Application of the NanoString nCounter System as an Alternative Method to Investigate Molecular Mechanisms Involved in Host Plant Responses to *Plasmodiophora brassicae*

Background

Clubroot, caused by ***Plasmodiophora brassicae***, is a major disease of Brassica crops, including canola, with significant economic impact in Canada. As new pathotypes of ***P. brassicae*** continue to emerge and overcome host resistance, there is a growing need to better understand host–pathogen interactions at the molecular level. While RNA-seq and qPCR have been widely used for transcriptomic studies and data validation, these methods can be resource-intensive, particularly when investigating defined gene sets across large sample cohorts. The nCounter platform offers a targeted approach that can streamline these efforts.

Research Questions

- Can the nCounter platform reliably validate RNA-seq data from clubroot-infected plant tissues?
- Which genes are consistently involved in clubroot resistance or susceptibility, and could be targeted in future expression panels?
- How does the performance of nCounter compare to RNA-seq and qPCR in terms of time, cost, and accuracy?

Results & Conclusions

- Expression results from the nCounter system showed strong correlations with both RNA-seq and qPCR ($R > 0.90$, $p < 0.01$), demonstrating its reliability and reproducibility in agricultural samples.
- Genes involved in salicylic acid, jasmonic acid, and auxin signaling pathways were differentially expressed and may contribute to disease resistance phenotypes.
- Candidate markers validated by nCounter include WRKY70, PNP-A, DMR6, CHI, and GH3.12, which are implicated in defense regulation and hormone signaling.
- nCounter successfully profiled 29 genes across multiple time points and cultivars, capturing consistent expression trends with fewer processing steps, lower costs, and less technical variability than qPCR or RNA-seq.
- The robustness and efficiency of results generated with the nCounter platform support its adoption as a single end-to-end solution for medium-scale targeted expression studies.

Experimental Setup

Sample Type	Total RNA extracted from pooled rutabaga root tissue
Tissue Type	Root tissue from resistant and susceptible *B. napus* cultivars
Assay	nCounter Custom CodeSet targeting 29 genes of interest and 4 reference genes
Analyte	RNA
Instrument	nCounter Analysis System

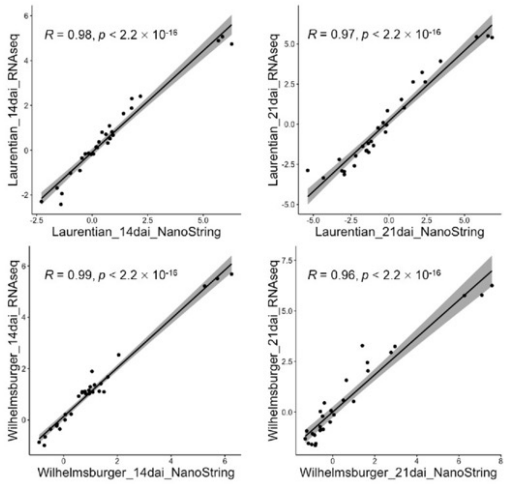

Figure 1: Correlation plots comparing of gene expression measurements between the nCounter platform and RNA-seq at several timepoints, demonstrating high concordance between the methods.

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