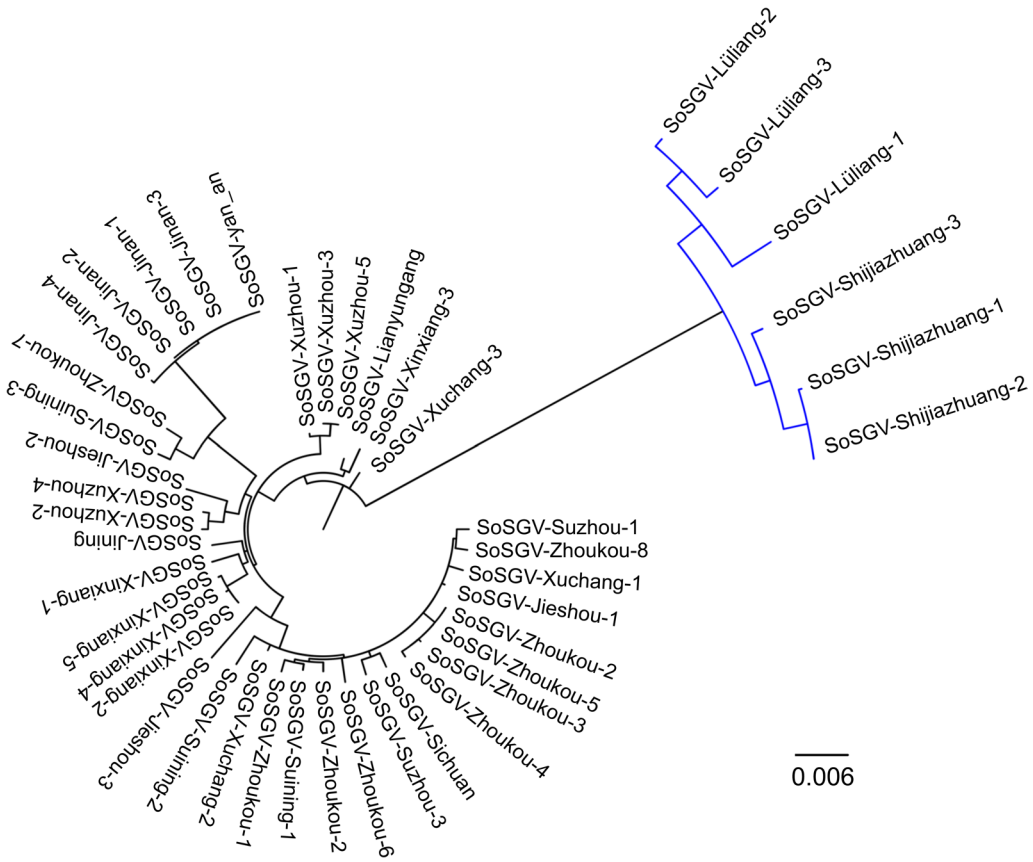

Fig. 2 Phylogenetic tree based on forty-four full genome sequences of SoSGV from 15 regions in 8 provinces. The tree was constructed using the maximum-likelihood (ML) method in the MEGA X software. The bootstrap confidence values generated by 1000 replications and separate clades were marked with blue color

and *D. stramonium* (Cheng et al. 2022). To expand our understanding of the host range of SoSGV, particularly in crops that are grown in the same area as soybean in the Huang-Huai-Hai region, we conducted further tests using the infectious clone of SoSGV. The results, presented in Table 2, Fig. 3, and Additional file 1: Figure S2, showed that SoSGV can also infect peas, chickpeas, and Chinese milk vetch in the legume family. Among these crops, pea is a widely cultivated crop globally, and Chinese milk vetch is valued for its role as a green manure and feed for livestock. SoSGV infection in pea plants resulted in a stay-green phenotype similar to that seen in soybean (Fig. 3a). In contrast, Chinese milk vetch infection caused the leaves to noticeably shrivel (Fig. 3b). Chickpea plants infected with SoSGV exhibited weaker growth compared to healthy plants (Fig. 3c). Notably, we found that SoSGV was unable to infect potatoes, a member of the Solanaceae family (Table 2). Furthermore, we tested five maize cultivars, a graminaceous crop grown alongside soybean, and found that SoSGV was unable to infect maize (Table 2).

Identification of insect vector transmitting SoSGV

To determine the insect(s) responsible for the transmission of SoSGV, we captured insects with sucking or piercing-sucking mouthparts, such as aphids (*Aphis glycines*), leafhoppers, bean bugs (*Riptortus pedestris*), and whiteflies (*B. tabaci*), from a soybean field in Fuyang, Anhui Province, where all soybean samples from this field were tested positive for SoSGV. Then, we placed 15 healthy soybean plants in cages containing insects that had been feeding on soybeans infected with SoSGV. We then tested leafhoppers (number = 12), whiteflies (number = 9), aphids (number = 8), and bean bugs (number = 14) for the presence of the virus using PCR. The results showed that all four types of insects tested positive for the virus (Additional file 1: Figure S3). Four weeks after infestation, all 15 plants were tested for the presence of SoSGV, and the results showed that they were infected, indicating that the insects containing the vector(s) transmitting the virus (Table 3). We then conducted laboratory transmission tests using each insect species to identify which could transmit SoSGV. In each assay, three insects were

Table 2 Determination of the natural hosts of SoSGV

	Species	Number of plants inoculated	Number of plants infected	Infection efficiency (%)
legume	*Pisum sativum*	12	9	75
	Cicer arietinum[a]	37	12	32.4
	Chinese milk vetch	16	12	75
	Vigna unguiculata	12	0	0
	Vicia faba	16	0	0
	Medicago sativa L.	11	0	0
Solanaceae	*Solanum tuberosum*	14	0	0
Gramineae *Zea mays*[b]	Suyunuo	10	0	0
	Suyu 29	10	0	0
	Runyangyu	10	0	0
	Suyu 39	10	0	0
	B73	10	0	0

[a], [b]Hairy root transformation inoculation was used

transferred to a healthy soybean plant, and the inoculation access period was set at 2 days. Four weeks later, leaf samples were collected and tested for the presence of SoSGV. The results showed that only leafhoppers were able to transmit SoSGV to soybean, while all other insects failed (Table 3). Two cultivars, 'Williams 82' and 'Zhonghuang 13', were used for the transmission assay, and the results were consistent. After 3 months of transmission by leafhoppers, the infected plants exhibited several symptoms that are characteristic of stay-green symptoms, including thicker and darker-green foliage, numerous flat pods, and seeds with abnormal pod-filling (Fig. 4b). The agronomic traits of the infected cv. 'Zhonghuang 13' were recorded and are presented in Fig. 4c.

Compared to the green leafhoppers, *Cicadella viridis* (Linnaeus) and *Empoasca flavescens* (Fabricius), the leafhoppers we collected from the field exhibited distinctive brown morphological characteristics (Fig. 4a). These leafhoppers are the second-largest insect population after whiteflies in the samples we collected. The adults were approximately 2.8–3.5 mm in length, with a pale-yellow head marked by an irregular dark-brown pattern, dark-brown eyes, and a pronotum displaying a pale yellow anterior third and a grey posterior two-thirds speckled with transverse dark-brown markings (Fig. 4a). These morphological features suggested that the leafhoppers belonged to the genus *Orosius*. To confirm our hypothesis, we designed primers targeting the *mitochondrial cytochrome oxidase subunit 1* (*COI*) gene of the *Orosius* genus, based on a previous study (Fletcher et al. 2017). We extracted DNA from ten individual adult leafhoppers, and their *COI* nucleotide sequences were found to be 100% identical. Therefore, we selected one sample for the construction of a phylogenetic tree, as shown in Fig. 4d. Our leafhopper clustered with haplotypes of *O. orientalis* (Matsumura) (Homoptera: Cicadellidae), and was closely related to *O. orientalis* haplotypes collected from China (Song et al. 2017). Remarkably, the COI sequence of our *O. orientalis* (Fuyang) sample was 100% identical to that of a Henan haplotype (Genbank accession no. KY039146.1). This finding represents the first evidence that *O. orientalis* acts as the natural transmission vector of SoSGV.

Discussion

In our previous study, we fulfilled Koch's postulates and identified SoSGV as a causative agent of SGS for the first time. In this current study, we collected and analyzed 368 soybean stay-green samples from 17 regions in 8 provinces. Our results revealed that SoSGV was present in 61.96% of the samples, suggesting that it is the leading cause of soybean green stem syndrome (SGS) in the Huang-Huai-Hai region at present. Notably, samples were collected objectively based on plants remaining green, without relying on the presence of virus symptoms. During the harvest season, individual soybean plants with delayed leaf senescence (remained green) were particularly noticeable. SoSGV was found to be sporadically distributed in the field, and it is spreading to other soybean producing areas (Fig. 1a). Furthermore, SoSGV is still mutating and evolving, with isolates from Shijiazhuang and Lüliang forming distinct categories (Fig. 2). In the future, we will be conducting a comparative analysis of the pathogenicity of different isolates, providing insights into the mechanisms that contribute to their virulence.

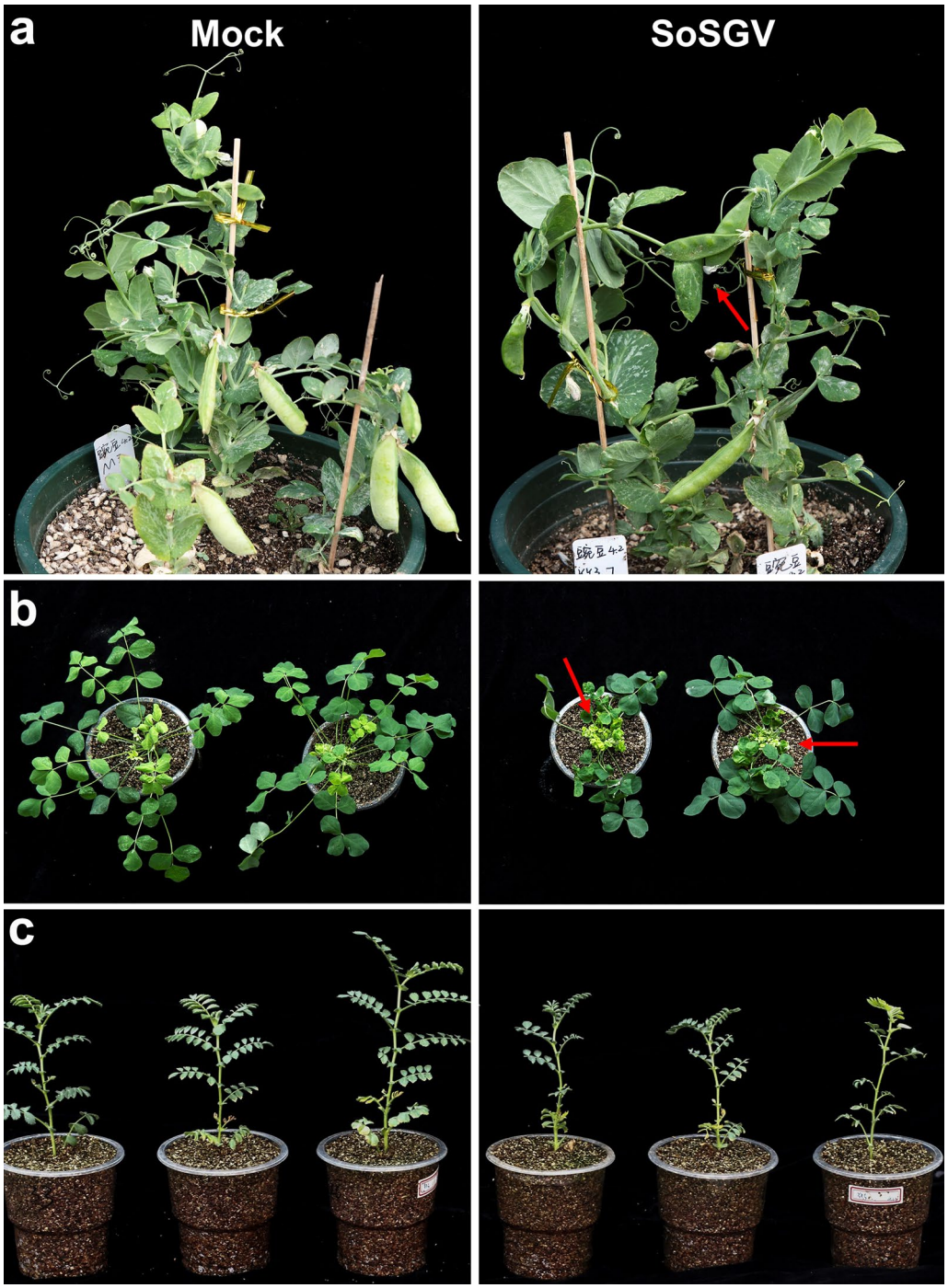

Fig. 3 Symptoms associated with SoSGV infection of peas, chickpeas, and Chinese milk vetch. Peas (**a**), chickpeas (**b**), and Chinese milk vetch (**c**) were inoculated with SoSGV infectious clone at 3 weeks post sowing, respectively. The symptoms were photographed at 35 days post inoculation of SoSGV. Red arrows in (**a**) indicated clear flat pod symptom and in (**b**), noticeably shriveled leaves are observed, also marked by red arrows

In addition, our study examined the natural host range of SoSGV by testing several crops that are commonly grown alongside soybeans. Our results showed that leguminous plants such as peas, chickpeas, and Chinese milk vetch can also be infected by SoSGV, in addition to soybeans (Table 2 and Additional file 1: Figure S2). In the Huang-Huai-Hai region, where peas are frequently planted during spring and early summer, their harvest

Table 3 Determination of the insect vector of SoSGV

Insect species	No. of plant infected with SoSGV/No. of plant tested	
	Williams 82	Zhonghuang 13
Mixed insects	15/15	15/15
Bean bug	0/15	0/15
Whiteflies	0/15	0/15
Aphid	0/10	0/10
Leafhopper	10/10	10/10

season coincides with the soybean planting season, making peas a potential intermediate host of SoSGV. Chinese milk vetch, a commonly used green manure, was also found to be a potential host of SoSGV. By identifying the host range of SoSGV, our study sheds light on the occurrence pattern of SoSGV in the field, providing a scientific basis for more effective control of soybean stay-green disease.

Orosius orientalis (Matsumura) (Homoptera: Cicadellidae) is a significant vector of various viruses and phytoplasmas globally. It can transmit phytoplasmas that cause several economically important diseases, such as legume

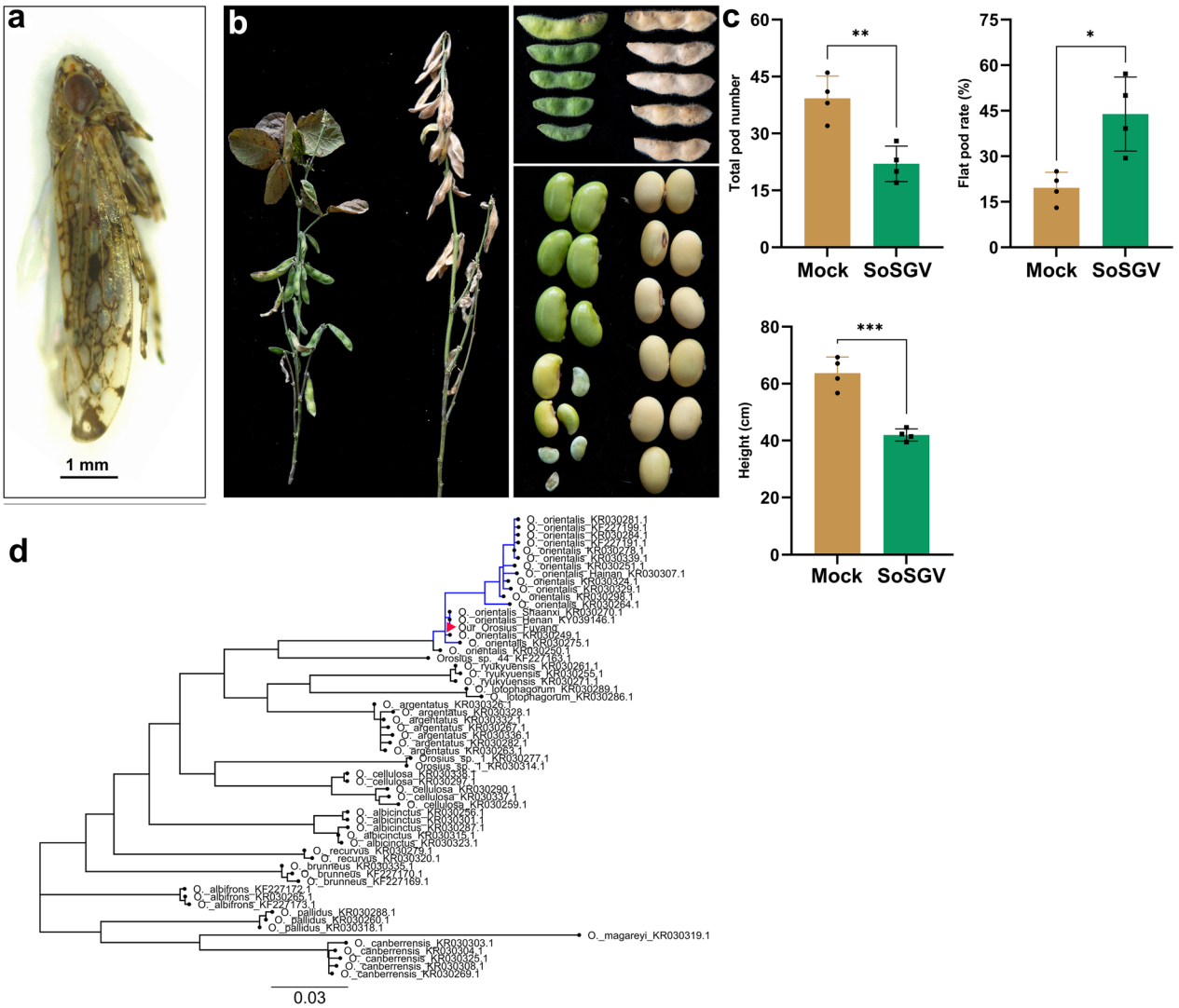

Fig. 4 Identification of *O. orientalis* as the transmission vector of SoSGV. **a** Habitus picture of an adult common brown leafhopper (*O. orientalis*) collected in the field. **b** Soybean plants that were fed on by SoSGV-viruliferous *O. orientalis* developed thicker and darker-green foliage, numerous flat pods, and seeds with abnormal pod-filling at 4 months post infestation. The left panel in each figure represents plants infested with leafhopper. **c** The agronomic traits were counted at 4 months post infestation. **d** *COI* DNA barcoding sequences of 58 *Orosius* reference species including all 12 known *Orosius* species and two indeterminate *Orosius* (sp. # 1; sp. # 44) were retrieved from NCBI (https://www.ncbi.nlm.nih.gov). Phylogenetic tree constructed by the ML method showing relationships among these reference *Orosius* species and *O. orientalis*. The bootstrap confidence values were generated by 1000 replications. The GenBank accession numbers of all the reference sequences used are listed

little leaf and tomato big bud (Hutton and Grylls 1956), lucerne witches broom (Helson 1951), potato purple top wilt (Harding and Teakle 1985), Australian lucerne yellows (Pilkington et al. 2004), and sesame phyllody. Additionally, *O. orientalis* is responsible for transmitting tobacco yellow dwarf virus (TYDV, genus *Mastrevirus*, family *Geminiviridae*) to beans, leading to bean summer death disease, and to tobacco, causing tobacco yellow dwarf disease (Thomas and Bowyer 1980). Moreover, this leafhopper transmits chickpea chlorotic dwarf virus (genus *Mastrevirus*, family *Geminiviridae*), which is one of the viruses associated with chickpea stunt disease (Horn et al. 1994). In this study, we demonstrated for the first time that *O. orientalis* is the natural transmitting vector of SoSGV. Notably, our previous research revealed that the CP structure of SoSGV is highly similar to that of CP from *Mastrevirus*, leading us to hypothesize that leafhoppers might transmit SoSGV (Cheng et al. 2022). Our experimental results are consistent with this hypothesis. Although the common brown leafhopper is not native to China, it has been found in various locations worldwide, including mainland Australia and central Pacific islands, Malaysia, and the Philippines (Fletcher et al. 2017). Further research is needed to investigate the distribution, host range, and the relationship between the distribution of *O. orientalis* and the incidence of SGS in China.

Conclusions

In the present study, we conducted an overall survey that collected and tested stay-green soybean plants at harvest from 17 regions across 8 provinces of China. Our epidemiological investigation of SoSGV revealed that it is currently undergoing geographical expansion and genetic differentiation. Additionally, we were able to confirm the natural hosts of SoSGV, and for the first time, identified *O. orientalis* as the natural vector for its transmission. With a better understanding of the transmission and its epidemiology of SoSGV, we can develop more effective strategies to manage and mitigate its impact on soybean yields.

Methods

Field survey and sampling

Whole plant samples showing delayed senescence were collected during the harvest period from September to November 2022 from 17 distinct geographic regions across 8 provinces in China (Fig. 1a). Samples were collected objectively based on plants remaining green, without relying on virus symptoms. Overall, 368 samples were collected in these 17 regions and all samples (weeds and pea) were grouped into lots of three, respectively prior to virus testing. Details of the collection site and numbers of collected samples are summarized (Table 1). Samples were stored frozen at − 80 °C until subsequent DNA extraction and PCR testing.

DNA extraction and PCR

The total DNA of plant samples was extracted with CTAB method-based extraction procedure (Murray and Thompson 1980). To extract DNA from a single insect, we placed it in a PCR tube and added lysis buffer consisting of 10 mM tris–HCl, 0.45% NP40, 50 mM KCl, 0.45% Tween-20, 0.2% gelatin, 60 mg/L protease K, with pH adjusted to 8.4. Next, we added 6–8 glass beads with a diameter of 1 mm and ground the mixture on a grinder at 60 Hz for 240 s. After rapid centrifugation, the sample was incubated in a 65°C water bath for 60 min. To inactivate the protease, we boiled the sample in water for 10 min. Finally, the supernatant was collected by rapid centrifugation and used for PCR detection or stored at −20°C.

PCR was conducted with the specific primer designed for CP (CP-F: 5′-ATGGATTACAGCAGGAAGAGG-3′; CP-R: 5′- TTACAATTTGCTCTTGAAATACGT-3′) to detect the presence of SoSGV in each sample. The full-length SoSGV genome of were amplified using a pair of back-to-back primers, FL-F (5′-CGCTGTTAAGCG CCTTGGCGTAAGC-3′) and FL-R (5′-CGGCAGTAA GTCAGAGGCTCTTAA-3′). For cloning the partial *COI* gene of the leafhopper, a pair of degenerate primer was designed based a previous study with minor modification (Fletcher et al. 2017), and COI-F (5′-AACTTTATA CTTTATmTTTGGTATTTGATCAGG-3′) and COI-R (5′-AAwACTGGTAGTGACAryAATArTAG-3′). All the PCR products were separated using 1% agarose gel, and gel extraction and sequencing were subsequently performed. The partial CDS of *COI* gene cloned from the leafhopper *Orosius orientalis* collected from Fuyang has been deposited to NCBI with GenBank accession number 2674804.

Agrobacteria and agroinfiltration

Peas, chickpeas, cowpeas, and broad beans are owned by our lab. Potato is a laboratory-owned Desiree variety. As for corns, all experimental cultivars including 'B73', 'Suyu Nuo', 'Suyu 29', 'Run Yangyu', and 'Suyu 39' were provided by Dr. Qin Gu from Nanjing Agricultural University. Chinese milk vetch and lucerne (*Medicago sativa* L.) were kindly provided from Dr. Bo Yang from Nanjing Agricultural University. All the plants were grown in a greenhouse at 26°C–28°C and 65% relative humidity under 16 h/8 h day/night conditions. For infectivity, the plasmids containing SoSGV infectious clone were transformed into *A. tumefaciens* strain EHA105 by electroporation method. Then agrobacteria harbored SoSGV infectious clone was suspended with buffer (10 mM

MES, 10 mM $MgCl_2$, 200 μM acetosyringone) for 2 h at $OD_{600}=1.0$, then infiltrated into plant leaves with 4–6 true leaves. Since chickpea and maize are difficult to infiltrate with leaves, we utilized the *A. rhizogenes* strain K599-mediated hairy root transformation inoculation method, as previously described in our publication (Cheng et al. 2022). Agrobacterium transformed with pBINPLUS empty vector were infiltrated into plant as negative control.

Phylogenetic analysis

Using the samples collected from Huang-Huai-Hai region in China, full-length of 44 SoSGV sequences were cloned and sequenced. Whole genome sequences of representative members of each of the fifteen regions that SoSGV was present. Sequences were aligned by ClustalW implemented in MEGA software version X (Kumar et al. 2018), and phylogenetic trees were constructed by the Maximum Likelihood method (Kumar et al. 2018). One thousand replications were analyzed to enhance robustness. To identify the taxonomy of leafhopper, we designed primers targeting the mitochondrial cytochrome oxidase subunit 1 (*COI*) gene of the *Orosius* genus and performed sequence analyses and integrative taxonomy based on a previous study (Fletcher et al. 2017). Then a phylogenetic tree was also constructed by the ML method with 1000 replications for each bootstrap values using MEGA X.

Insect capture and transmission assay

Insects with sucking or piercing-sucking mouthparts including aphids, leafhoppers, bean bugs, and whiteflies were captured from a soybean field in Fuyang, Anhui Province where all soybean samples tested positive for SoSGV. A portion of each insect samples collected was stored at −20°C for DNA extraction and for SoSGV testing by PCR and the remaining was cage-reared along with soybean plants collected from the same field sesame plants under greenhouse conditions with 26°C–28°C and 65% relative humidity under 16 h/8 h day/night conditions. Insect samples were identified to species by morphological characteristics using a stereomicroscope. Three adult aphids, whiteflies, leafhoppers, or bean bugs rearing on SoSGV-infected soybean plants were then transferred to each caged healthy soybean (4–5-leaf stage), respectively. The insects were allowed a 48-h inoculative feed to transmit the virus. After inoculation, the plants were treated with an insecticide and placed in an insect-proof greenhouse. Four weeks later, we tested the plants for infection using PCR. Those that tested positive were kept in the greenhouse, monitored for the appearance of symptoms for up to 4 months. Two *Glycine max* cultivars 'Williams 82' and 'Zhonghuang 13' were used in the experiments.

Abbreviations

CDS	Coding DNA sequence
COI	Cytochrome oxidase subunit 1
CP	Coat protein
PCR	Polymerase chain reaction
SoSGV	Soybean stay-green associated virus
SGS	Soybean stay-green syndrome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-023-00177-x.

Additional file 1: Figure. S1. SoSGV infection causes stay-green symptoms in different soybean varieties. Symptoms of two soybean cultivars infected with SoSGV in mature stage collected from fields. **Figure. S2.** Detection of the infection of SoSGV by PCR in other natural hosts. The systemically infected leave from Pisum sativum,; Cicer arietinum,; Chinese milk vetch,; Vigna unguiculata,; Vicia faba,; Medicago sativa,; Solanum tuberosum,; and different corn cultivarswere used for extraction of DNA, respectively. DNA extracted from SoSGV-infected soybean was used as positive control and mock-inoculated plant was used as negative control. **Figure. S3.** Detection of the presence of SoSGV by PCR in different insects collected from the field. Orosius orientalis,; Bemisia tabaci,; Aphis glycines, and Riptortus pedestris. DNA extracted from SoSGV-infected soybean was used as positive control and mock-inoculated plant was used as negative control.

Additional file 2: Table S1. Estimates of evolutionary divergence among 44 SoSGV isolates.

Additional file 3: Nucleotide sequence alignment of SoSGV whole genome sequence from Lüliang and Shijiazhuang isolates with those of other representative isolates. The identical sequences shared among these isolates are highlighted in red.

Acknowledgements

We would like to express our great gratitude to China Agriculture Research System (CARS-004) for their assistance in collecting the soybean stay-green samples. These stations and institutes include Xuzhou, Zhengzhou, Shangqiu, Fuyang, Suzhou, Jining, Jinan, Fenyang, Yan'an, Cangzhou, Nancong, Zhoukou Academy of Agricultural Sciences, Anhui Academy of Agricultural Sciences and Hebei Academy of Agricultural Sciences. I am immensely grateful to my kid Ethan for his unwavering dedication in assisting me with the collection of leafhoppers from the fields.

Authors' contributions

YX, XPZ, XRT, and YCW conceived the project. RXC, RY, RXM, YDW, WN, HA, SJQ, MJX, WY, and WWY performed the experiments. YX and RY wrote the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the National Key Research and Development Program of China (2022YFC2602000, 2022YFF1001500), the Open Research Fund from State Key Laboratory for Biology of Plant Diseases and Insect Pests (SKLOF202205), and Jiangsu Agricultural Science and Technology Innovation Fund (CX(22) 2039).

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Received: 19 February 2023 Accepted: 13 April 2023
Published online: 30 May 2023

References

Ayre BG. Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. Mol Plant. 2011;4:377–94. https://doi.org/10.1093/mp/ssr014.

Cheng R, Mei R, Yan R, Chen H, Miao D, Cai L, et al. A new distinct geminivirus causes soybean stay-green disease. Mol Plant. 2022;15:927–30. https://doi.org/10.1016/j.molp.2022.03.011.

Fletcher M, Locker H, Mitchell A, Gopurenko D. A revision of the genus *Orosius* Distant (Hemiptera: Cicadellidae) based on male genitalia and DNA barcoding. Austral Entomol. 2017;56:198–217. https://doi.org/10.1111/aen.12224.

Harding R, Teakle DS. Mycoplasma-like organisms as causal agents of potato purple top wilt in Queensland. Austral J Agric Res. 1985;36(3):443–9. https://doi.org/10.1071/AR9850443.

Helson G. The transmission of witches broom virus disease of lucerne by the common brown leafhopper, *Orosius argentatus* (Evans). Aust J Biol Sci. 1951;4:115–24. https://doi.org/10.1071/BI9510115.

Horn NM, Reddy SV, Reddy DVR. Virus-vector relationships of chickpea chlorotic dwarf geminivirus and the leafhopper *Orosius Orientalis* (Hemiptera, Cicadellidae). Ann Appl Biol. 1994;124:441–50. https://doi.org/10.1111/j.1744-7348.1994.tb04149.x.

Hutton E, Grylls N. Legume 'little leaf', a virus disease of subtropical pasture species. Aust J Agric Res. 1956;7:85–97. https://doi.org/10.1071/AR9560085.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol. 2018;35:1547–9. https://doi.org/10.1093/molbev/msy096.

Kumar R, Bishop E, Bridges WC, Tharayil N, Sekhon RS. Sugar partitioning and source-sink interaction are key determinants of leaf senescence in maize. Plant Cell Environ. 2019;42:2597–611. https://doi.org/10.1111/pce.13599.

Li QL, Zhang YY, Lu WG, Han XY, Yang LL, Shi YJ, et al. Identification and characterization of a new geminivirus from soybean plants and determination of V2 as a pathogenicity factor and silencing suppressor. Bmc Plant Biol. 2022;22:362. https://doi.org/10.1186/s12870-022-03745-z.

Murray MG, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res. 1980;8:4321–5. https://doi.org/10.1093/nar/8.19.4321.

Pilkington LJ, Gurr GM, Fletcher MJ, Nikandrow A, Elliott E. Vector status of three leafhopper species for Australian lucerne yellows phytoplasma. Aust J Entomol. 2004;43:366–73. https://doi.org/10.1111/j.1440-6055.2004.00419.x.

Song N, Cai WZ, Li H. Deep-level phylogeny of Cicadomorpha inferred from mitochondrial genomes sequenced by NGS. Sci Rep. 2017;7:10429. https://doi.org/10.1038/s41598-017-11132-0.

Thomas J, Bowyer J. Properties of tobacco yellow dwarf and bean summer death viruses. Phytopathology. 1980;70:214–7. https://doi.org/10.1094/Phyto-70-214.

Wang X, Wang M, Wang L, Feng H, He X, Chang S, et al. Whole-plant microbiome profiling reveals a novel geminivirus associated with soybean stay-green disease. Plant Biotechnol J. 2022;20:2159–73. https://doi.org/10.1111/pbi.13896.

Wei Z, Guo W, Jiang S, Yan D, Shi Y, Wu B, et al. Transcriptional profiling reveals a critical role of GmFT2a in soybean stay-green syndrome caused by the pest *Riptortus pedestris*. New Phytol. 2023;237:1876–90. https://doi.org/10.1111/nph.18628.

Xu C, Han T, Wu C. Discussion on the causes of stay-green syndrome for summer soybean and its preventive methods in the Huang-Huai-Hai region. Soybean Sci Technol. 2019;160:22–8.

Zhang XX, Wang M, Wu TT, Wu CX, Jiang BJ, Guo CH, et al. Physiological and molecular studies of stay-green caused by pod removal and seed injury in soybean. Crop J. 2016;4:435–43. https://doi.org/10.1016/j.cj.2016.04.002.