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Comparative analysis of virus-specific small RNA profiles of three biologically distinct strains of Potato virus Y in infected potato (*Solanum tuberosum*) cv. Russet Burbank

Khalid Naveed[a], Neena Mitter[b], Artemus Harper[c], Amit Dhingra[c], Hanu.R. Pappu[a,*]

[a] Department of Plant Pathology, Washington State University, Pullman, WA, USA
[b] Queensland Alliance for Agriculture and Food Innovation, The University of Queensland, St. Lucia, QLD, Australia
[c] Department of Horticulture, Washington State University, Pullman, USA

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ABSTRACT

Deep sequencing technology has enabled the analysis of small RNA profiles of virus-infected plants and could provide insights into virus–host interactions. Potato virus Y is an economically important viral pathogen of potato worldwide. In this study, we investigated the nature and relative levels of virus-derived small interfering RNAs (vsiRNAs) in potato cv. Russet Burbank infected with three biologically distinct and economically important strains of PVY, the ordinary strain (PVY-O), tobacco veinal-necrotic strain (PVY-N) and tuber necrotic strain (PVY-NTN). The analysis showed an overall abundance of vsiRNAs of 20–24 nt in PVY-infected plants. Considerable differences were present in the distribution of vsiRNAs as well as total small RNAs. The 21 nt class was the most prevalent in PVY-infected plants irrespective of the virus strain, whereas in healthy potato plants, the 24 nt class was the most dominant. vsiRNAs were derived from every position in the PVY genome, though certain hotspots were identified for each of the PVY strains. Among the three strains used, the population of vsiRNAs of different size classes was relatively different with PVY-NTN accumulating the highest level of vsiRNAs, while PVY-N infected plants had the least population of vsiRNAs. Unique vsiRNAs mapping to PVY genome in PVY-infected plants amounted to 3.13, 1.93 and 1.70% for NTN, N and O, respectively. There was a bias in the generation of vsiRNAs from the plus strand of the genome in comparison to the negative strand. The highest number of total vsiRNAs was from the cytoplasmic inclusion protein gene (CI) in PVY-O and PVY-NTN strains, whereas from PVY-N, the NIb gene produced maximum total vsiRNAs. These findings indicate that the three PVY strains interact differently in the same host genetic background and provided insights into virus–host interactions in an important food crop.

1. Introduction

Potato virus Y (PVY) is an economically important pathogen of potato worldwide causing significant losses to both yield and quality (Gray et al., 2010; Karasev and Gray, 2013; Nie et al., 2004). PVY belongs to the family *Potyviridae*, and genus *Potyvirus*. The genus is one of the largest genera of plant-infecting viruses and has 128 approved and 89 tentative species (Fauquet et al., 2005). PVY is a complex of different strains which differ from one another in the symptoms they produce and in their genomic sequences. PVY strains include the ordinary strain (PVY-O), stipple streak strain (PVY-C) and necrotic strains: tobacco veinal-necrotic strain (PVY-N), necrosis tuber-necrotic strain (PVY-NTN), necrotic-wigla (PVY-N:Wi) and a recombinant between N and O (PVY-N:O) (Singh et al., 2008; Kerlan, 2006; Karasev and Gray, 2013). PVY-O is the most prevalent strain in Europe and the USA and it produces foliar symptoms including mosaic, mottling, leaf drop and premature leaf senescence in majority of potato cultivars (Gray et al., 2010).

The necrotic strain, PVY-N, induces systemic veinal necrosis in tobacco and but mostly without any foliar symptoms in majority of the potato cultivars. The emergence of necrotic strains has made the control of PVY particularly difficult, since these necrotic strains do not produce foliar symptoms in potato and this results in disease escapes. However, necrotic strains impact the potato yield and produce external and internal necrotic rings on the tubers of sensitive potato cultivars such as Highland Russet, resulting in the development of disease known as “Potato tuber necrotic ring spot

* Corresponding author. Tel.: +1 509 335 3752; fax: +1 509 335 9841.
E-mail address: hrp@wsu.edu (Hanu.R. Pappu).

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disease" (PTNRD) (Beczner et al., 1984). However, PVY-NTN does not always produce PTNRD. Isolates from the PVY-N strain group induce systemic veinal necrosis in tobacco, whereas potato cultivars carrying Nc or Ny genes do not show systemic veinal necrosis due to PVY-N infection. Production of different kinds of disease symptoms indicates that different PVY strains interact differently in different potato cultivars (Karasev et al., 2008; Singh et al., 2008).

The genome of PVY is a single-stranded, positive sense RNA, 9.7 kb in length and is translated into a single large polyprotein that is subsequently cleaved into 10 mature functional proteins by the action of three virally coded proteases (Hc-Pro, P1 and Nib). PVY genome has an untranslated region at the 5′ end and a poly A-tail at the 3′ end. The virions are 680–900 nm in length and 11–15 nm in width and has one major species of 29-kDa capsid protein, whereas Hc-Pro is also present in virus particles along with CP, and a VPg that is covalently attached to the genomic RNA (Edwardson, 1966; Kerlan, 2006). Infection by potyviruses usually is associated with inclusion bodies that could be used as a diagnostic feature for potyvirus identification (Edwardson, 1966; Kerlan, 2006; Torrance et al., 2006).

Compatible virus–plant interactions usually result in the accumulation of virus-derived small interfering (vsiRNAs) in infected plant cells as a result of host-induced defense mechanism (Dunoyer and Voinnet, 2005). The vsiRNAs generated as a result of virus infection target homologous viral genes as part of host's antiviral defense mechanism. Virus-specific dsRNA in an infected host cell can result from different mechanisms including processing by virus encoded RNA polymerases, base pairing between plus and minus strands of viral RNAs, imperfect folding of self-complementary sequences and action of host encoded RDRs (Kasschau et al., 2007). RNA interference (RNAi) is a conserved mechanism among eukaryotes that involves the processing of dsRNA into small interfering (si) RNAs or micro (mi) RNAs by RNAse III Dicer-like proteins (DCLs) and formation of RNA-induced silencing complex mediated by argonautes (AGO) protein family to facilitate the cleavage of target RNA (Elbashir et al., 2001; Baulcombe, 2004; Deleris et al., 2006; Blevins et al., 2006). During virus–host interactions, the production of vsiRNAs not only requires DCLs and AGOs, but it is also dependent on virally coded RNA dependent RNA polymerases (RdRp) that play role in synthesis of dsRNA by using RNA as a template toward amplification of silencing (Brodersen and Voinnet, 2006; Wang et al., 2010). Studies conducted with loss-of-function mutants in *Arabidopsis* shows that in case of RNA viruses DCL4, DCL2 and DCL3 processing of viral dsRNA leads to 21, 22 and 24 nt vsiRNAs, respectively (Deleris et al., 2006). In the absence of DCL 4, DCL2 processing of dsRNA results in increased levels of 22 nt vsiRNAs and both 21 and 22 nt vsiRNAs are efficient inducers of antiviral response of the host (Ruiz-Ferrer and Voinnet, 2009). Mi et al. (2008) and Montgomery et al. (2008) have shown that multiple AGO genes might be involved in antiviral defense and the identity of 5′ nucleotide of the siRNA may dictate the binding of AGO protein to siRNAs.

Recent high-throughput sequencing of vsiRNAs in different host–virus infection systems along with functional characterization have provided insights into the origin and composition of vsiRNAs and their potential for controlling gene expression (Prabha et al., 2013). Studies on various host-virus systems support the evidence that 21 nt class processed through the action of DCL4 is the most predominant amongst vsiRNAs with a few exceptions. In addition to the variation in characteristics of vsiRNAs, host small RNAs population also varies in response to virus infection and may differ for same virus infecting different hosts or same host infected by different viruses. Mitter et al. (2013) showed significant differences in vsiRNA profiles as well as host endogenous small RNAs in case of tomato spotted wilt virus-infected *Nicotiana benthamiana* and tomato. While much is known about the biology, strain diversity, epidemiology and molecular biology of PVY, to the best of our knowledge, no information is available about the composition of vsiRNAs in potato infected with PVY. In this study, we hypothesized that, during the infection process, different strains of PVY interact differently in potato plants of the same cultivar, thereby resulting in differential expression of virus-specific siRNAs. To test this hypothesis, small RNA populations were obtained from PVY-infected potato plants that were separately inoculated with three most prevalent and economically important strains of PVY. Potato cv. Russet Burbank was used in the study due to its wide adoption and cultivation in the Pacific Northwestern USA and Canada. It is a multipurpose potato cultivar and is suitable for fresh market and is excellent for baking and French fries (The potato association of America, 2009). We report here, a comparative description of the small RNA profiles produced by three biologically distinct, economically important, and most prevalent strains of PVY in the same genetic background of a popular potato cultivar, Russet Burbank.

2. Materials and methods

2.1. Plant materials and PVY strains

Three strains of PVY, ordinary strain (PVY-O) isolate ID243, tobacco veinal-necrotic strain (PVY-N) Montana isolate and necrosis tuber-necrotic strain (PVY-NTN) isolate L26 were provided by A. Karasev, University of Idaho, Moscow, ID. These were maintained in *Nicotiana tabacum* in a greenhouse. The isolates were characterized on the basis of their symptoms in indicator hosts (Lorenzen et al., 2006; Karasev et al., 2011; Hu et al., 2009). Healthy plants of potato cultivar Russet Burbank were grown through tissue culture from virus-free potato plants. Two-week-old potato seedlings from tissue culture medium were transferred to pots containing LC1 potting mixture and were maintained in a greenhouse. Six-week-old plants were inoculated with virus inocula by grinding the leaves of *N. tabacum* plants that were separately infected with three strains of PVY (O, N and NTN) in 0.1 M phosphate buffer (pH 7.2) containing 0.4% beta-mercaptoethanol (Hu et al., 2009). Before inoculation with different strains of PVY, plants were tested to ensure their virus-free status by ELISA using a commercially available kit (Agdia Inc., Elkhart, IN). Four to five leaves of healthy potato plants were dusted with carborundum powder-320 mesh (Fisher) prior to applying the inoculum using a cotton swab. Following inoculations, each group of plants were kept in separate compartments of a greenhouse with same environmental conditions. In each treatment, ten plants of Russet Burbank were inoculated with each of the three strains of PVY and four healthy controls were included in each treatment.

2.2. Virus infection

Following inoculations, plants were observed daily for symptom development. Symptoms of PVY infection started to develop two weeks post-inoculation (WPI). Three WPI, two younger, uninoculated leaves were taken from the inoculated plants and tested by DAS-ELISA. Leaves from uninoculated potato plants were included in the ELISA assay as negative (healthy) controls and only buffer was used as a buffer control. Samples were tested in duplicate wells by using equal amount of leaf sample (0.50 g) in 1 ml of extraction buffer. The plant samples that gave absorbance values three times higher than the healthy controls were considered positive to infection with PVY. Leaves from *N. tabacum* plants infected with PVY were included as a positive control. Four WPI, PVY-infected plants which showed similar virus levels as determined by ELISA were sampled and 4–5 uninoculated, systemically infected leaves were harvested from each plant infected with different strains of PVY.