The role of plant defense proteins during early symbiotic and pathogenic infection in the model legume *Medicago truncatula*

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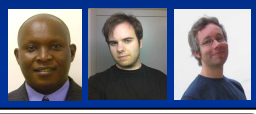


Background

Legumes are among the most economically important crops worldwide. They establish mutualistic symbioses and pathogenic interactions with microbes. Their infection physiology involves protein-protein interactions with the host plant generating an initial defense response, presumed to be similar at the very early infection phase but later split into symbiotic and pathogenic specific cellular defense responses.

We characterized the two host defense response patterns, using the model legume *Medicago truncatula* as the host for nitrogen-fixing rhizobial bacteria *Sinorhizobium meliloti* and arbuscular mycorrhizal fungi *Glomus intraradices* as well as root rot oomycete pathogen *Aphanomyces euteiches*. To functionally characterize the role of defense proteins during the very early phases of infection, we used plants deficient of Rac1 protein. Rac1 is a small monomeric GTP binding protein and a key signalling factor in eukaryotic cells. It regulates the membrane-bound Rboh (respiratory burst oxidase homologue) which catalyses the generation of reactive oxygen species (ROS) in plants in response to microbial infection.

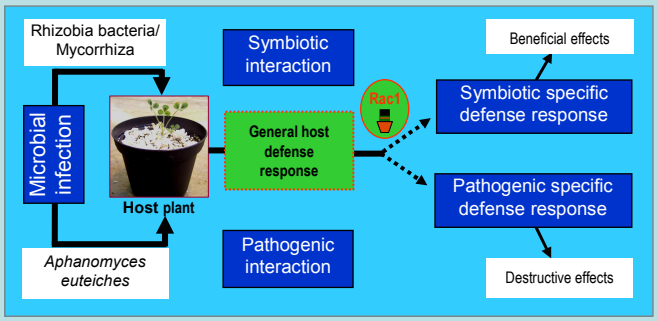


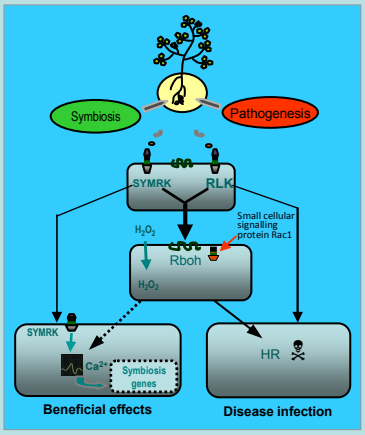
Fig 1: Symbiotic and pathogenic host plant defense response pathways

Conclusion and Outlook

- Retarded growth and low nodulation of MtRac1-deficient lines shows the role of Rac1 protein in plant growth and development
- Activation of Rac1 protein confirmed its role as an early signalling molecule involved in host defense against microbes
- MtRac1 protein is not only involved in host plant defense but also positively influence plant development
- For identification, functional analysis and evaluation of protein profiles, proteomic analysis and mass spectrometry are currently carried out

Fig 2: Proposed model of infection network of *M. truncatula* involving symbiosis and pathogenesis

Note:
During the initial infection phase, the host membrane receptors perceive extra-cellular stimuli from both the symbiont and the pathogen triggering a host cellular response. Rboh is activated by binding of Rac1 leading to generation of ROS, which can result to cell death or initiation of second signalling cascade and downstream activation of symbiosis specific genes



Results and Discussion

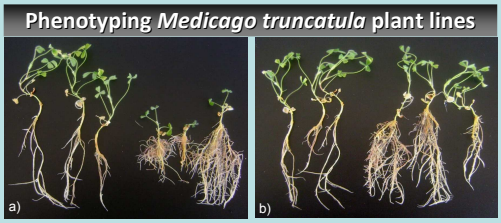


Fig 3: (a) Wild type vs. MtRac1- deficient lines and (b) wild type vs. vector control plants

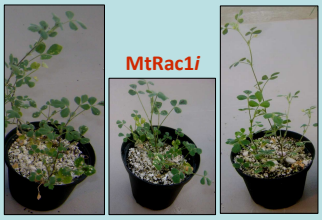


Fig 4: Comparisons between (a) Wild type (b) MtRac1-deficient and (c) vector control plants in pot system

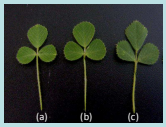


Fig 5: (a) Vector control (b) w-type (c) MtRac1-deficient

- Stunted growth in MtRac1-deficient plants compared to wild type plants but the vector control plants developed normally
- More hairy roots/cm root-length in MtRac1-deficient plants compared to vector control
- Tapering and/or deformed leaves in MtRac1-deficient plants but normal development in wild type and vector control lines

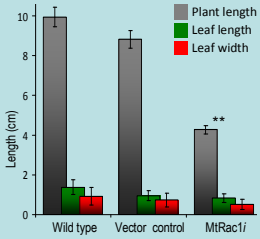


Fig 6: Comparisons of different parameters for plants cultured under *in vitro* conditions

- Difference in plant length between MtRac1-deficient plants, vector control and wild type plants was significantly high
- MtRac1-deficient lines had smaller leaf size than the wild type and vector control lines
- Significantly higher nodulation in wild type than MtRac1-deficient and vector control lines

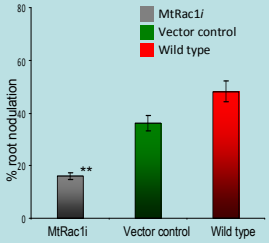


Fig 7: Influence of Rac1 protein on nodule formation for *M. truncatula* plants cultivated under *in vivo* conditions

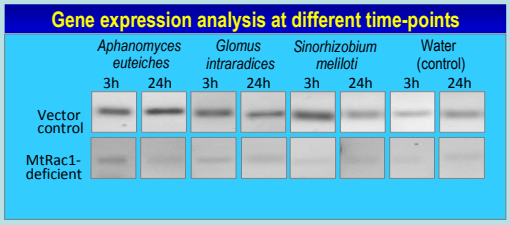


Fig 8: Gene expression analysis at 3 and 24hrs post inoculation via semi -quantitative RT-PCR using Rac1 primer system

- Expression level of Rac1 was clearly suppressed in MtRac1 -deficient plants compared to vector control and even after infection with *Aphanomyces euteiches*

Notes on Materials and Methods

- Generation of transgenic plants via *Agrobacterium rhizogenes*-mediated transformation: RNAi – gene silencing approach
- Phenotyping of MtRac1-deficient, vector control and wild type plants under *in vitro* and *in vivo* conditions
- Microbial infection and evaluation of infection profiles
- Identification and characterization of protein profiles via 2D-Gel electrophoresis and mass spectrometry

Fig 9: *M. truncatula* transgenic root cultures and as observed under a microscope showing red florescence-dsRED to distinguish transgenic hairy roots

Reference: Kiirika Leonard M[1], Bergmann H F[2], Schikowsky C[1], Wimmer D[1], Korte J[1], Udo Schmitz[1], Niehaus K[2] and Colditz F[1]* 2011. Silencing of the Rac1 GTPase MtROP9 in *Medicago truncatula* stimulates early mycorrhizal and oomycete root rot colonizations but negatively affects rhizobial infection (*Submitted for publication*)

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