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

Hajimorad MR***, Halter MC¹, Wang Y¹, Staton ME¹ and Hershman DE²

¹Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN 37996, USA
²Department of Plant Pathology, University of Kentucky Research and Educational Center, Princeton, KY 42445, USA

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 family Tomatospirita [6,8]. Tospovirus

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 KJ935706 to KJ935718.

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

*Corresponding author: Hajimorad MR, Department of Entomology and Plant Pathology, The University of Tennessee, Knoxville, TN 37996, USA, Tel: (865) 974 1620; E-mail: mhr@utk.edu

Received April 29, 2015; Accepted June 22, 2015; Published June 27, 2015

Citation: Hajimorad MR, Halter MC, Wang Y, Staton ME, Hershman DE (2015) Evaluation of Seed Transmissibility of Soybean Vein Necrosis-Associated Virus in Two Soybean Cultivars Grown Under Field Conditions. J Plant Pathol Microb 6: 278. doi:10.4172/2157-7471.1000278

Copyright: © 2015 Hajimorad MR, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
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 fence-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 ºC 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 per 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 ºC ± 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’-AGCCTGGCATGTTAGTACC-3’) and R2 (5’-TCACACCTGTGATTGATGC-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/.

Cultivar AG4832

Table 1: Evaluation by ELISA of 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.

| Soybean plants   | No. of seeds planted | No. of harvested seedlingb | ELISA outcomec |
|------------------|----------------------|---------------------------|----------------|
| Cultivar KY4832  |                      |                           |                |
| 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                       | 1/100          |
| KY42             | 105                  | 100                       | 0/100          |
| KY43             | 105                  | 100                       | 0/100          |
| KY44             | 90                   | 86                        | 0/86           |
| Cultivar KY386RR2y/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           |

bSeeds 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 26.7ºC ± 1.

Seeds were harvested 4-5 weeks post-emergence individually by cutting stems above the unifoliolate leaves.

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 stored at 26.7 ºC ± 1.

Seeds were harvested 4-5 weeks post-emergence individually by cutting stems above the unifoliolate leaves.

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 of soybean mother plants infected with Soybean vein necrosis-associated virus for possible virus transmission.
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 pods were harvested. A total of 100 seeds from each of five pods (approximately 20 seeds from each pod) 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 Kentucky and photos were taken about 3 months post-planting.

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.

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 soybean 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.
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

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

| Isolates | Positions | 15 | 22 | 35 | 47 | 146 |
|----------|-----------|----|----|----|----|----|
| KY29     | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY34     | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY35     | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY43     | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY44     | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY102    | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY105    | Arg       | Lys| Asn| Ile| Asn| Lys |
| KY108    | Arg       | Arg| Ser| Thr| Lys |
| KY112    | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY113    | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY115    | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY117    | Arg       | Arg| Arg| Asn| Thr| Lys |
| KY124    | Ile       | Arg| Arg| Asn| Thr| Lys |

1Distinct amino acids are shown bold-faced.
2The 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.

Figure 3: Neighbor joining trees generated from the multiple sequence alignments of nucleotide (A) or amino acids (B) of nucleocapsid protein (NP) gene of Soybean vein necrosis-associated virus isolates from the infected mother plants. The soybean isolates were aligned with sequences from NP gene of TN isolate, the type member, and Bean necrotic mosaic virus (BeNMV), an outgroup NP gene sequence from another tospovirus species. Bootstrap values appear at branch points.
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 CF386R2y/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 and time of infection; whereas, 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 tests 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.

Acknowledgement

Dr. Y. Wang wishes to express his gratitude to China Scholarship Council and Jilin provincial government for partial financial support. This project was supported in part by Kentucky Soybean Promotion Board and The University of Tennessee Agricultural Experimental Station, Knoxville.

References

1. Tzanetakis I, Wen R, Newman M, Hajimorad R (2009) Soybean vein necrosis virus: a new threat to soybean production in Southeastern United States? Phytopathology 99: S131.
2. Ali A, Abdalla OA (2013) First report of Soybean vein necrosis-associated virus in soybean fields of Oklahoma. Plant Dis. 97: 1664.
3. Conner K, Sikora EJ, Zhang L, Burmester C (2013) First report of Soybean vein necrosis-associated virus suffering soybeans in Alabama. Plant Health Progress. Online publication, doi: 10.1094/PHP-2013-0729-03-BR.
4. Han J, Domier LL, Dorrance AE, Qu F (2013) First report of Soybean vein necrosis-associated virus in Ohio soybean fields. Plant Dis. 97: 693.
5. Jacobs JL, Chilvers M (2013) First report of Soybean vein necrosis-associated virus on soybeans in Michigan. Plant Dis. 97: 1387.
6. Khatibi B, Wen R-H, Hershman DE, Kennedy BS, Newman MA, et al. (2012) Generation of polyclonal antibodies and serological analyses of nucleocapsid protein of Soybean vein necrosis-associated virus: A distinct soybean infecting tospovirus serotype. Eur. J. Plant Pathol. 133: 783-790.
7. Smith DL, Fritz C, Watson Q, Willis DK, German TL, et al. (2013) First report of soybean vein necrosis disease caused by Soybean vein necrosis-associated virus in Wisconsin and Iowa. Plant Dis. 97: 693.
8. Zhou J, Kantartzis SK, Wen RH, Newman M, Hajimorad MR, et al. (2011) Molecular characterization of a new Tospovirus infecting soybean. Virus Genes 43: 289-295.
9. Zhou J, Tzanetakis IE (2013) Epidemiology of Soybean vein necrosis-associated virus. Phytopathology 103: 966-971.
10. Tenuta A (2012) First confirmation of soybean vein necrosis virus in Ontario. http://fieldcropnews.com/2012/09/first-confirmation-of-soybean-vein-necrosis-virus-in-ontario.
11. Pappu HR, Jones RA, Jain RK (2009a) Global status of tospovirus epidemics in diverse cropping systems: success achieved and challenges ahead. Virus Res. 141: 219-236.
12. Bloomington C, Smith D, Groves RL (2014) Thrips dispersal and soybean vein necrosis virus (SVNV) in Wisconsin soybean. Proceeding of the 2014 Wisconsin Crop Management Conference 53: 95-98.
13. Hassani-Mehraban A, Botermans M, Verhoeven JT, Meekes E, Saaier J, et
13. Wang Y, Khatabi B, Hajimorad MR (2015) Amino acid substitution in P3 of Soybean mosaic virus to convert avirulence to virulence on Rsv4-genotype soybean is influenced by the genetic composition of P3. Mol Plant Pathol 16: 301-307.

14. Hull R (2014) Plant Virology. Fifth edition. Academic Press, New York.

15. Johansen IE, Edwards MC, Hampton RO (1994). Seed transmission of viruses: current perspectives. Ann Rev Phytopathol 32: 363-386.

16. Mink GL (1993) Pollen and seed-transmitted viruses and viroids. Annu Rev Phytopathol 31: 375-402.

17. Sastry KS (2013) Seed-borne plant virus disease. Springer, New York.

18. Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Series 41: 95-98.

19. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23: 2947-2948.

20. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30: 2725-2729.

21. Domier LL, Hobbs HA, McCoppin NK, Bowen CR, Steinlage TA, et al. (2011) Multiple loci condition seed transmission of Soybean mosaic virus (SMV) and SMV-induced seed coat mottling in soybean. Phytopathology 101: 750-756.

22. Hanada K, Harrison BD (1977) Effects of virus genotype and temperature on seed transmission of nepoviruses. Ann. Appl Biol 85: 79-92.

23. He B, Fajolu OL, Wen R-H, Hajimorad MR. (2010) Seed transmissibility of Alfalfa mosaic virus in soybean. Plant Health Progress doi: 10.1094/PHP-2010-1227-01-BR.

24. Johansen IE, Dougherty WG, Keller KE, Wang D, Hampton RO (1996) Multiple viral determinants affect seed transmission of pea seedborne mosaic virus in Pisum sativum. J Gen Virol 77 : 3149-3154.

25. Jossey S, Hobbs HA, Domier LL (2013) Role of Soybean mosaic virus-encoded proteins in seed and aphid transmission in soybean. Phytopathology 103: 941-948.

26. Maule AJ, Wang D (1996) Seed transmission of plant viruses: a lesson in biological complexity. Trends Microbiol 4: 153-158.

27. Pathipanawat W, Jones RA, Sivasithamparam K (1997) Factors influencing transmission of alfalfa mosaic virus through seed of annual medics (Medicago spp.) and the genetic control of seed transmission rate. Aust. J Agric Res 48: 989-997.

28. Cabulbreath AK, Todd JW, Brown SL (2003) Epidemiology and management of tomato spotted wilt in peanut. Annu Rev Phytopathol 41: 53-75.

29. Giesler LJ, Ghabrial SA, Hampton RO, McCoppin NK, Bowen CR, Steinlage TA, et al. (2011) Multiple loci condition seed transmission of Soybean mosaic virus (SMV) and SMV-induced seed coat mottling in soybean. Phytopathology 101: 750-756.

30. Tu JC (1989) Effect of different strains of soybean mosaic virus on growth, maturity, yield, seed motting and seed transmission in several soybean cultivars. J Phytopathol 126: 231-236.

31. Wang Y, Khatabi B, Hajimorad MR (2015) Amino acid substitution in P3 of Soybean mosaic virus to convert avirulence to virulence on Rsv4-genotype soybean is influenced by the genetic composition of P3. Mol Plant Pathol 16: 301-307.

32. Wen RH, Khatabi B, Ashfield T, Saghai Maroof MA, Hajimorad MR (2013) The HC-Pro and P3 cistrons of an avirulent Soybean mosaic virus are recognized by different resistance genes at the complex Rsv locus. Mol Plant Microbe Interact 26: 203-215.

33. Wang D, Maule AJ (1994) A model for seed transmission of a plant virus: genetic and structural analyses of pea embryo invasion by Pea seed-borne mosaic virus. Plant Cell 6: 777-787.

34. Feng X, Poplowsky AR, Nikolaeva OV, Myers JR, Karasev AV (2014) Recombinants of Bean common mosaic virus (BCMV) and genetic determinants of BCMV involved in overcoming resistance in common bean. Phytopathology 104: 786-793.

35. Ahrent DK, Caviness CE (1994) Natural cross-pollination of twelve soybean cultivars in Arkansas. Crop Sci 34: 376-378.

36. de Assis Filho FM, Sherwood JL (2000) Evaluation of Seed Transmission of Turnip yellow mosaic virus and Tobacco mosaic virus in Arabidopsis thaliana. Phytopathology 90: 1233-1238.

37. Owusu GK, Crowley NC, Franci RIB (1968) Studies of the seed-transmission of tobacco ringspot virus. Ann. Appl Biol 61: 195-202.

38. Ren Q, Pfeiffer TW, Ghabrial SA (1997) Soybean mosaic virus incidence level and infection times: interaction effects on soybean. Crop Sci 37: 1706-1711.

39. Bulajic A, Djekic I, Jovic J, Krnjajic S, Vucurovic A, et al. (2009) Incidence and distribution of Iris yellow spot virus on Onion in Serbia. Plant Dis. 93: 976-982.

40. Kritzman A, Lampel M, Raccah B, Gera A (2001) Distribution and transmission of Iris yellow spot virus. Plant Dis 85: 838-842.

41. Mumford RA, Barker I, Wood KR (1996) The biology of tospoviruses. Ann. Appl Biol 128: 159-183.

42. Thakur MP, Verma KP, Agrawal KC (1998) Characterization and management of bud blight disease of soybean in India. Int J Pest Manag 44: 87-92.

43. Pappu SS, Pappu HR, Cutbreathe AK, Todd JW (1999b) Localization of Tomato spotted wilt virus (Genus Tospovirus, Family Bunyaviridae) in peanut pods. Peanut Sci 26: 98-99.

44. Matsuura S, Ishikura S, Shigemoto N, Kajihara S, Hagiwara K (2004) Localization of Tomato spotted wilt virus in Chrysanthemum stock plants and efficiency of viral transmission from infected stock plants to cuttings. J Phytopathol 152: 219-223.

45. Bulajic AR, Stankovic IM, Vucurovic AB, Ristic DT, Milojcic KN, et al. (2014) Tomato spotted wilt virus - potato cultivar susceptibility and tuber transmission. A J Pot Res 91: 189-194.