

# Evaluation of Seed Transmissibility of *Soybean Vein Necrosis-Associated Virus* in Two Soybean Cultivars Grown Under Field Conditions

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## Abstract

*Soybean vein necrosis-associated virus* (SVNaV), originally found in Tennessee in 2008, has recently been reported from other soybean-growing regions of the United States as well as Canada. To provide direct evidence for lack of seed transmissibility of SVNaV in soybean, cultivars CF386RR2y/stsn and AG4832 were planted in a field in Kentucky during the 2013 growing season. Typical SVNaV induced symptoms emerged in early August. Tissues were collected from 150 symptomatic and asymptomatic plants of both cultivars and assayed by ELISA. SVNaV was detected in 94% of symptomatic, but none of asymptomatic plants. Seeds were harvested independently from 10 selected infected plants of each cultivar that exhibited the highest ELISA readings. A total of 2085 seeds from both cultivars were planted under greenhouse conditions and subsequently 1955 asymptomatic seedlings were harvested 4-5 weeks post-emergence and assayed individually by ELISA. Sap from only one of the seedlings exhibited an absorbance reading higher than background. However, when all the seeds from the same mother plant were grown and tested, none was positive. This suggests higher than background absorbance value for this single seedling is an anomaly. To search for probable genetic variation among SVNaV isolates from infected mother plants, whose seeds were subjected to grow out test, nucleocapsid protein gene was RT-PCR amplified from 13 mother plants representing both cultivars and sequenced. The analysis of sequences revealed the presence of distinct variants. Lack of potential for SVNaV to be seed transmissible in soybean is discussed.

**Keywords:** Soybean vein necrosis disease; Tospovirus; *Glycine max*; ELISA; RT-PCR; Nucleocapsid protein gene

## Introduction

*Soybean vein necrosis-associated virus* (SVNaV) was first isolated from field-grown soybeans (*Glycine max* (L.) Merrill) in Tennessee in 2008 [1]. Since then SVNaV has been detected in field grown soybeans from other soybean-growing areas of the United States including Alabama, Arkansas, Illinois, Iowa, Kansas, Kentucky, Michigan, Missouri, Mississippi, Oklahoma and Wisconsin [2-9]. Outside the United States, the virus has only been reported in field-grown soybeans from Canada [10]. Symptoms of the soybean vein necrosis diseases caused by SVNaV include chlorosis of leaf tissues adjacent to small and major veins which eventually progresses to necrosis and occasionally extends to a large portion of the affected leaves [6,8,9]. SVNaV is genetically and serologically a distinct species of the genus *Tospovirus* within the family *Bunyaviridae* [6,8]. *Tomato spotted wilt virus* (TSWV), a subject of extensive studies, is the type member of the genus [11].

Limited studies have been conducted on SVNaV [6,8,9]. As such, there is a significant gap in our knowledge regarding both biological and molecular aspects of SVNaV in soybean. Despite widespread presence within the United States, not much is known about its impact on seed quality or yield [12]. Similar to the other tospoviruses, SVNaV is thrips transmissible [9]. However, transmission of SVNaV mechanically from soybean to soybean or from experimental hosts to soybean has been difficult and inefficient [6,8,9]. It should be noted that SVNaV is not an exception in this regard as difficulties in mechanical inoculation of *Alstroemeria necrotic streak virus*, another tospovirus, to its respective natural host have also been reported [13]. The nucleotide sequence data corresponding to complete nucleocapsid protein gene of 13 isolates reported in this article have been submitted to GenBank and assigned the accession numbers KJ955706 to KJ955718.

Seed transmission of plant viruses is one of the most important routes for long-distance dissemination and about one-seventh of the known plant viral species are seed-transmitted through the seed of at least one of their infected host plants [14-17]. Ramifications of seed transmissibility include potential seed/grain importation restrictions into countries where specific seed-transmitted viruses do not occur. Additionally, infected seedlings derived from infected seeds can serve as the initial local source of inoculum for the spread of a virus within a field [14]. Thus, in the case of new emergent viruses, such as SVNaV in soybean, information on possible seed transmissibility is essential for directing management efforts.

In general tospoviruses are not seed transmissible; however, SVNaV represents genetically and serologically a distinct new species [6,8]. Our main objective in this study was to determine whether natural infection of soybean plants from two cultivars with SVNaV population under field conditions results in seed transmission of the virus.

## Materials and Methods

### Soybean cultivars

Seeds of commercial soybean cultivars CF386RR2y/stsn, late

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group maturity 3, and AG4832, late group maturity 4, were planted no-till into corn stubble in two adjacent blocks in a field located at the University of Kentucky Research and Education Center in Princeton, Kentucky (KY) in the middle May of 2013. Blocks were surrounded by a border of at least 15-30 m of tall fescue-based pasture; however, other soybean fields were in the vicinity. Row spacing was 30 cm and the seeding rate was four to five per 30 cm of row at a planting depth of 3.8 cm. Blocks were treated with phosphorus and potassium as determined by pre-plant soil sampling. Glyphosate was applied pre-planting as well as 35 days post-planting to keep blocks weed-free. No fungicide or insecticide was applied.

### Detection of SVNaV in field-grown soybeans

Two to three symptomatic trifoliolate leaves were harvested from each of 50 arbitrarily-selected symptomatic plants of soybean cultivar CF386RR2y/stsn at Mid-R6 growth stage (pods reached full size) in early September. At the same time, two to three trifoliolate leaves were also harvested from 50 asymptomatic plants of the same cultivar. Samples also were collected similarly from cultivar AG4832 in late September except 25 symptomatic and 25 asymptomatic plants were selected for sampling. Plants sampled were all tagged. Leaf samples were transferred by overnight delivery to the University of Tennessee, Knoxville, where they were tested for the presence of SVNaV using ELISA (described below).

### ELISA

Sap was extracted in the presence of carbonate buffer, pH 9.6 with a ratio of 1:5 (w/v) and stored at -80 °C until tested. Whenever applicable, sap was extracted from symptomatic portions of leaves only. Antigen-coated indirect ELISA to detect SVNaV was done as previously described [6]. Absorbance values exceeding mean value of background (i.e. reaction with sap extract from healthy soybean) + 3X standard deviation were considered positive.

### Harvesting seed and maintenance

Seeds were hand-harvested from 20 symptomatic infected mother plants with the highest ELISA readings, 10 plants per cultivar, within 2 weeks of postharvesting plants from the field. The two weeks delay allowed the plants and seed to dry down to harvest moisture of about 11%. Seeds were cleaned by hand and stored under typical laboratory conditions in small envelopes until planting. Seeds were visually examined for the presence of any virus-induced symptoms such as mottling, etc.

### Seed-growth test

Seeds derived from 10 infected mother plants of each cultivar with highest ELISA readings were planted 12.7 mm deep into Promix Biofungicide Professional Growing Medium (Premier Horticulture, Inc. Quakertown, PA) contained in 38.1-mm-diameter X 203.2-mm-deep Cone-tainer Cells (Stuewe and Sons, Tangent, OR). 105 seeds from each mother plant were planted, except seeds from plant KY44 that yielded a total of only 90 seeds (Table 1). Planted cells were contained in 50-hole racks specially designed to hold Cone-tainer Cells and placed on benches of a greenhouse maintained at 26.7 °C ± 1. Cells were watered with tap water once in the first week following sowing and then every other day from emergence until plants had two fully and a third partially expanded trifoliolate leaves (4-5 weeks post-emergence). No fertilizer was applied. Plants were harvested individually by cutting

stems above the unifoliolate leaves in the morning and shipped to Knoxville, TN, by overnight delivery to be tested for the presence of the virus by ELISA.

### Reverse transcription-polymerase chain reaction (RT-PCR) and nucleotide sequencing

Total RNA was extracted from symptomatic leaf tissues of infected soybean mother plants whose seeds were subjected to grow-out by RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and served as template to generate complementary DNA in the presence of 10-mer random primers and SuperScript II (Invitrogen, Carlsbad, CA) following manufacturer's instructions. PCR was done in the presence of SVNaV primers F1 and R1 and *Ex Taq* polymerase (Takara Bio, Madison, WI) according to a protocol previously described [6]. Amplicons were purified using QIAquick-PCR Purification Kit (Qiagen) and sequenced using primers F2 (5'-AGCTTGTGCAATGATTTAGC-3') and R2 (5'-TCACAACCTGTGATTGATGC-3') corresponding to nucleotide sequences 2136-2155 and 2041-2022 of small RNA of the SVNaV-TN isolate (GenBank accession number HQ728387), respectively. Sequencing was done at the University of Tennessee DNA Sequencing Facility. The nucleotide sequences were edited using BioEdit [18], at <http://bioedit.software.informer.com/7.1/>.

Soybean plants	No. of seeds planted	No. of harvested seedling <sup>b</sup>	ELISA outcome <sup>c</sup>
Cultivar AG4832			
KY8	105	100	0/100
KY27	105	100	0/100
KY29	105	100	0/100
KY34	105	100	0/100
KY35	105	100	0/100
KY36	105	100	0/100
KY37	105	100	1 <sup>d</sup> /100
KY42	105	100	0/100
KY43	105	100	0/100
KY44	90	86	0/86
Cultivar CF386RR2y/stsn			
KY102	105	99	0/99
KY105	105	98	0/98
KY108	105	98	0/98
KY112	105	95	0/95
KY113	105	99	0/99
KY114	105	100	0/100
KY115	105	89	0/89
KY117	105	100	0/100
KY120	105	92	0/92
KY124	105	99	0/99

<sup>a</sup>Seeds obtained from selected infected mother plants that exhibited highest ELISA readings when derivative symptomatic leaf tissues were assayed. Seeds were planted in a greenhouse that was maintained at 26.7 °C ± 1.

<sup>b</sup>Seedlings were harvest 4-5 weeks post-emergence individually by cutting stems above the unifoliolate leaves.

<sup>c</sup>Total positive seedlings/total seedlings tested. Sap was extracted from trifoliolate leaf tissues in the presence of carbonate buffer, pH 9.6 with a ratio of 1:5 (w/v) and tested by ELISA.

<sup>d</sup>Absorbance value was 3X that of background. It should be noted that a total of 116 seedlings derived from seeds of the same mother plant (100 grown under greenhouse conditions while 16 in growth chamber) were all tested negative in ELISA.

**Table 1:** Evaluation by ELISA of seedlings derived from seeds from soybean mother plants infected with *Soybean vein necrosis-associated virus* for possible virus transmission<sup>a</sup>.

## Sequence analysis and bioinformatics

The predicted amino acid sequences were deduced using BioEdit. The sequences were aligned and compared using the Clustal W function [19]. Sequence similarity calculations were done using the SIAS sequence identity/similarity calculator at <http://imed.med.ucm.es/Tools/sias.html>. Using the nucleocapsid protein (NP) gene sequences of 13 isolates from infected mother plants representing both cultivars, two multiple sequence alignments were created; one for the nucleotide and one for the deduced amino acid sequences. The sequences were imported into the software Mega6.06 [20] and a multiple sequence alignment (MSA) was generated using the Clustal W with a gap opening penalty of 25 for both pairwise and multiple alignment steps. Numbers of variable sites were counted at this stage. After adding the sequence from the virus type member (TN) and a distant outgroup virus species (*Bean necrotic mosaic virus*; BeNMV) (GenBank accession No. JN587268), the MSA was recalculated with the same parameters. Phylogeny reconstruction was conducted using the neighbor-joining statistical method with 1000 bootstrap tests and a p-distance model. The resulting trees were condensed at a cut-off value of 70% bootstrap reliability to hide unsupported branches.

## Results

### SVNaV incidence in field-grown soybeans

Plants were monitored for appearance of SVNaV-induced symptoms. Typical symptoms emerged in early August when plants were at the R5.5 growth stage (pods full sized but beans half filling pods). A total of 100 plants from cultivar CF386RR2y/stsn were sampled and individually tested for the presence of SVNaV by ELISA. Of 50 symptomatic plants, 47 were positive whereas all 50 asymptomatic plants were negative. In the case of soybean cultivar AG4832, a total of 50 plants were sampled. Of 25 symptomatic plants, all were positive while 25 asymptomatic plants were negative in ELISA. From symptomatic leaves sap was extracted only from tissues displaying symptoms of SVNaV infection (Figure 1A and 1B). Thus, our observations corroborate previous suggestions that there is a direct association between symptoms and the presence of SVNaV in leaf tissues [6,8]. However, in general it should be noted that symptoms alone are not a reliable mean for detection of viruses in plants [14].

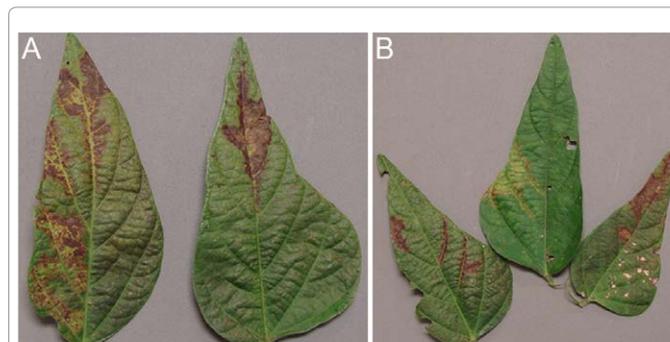
### Seed transmission assay

Of 2107 seeds derived from 20 selected SVNaV infected mother plants of soybean cultivars CF386RR2y/stsn and AG4832, none exhibited any mottling or other viral-like symptoms. Of these, a total of 2085 seeds were sown under greenhouse conditions where 1955 seedlings were harvested 4-5 weeks post-emergence (Table 1). None of the seedlings exhibited symptoms similar to those induced by SVNaV. When assayed individually by ELISA, sap from only one of the seedlings derived from a mother plant of cultivar AG4832 yielded absorbance value equal to 3X that of background. Unfortunately, no further tissue was available to re-test this seedling by RT-PCR. However, the remaining seeds from the mother plant that yielded this seedling (a total of 22 seeds) were grown in a growth chamber. When tested by ELISA, sap from none of the 17 emerged seedlings was positive. We also tested selected seedlings derived from various mother plants by RT-PCR; however, none yielded amplicons corresponding to those of SVNaV (data not shown).

### Variation among SVNaV isolates from mother plants

The complete nucleotide sequences of NP gene from a total of 13 isolates from 13 individual mother plants of both cultivars were

obtained. Pairwise comparison of the NP gene of these isolates, with each other as well as with that of the TN isolate, revealed identities of 98.6 to 100% at both the nucleotide and at amino acid levels (Figure 2). This is in agreement with low diversity of SVNaV isolates reported previously [6,8]. While sequences of NP gene from all isolates generated in this study showed a high degree of similarity, a number of nucleotide differences resulting in amino acid differences were observed. The MSA yielded 24 variable nucleotide sites out of 834 total bases and 5 variable amino acid sites out of 277 total amino acids (data not shown). It should be noted that polymorphisms in viral population is a common feature of RNA viruses [14]. Isolates KY105, KY108 and KY124, all infecting



**Figure 1:** Symptoms induced by *Soybean vein necrosis-associated virus* on leaflets of two independent soybean mother plants of cultivar AG4832 by (A) KY42 and (B) KY43 isolates. Seeds were planted in a field located in Kentucky and photos were taken about 3 months post-planting.

A												
KY 29	99.2											
KY 34	99.4	99.8										
KY 35	99.4	99.8	100									
KY 43	99.4	99.8	100	100								
KY 44	99.2	99.5	99.8	99.8	99.8							
KY 102	99.4	99.8	100	100	100	99.8						
KY 105	98.6	98.7	98.9	98.9	98.7	98.9						
KY 108	99	99.4	99.6	99.6	99.6	99.4	99.6	98.6				
KY 112	99.4	99.8	100	100	100	99.8	100	98.9	99.6			
KY 113	99.2	99.5	99.8	99.8	99.8	99.5	99.8	98.7	99.4	99.8		
KY 115	99.2	99.5	99.8	99.8	99.8	99.5	99.8	98.7	99.4	99.8	99.8	
KY 117	99.3	99.6	99.9	99.9	99.9	99.6	99.9	98.8	99.5	99.9	99.6	99.6
KY 124	98.8	98.9	99.2	99.2	98.9	99.2	98.6	98.9	99.2	99	98.9	99
TN												
KY 29												
KY 34												
KY 35												
KY 43												
KY 44												
KY 102												
KY 105												
KY 108												
KY 112												
KY 113												
KY 115												
KY 117												
KY 124												
TN												

B												
KY 29	98.9											
KY 34	98.9	100										
KY 35	98.9	100	100									
KY 43	98.9	100	100	100								
KY 44	98.9	100	100	100	100							
KY 102	98.9	100	100	100	100	100						
KY 105	98.6	98.9	98.9	98.9	98.9	98.9	98.9					
KY 108	98.6	99.6	99.6	99.6	99.6	99.6	99.6	98.6				
KY 112	98.9	100	100	100	100	100	100	98.9	99.6			
KY 113	98.9	100	100	100	100	100	100	98.9	99.6	100		
KY 115	98.9	100	100	100	100	100	100	98.9	99.6	100	100	
KY 117	98.9	100	100	100	100	100	100	98.9	99.6	100	100	100
KY 124	99.3	99.6	99.6	99.6	99.6	99.6	99.6	98.6	99.3	99.6	99.6	99.6
TN												
KY 29												
KY 34												
KY 35												
KY 43												
KY 44												
KY 102												
KY 105												
KY 108												
KY 112												
KY 113												
KY 115												
KY 117												
KY 124												
TN												

**Figure 2:** Percent similarity of (A) nucleotide and (B) amino acid sequences of nucleocapsid protein gene of *Soybean vein necrosis-associated virus* isolates recovered from infected mother plants. KY29, KY34, KY35, KY43 and KY44 were isolated from independent soybeans plants of cultivar AG4832 while KY102, KY105, KY108, KY112, KY113, KY115, KY117 and KY124 from independent soybean plants of cultivar CF386RR2y/stsn. The NP gene of SVNaV-TN isolate (GenBank accession No. HQ728387) served as the type member.

Isolates	Positions <sup>2</sup>				
	15	22	35	47	146
KY29	Arg	Arg	Asn	Thr	Lys
KY34	Arg	Arg	Asn	Thr	Lys
KY35	Arg	Arg	Asn	Thr	Lys
KY43	Arg	Arg	Asn	Thr	Lys
KY44	Arg	Arg	Asn	Thr	Lys
KY102	Arg	Arg	Asn	Thr	Lys
KY105	Arg	<b>Lys</b>	Asn	<b>Ile</b>	<b>Asn</b>
KY108	Arg	Arg	<b>Ser</b>	Thr	Lys
KY112	Arg	Arg	Asn	Thr	Lys
KY113	Arg	Arg	Asn	Thr	Lys
KY115	Arg	Arg	Asn	Thr	Lys
KY117	Arg	Arg	Asn	Thr	Lys
KY124	<b>Ile</b>	Arg	Asn	Thr	Lys

<sup>1</sup>Distinct amino acids are shown bold-faced.

<sup>2</sup>The position of the amino acids are based on the full-length deduced amino acid sequences of the nucleocapsid protein of TN isolate (GenBank accession No. HQ728387). Except for the above positions, all other encoded amino acids are identical among all 13 isolates.

**Table 2:** Positions of the unique amino acids of nucleocapsid protein of distinct variants from soybean mother plants infected with *Soybean vein necrosis-associated virus*<sup>1</sup>.

soybean mother plants of cultivar CF386RR2y/stsn, were distinct as they encoded unique residues not shared with the NP from other isolates infecting other mother plants (Table 2). KY108 encodes serine at position 35 instead of asparagine whereas KY124 encodes isoleucine at position 15 instead of arginine. Interestingly, KY105 encodes lysine instead of arginine at position 22, isoleucine instead of threonine at position 47, and asparagine instead of lysine at position 146. The phylogenetic tree generated from nucleotide sequence alignments supports BeNMV, TN, KY124 and KY105 as outgroups to the other SVNaV isolates (Figure 3A). On the other hand, the phylogenetic tree constructed from the amino acid alignments with the TN type member and the BeNMV outgroup sequences show KY105 branched separately from the other samples with a bootstrap value of 72 (Figure 3B).

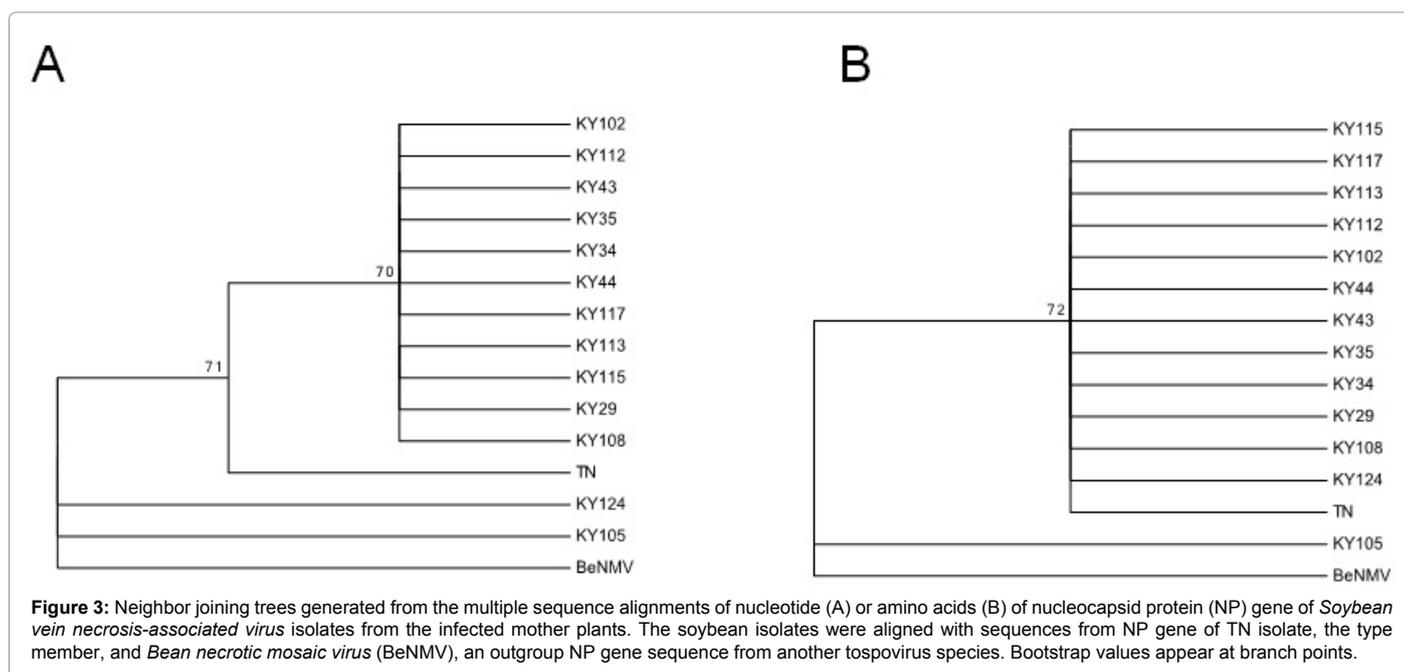
## Discussion

The main objective of this study was to determine whether SVNaV infection of soybean under field conditions results in seed transmission. To the best of our knowledge, a study addressing possible seed transmissibility of SVNaV in soybean has not been previously documented. Seed transmission is a major route for long-distance dissemination of plant viruses and also serves as the initial local source of inoculum in many viral pathosystems [14]. The underlying mechanism of seed transmissibility is complex and is influenced by many factors, including the genetics of viruses and hosts [15,21-27].

In this study we utilized two different soybean genotypes of CF386RR2y/stsn and AG4832. Seeds derived from 20 independent infected mother plants of the two cultivars did not exhibit any mottling or other viral-like symptoms. Hence, this study provided no indication that SVNaV infection in soybean results in symptomatic seeds. This is in contrast to infection of peanut with TSWV, which results in reddish discoloration and cracking of the seed coat [28]. It should be noted that infection of soybean with *Soybean mosaic virus* or *Bean pod mottle virus* results in mottling of the seed coat that has a negative impact on seed quality [29,30].

All seedlings that originated from seeds derived from infected mother plants grown under the greenhouse conditions were asymptomatic. Sap derived from only a single asymptomatic seedling of cultivar AG4832 yielded absorbance value that was higher than threshold for negative samples in ELISA. However, 116 additional seedlings derived from the same mother plant were all negative in ELISA. Furthermore, 886 seedlings from 9 other independent mother plants of the same cultivar as well as 969 seedlings of cultivar CF386RR2y/stsn from 10 independent infected mother plants were also all negative in ELISA. Based on these observations, we interpret the absorbance value for this single seedling as an anomaly and not evidence for seed transmissibility of SVNaV in soybean.

No distinct strain of SVNaV has been described to date. However, based on deduced NP amino acid sequences of the isolates recovered



from the infected mother plants, the presence of a few distinct variants was established. This is solely based on unique amino acids encoded by these isolates not shared with the other isolates infecting the other mother plants. The unique amino acids are not experimental artifacts as other isolates reported from elsewhere also encode these amino acids at similar positions. For example, the unique amino acid encoded by KY108 at position 35 (serine) is also encoded by KS2 isolate from Kansas (HQ728382). The unique amino acid at position 15 of KY124 (isoleucine) is also encoded by isolate IL6 from Illinois (HQ728369). KY105 amino acids at positions 22 (lysine) and 146 (asparagine) are also encoded by KS3 isolate from Kansas at the same positions (HQ728383). However, the amino acid at position 47 of KY105 (isoleucine) is not shared by any other isolate infecting other mother plants or other isolates reported from elsewhere whose sequences are available in GenBank. The uniqueness of KY105 is also supported by the bioinformatic analysis where it branched separately from other isolates infecting other mother plants with a bootstrap value of 72 (Figure 3B). These observations suggest that mother plants were infected with a virus population that represents known diversity of SVNaV. Regardless, the biological significance of unique amino acids encoded by these variants, all recovered from infected soybean plants of cultivar CF386RR2y/stsn (Table 1), is unknown. This is mainly due to lack of knowledge on the role of NP gene of SVNaV in interaction with soybean. In other viral pathosystems, the significance of a single amino acid substitution in viral encoded proteins is well documented [31,32]. Collectively, these observations suggest that we used different soybean genotypes as well as a representative diverse virus population and demonstrated lack of seed transmissibility of SVNaV in soybean.

It should be noted that there are two distinct routes leading to seed transmissibility of a particular virus in a particular plant host; infected embryo from an infected mother plant or fertilization of an ovule of a virus free mother plant with infected pollen originating from another infected plant [15,16,26,33]. Regardless of the route, a virus must be capable of crossing the boundary between the non-productive and productive tissues in an infected plant in order for seed transmission to occur [26]. The exact mechanism(s) by which viruses cross this boundary is not understood [14]; however, a particular virus must be capable of moving systemically, and efficiently, in a host. It has been shown that *Bean common mosaic virus*, a seed-transmissible potyvirus in bean, is not seed transmissible in *I*-gene carrying genotypes where infection remains restricted to inoculated tissues [34]. Interestingly, SVNaV is incapable of efficient systemic movement within an infected soybean plant [6,8]. Thus, it is very unlikely that in an infected soybean plant, SVNaV can move from non-productive to productive tissues to infect an embryo. On the other hand, a high rate of outcrossing is required for infected pollen to infect an ovule in a virus free mother plant. The cultivated soybeans are known to be predominantly self-pollinating with a very low outcrossing rate [35]. One should also note that not all plant viruses are pollen-borne [36]. Altogether, these limitations further support the unlikelihood of seed transmissibility of SVNaV in soybean.

For viruses that are seed transmissible, there is a direct correlation between the rate of seed transmission and time of infection; earlier infection of a mother plant results in a higher percentage of seed transmission [37,38]. Seasonal timing of appearance of SVNaV symptoms in field-grown soybeans varies among soybean-growing areas; however, in general, the virus seems to infect soybean in the late stage of growth in the United States. For example, in Arkansas and Illinois SVNaV-induced symptoms appear early in June, in KY in late June to early August, in Michigan in late August and September, in

Wisconsin and Iowa in September and in Alabama during October [3, 5-8]. Hence, late growth stage infection of soybean with SVNaV, where pods are already formed, further reduces the chance of SVNaV for being seed transmissible. One should also take into account the lack of evidence for seed transmissibility of all known tospoviruses in general [39-42]. Interestingly, TSWV is detectable in pod shells and seed testa of infected peanut; however, sowing such seeds did not result in virus transmission [28,43]. It should be noted that tospoviruses are transmitted through vegetative propagation such as growth from cuttings [44] or potato tubers [45]. However, tubers are not true seeds and this type of transmission is considered vegetative.

Collectively, our study provided direct evidence for lack of seed transmission of SVNaV in soybean. Considering our results and the lack of seed transmissibility typical of tospoviruses, seed transmission of SVNaV in soybean is highly unlikely. As such, the management of SVNaV in soybean should focus on the natural vector of the virus, thrips, as well as the secondary plant hosts as the main local sources of inoculum for spread of SVNaV to and within soybean fields and search for sources of resistance or tolerance against the virus.

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