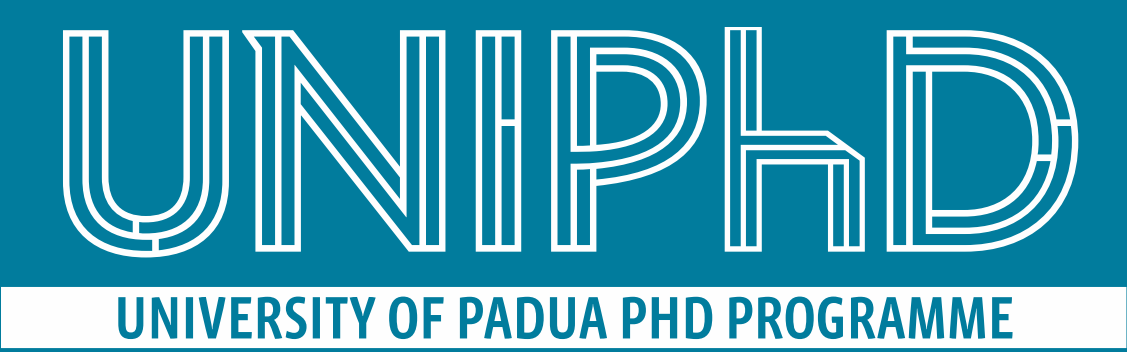


Ionomics, transcriptional and mutational profiling of *Solanum lycopersicum* L. infected with Tomato Brown Rugose Fruit Virus (ToBRFV)

Aditi Padmakar Thakare, Maria Cristina Della Lucia, Chandana Mulagala, Giovanni Bertoldo, Massimo Cagnin and Piergiorgio Stevanato
DAFNAE, University of Padova, Italy
e-mail: aditipadmakar.thakare@studenti.unipd.it



ABSTRACT

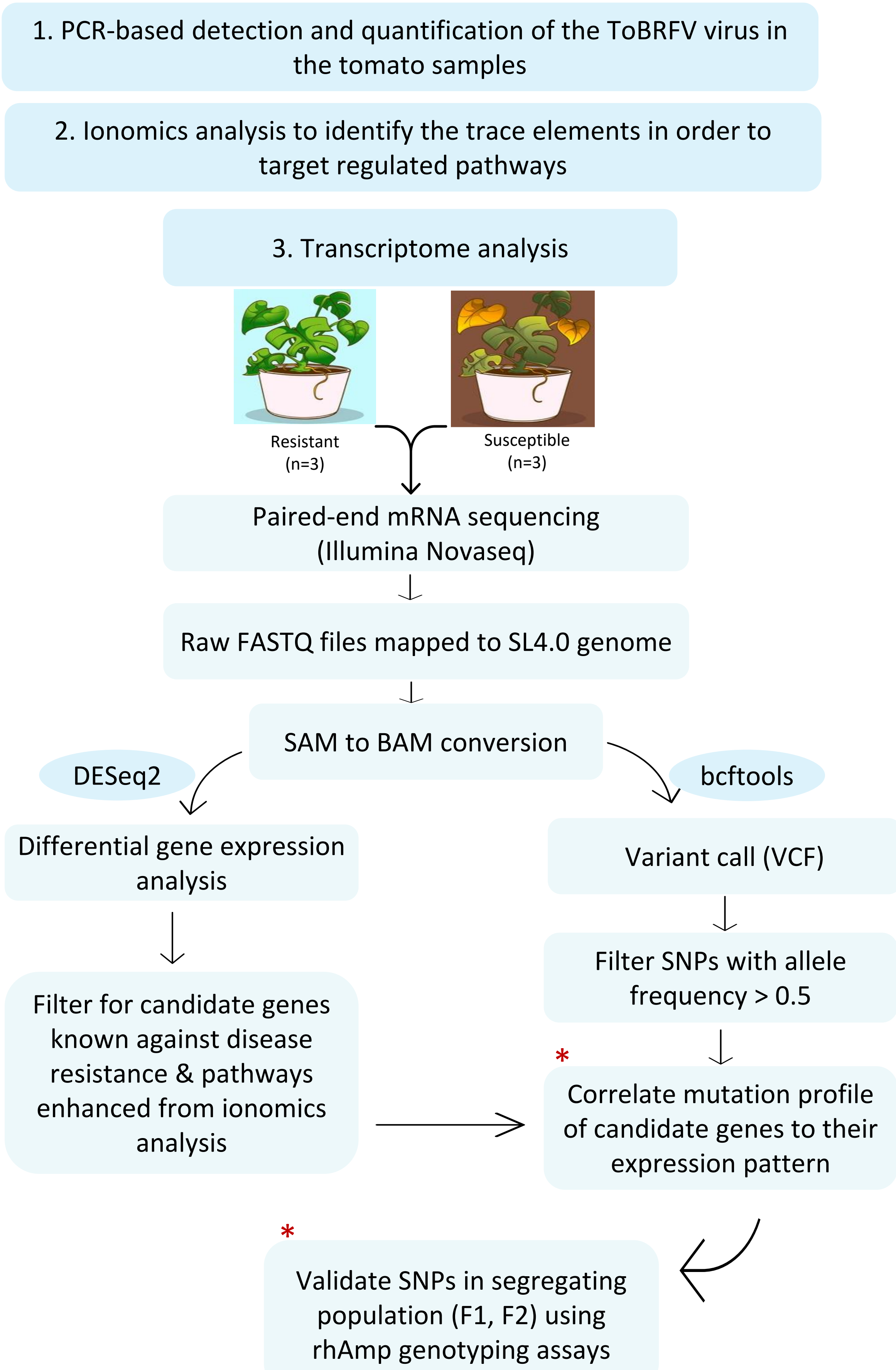
The Tomato Brown Rugose Fruit Virus (ToBRFV) is a plant pathogen that mostly affects tomatoes and peppers. This virus has a single-stranded positive RNA genome measuring about 6.3 kilobases in length. The R genes Tm1, Tm2, and Tm2² that were initially introduced in commercial cultivars to fight TMV can be overcome by this virus. The fruits and foliage of the plants display symptoms after ToBRFV infection. The leaves exhibit mosaic discoloration and leaf blade shrinking. While the tomato fruit develops wrinkling and brown / black necrotic patches on its surface. In this study, we attempt to examine the gene expression profile in resistant and susceptible tomato lines upon infection using an omics-based approach integrating ionomics, transcriptomics, and SNP identification. It is critical to validate these SNPs using different PCR techniques in order to better understand the possible mechanism underlying disease resistance and to aid in the detection of a mutational landscape that can evaluate plant phenotypic traits. The targeted selection of these genetic determinants can further assist in improvising plant breeding and crop yield management techniques.

MATERIALS AND METHODS



The plant material used in this study belongs to a collection of the DAFNAE at the University of Padova. Susceptible and resistant pre-breeding lines to Tomato Brown Rugose Fruit Virus (ToBRFV) were selected for this study. Tomato lines were grown in pots under uniform conditions. Upon reaching 60 days, the leaves were artificially infected. After 96 h from the treatment, fresh leaf samples were collected and evaluated using an omics-based approach integrating ionomics, transcriptomics, and SNP identification.

WORKFLOW



FINDINGS

1. Ionomics: leaf tissue

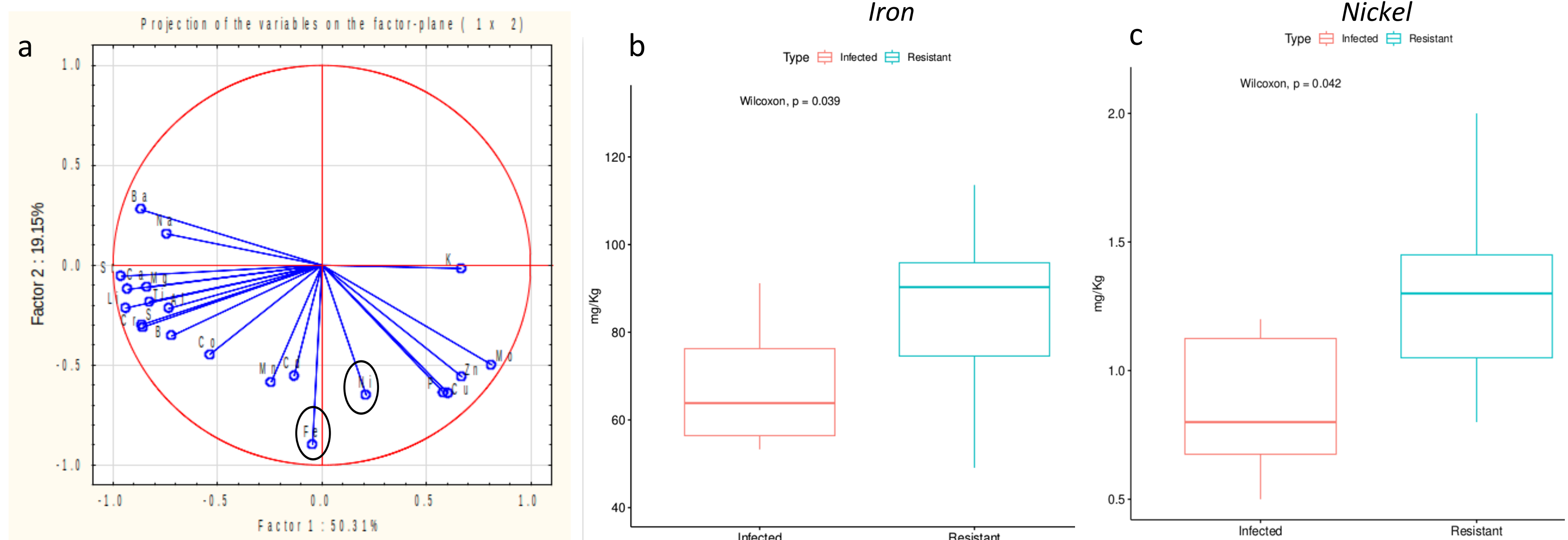


Fig 1: (a) The PCA plot obtained from ionomics analysis of leaf tissues show that the element Iron (Fe) and Nickel (Ni) are significant. (b) Iron is known to produce ROS and trigger hypersensitive response against pathogens. This can help target transcription factors with HLH structure, which are known to regulate genes signaling for Fe uptake from the rhizosphere (Min. requirement 60-300 mg/kg). (c) Nickel helps in the synthesis of phytoalexins, as a defence response against pathogens & is directly correlated with lignin production for strengthening of the cell wall (Min. requirement 0.5-1 mg/kg).

2. Transcriptome Analysis

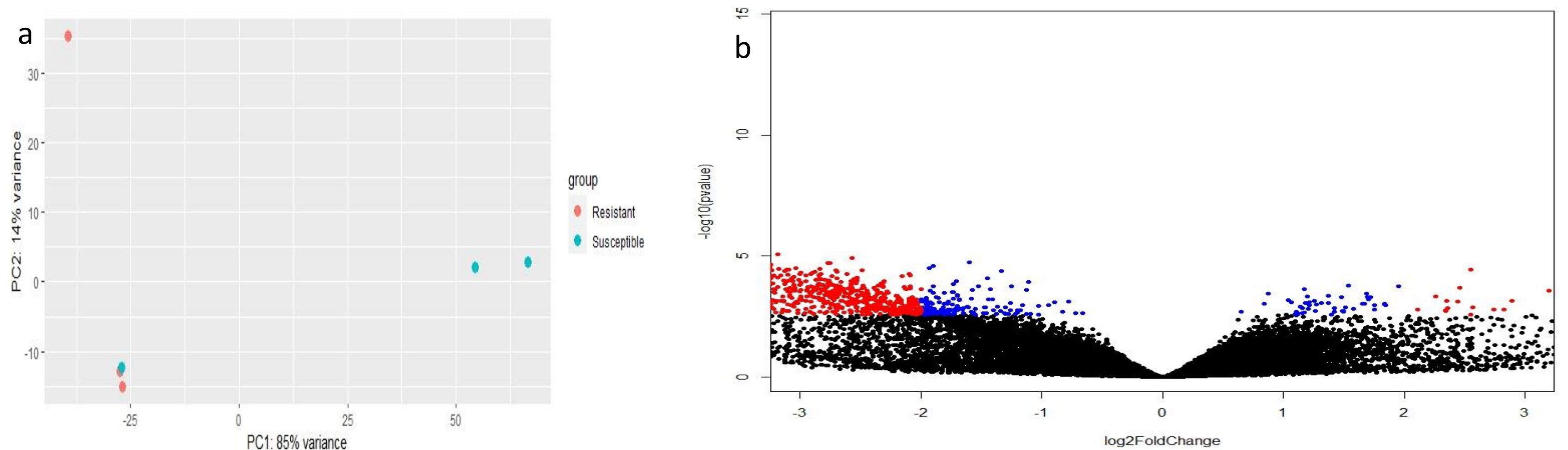


Fig 2a: PCA analysis of the transcriptome samples revealed two distinct clusters of resistant and susceptible samples with two samples belonging to each cluster exclusively.

Fig 2b: Volcano plot showing all the differentially expressed genes. Blue dots are the significant ones with a p-value of less than 0.05. Red dots represents the significant genes with log2fold change \geq 2.

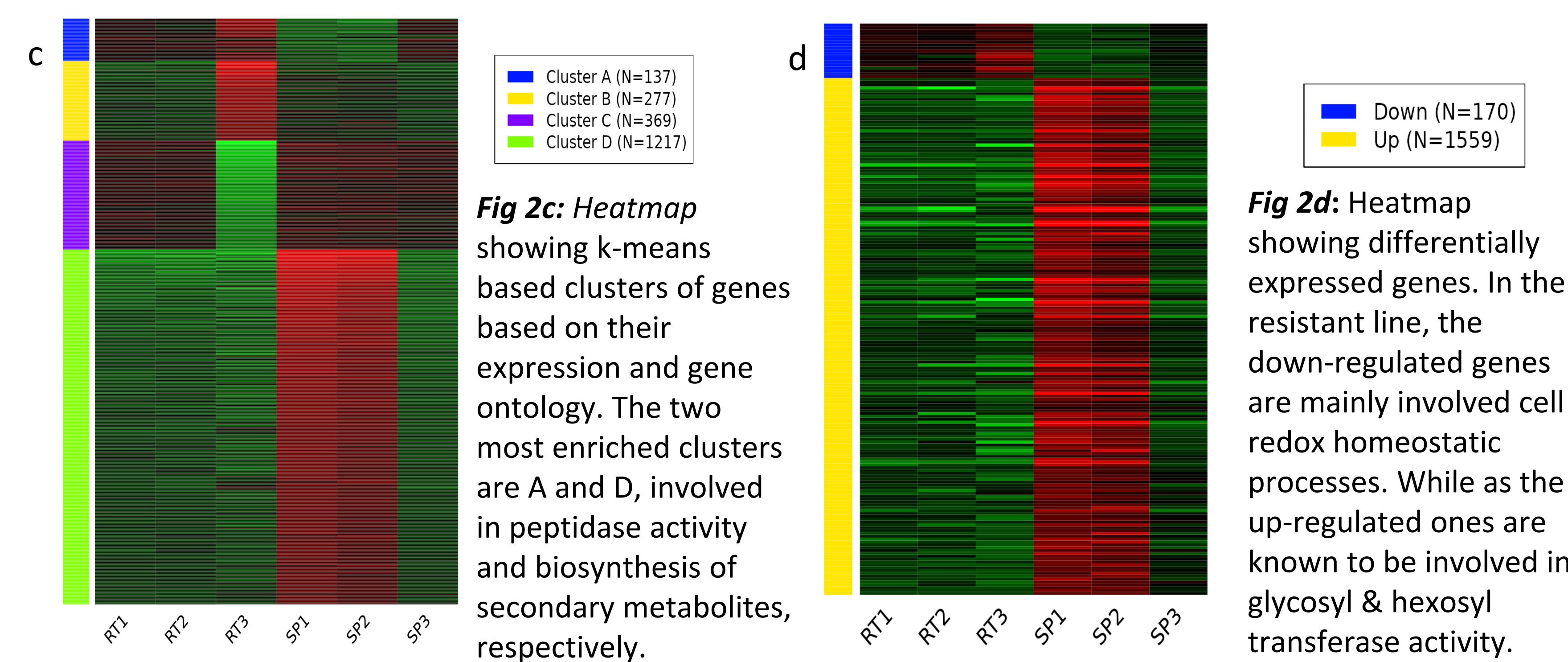


Fig 2c: Heatmap showing k-means based clusters of genes based on their expression and gene ontology. The two most enriched clusters are A and D, involved in peptidase activity and biosynthesis of secondary metabolites, respectively.

Fig 2d: Heatmap showing differentially expressed genes. In the resistant line, the down-regulated genes are mainly involved cell redox homeostatic processes. While as the up-regulated ones are known to be involved in glycosyl & hexosyl transferase activity.

FUTURE PROSPECTS

- Investigate the gene profiles unique to susceptible and resistant lines.
- Integrate transcriptome and variant analysis to screen for targetable biomarkers, and further validate it in F2 segregating populations using SNP genotyping assays.
- Identify breeding and disease resilient crops for the future.

REFERENCES

- Love, M. I., Huber, W., & Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12), 1–21.
- Zhang, S., Griffiths, J. S., Marchand, G., Bernards, M. A., & Wang, A. (2022). Tomato brown rugose fruit virus: An emerging and rapidly spreading plant RNA virus that threatens tomato production worldwide. *Molecular Plant Pathology*, 23(9), 1262–1277.
- Zinger, A., Lapidot, M., Harel, A., Doron-Faigenboim, A., Gelbart, D., & Levin, I. (2021). Identification and Mapping of Tomato Genome Loci Controlling Tolerance and Resistance to Tomato Brown Rugose Fruit Virus. *Plants*, 10(1), 179.
- Ge, S.X., Son, E.W. & Yao, R. iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. *BMC Bioinformatics* 19, 534 (2018).

