Natural Infection of Soybean with *Soybean vein necrosis-associated virus* Grown under Greenhouse Conditions: An Accidental Observation

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Accepted for publication 5 October 2015. Published 14 October 2015.

Hajimorad, M. R., Halter, M. C., and Mengistu, A. 2015. Natural infection of soybean with *Soybean vein necrosis-associated virus* grown under greenhouse conditions: An accidental observation. Plant Health Progress doi:10.1094/PHP-BR-15-0025.

Soybean vein necrosis-associated virus (SVNaV), a distinct *Tospovirus* species within the family *Bunyaviridae*, was first detected in field-grown soybeans (*Glycine max* (L.) Merrill) in Tennessee and Arkansas in 2008; however, it is now considered to be the most widespread soybean disease in the United States, with a presence in Canada as well (Zhou et al. 2011; Zhou and Tzanetakis 2013).

In late February 2014, leaves of soybean lines LS98-0358, Pharaoh, and DT97-4290 grown in a greenhouse in Jackson, TN, devoid of any SVNaV-infected plants started to exhibit various symptoms when plants were still in the vegetative stage (between R3 and R4 growth stages) 44 to 50 days post-sowing. Seeds originated from field-grown soybeans and had been sown in early January and maintained in a greenhouse with an average temperature of 27°C with humidity ranging between 71 to 88%. Symptoms on leaves initially resembled those of downy mildew infection, but were not uniformly spread. Later, leaves began to exhibit viral-like symptoms including mosaic and yellowing with limited veinal necrosis (Fig. 1A-B).

To search for possible involvement of SVNaV, symptomatic portions of leaves from selected plants of each line were harvested late in March, at R5 growth stage, and assayed by ELISA (Khatabi et al. 2012). SVNaV was detected in three out of four and one out of two plants from lines LS98-0358 and Pharaoh, respectively, but in none of four plants from line DT97-4290. Total RNA was extracted from symptomatic tissues of the same plants by RNeasy Plant Mini Kit (Qiagen, Valencia, CA) and used to generate complementary DNA in the presence of random primers and SuperScript II (Invitrogen, Carlsbad, CA). 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 (Khatabi et al. 2012). Analysis on agarose-gel revealed amplicons corresponding to those of SVNaV from three out of four and one out of four soybean lines LS98-0358 and DT97-4290, respectively. No amplicon was obtained from two plants of line Pharaoh.

The amplicons derived from two plants of line LS98-0358 were purified using QIAquick-PCR Purification Kit (Qiagen) and

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doi:10.1094/PHP-BR-15-0025

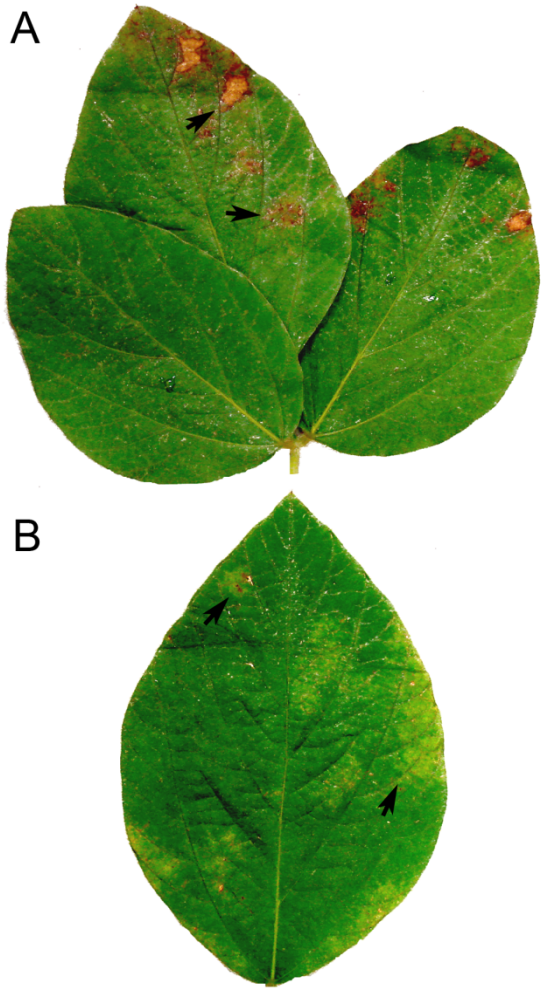

FIGURE 1

Symptomatic leaves derived from two soybean plants from breeding line LS98-0358 (A-B) grown in a greenhouse in Jackson, TN. Seeds originated from fields, but were planted and maintained under greenhouse conditions. *Soybean vein necrosis-associated virus* was detected by both ELISA and RT-PCR from symptomatic portions of leaves, indicated by arrows .

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 Tennessee isolate (GenBank Accession No. HQ728387), respectively. The full-length sequences of the nucleocapsid protein (NP) gene of both isolates were obtained and deposited in GenBank (KJ955719 and KJ955720). NP genes of both isolates were each 844 nucleotides long and identical, except at two polymorphic positions. Comparative analysis with the NP gene of the Tennessee isolate showed 99% sequence identity at both nucleotide and amino acid levels.

Our data demonstrate occurrence of natural infection of SVNaV in soybean under greenhouse conditions during winter in the absence of field-grown soybeans or any infected host plant within the greenhouse. This observation raises an interesting question regarding the source of the inoculum. SVNaV, similar to all other known tospoviruses, is not seed transmissible and mechanical transmission is highly inefficient in soybean (Hajimorad et al. 2015; Zhou and Tzanetakis 2013). Ivy leaf morning-glory (*Ipomoea hederaces* Jacq.), the only other known natural host of the virus (Zhou and Tzanetakis 2013), is an annual plant and absent during winter in fields in Jackson, TN, and our greenhouses were devoid of this plant as well. However, our greenhouse is not insect-proof, serving as "semi-open environment" and thrips were observed in contact with infected plants. It should be noted that SVNaV transmission by soybean thrips has been demonstrated, but the specific species has not been identified (Zhou and Tzanetakis 2013). Even though we did not evaluate thrips associated with the infected plants for ability to transmit SVNaV, it is very likely that viruliferous adult thrips served as the original inoculum source. Although tospoviruses replicate in thrips, transovarian transmission does not occur (Wijkamp et al. 1996). However, adult thrips can survive at low temperature up to 87 days (Murai 2000).

Collectively, these observations point to the likelihood that overwintering viruliferous adult thrips may have the potential to serve as a local source of SVNaV; however, the epidemiological significance needs to be determined.

ACKNOWLEDGMENTS

This project was supported in part by Kentucky Soybean Promotion Board.

LITERATURE CITED

Hajimorad, M. R., Halter, M. C., Wang, Y., Staton, M. E., and Hershman, D. E. 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.

Khatabi, B., Wen, R.-H., Hershman, D. E., Kennedy, B. S., Newman, M. A., and Hajimorad, M. R. 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.

Murai, T. 2000. Effect of temperature on development and reproduction of the onion thrips, *Thrips tabaci* Lindeman (Thysanoptera: Thripidae), on pollen and honey solution. Appl. Entomol. Zool. 35:499-504.

Zhou, J., Kantartzi, S. K., Wen, R.-H., Newman, M., Hajimorad, M. R., Rupe, J. C., and Tzanetakis, I. E. 2011. Molecular characterization of a new tospovirus infecting soybean. Virus Genes 43:289-295.

Zhou, J., and Tzanetakis, I. E. 2013. Epidemiology of Soybean vein necrosis-associated virus. Phytopathology 103:966-971.

Wijkamp, I., Goldbach, R., and Peters, D. 1996. Propagation of tomato spotted wilt virus in *Frankliniella occidentalis* does neither result in pathological effects nor in transovarial passage of the virus. Entomol. Exp. Appl. 81:285-292.