Host responses elicited by a satellite virus-associated synergism in multiple *Brachypodium distachyon* accessions

Jesse D. Pyle[1,2], Kranthi K. Mandadi[2], Christopher W. P. Lyons[2], and Karen-Beth G. Scholthof[1,2]

[1]Bioenvironmental Sciences Undergraduate Honors Program,

[2]Department of Plant Pathology & Microbiology, Texas A&M University, College Station, TX 77843

ABSTRACT

Panicum mosaic virus **(PMV) and its satellite virus (SPMV) infect several species of grasses (*Poaceae*). SPMV is an 824-nucleotide single-stranded RNA virus that is dependent upon PMV, its helper virus, for replication and movement in host plants. The 17-kDa capsid protein of SPMV (SPCP) is the main effector of a distinct disease synergism with its helper virus, causing pronounced disease symptoms. Our previous studies in millets and Brachypodium, a model grass species in the family *Poaceae*, have shown that SPCP localizes to the nucleus/nucleolus and plasmodesmata. The PMV+SPMV co-infection mis-regulates the expression of several genes involved with metabolism, photosynthesis, and pathogen defense. We performed a screen of 189 geographically diverse inbred accessions of Brachypodium by co-infecting with PMV+SPMV. Plants were scored for disease symptoms at 7, 14, and 21 days post inoculation (dpi) and subsequently analyzed for accumulation of the 26-kDa PMV capsid protein and SPCP, as markers for mixed virus infections, by immunoblot analyses. At 21 dpi, analysis of virus accumulation on upper-non-inoculated leaves of the 189 accessions revealed that only 24 accessions consistently supported SPMV, characterized by the typical severe disease symptoms of PMV+SPMV-infected plants, while the others did not. Plants that did not support SPMV had milder symptoms. The future goal of this study is to identify genetic factors that contribute to the PMV and SPMV-synergism, thus facilitating the engineering of new strategies for host plant resistance.**

INTRODUCTION

- ***Panicum mosaic virus*** **(PMV) alone or in a co-infection with its satellite virus (SPMV) is responsible for the diseased condition of St. Augustinegrass, referred to as St. Augustine decline.**

- **Previous research in our lab revealed a unique disease synergism between PMV and SPMV during a co-infection in millet species[1, 2].**

OBJECTIVE

- **The primary objective of this study is to establish Brachypodium[3] as a disease model for viral infections of PMV and SPMV, with a particular focus on understanding SPMV-imparted synergism.**

METHODOLOGY

- **Brachypodium accession Bd21-3 was mechanically inoculated with infectious RNA of PMV alone and PMV+SPMV. Disease symptoms were scored and infected plants were analyzed by immunoblotting technique using PMV and SPMV anti-serum.**

- **The genetic screen for PMV and SPMV resistance or susceptibility was performed on 189 geographically diverse Brachypodium accessions[4]. Disease symptoms were scored visually and infected plants were analyzed by immunoblotting methods at 7, 14, and 21 days post-inoculation to detect PMV and SPMV.**

- **Transcriptome analysis of PMV and PMV+SPMV infected Brachypodium was performed to identify molecular pathways affected by PMV and SPMV[5].**

RESULTS AND DISCUSSION

Fig. 1A

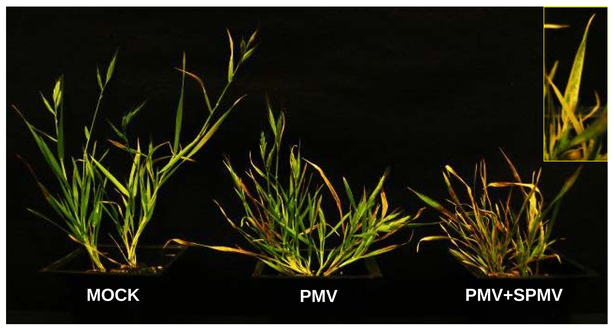

Fig. 1B

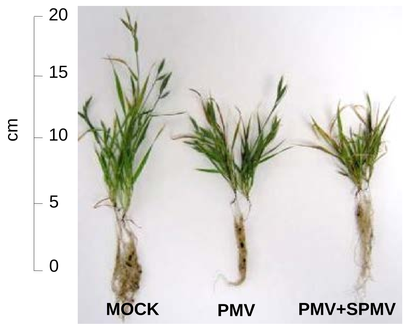

Fig. 1C

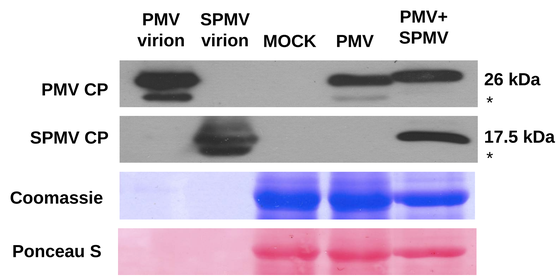

Figure 1. Phenotypic and molecular characterization of PMV and PMV+SPMV infection in Brachypodium. Brachypodium plants infected with PMV and PMV+SPMV displays chlorosis and necrosis of shoots and are stunted (A, B). Inset picture in Figure 1A shows the deep chlorosis symptoms typical issociated with the co-infection of PMV+SPMV compared to PMV alone infection. Immunoblotting using PMV and SPMV anti-serum consistently detected PMV and SPMV coat protein (CP) in the infected plants (C). Coomassie- and Ponceau S-stained gel and membrane, respectively, are shown to demonstrate approximately equal loading in the lanes. The asterisk (*) represents lower molecular-weight CPs, possibly produced from leaky scanning or post-translational modifications.

Fig. 2A

Fig. 2B

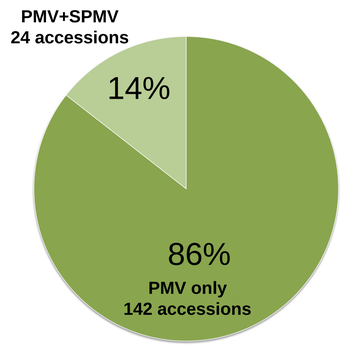

Fig. 2C

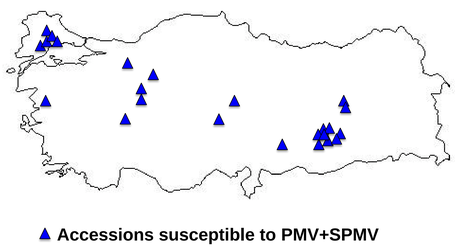

Figure 2. Genetic screen of 189 Brachypodium accessions following inoculation with PMV+SPMV. Brachypodium accessions (A) were collected from different geographical locations in Turkey[4] . A representative map of the few accessions are indicated in Figure 2C. Accessions were categorized based on the presence or absence of PMV and SPMV upon infection, as determined by immunoblotting using PMV and SPMV anti-serum and disease symptoms.

Table 1. Microarray analysis of PMV and PMV+SPMV infection. Expression of putative splicing factors and transcription factors uniquely altered during a PMV+SPMV infection. The relative fold-change in gene expression of PMV and PMV+SPMV compared to mock is indicated. Statistical significance was determined using Student's t-test and was corrected for false discovery rate using Benjamini and Hochberg method (q-value < 0.05).

Gene ID	PMV	PMV+ SPMV	Description
Bradi2g09850	1.11	1.58	Pre-mRNA splicing factor PRP21 like protein, splicing factor 3A subunit 1
Bradi5g07850	1.11	2.09	Splicing factor 1/branch point binding protein (RRM superfamily), Zinc knuckle domain
Bradi3g06560	1.16	1.55	AP2 domain
Bradi4g34700	1.02	1.63	Unknown

CONCLUSIONS AND FUTURE EXPERIMENTS

- **Brachypodium is a supercalifragilisticexpialidocious (good) genetic model for studying host:virus interactions and synergism between PMV and SPMV.**
- **Genetic diversity among Brachypodium accessions will allow further molecular characterization of the PMV and SPMV synergism.**
- **SPMV specifically affects expression of transcription and splicing factors.**
- **Expression analysis of SPMV-specific nuclear factors among the Brachypodium accessions using quantitative RT-PCR will allow us to determine if their regulation is conserved among different Brachypodium accessions that support SPMV.**

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