Review Article

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Analysis of the potato virus Y transcriptome reveals the synergistic pathways between two strains in Solanum tuberosum.

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ABSTRACT

Superinfection exclusion (SIE) is a mechanism that many viruses utilize to prevent other viruses, or viruses that are closely related to them, from entering or replicating in the cells they occupy. When a host plant infected with a weak strain of a virus or viroid develops immunity against a more severe strain that is closely similar to the first infectant, this phenomenon is known as SIE, also known as cross-protection. It is unclear how cross-protection works in its entirety. We conducted a comparative transcriptome analysis of potato (Solanum tuberosum L.) leaves in this investigation. We will henceforth refer to the strains PVYN− Wi-HLJ-BDH-2 and PVYNTN− NW-INM-W-369-12 as BDH and 369, respectively. Between the Control and JZ, 806 differentially expressed genes (DEGs) were found.(BDH preinfection and 369 treatment challenge). The response to external biological stimulation, signal transduction, kinase, immune, and redox pathways were all considerably enriched, according to a Gene Ontology (GO) analysis. We found a large number of metabolites that were expressed differently along these routes.connected to a viral illness. Furthermore, a small group of genes that are probably crucial for the development of cross-protection were also found in our data. In particular, we found significant differences in the expression of the subtilisin-like protease StSBT1.7 gene, elongation factor 1-alpha-like gene, and A1-II gamma-like gene; of these, StSBT1.7 was the most important in our transcriptome data. These genes have the ability to generate chemical defense in plants, promote the expression of defensive plant genes, and contribute to the generation of harmful microorganisms and trauma.

INTRODUCTION

After rice, wheat, and corn, potatoes are the fourth most widely cultivated food crop worldwide. However, potato virus Y (PVY) significantly hinders potato growth and output. PVY is a common and economically harmful potato disease that belongs to the family Potyviridae and genus Potyvirus. There are various strain groupings within PVY, including as the traditional PVYO, PVYN, and PVYC strains. There are other strains and sub-strains of the virus, such as PVYO, PVYN, and PVYC (Jones, 1990; Zaitlin, 1976; Valkonen and Jari, 2015) and the recently emerged recombinant strains PVYN:O, PVYN− Wi, Eu-PVYNTN, and PVYNTN− NW (Karasev and Gray, 2013).
On the other hand, in most, if not all, potato cultivars, they show wildly disparate levels of virulence, with PVYN− Wi typically producing mild symptoms and PVYNTN− NW-SYR-II producing severe symptoms (Bai et al., 2019; Chikh-Ali et al., 2010; Kamangar et al., 2014; Anfoka et al., 2014; Folimonova, 2013). PVYNTN− NW-SYR-II can cause potato tuber necrotic ringspot disease (PTNRD) in susceptible cultivars in addition to the foliar symptoms. This can have a disastrous effect on the yield and quality of potatoes (Mackenzie et al., 2019; McKinney, 1941; Nanayakkara et al., 2012).

Abdalla et al. (2018), Atta et al. (2019), and Zhou and Zhou (2012) described cross-protection, a mechanism where host plants infected by a mild virus or viroid strain build immunity against more severe, closely related strains of the same pathogen. Fulton (1986), Atta et al. (2019), and Ziebell and Carr (2010) have all pointed out how little is known about the fundamental mechanics of this process. Cross-protection has been shown to be effective in protecting a variety of economically significant viruses in a number of horticulture crops, including Cucurbita crops (Lecoq and Katis, 2014; Huang et al., 2019). For instance, utilizing a moderate strain of the pepino mosaic virus (PepMV; genus Potexvirus, family Alphaflexiviridae), cross-protection against the virus that infects tomatoes has been proven (Agüero et al., 2018; Harper et al., 2017). Likewise, a slight strain, We show that PVYN− Wi (HLJ-BDH-2) can cross-protect potato plants against PVYNTN− NW-SYR-II (INM-W-369-12), reducing quality and yield losses brought on by the latter in cultivars of potatoes that are popular yet susceptible to the virus. The functional categorization of differentially expressed genes in cross-protected plants is greatly aided by the information our

work offers.Furthermore, the findings suggest that cross-protection could be used as a substitute method for potato growing, especially in regions where seed potato certification is not available or where environmental variables make virus control difficult. The molecular underpinnings of cross-protection and its possible uses in agriculture are clarified by this work.

MATERIALS AND METHODS

Plant materials and PVY isolates

The Heilongjiang Academy of Agricultural Sciences in Harbin provided virus-free mini-tubers of the potato variety "Kexin 13," which were then planted in mixed loam soil that had been ready for potting. The plants were grown in a controlled environment chamber with a 16/8 h light/dark cycle and 20–22 □C temperature range. The humidity was regulated between 70 and 80%, while the light was kept at 1500 lux of intensity. Each plant's penultimate leaves were covered with carborundum when it reached the 4-leaf stage, and they were then gently rubbed with a pestle. This was carried out using an inoculation buffer or 0.5 mL of BDH or 369 leaf extract, with a leaf to buffer ratio of 1:10 (wt/vol).

There were four treatment groups created

mild strain (PVYN− Wi-HLJ-BDH-2) and buffer inoculation (CK). obtained from HAAS)inoculation (RD), challenge inoculation (JZ: preinfected with BDH and challenge with 369), and severe strain (PVYNTN− NW-INMW-369-12 obtained from HAAS) inoculation (QD). There were five duplicates of each therapy. Each copy has three plants. On the same leaflets, the plants were manually inoculated with the challenging PVY isolate of 369 fifteen days following the pre-inoculation with HLJ-BDH-2 or buffer. Following inoculation, samples of Kexin 13 leaves were taken at various periods, and they were promptly refrigerated at -80°C until they were needed for protein and RNA extraction.

Physicochemical property analysis after virus inoculation

Fresh leaf tissue weighing about 0.5 g was ground up and combined with 0.1 mM EDTA, 1% PVP (w/v), 0.1 mM PMSF, and 0.2% Triton X-100 (v/v) in a blender. After that, this mixture was centrifuged for 15 minutes at 12,000 rpm and 4 °C. Following centrifugation, 1 mL of the transparent upper layer was removed and mixed with 1 mL of hydroxylamine hydrochloride, β-aminobenzene sulfonic acid, and α-naphthylamine in equal amounts. After that, this new solution was incubated for 20 minutes at 25 □C. Using a $NaNO_2$ reference curve for calibration, the absorbance of this final mixture was measured at 530 nm to quantify the quantity of the superoxide radical.

Reactive oxygen species (ROS) in leaf tissue can be seen by using a mixture of 0.1% NBT (nitroblue tetrazolium chloride) and 0.1% DAB (3,3'-diaminobenzidine tetrahydrochloride) solutions in addition to 0.1% NBT solutions, were used. In order to prepare these solutions, they were dissolved in a 10 mM KH_2PO_4 buffer (pH 7.8). Samples of leaves from plants that were both infected and controlled were vacuum-infiltrated with the corresponding solutions for five minutes at a pressure of 100–150 bar. The leaves were vacuum-infiltrated, then left in darkness for the whole night before being exposed to 1500 lux of light for an additional 8 hours. The stains were removed from the leaves by soaking them in a bleaching solution including glycerol, acetic acid, and methanol.

RNA-seq and transcriptome analyses

For RNA extraction and RNA-seq, samples of the potato cultivar Kexin 13 leaves were taken 12 hours, 24 hours, and 10 days after inoculation. The quality of the RNA of the leaf samples was verified, and Zhejiang Annoroad Biotechnology Co., Ltd. received the materials for RNA sequencing (Das et al., 2019). For the purpose of preparing RNA samples, 2 μg (μg) of RNA per sample was used as the input material. Sequencing libraries were produced using the NEBNext UltraTM RNA Library Prep Kit for Illumina (#E7530L, NEB, USA), in accordance with the manufacturer's instructions. To allocate sequences to each unique sample, index codes were added. In summary, poly-T oligo-attached magnetic beads were used to separate mRNA from total RNA, and Fragmentation was carried out in the presence of divalent cations at a high temperature in the NEBNext First Strand Synthesis Reaction Buffer (5×). First strand cDNA was synthesized using RNase H and a random hexamer primer in the first step. Next, dNTPs, RNase H, DNA polymerase I, and a buffer were used to synthesise cDNA on the second strand. Using QiaQuick PCR kits, the resultant library fragments were purified and then eluted using EB buffer. The addition of adapters, A-tailing, and terminal repair came next. After obtaining the appropriate products, PCR was carried out to finish the library.

RESULTS

The PVY isolate BDH, which we refer to as RD, was first introduced into the Kexin 13 variety of Solanum tuberosum L in our investigation. This BDH isolate shares 94.3% of its sequence identity with another isolate, 369 (referred to as QD), demonstrating a high degree of similarity. Additionally, according to Bai et al. in 2019, they share the same serological traits. The plants were mechanically injected with BDH, 369, or a mimic buffer solution when they reached the 4-leaf stage. The same leaflets were exposed to a difficult isolate of 369 in the RD therapy (referred to as JZ) fifteen days later (Fig. 1).

Regarding Kexin 13, the plants that had a pre-inoculation with the dummy When the buffer was then challenged with the 369 isolate (QD), it showed mosaic and severe necrotic signs. On the other hand, plants pre-infected with BDH and subsequently challenged with 369 showed symptoms similar to those seen in plants that were inoculated with BDH alone (Fig. 1). The fact that 369 did not cause new symptoms in plants that were already BDH-infected suggests that the original BDH infection served as cross protection against the 369 strain.

staining the infected seedlings' and control seedlings' leaves (Fig. 1). When we measured the O2− and H2O2 levels in the leaves, we discovered that there was little buildup in the leaves of the seedlings treated with challenge inoculation and control (Fig. 1). On the other hand, the QD-injected seedlings had noticeably greater amounts of ROS buildup. More specifically, compared to the other groups, the leaves injected with QD showed the deepest staining, suggesting that the virus inflicted the greatest harm to the leaves. The staining experiment's findings revealed that the leaves inoculated with 369 had the deepest staining, indicating the highest degree of leaf damage.

In order to study the molecular reactions of potato plants to various virus inoculations, we separated the samples into three groups: control, QD, RD, and JZ, which were each given a treatment for 12 hours, 24 hours, and 10 days. At 12 hours, Group 1 showed fragmented samples that suggested early molecular differential expression in response to the virus infection. Group 1 also included the control and all infected groups. JZ and RD, who were infected for 24 hours and 10 days, were included in Group 2, which clustered with the control group, showing the plants had developed a strong defense mechanism and resumed normal growth. Potatoes in Group 3 had been infected with QD for 24 hours and 10 days, and they displayed notable molecular alterations that set them apart from the other groups. Our findings showed that, in contrast to the control group, potatoes were unable to successfully fend off QD infection and were unable to reach their normal growth stage (Fig. 2). It is noteworthy that the expression aggregation of the JZ and RD groups was identical, suggesting that the plants had built a strong defense mechanism against RD infection, which enabled them to withstand QD infection even in the presence of RD infection.

Under all PVY stain infections, significant gene co-expression response modules are seen. For instance, the modules that respond strongly to QD, RD, and JZ treatments are green-yellow, black, blue, turquoise, and especially magenta (correlation coefficient: 0.78 and P = 3e− 7). Following RD infection, magenta is likewise the most significantly associated module (correlation coefficient: 0.71 and P = 1e−5). Under QD infection, turquoise (correlation coefficient: 0.59 and P = 7e− 4) is the most significant response module. Greater similarity is seen in the common response modules between JZ and RD infections than those of QD infection, which is in line with the findings of the earlier gene expression study (Fig. 3) and phenotypic data. The most important response module under JZ infection, in contrast to RD, is green-yellow, which may suggest a "composite" infection with QD. Compared to RD, this module makes a stronger contribution. Under RD and JZ infections, light green is the most significant module (correlation coefficient: 0.65 and P = 1e− 4). The important

DISCUSSION

PVY is a significant issue that restricts potato production, leading to significant reductions in both tuber yield and quality. Potato damage caused by PVY has been demonstrated to be greatly reduced by cross protection; nevertheless, the molecular processes driving cross protection remain poorly understood (Wang et al., 1991; Zhang and Qu, 2016). The current work investigated the variation in the transcriptome linked to cross-protection using RNA-seq technologies. Our investigation uncovered a large number of resistance genes that are involved in plant-virus interactions, such as A1-I gamma 1, SBT, and ERF transcription factor genes. A1-I gamma 1 has a strong positive correlation with QD, RD, and JZ, and it can hydrolyze methyl jasmonate (MeJA) to create jasmonate (JA). Serine similar to subtilisin Extracellular proteases known as proteases (SBTs) depend on certain characteristics of zymogens to mature and activate them. We hypothesize that StSBT1.7 may work through the cell wall to stop virulent toxin from invading more areas; however, more research is needed to determine the precise molecular mechanisms.

More recent studies have questioned the function of some viruses, as noted by Ratcliff et al. (1999) and Kurihara and Watanabe (2003), in the SIE of different RNA viruses. Rather, these investigations indicate different pathways, as noted by Zhang et al. (2018), Zhang and Qu (2016), and Ziebell and Carr (2010). Our work proposes a novel mechanical explanation for superinfection exclusion and presents a comprehensive model that incorporates these results. Our most recent research, which used PVY as a model for SIE, has shown a mechanism that is centered on proteins. The virus may effectively leverage the host transcription and translation system (magenta module) during RD processing to multiply itself without causing substantial damage to the host. As a result, the host can grow normally and has a minimal immunological reaction to RD. Upon initial exposure to RD, the host develops increased resistance to QD (also known as "JZ") infection. While the host (green-yellow module) does respond to QD to some extent, this is mostly because the host has a high number of

RD copies, which gives the host a population advantage and helps to refocus the response on host defense.
could cause the turquoise module's scheduled cell death. Sequence analysis revealed that the only genes different between the moderate strain PVYN− Wi-HLJ-BDH-2 (RD) and the severe strain PVYNTN− NW-INM-W-369 (QD) were the coat and Nlb genes. This shows that the proteins from QD may be more hazardous to the host (light green module). To find out more about the many host reactions that these two genes cause, more research will be done.

CONCLUSION

Since potatoes are mostly cultivated by vegetative methods, they are naturally susceptible to a range of viral illnesses. A viable substitute strategy for mitigating the severe yield losses and quality degradation that more virulent strains in potato production usually cause is the application of modest strain-mediated cross protection. To be more precise, using PVYN− Wi to provide cross-protection against the more aggressive PVYNIN-NW SYR-II strain in potatoes may be a useful tactic, especially when the latter applies significant pressure and traditional control. Measures are insufficient. Nonetheless, more investigation is necessary to fully assess the safety and efficacy of applying this cross-protection method in real-world deployment scenarios.

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