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Review Article

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Integration of Transcriptomic approaches towards understanding Begomovirus Infection in Plants

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A B S T R A C T

Begomoviruses are considered as the destructive plant virus genus. During plant-virus interactions, begomovirus alters several cellular and physiological pathways by changing the gene expression. To understand these interactions with host plants, transcriptomic methods are adopted by many researchers. Significant changes in the expression of transcripts are found to be associated with biochemical pathways. Viral infection begins a complex interaction between the virus and the host. Unravelling these interactions can be helpful in the development of effective strategies for the virus control. In recent years, RNA sequencing and microarray studies have been applied extensively to know the response of plant hosts to viral infection. We aimed to identify important differentially expressed genes in host plants under begomovirus infection. In this present review, we focused on begomovirus-plat interactions with the help of transcriptomic analysis which will be helpful for scientific community to better understand the mechanisms of host against begomovirus species.

Introduction

Plant viruses have been evolved with massive potential of utilizing and regulating host cells to cause infection. They need host machinery for their replication, transcription and movement (Czosnek *et al.*, 2013). During pathogen invasion, plants have developed various defense systems (Kachroo *et al.*, 2006). Host-Virus specific interactions determine the severity of infection. *Begomovirus* (Family *Geminiviridae*) causes severe threat to plants and major economic loss in tropical, sub-tropical and temperate regions. Geographical range of this genus increases rapidly and spread all over the world. According to International Committee on Taxonomy of Viruses (ICTV), *Geminiviridae* family is classified into nine genera viz. *Becurtovirus, Begomovirus, Capulavirus, Curtovirus, Eragovirus, Grablovirus, Mastrevirus, Topocuvirus* and *Turncutovirus* (Boulton, 2005). Among the nine

genera, *Begomovirus* is the largest plant virus genus and has 445 species according to ICTV 2020 report. It is known to infect mostly dicotyledonous plants. The transmission of the virus is mediated by whitefly (*Bemisia tabaci)* vector (Morales and Jones, 2004; Seal *et al.,* 2007). It is considered as the causal agent of destructive diseases like leaf curl in chilli (Kumar *et al.,* 2011), mosaic of cassava (Mittal *et al.,* 2008), cotton (Monga *et al.,* 2009), okra (Sheikh *et al.,* 2013), potato (Kumar *et al.,* 2021), tomato (Kumar *et al.,* 2008), zucchini, beans (Kumar *et al.,* 2009; Zaim *et al.,* 2011), pulses (Raj *et al.,* 2005a), cucurbits (Guzman *et al.,* 2000; Brown *et al.,* 2001; Dasgupta *et al.,* 2003), flowering plants (Kumar *et al.,* 2010), chlorosis in *Cordyline fruticosa* (Lager *et al.,* 2022) and etc. Their genome is made up of either DNA-A component (monopartite) or both DNA-A or DNA-B (bipartite), covalently closed circular (ccc) single stranded DNA. The genome size is approximately 2.6-2.8kb in size and encapsidated in geminate quasi-isometric virion particles. The component DNA-A has six open reading frames (ORFs), four in the complementary sense (AC1, AC2, AC3 and AC4) and two in the virion sense (AV1 and AV2). The DNA-B component has two ORFs, the virion-sense BV1 and complementary-sense BC1. The nucleotide segment (~200 bp) of the intergenic region (IR), designated the common region (CR). The CR region contains an origin of replication (ori), including a stem-loop structure containing the invariant nonanucleotide 'TAATATTAC' sequence, this sequence is used for the cleavage and for viral DNA replication. The monopartite begomoviruses have the complementary-sense C1, C2, C3 and C4genes and the virion-sense V1and V2 genes.

Monopartite and some bipartite begomovirus infection are usually associated with circular ssDNA satellites, referred to as alpha-satellite and beta-satellite. It depends on the helper virus for encapsidation and systemic infection. Alpha-satellite encodes a Rep gene and is replicated autonomously in host plant cells (Saunders and Stanley, 1999). Beta-satellite encodes a βC1 protein, dependent on the helper virus for replication (Cui *et al.,* 2005) and plays an important role in the development of symptoms of their helper viruses (Jyothsna *et al.,* 2013; Kumar *et al.,* 2008). In last few years, a large number of begomoviruses have been reported all over the world, attacking fibre crops, vegetables, root crops, pulses and legumes (Sharma *et al.,* 2021).

Importance

The invention and development of new technology creates new opportunities to improve the knowledge regarding virus-host interactions. The development of genomic technologies is now useful and providing virus-host interactions. Now virology is not restricted to examining the detection of viruses, molecular characterization of the virus, their replication in host plant and in the vector. Now-a-days gene expression studies which are based on the transcriptome and proteome level is helpful and tremendously potential.

Purpose of this study

Transcriptomic studies have been used tremendously to unravel the host response to virus infection (Babu *et al.,* 2008). In this study, we have discussed for the first time begomovirus and host plant interactions. Our study highlighted the differential expression of several genes involved in defense, DNA organization, replication transcription, translation process, resistance response against begomovirus. The invention and development of new technology creates new opportunities to improve the knowledge regarding virus-host interactions.

Background and current research

To study gene expression, quality of genome greatly influences its usefulness. The whole transcriptome study began in the early 1990s. There are two main technologies in the field of transcriptomes and are microarray and RNA sequencing. It gives information of how genes are regulated and reveals details of an organism`s biology by measuring the expression of an organism`s genes in different

tissues, conditions and time. It gives valuable information by using less than 1µg RNA samples. RNA-Sequencing experiments generate a large volume of raw sequence reads and are required to process to yield useful information. For data analysis, a set of bioinformatics software tools are required. The processing of data is divided into four stages: quality control, alignment, quantification and differential expression (Van Verk *et al.,* 2013). Mostly RNA-Seq programs run from a command-line interface, either in a Unix environment or within R/Bioconductor statistical environment (Huber *et al.,* 2015). The transcript abundance for each of the probe sequence is determined by fluorescent intensity (Barbulovic-Nad *et al.,* 2006). Microarray requires some prior knowledge of the organism of interest (annotated genome sequence/library of expressed sequence tags).

RNA extraction is essential to perform all transcriptome methods. It requires the lysis of cells or tissues, disruption of RNase, lysis of macromolecules and nucleotides, extraction of RNA from other undesired molecules such as DNA (using DNase) and elution of RNA from a solid matrix and precipitation from solution. For transcriptome analysis, mRNA is required so it is necessary to remove ribosomal RNA (rRNA). To enrich mRNA, poly-A affinity methods or depletion of ribosomal RNA (total RNA contains 98% rRNA) using sequence-specific probes are used. Expressed sequence tags (ESTs) are short nucleotides (200-800 bases length) of mRNA sequences derived from cDNA libraries. With the help of reverse transcriptase enzyme cDNA is synthesized from mRNA. The oligonucleotide probes are used for the separation of mRNA molecules which bind their poly-A tails. Sometimes ribo-depletion can be used to specifically remove abundant. The cDNA copies of transcripts are amplified by PCR for the enrichment of fragments that contain the expected 5A' and 3 A' adapter sequences.

Initially sequencing based transcriptomic method, Serial analysis of gene expression (SAGE) was developed in 1995 (Pietu *et al.,* 1999). It is based on Sanger sequencing of concatenated random transcript fragments. With the help of reverse transcriptase enzyme, cDNA is constructed from RNA. The cDNA is then digested into 11 bp tag fragments by using restriction enzymes. These cDNA tags are concatenated head-to-tail into >500bp long stands and sequenced. The tags are then aligned to identify their corresponding genes if the reference genome is available. The tags can be directly used as diagnostic markers if the reference genome is unavailable. The modify method of SAGE called Cap analysis of gene expression (CAGE) which sequences tags from the 5` end of an mRNA transcript (Shiraki *et al.,* 2003). Limitation of these methods is sample preparation, data analysis and labour intensive. Another technique called Massively parallel signature sequencing (MPSS) based on generating 16-20 bp sequences through a complex series of hybridization was developed (Brenner *et al.,* 2000). In the mid-1990s and 2000s microarray and RNA sequencing methods were developed. Microarray consists of short nucleotide oligomers (probes) which are arrayed on a solid substrate such as glass. The gene expression in organisms can be detected after fluorescent labelling and hybridization to the corresponding probes on the microarray. The transcriptome abundance is determined by checking the fluorescence intensity at each probe location on the array. Prior information of the organism of interest is required. Its sensitivity is 10^{-3} limited by fluorescence detection. Main drawback of microarray analysis includes poor quantification, required prior knowledge of sequence and cross-hybridization artifacts.

Due to these problems transcriptomics advanced to RNA-Seq methods. It requires cDNA libraries and then sequenced into a computer-readable format. Recently many sequencing technologies developed for cDNA sequencing such as Illumina, Thermo Fisher, BGI/MGI, PacBio and Oxford Nanopore Technologies (Oikonomopoulos *et al.,* 2020). After cDNA sequencing, tools like Sailfish, RSEM and BitSeq13 quantify transcription levels. The software FAST QC and FaQCs are used for sequence quality

analysis and removed the abnormalities identified in the sequences (de Sena and Smith, 2019). To read raw sequence reads De novo and gene guided methods are used. When the genome is incomplete or unknown then De novo method is used because it does not require the reference genome to reconstruct transcriptome. Assemblers are used- Bridger (Chang *et al.,* 2015), rna SPAdes (Bushmanova *et al.,* 2019) and Trinity (Grabherr *et al.,* 2011). Alternatively the genome guided method which is based on the DNA alignment method with the additional complexity of aligning reads that cover non-continuous portions of the reference genome. Software tools include Bowtie, STAR, Subread, HISAT2 and GMAP (Langmead *et al.,* 2009; Dobin *et al.,* 2013; Liao *et al.,* 2013; Kim *et al.,* 2015; Wu and Watanabe, 2005). Gene expression is measured by quantifying the levels of the gene product and exon. Gene and exon count expression can be quantified using contigs or reference transcript annotations. Tools that determine read counts from aligned RNA-Seq data are HTSeq (Anders *et al.,* 2015), Rcount (Schmid and Grossniklaus, 2015), Cuffquant and FIXSEQ (Hashimoto *et al.,* 2014). For alignment free counts Sailfish (Patro *et al.,* 2014) and Kallisto (Bray *et al.,* 2016) tools are used. To find the differences between gene expression of two or more states of the conditions (healthy and infected plant), Differential gene expression (DEG) is measured (up-regulation/down-regulation). Many tools are used to study DEGs that find the up/down-regulation of the genes between two or more states. Commonly used tools are DESeq (Anders and Huber, 2010) and voom+limma (Ritchie *et al.,* 2015). Transcriptomic analysis is validated by performing quantitative PCR (qPCR). Gene expression measurement by using qPCR is similar to result obtained from RNA-Seq. For obtaining higher-level biological understanding of the results, gene set enrichment analysis is used. Gene set enrichment determines the overlap between two gene sets is statistically significant and are determined by databases or pathways (Gene Ontology, KEGG, Human Phenotype Ontology) or from complementary analyses in the same data (like co-expression networks). This review collates the research work performed by many researchers to unravel their interactions with the host plants. Begomovirus-host plant interaction studies described below:

Chilli leaf curl virus

Virus invasion into the host plant causes many physiological and cellular changing the gene expression (Sahu *et al.,* 2010). The study *Chilli leaf curl virus* (ChiLCV) disease infection and their interactions with host plant (Kushwaha *et al.,* 2015). ChiLCV is a monopartite begomovirus and causes major destruction of chilli crops (Kumar *et al.,* 2006). They used ChiLCV chilli variety Punjab Lal to study the differential expression of several genes. They hypothesized that the resistance character of chilli variety Punjab Lal against ChiLCV might have some correlation with the upregulation in the expression of genes which are essential for the virus replication, transcription and movement at an early phase of infection. The host plant started expressing defense-related genes when the level of virus titer reached to threshold in the host infected plant cells. Proteins that were involved in ChiLCV infected resistant plants were played role in diverse cellular and physiological pathways. The comparative gene expression studies in resistant and susceptible plants showed up-regulation of defense-related genes upon infection. Nucleoside-binding site Leucine-rich repeat (NBS-LRR) is a conserved domain present in proteins involved in conferring resistance against pathogens (Meyers *et al.,* 2003). NBS-LRR genes actively involved in the resistant plants and >5-fold upregulation of genes in resistant chilli plants as compared to susceptible plants. Polyphenol oxidase (PPO) is a tetrameric copper containing protein which catalyses the o-hydroxylation of phenols produced during the oxidative burst during pathogen attack. PPO acts as a scavenger and protects the cell from reactive oxygen species. During infection in resistant chilli var. Punjab Lal plants activates the basal defense response. Their study also revealed the upregulation of an ATP/ADP transporter in resistant plants. These transporters catalyse the highly specific transport of ATP across membrane such as chloroplast and mitochondria in an exchange mode

with ADP. Thionin has antimicrobial protein properties and provide a basal defense role in resistant chilli plants. The enhanced level of histone H1 transcripts found in the plants and these host proteins are required for the formation of minichromosomes, replication, transcription and symptom development.

Cotton leaf curl disease

Cotton leaf curl disease (CLCuD) causes huge loss to cotton fields. Against CLCuD, the diploid species of *Gossypium arboreum* is a natural host resistant than susceptible tetraploid *Gossypium hirsutum*. Mild symptoms (only a few leaves developed symptoms) were observed in *G. arboretum* having scion of *G. hirsutum* (infected CLCuD). The study revealed that the changes in gene expression of resistant variety in response to disease. Transcriptomic analysis found 563 DEGs were up-regulated and 499 DEGs were down-regulated (total 1062 DEGs). To validate transcriptome data, 17 DEGs that might confer disease resistance were selected and qPCR primers designed and result shown qPCR data strongly correlated with the expression data of RNA-Seq. Some transporter genes such as a boron transporter gene (helps to induce necrosis or modulate R gene mediated defense response) were up-regulated while a SWEET 17 gene was down-regulated in asymptomatic plants of *G. arboreum*. The up-regulation of the boron transporter gene in *G. arboretum* indicates the protection of the plant from boron toxicity. SWEETs are bidirectional vacuolar fructose transporters (maintained sugar homeostasis) and play an important role in plant-pathogen interaction. The downregulation of *G. arboreum* SWEET transporter suggested its involvement in reduced pathogen growth and disease resistance. Several transcription factors play a key in resistant in *G. arboretum* plants such as RADIALS, REVEILLE, bHLH, oxidative stress related genes, Ethylene response factor (ERF), R-genes, protein kinases etc. During infestation of CLCuD in *G. arboreum* plants phytohormones such as auxin, cytokinin, abscisic acid and brassinosteroid related genes were up-regulated while ethylene and salicylic acid phytohormones were down-regulated (Naqvi *et al.,* 2017).

Tobacco curly shoot virus

Study on *Nicotiana benthamiana* plants infected with *Tobacco curly shoot virus* (TbCSV, monopartite begomovirus) and Tobacco curly shoot beta-satellite (TbCSB) using RNA sequencing method. Transcriptomic data showed 4081 DEGs were identified in TbCSB samples and 3196 DEGs were identified in TbCSV plants. KEGG analysis revealed that DEGs of TbCSB samples involved in carbon metabolism, glyoxylate, photosynthesis, carbon fixation and dicarboxylate metabolism, porphyrin and chlorophyll metabolism, DNA replication, pentose phosphate pathway and nitrogen metabolism. The DEGs of TbCSV samples involved in ribosome, glyoxylate and dicarboxylate metabolism, DNA replication, circadian rhythm-plant, photosynthesis-antenna proteins and nitrogen metabolism. Metabolic pathway was the major pathway containing the large number of DEGs in both TbCSV and TbCSB infected plants. Pathogenesis related (PR) eight genes were significantly up-regulated and 44 Leucine-rich repeat receptor-like protein kinases (LRR-RLKs) genes were significantly differentially expressed in TbCSV or in TbCSB treatment. The biosynthesis and signal transduction pathway of BR and JA were significantly changed (Li *et al.,* 2018).

Pepper golden mosaic virus

Pepper golden mosaic virus (PepGMV) is a bipartite begomovirus. Differential expression analysis of healthy, symptomatic and recovered pepper leaves was studied. Transcriptomic analysis revealed that the recovery process of plant which was infected by pepper-PepGMV had been associated with transcriptional and post-transcriptional gene silencing. Using Roche/454 pyrosequencing method for deep transcriptome sequencing technology (used for the identification of transcripts and transcript variation in plant-pathogen interaction), a total of

309 (168 up and 141 down-regulated) DEGs were identified which showed major differences in up and down-regulated mechanism. Out of 309 DEGs, 246 have a known function which was associated with biological and metabolic pathways. Gene Ontology (GO) analysis revealed catabolic process related genes and photosynthetic process related genes were down-regulated in both symptomatic and recovered leaves. RRP1 gene was found in recovered tissues (role in plant defense mechanism upon virus infection). The novel genes such as Pepper-RRP1 and histone proteins, were identified which may have a role in plant defense (Góngora-Castillo *et al.*, 2012).

PepGMV and *Tomato chino La Paz virus* (ToChLPV)

RNA interference (RNAi) is a tool that is used to induce pathogen-derived resistance against begomoviral disease in plants. Its reliability and specificity serve as a guide and down-regulate gene expression and viral DNA accumulation. Genes of begomovirus AC1 (replication gene), AV1 (coat protein) and the non-coding intergenic region (IR) entire, partial or mutated sequences used to develop strategies by applying RNAi. Two widely distributed begomovirus-PepGMV and ToChLPV were used to develop strategies against them. The AC1-IR-AV1 region of PepGMV and ToChLPV were used to construct RNAi using microarray hybridization technique. Evidences proved that both the constructs were highly efficient for the suppression of multiplication of viral genome, cross protection and important for the control of PepGMV and other begomovirus diseases (Medina-Hernández *et al.*, 2013).

Tomato yellow leaf curl virus (TYLCV)

Previous studies showed that five major loci (Ty-1, Ty-2, Ty-3, Ty-4, Ty-5) present in wild type tomato plants which are resistant to TYLCV infection. DEGs levels in two tomato lines- resistant(R) and susceptible (S) tested which contained 209 DEGs in R-line and 807 DEGs in S-lines. R-line plants showed higher proportion of up-regulated DEGs than S-line with a difference of 49.2%. To study different biological, cellular and metabolic processes, GO analysis was performed. For this analysis, blast2go software was used and annotated 67.46% and 63.89% of DEGs in the R and S-lines respectively. Differential expressed genes which are involved in the cellular process were higher in the S-line (30.5%) than that in the S-line (23.04%). Some genes were involved in the protein binding transcription factors activity were only found in R-line while antioxidant, nucleic acid binding transcription factor activity were specific to the S-line. Total 40 up-regulated annotated genes (out of 122) were found involved in the defense mechanism related genes. Virus induced gene silencing (VIGS) treated R-plants showed no symptoms such as leaf curling and yellowing. This journal provides comprehensive knowledge about the molecular mechanism underlying the resistant (R) gene network (Chen *et al.*, 2013).

Another method, ionising radiation and has been used in diverse commercial areas such as food industry, plant breeding studies (Zhou *et al.*, 2019). Neutron mutagenesis is a highly efficient method to develop deletion mutant populations in diverse species of plants. By using this method, phenotypic changes in plant characteristics such as seed germination rate, plant height, leaf number have been observed. Neutron irradiation interfere gene expression in plants. They examined tomato seed germination rate and RNA sequencing method to study transcriptome analysis against TYLCV infection. Neutron irradiated tomato mutants were resistant or tolerant to TYLCV infection as compared to wild type (WT) plants. Transcriptome analysis showed that the number of up-regulated nuclear genes were much higher than that of down-regulated genes in all irradiated mutants as compared to the WT plants. Neutron irradiation of up-regulated nuclear gens involved in many biological processes and down-regulated genes were targeted to cellular organelles such as chloroplast and mitochondria. In this experiment, they set two different seed conditions (pre-soaked and dried

seeds) and two different irradiation intensities (30 and 90 min) have been used for seed germination and transcriptome analysis. After neutron irradiation pre-soaked seeds showed lower germination rates as compared to dry seeds (due to the inhibition of seed germination). Neutron irradiated eight mutants (as compared to WT) showed higher number of SNPs (Single nucleotide polymorphisms). The study suggested that neutron irradiation strongly impacts RNA editing and insertion/deletion which might be correlated with the experiment of the corresponding genes. The Ts/Tv (transition/transversion) ratio values remain constant in all mutants but lower than that of the WT. The two different irradiation intensities showed that sample irradiated for 30 minutes were higher than those in the sample irradiated for 90 minutes. Results based on this article suggested that higher intensity of irradiation could cause more effects in the experiment of host genes which might be required for infection. This article helps us to understand the application of neutron irradiation in plant-virus interaction.

Future approaches

A large number of begomoviruses have been reported worldwide. As is the case with much of science, new technology leads to new opportunities to improve the knowledge base regarding plant-virus interactions. Transcriptome analysis is an efficient method to understand how genomes are expressed and can be used without genomic reference. Many new technologies have developed to investigate and other omics technologies are giving an increasing integrated view of the complexities of cellular life. Plant-virus interactions comparing transcriptomes gives us huge knowledge to understand cellular changes in plants during infection.

The development of new scientific approaches with existing methods will bring us to understand the interactions between them and will let us know the biological basis for many physiological functions, biochemical pathways, virus-vector interactions. In the future, research based on transcriptome data will promote the discovery of new functional genes, secondary metabolic pathways, and virus-host and virus-vector interactions. In this review, we discussed the molecular basis of begomovirus-host plant infection and will help us to develop the durable resistant strategies against begomovirus.

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References

Anders, S. and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol*, 11(10): R106. https://doi.org/10.1186/gb-2010-11-10-r106

Anders, S., Pyl, P. T. and Huber, W. (2015). HTSeq-a Python framework to work with high-throughput sequencing data. *Bioinformatics*, 31(2): 166-9. https://doi.org/10.1093/bioinformatics/btu638

Babu, M., Gagarinova, A. G., Brandle, J.E. and Wang, A. (2008). Association of the transcriptional response of soybean plants with *Soybean mosaic virus* systemic infection. *J Gen Virol*, 89(Pt 4): 1069-80. https://doi.org/10.1099/vir.0.83531-0

Barbulovic-Nad, I., Lucente, M., Sun, Y., Zhang, M., Wheeler, A. R. and Bussmann, M. (2006). Bio-microarray fabrication techniques--a review. *Crit Rev Biotechnol*, 26(4): 237-59. https://doi.org/10.1080/07388550600978358

Boulton, MI. (2005). Geminiviruses: major threats to world agriculture. *Ann. Appl. Bio,* 142(2): 143. https://doi.org/10.1111/j.1744-7348.2003.tb00239.x

Bray, N.L., Pimentel, H., Melsted, P. and Pachter, L. (2016). Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol*, 34(5): 525-7. https://doi.org/10.1038/nbt.3519

Brenner, S., Johnson, M., Bridgham, J., Golda, G., Lloyd, D. H., Johnson, D., Luo, S., McCurdy, S., Foy, M., Ewan, M., Roth, R., George, D., Eletr, S, Albrecht, G., Vermaas, E., Williams, S. R., Moon, K., Burcham, T., Pallas, M., DuBridge, R. B., Kirchner, J., Fearon, K., Mao, J. and Corcoran, K. (2000). Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. *Nat Biotechnol*, 18(6): 630-4. https://doi.org/10.1038/76469

Brown, J. K., Idris, A. M., Rogan, D., Hussein, M. H. and Palmieri, M. (2001). *Melon chlorotic leaf curl virus*, a New Begomovirus Associated with *Bemisia tabaci* Infestations in Guatemala. *Plant Dis*, 85(9): 1027. https://doi.org/10.1094/PDIS.2001.85.9.1027C

Bushmanova, E., Antipov, D., Lapidus, A., Prjibelski, A. D. (2019). rnaSPAdes: a de novo transcriptome assembler and its application to RNA-Seq data. *Gigascience*, 8(9): giz100. https://doi.org/10.1093/gigascience/giz100

Chang, Z., Li, G., Liu, J., Zhang, Y., Ashby, C., Liu, D., Cramer, C. L., Huang, X. (2015). Bridger: a new framework for de novo transcriptome assembly using RNA-seq data. *Genome Biol*, 16(1): 30. https://doi.org/10.1186/s13059-015-0596-2

Chen, T., Lv, Y., Zhao, T., Li, N., Yang, Y., Yu, W., He, X., Liu, T. and Zhang, B. (2013). Comparative transcriptome profiling of a resistant vs. susceptible tomato (*Solanum lycopersicum*) cultivar in response to infection by *Tomato yellow leaf curl virus*. *PLoS One*, 8(11): e80816. https://doi.org/10.1371/journal.pone.0080816

Cui, X., Li, G., Wang, D., Hu, D. and Zhou, X. (2005). A Begomovirus DNA beta-encoded protein binds DNA, functions as a suppressor of RNA silencing, and targets the cell nucleus. *J Virol*, 79(16): 10764-75. https://doi.org/10.1128/JVI.79.16.10764-10775.2005

Czosnek, H., Eybishtz, A., Sade, D., Gorovits, R., Sobol, I., Bejarano, E., Rosas-Diaz, T. and Lozano-Duran, R. (2013). Discovering host genes involved in the infection by the *Tomato yellow leaf curl virus* complex and in the establishment of resistance to the virus using *Tobacco Rattle Virus* based post transcriptional gene silencing, *Viruses*, 5(3): 998–1022. https://doi.org/10.3390/v5030998

Dasgupta, I., Malathi, V. G. and Mukherjee, S. 2003. Genetic engineering for virus resistance. *Curr Sci*, 84(3): 341-54.

de SenaBrandine, G., Smith, A. D. (2019). Falco: high-speed FastQC emulation for quality control of sequencing data. *F1000Re*, 8: 1874. https://doi.org/10.12688/f1000research.21142.2

Dobin, A., Davis, C. A., Schlesinger, F., Drenkow, J., Zaleski, C., Jha, S., Batut, P., Chaisson, M., Gingeras, T. R. (2013). STAR: ultrafast universal RNA-seq aligner. *Bioinformatics*, 29(1): 15-21. https://doi.org/10.1093/bioinformatics/bts635

Góngora-Castillo, E., Ibarra-Laclette, E., Trejo-Saavedra, D. L. and Rivera-Bustamante, R. F. (2012). Transcriptome analysis of symptomatic and recovered leaves of geminivirus-infected pepper (*Capsicum annuum*). *Virol J*, 9: 295. https://doi.org/10.1186/1743-422X-9-295

Grabherr, M. G., Haas, B. J., Yassour, M., Levin, J. Z., Thompson, D. A., Amit, I., Adiconis, X., Fan, L., Raychowdhury, R., Zeng, Q., Chen, Z., Mauceli, E., Hacohen, N., Gnirke, A., Rhind, N., di Palma, F., Birren, B. W., Nusbaum, C., Lindblad-Toh, K., Friedman, N. and Regev, A. (2011). Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotechnol*, 29(7): 644-52. https://doi.org/10.1038/nbt.1883

Guzman, P., Sudarshana, M. R., Seo, Y. S., Rojas, M. R., Natwick, E., Turini, T., Mayberry, K. and Gilbertson, R. L. (2000). A New

Bipartite Geminivirus (Begomovirus) Causing Leaf Curl and Crumpling in Cucurbits in the Imperial Valley of California. *Plant Dis*, 84(4): 488. https://doi.org/10.1094/PDIS.2000.84.4.488C

Hashimoto, T. B., Edwards, M. D. and Gifford, D. K. (2014). Universal count correction for high-throughput sequencing. *PLoS Comput Biol*, 10(3): e1003494. https://doi.org/10.1371/journal.pcbi.1003494

Huber, W., Carey, V. J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B. S., Bravo, H. C., Davis, S., Gatto, L., Girke, T., Gottardo, R., Hahne, F., Hansen, K. D., Irizarry, R. A., Lawrence, M., Love, M. I., MacDonald, J., Obenchain, V., Oleś, A.,, Pagès, H., Reyes, A., Shannon, P., Smyth, GK., Tenenbaum, D., Waldron, L. and Morgan, M. (2015). Orchestrating high-throughput genomic analysis with Bioconductor. *Nat Methods*, 12(2): 115-21. https://doi.org/10.1038/nmeth.3252

Jyothsna, P., Haq, Q. M., Singh, P., Sumiya, K. V., Praveen, S., Rawat, R., Briddon, R. W. and Malathi, V. G. (2013). Infection of *Tomato leaf curl New Delhi virus* (ToLCNDV): a bipartite begomovirus with betasatellites, results in enhanced level of helper virus components and antagonistic interaction between DNA B and betasatellites. *Appl Microbiol Biotechnol*, 97(12): 5457-71. https://doi.org/10.1007/s00253-012-4685-9

Kachroo, P., Chandra-Shekara, A. C. and Klessig, D. F. (2006). Plant signal transduction and defense against viral pathogens. *AdvVirus Res*, 66: 161-91. https://doi.org/10.1016/S0065-3527(06)66004-1

Kim, D., Langmead, B., Salzberg, S.L. (2015). HISAT: a fast spliced aligner with low memory requirements. *Nat Methods*, 12(4): 357-60. https://doi.org/10.1038/nmeth.3317

Kumar, R., Tiwari, R. K., Jeevalatha, A., Siddappa, S., Shah, M. S., Sharma, S., Sagar, V., Kumar, M., and Chakrabarti, S. K. (2021). Potato apical leaf curl disease: Current status and perspectives on a diseased caused by *Tomato leaf curl New Delhi virus*. *Journal of plant diseases and protection*, 128: 897-911. https://doi.org/10.1007/s41348-021-00463-w

Kumar, S., Kumar, S., Singh, M., Singh, AK. and Rai, M. (2006). Identification of host plant resistance to *Pepper leaf curl virus* in chilli (Capsicum species). *Scientia horticulturae*, 110: 359-361. https://doi.org/10.1016/j.scienta.2006.07.030

Kumar, Y., Bhardwaj, P., Hallan, V. and Zaidi, A. A. (2010). Detection and characterization of *ageratum enation virus* and a nanovirus-like satellite DNA1 from zinnia causing leaf curl symptoms in India. *Journal of General Plant Pathology*, 76: 395-98. https://doi.org/10.1007/s10327-010-0270-1

Kumar, Y., Hallan, V. and Zaidi, A. A. (2008). Molecular characterization of a distinct begomovirus species infecting tomato in India. *Virus Genes*, 37(3): 425-31. https://doi.org/10.1007/s11262-008-0286-1

Kumar, Y., Hallan, V. and Zaidi, A. A. (2009). Identification and characterization of *Bean yellow mosaic virus* infecting Freesia. *J. Plant Biochem. Biotechnol*. 18: 253-55. https://doi.org/10.1007/BF03263331

Kumar, Y., Hallan, V. and Zaidi, A. A. (2011). *Chilli leaf curl Palampur virus* is a distinct begomovirus species associated with a betasatellite. *Plant Pathology*, 60(6): 1040-47. https://doi.org/10.1111/j.1365-3059.2011.02475.x

Kushwaha, N., Sahu, P. P., Prasad, M. and Chakraborty, S. (2015). *Chilli leaf curl virus* infection highlights the differential expression of genes involved in protein homeostasis and defense in resistant chilli plants. *Appl Microbiol Biotechnol*, 99(11): 4757-70. https://doi.org/10.1007/s00253-015-6415-6

Lager, P., Sharma, J. and Kumar, Y. (2022). First report of a begomovirus infecting *Cordyline fruticosa*. *New Disease Reports*, 45: e12068. https://doi.org/10.1002/ndr2.12068

Langmead, B., Trapnell, C., Pop, M. and Salzberg, S. L. (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol*, 10(3): R25. https://doi.org/10.1186/gb-2009-10-3-r25

Li, K., Wu, G., Li, M., Ma, M., Du, J., Sun, M., Sun, X. and Qing, L. (2018). Transcriptome analysis of *Nicotiana benthamiana* infected by *Tobacco curly shoot virus*. *Virol J*, 15(1): 138. https://doi.org/10.1186/s12985-018-1044-1

Liao, Y., Smyth, G. K. and Shi, W. (2013). The Subread aligner: fast, accurate and scalable read mapping by seed-and-vote. *Nucleic Acids Res*, 41(10): e108. https://doi.org/10.1093/nar/gkt214

Medina-Hernández, D., Rivera-Bustamante, R. F., Tenllado, F. and Holguín-Peña, R. J. (2013). Effects and effectiveness of two RNAi constructs for resistance to *Pepper golden mosaic virus* in *Nicotiana benthamiana* plants. *Viruses*, 5(12): 2931-45. https://doi.org/10.3390/v5122931

Meyers, B. C., Kozik, A., Griego, A., Kuang, H. and Michelmore, R. W. (2003). Genome-wide analysis of NBS-LRR-encoding genes in Arabidopsis. *Plant Cell*, 15(4): 809-34. https://doi.org/10.1105/tpc.009308

Mittal, D., Borah, B. K. and Dasgupta, I. (2008). Agroinfection of cloned *Sri Lankan cassava mosaic virus* DNA to *Arabidopsis thaliana*, *Nicotiana tabacum* and cassava. *ArchVirol*, 153(11): 2149-55. https://doi.org/10.1007/s00705-008-0238-z

Monga, D., Chakrabarty, P. K. and Kranthi, K. R. (2009). Cotton leaf curl virus disease in India: Recent status and management strategies. Central Institute for Cotton Research (C.I.C.R.): Regional Station, Sirsa, Haryana and C.I.C.R., Nagpur.

Morales, F. J. and Jones, P. G. (2004). The ecology and epidemiology of whitefly transmitted viruses in Latin America. *Virus Res*, 100(1): 57-65. https://doi.org/10.1016/j.virusres.2003.12.014

Naqvi, R. Z., Zaidi, S. S., Akhtar, K. P., Strickler, S., Woldemariam, M., Mishra, B., Mukhtar, M. S., Scheffler, B. E., Scheffler, J. A., Jander, G., Mueller, L. A., Asif, M. and Mansoor, S. (2017). Transcriptomics reveals multiple resistance mechanisms against cotton leaf curl disease in a naturally immune cotton species, *Gossypium arboreum*. *Sci Rep*, 7(1): 15880.

Oikonomopoulos, S., Bayega, A., Fahiminiya, S., Djambazian, H., Berube, P. and Ragoussis, J. 2020. Methodologies for Transcript Profiling Using Long-Read Technologies. *Front Genet*, 11: 606. https://doi.org/10.3389/fgene.2020.00606

Patro, R., Mount, S. M. and Kingsford, C. (2014). Sailfish enables alignment-free isoform quantification from RNA-seq reads using lightweight algorithms. *Nat Biotechnol*, 32(5): 462-4. https://doi.org/10.1038/nbt.2862

Piétu, G., Mariage-Samson, R., Fayein, N. A., Matingou, C., Eveno, E., Houlgatte, R., Decraene, C., Vandenbrouck, Y., Tahi, F., Devignes, M. D., Wirkner, U., Ansorge, W., Cox, D., Nagase, T., Nomura, N. and Auffray, C. (1999). The Genexpress IMAGE knowledge base of the human brain transcriptome: a prototype integrated resource for functional and computational genomics. *Genome Res*, 9(2): 195-209.

Raj, S. K., Khan, M. S., Singh, R., Kumari, N. and Prakash, D. (2005a). Occurrence of yellow mosaic geminiviral disease on bitter gourd (*Momordica charantia*) and its impact on phytochemical contents. *Int J Food Sci Nutr*, 56(3): 185-92. https://doi.org/10.1080/09637480500103946

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W. and Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res*, 43(7): e47. https://doi.org/10.1093/nar/gkv007

Sahu, P. P., Rai, N. K., Chakraborty, S., Singh M., Chandrappa, P. H., Ramesh, B,

Chattopadhyay, D. and Prasad, M. (2010). Tomato cultivar tolerant to *Tomato leaf curl New Delhi virus* infection induces virus-specific short interfering RNA accumulation and defence-associated host gene expression. *Mol Plant Pathol*, 11(4): 531-44. https://doi.org/10.1111/j.1364-3703.2010.00630.x

Saunders, K. and Stanley, J. (1999). A nanovirus-like DNA component associated with yellow vein disease of *Ageratum conyzoides*: evidence for interfamilial recombination between plant DNA viruses. *Virology*, 264(1): 142-52. https://doi.org/10.1006/viro.1999.9948

Schmid, M. W. and Grossniklaus, U. (2015). Rcount: simple and flexible RNA-Seq read counting. *Bioinformatics*, 31(3): 436-7. https://doi.org/10.1093/bioinformatics/btu680

Seal, S. E., van den Bosch, F. and Jeger, M J. (2007). Factors influencing begomovirus evolution and their increasing global significance: Implications for sustainable control.*Crit. Rev. Plant Sci*, 25(1): 23-46. https://doi.org/10.1080/07352680500365257

Sharma, J., Lager, P. and Kumar, Y. (2021). First report of *Tomato leaf curl New Delhi virus* infecting *Ricinus communis*. *New Dis Rep*, 44: e12053. https://doi.org/10.1002/ndr2.12053

Sheikh, M., Khan, S. Z. and Mahmood, I. (2013). Effect of *Bhendi yellow vein mosaic virus* on yield components of okra plants. *Journal of Plant Pathology*. 95(2): 391-93.

Shiraki, T., Kondo, S., Katayama, S., Waki, K., Kasukawa, T., Kawaji, H., Kodzius, R., Watahiki, A., Nakamura, M., Arakawa, T., Fukuda, S., Sasaki, D., Podhajska, A., Harbers, M., Kawa, J., Carninci, P. and Hayashizaki, Y. (2003). Cap analysis gene expression for high-throughput analysis of transcriptional starting point and identification of promoter usage. *Proc Natl Acad Sci U S A*, 100(26): 15776-81. https://doi.org/10.1073/pnas.2136655100

Wu, T. D. and Watanabe, C. K. (2005). GMAP: a genomic mapping and alignment program for mRNA and EST sequences. *Bioinformatics*, 21(9): 1859-75. https://doi.org/10.1093/bioinformatics/bti310

Zaim, M., Kumar, Y., Hallan, V. and Zaidi, A. A. (2011). *Velvet bean severe mosaic virus*: a distinct begomovirus species causing severe mosaic in *Mucuna pruriens* (L.) DC. *Virus Genes*, 43(1): 138-46. https://doi.org/10.1007/s11262-011-0610-z

Zhou, Y., Cho, W. K., Byun, H. S., Kil, E. J., Bak, S. I., Moon, D. H., Chavan, V., Park, T. S., Lee, S. and Hong, S. W. (2019). Transcriptome profiles of tomato plants after neutron irradiation and infection with TYLCV. *Physiol Plant*, 165(2): 427-441. https://doi.org/10.1111/ppl.12913

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