

Novel crosstalk between ethylene and salicylic acid signaling in potato unraveled by network analysis

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Introduction

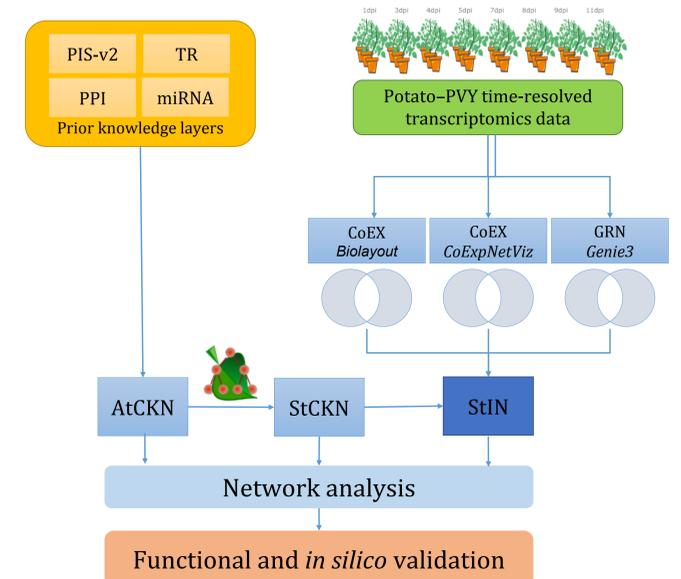
Infection of a plant by a pathogen initiates a complex interaction between both players involved, leading to changes in the complex signaling network, which result in gene activity changes and reprogramming of the cell metabolism. In order to understand the mechanisms and dynamics involved, a systems biology approach was adopted to model the complex biological processes associated in the interplay between potato and its defense components following the infection with potato virus Y (PVY). Such an approach has the potential to increase our understanding of the potato signaling network in response to viral infection, which is necessary for the development of more effective crop protection strategies.

Methods

A qualitative plant immune signaling (PIS) model was constructed, describing the biosynthesis and signal transduction pathways for three crucial phytohormones involved in plant defense: salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) [1]. The PIS model was expanded with publicly available dispersed knowledge of protein-protein and transcription factor networks, miRNA regulation and defense specific components. The resulting network, the *Arabidopsis thaliana* comprehensive knowledge network (AtCKN), was translated from Arabidopsis to potato using orthology information (www.gomapman.org) forming the potato comprehensive knowledge network (StCKN).

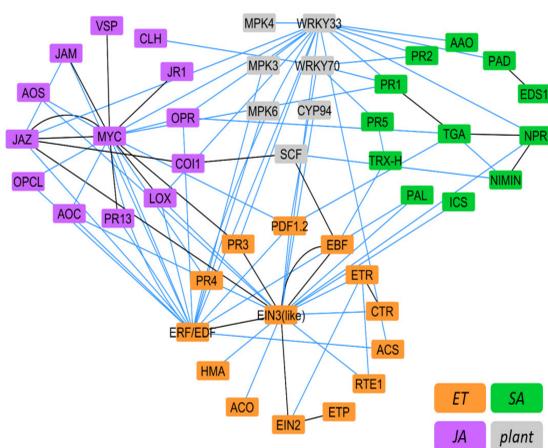
Additionally, data of two short transcriptomics time-series of potato infected with PVY has been generated in the lab [2,3] and were used for the generation of co-expression [4,5] and gene regulatory networks (GRN; [6]).

The StCKN was combined with the differential experimental networks into the potato integrated network (StIN). The resulting networks and subnetworks were analyzed topologically by the shortest paths and walk analyses between the hormone signaling pathways.



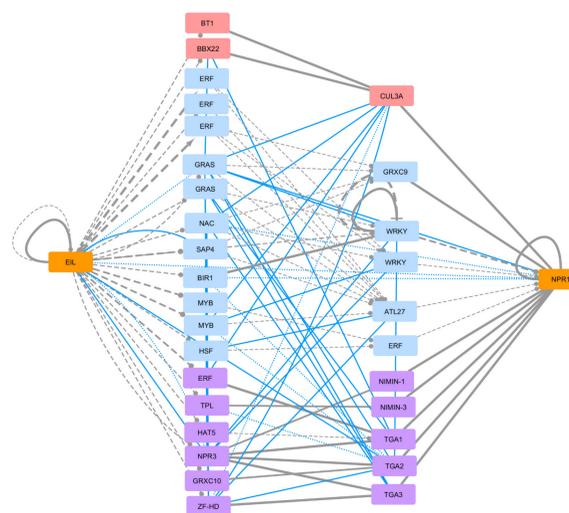
Results

Missing links in the PIS model: By extracting a subnetwork of the integrated network, we found new connections between the existing nodes.



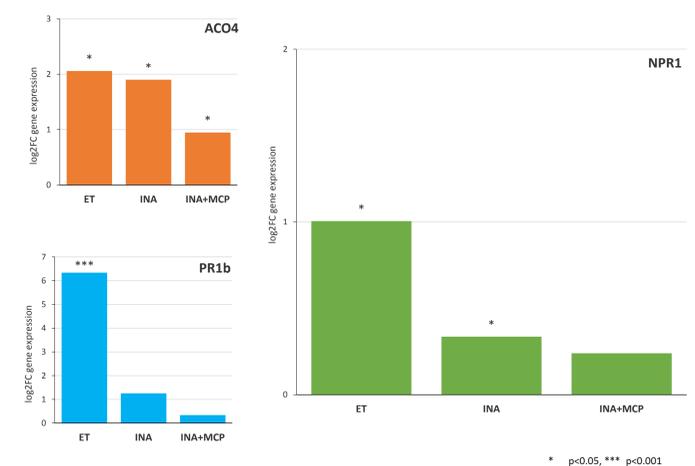
Integrated network subset of gene families involved in three hormonal signaling pathways (ethylene, jasmonic acid, salicylic acid) and some other components. The blue lines represent newly found connections from the comprehensive knowledge network (public datasets), whereas the black represent connections existing in the manually built PIS model.

Missing components of the immune signaling: Shortest path search between receptors and transmitters of hormones important for the plant immune system in the created networks.



Short path search from EIN3 (ET pathway) to NPR1 (SA pathway) in the potato network, combined with experimental data. Full black lines represents binding, dashed lines transcriptional regulation, full blue lines indicate GRN and dashed coexpression connections. Thickness of lines denotes the reliability of the connection (thicker is more reliable).

Experimental validation of network generated hypothesis of novel ET-SA crosstalk in potato.



Potato plant leaf samples (cv. Rywal) were sampled 24h after treatment ET, INA (SA analogue) or INA in combination with 1-MCP (ethylene pathway inhibitor). Gene expression of ACO4 (ethylene synthesis), PR1b (actuator in SA signaling) and NPR1 (signal transmitter in the SA pathway) were analysed. The results display a tight connection of SA-ET pathways, thus validation the connection between ET and NPR1.

References

[1] Miljković D, et al. *PLoS One* (2012) [2] Stare T, et al. *BMC Genomics* (2015) [3] Baebler Š, et al. *J Exp Bot* (2014) [4] Itkin M, et al. *Science* (2013) [5] Theocharidis A, et al. *Nat Protoc* (2009) [6] Huynh-Thu VA, et al. *PLoS One* (2010)

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