# Differential effect of a novel BAK1 allele on brassinosteroid, innate immunity and cell death signalling



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The potato late blight pathogen [illegible] RXLR-type effectors that are translocated inside host cells where they modulate plant immunity. Basal plant immunity is mediated by membrane-integral receptor-like kinases (RLK) that perceive non-self pathogen-associated molecular patterns (PAMPs) to initiate a defense response. This work aims at identification of RXLR effectors that target host cell membrane structures and to characterise their impact on alteration of basal immunity. We applied transient and stable *Agrobacterium tumefaciens*-mediated expression in *Nicotiana benthamiana* to analyse the localisation of RXLR effectors and their effect on *P. infestans* susceptibility and PAMP-triggered immunity. We found that overexpression of selected RXLR effectors confers enhanced susceptibility towards *P. infestans* infection. Some of these effectors associated with endomembrane compartments and/or showed focal accumulation at haustorial sites suggesting interference with the host machinery for secretion, maturation and quality control of secreted and membrane-integral proteins. Interference with the function of membrane-integral PAMP receptors is further supported by attenuated production of reactive oxygen species (ROS) upon expression of specific RXLR effectors. Importantly, we found that some *P. infestans* effectors are able to alter the cellular localisation of specific RLKs involved in PAMP-triggered immunity. Further research aims at defining the target specificity and to elucidate the molecular basis of altered RLK localisation.

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## 231 Differential effect of a novel BAK1 allele on brassinosteroid, innate immunity and cell death signalling

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The bacterial pathogen-associated molecular patterns (PAMPs) elf18 and flg22 are recognised by the Arabidopsis leucine-rich repeat receptor kinases (LRR-RKs) EFR and FLS2, respectively. To elucidate novel components of PAMP-triggered immunity (PTI) we performed a forward-genetic screen to identify *elfin* (*elf18-insensitive*) Arabidopsis mutants. Out of 103 non-*efr elfin* mutants, one was clearly impaired in elf18, as well as in flg22 responsiveness. Map-based cloning of the mutated gene revealed a mis-sense mutation leading to a single amino acid substitution in the kinase domain of the LRR-RK BAK1/SERK3. BAK1 forms hetero(di)mers with FLS2 and the LRR-RK brassinosteroid (BR) receptor BRI1 to control PTI and BR signalling, respectively. In addition, BAK1 is also involved in cell death signalling together with its paralog BKK1/SERK4. Unexpectedly, detailed phenotypic characterization revealed that the novel *bak1-5* allele is more impaired in PTI signalling than previously described null *bak1* alleles. Thus, the BAK1-5 protein seems to act in a dominant-negative manner. Importantly, *bak1-5* is hyper-susceptible to a wide range of adapted and non-adapted pathogens, suggesting that BAK1 is also involved in the sensing of yet unknown PAMPs. Interestingly, *bak1-5xbkk1/serk4-1* double mutants show no aberrant senescence or cell death phenotypes in contrast to known *bak1/serk4xbkk1/serk4* double mutants, revealing that cell death control is not impaired in *bak1-5*. Surprisingly, while previously described null *bak1* alleles are BR hyposensitive, *bak1-5* displays a wild-type-like phenotype.

Our detailed phenotypic analysis reveals the intriguing differential effect of a single amino acid change in BAK1-5 on three independent signalling pathways, namely PTI, BR and cell death signalling.
We are currently investigating the molecular mechanisms of BAK1-5 function. Co-immunoprecipitation experiments show an increased association of BAK1-5 with the main ligand-perceiving receptors. Furthermore, the BAK1-5 kinase activity seems to be required for *bak1-5* related phenotypes. We hypothesise that the increased association of BAK1-5 with FLS2/EFR and BRI1 in combination with their differential phosphorylation is causative for the *bak1-5* phenotype.

We will present novel insights into the trans-phosphorylation events revolving around BAK1/BAK1-5 and their implication for the different BAK1-dependent signalling pathways.

are perceived by highly specific receptors at t
as the flagellin receptor FLS2 (Flagellin Sens
Previously, we demonstrated that BAK1 inter
of the ligand flg22 and that this interaction is
physiological responses [2]. In the present w
analysis of receptor heteromerization and sh
with BAK1 within less than 1 s after stimulati
While FLS2 is responsible for ligand binding,
both FLS2 and BAK1 are believed to trigger
Using in vivo labeling with [33P]phosphate, v
phosphorylation events on FLS2 and BAK1
of the phosphorylated proteins over time. In
both, FLS2 and BAK1, are phosphorylated w
flg22. Thus, de novo phosphorylation within
precedes activation of other signaling steps
immune responses.

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## 232 Insights into PAMP-triggered events in *Nicotiana benthamiana*

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The first layer of plant innate immunity re
pathogen-associated molecular patterns
triggered immunity (PTI). Important featu
reactive oxygen species (ROS), activatic
kinase (MAPK) cascades, changes in int
and activation of defense genes. We use
silencing (VIGS)-based and pharmacolo
*benthamiana* to decipher the links betwe
the PAMPs flg22 and chitin. The calcium
calcium chelator EGTA suppressed the
concentration triggered by the two PAM
MAPKs activation and induction of defe
was not affected by the specific silencin
and Nb*WIPK*, indicating that this event
burst and MAPKs activation. Importantl
in plants silenced for Nb*SIPK*, Nb*WIPK*
these MAPKs are not required to activa
only Nb*SIPK* was necessary for defens
MAPKs were required for disease resis
*Pseudomonas syringae* and their *hrp* m
that the calcium burst triggered by PAM
signaling pathways, one leading to MA
defense genes, the other to ROS produ

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