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Constitutively active MPK4 helps to clarify its role in plant immunity

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Mitogen-activated protein kinase (MAPK) modules are often involved in stress responses and plant developmental processes. Among these MAPKs, MPK4 has a complex role in biotic stress signaling, cell division control and cytoskeletal organization. *mpk4* knockout (KO) plants are dwarfed and very sick, making it difficult to distinguish between cause and effect of its phenotype. To overcome this difficulty, we developed mutations triggering constitutive MPK4 activity and created transgenic lines allowing phenotypic studies on a WT-like plant. By this approach, we confirmed that MPK4 functions as a negative regulator of pathogen defense, but our work also suggests that MPK4 interferes with stress signaling pathways at several distinct steps in pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) as well as in effector-triggered immunity (ETI). This study shows that CA mutations are valuable complementary tools to study MAPK signaling pathways in planta.

Keywords: *Arabidopsis thaliana*, mitogen activated protein kinase 4, constitutive activity, biotic stress, *pseudomonas syringae*

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Plants can detect pathogens through the binding of conserved pathogen-associated molecular patterns (PAMPs) to receptors. This system, best exemplified by the interaction of bacterial flagellin with the FLS2 receptor, triggers calcium-dependent protein kinases (CDPKs) and MAPK modules (**Fig. 1**) to induce reactive oxygen species (ROS) production and gene expression.[1] This process is referred to as PAMP-triggered immunity (PTI). Pathogens can suppress PTI by injecting effectors in host cells. In some cases, plants dispose of resistance genes to detect microbial effectors and induce effector triggered immunity (ETI), which is characterized by a programmed cell death and systemic acquired resistance. MPK4 plays an important role in defense and *mpk4* knockout (KO) plants display constitutive stress responses and enhanced resistance to biotrophic pathogens.[2] Even in unchallenged conditions, plants with a defect in MPK4 are dwarfed, accumulate ROS and the stress-related hormone salicylic acid (SA). These data suggest that MPK4 plays a negative role in stress responses, but as the *mpk4* mutant is very sick, it is difficult to decide between the primary effects and the long-term consequences of the mutation.

We reported recently the identification and utilization of constitutively active (CA) forms of plant mitogen associated protein kinases (MAPKs):[3] using a randomly mutated library of Arabidopsis MPK6 in a yeast functional screen, we unveiled CA triggering mutations. We then used this tool to tackle the function of MPK4 in plant immunity. We generated transgenic lines expressing a *CA-MPK4* locus instead of the native *MPK4* gene. In contrast to the dwarfed *mpk4* KO plants, CA-MPK4 plants show no morphological phenotype. but modified susceptibility toward pathogens: for example, spraying leaves with the pathogen *Pseudomonas syringae* DC3000 resulted in higher bacterial proliferation rates in the apoplast of CA-MPK4 lines than in WT plants. We also showed that CA-MPK4 plants produce less SA and ROS when challenged by pathogens or PAMPs than WT plants. In agreement with the studies performed on *mpk4* KO plants, these data confirm that MPK4 has a negative function in plant immunity.

However, the phenotypically normal CA-MPK4 lines also allowed us to

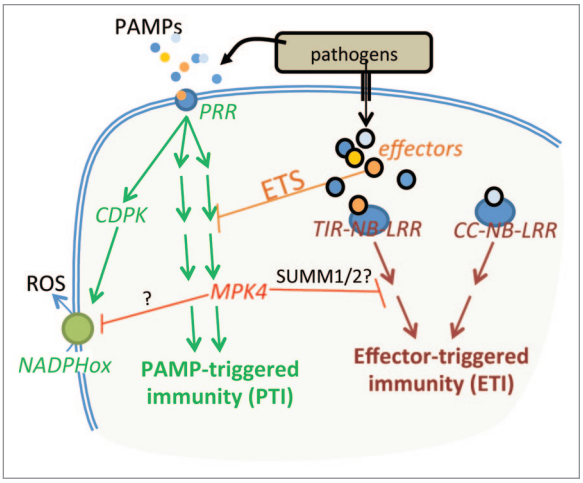

Figure 1. Arabidopsis MPK4 is involved in PTI signaling and influence ETI.

address more subtle traits. Contrary to spraying of the leaves with *Pseudomonas syringae*, direct infiltration of the bacteria into the leaf apoplast resulted in similar proliferation rates in CA-MPK4 and WT plants. Different bacterial proliferation rates upon spraying- and injection-based assays in leaves usually indicate a stomatal function and in our case suggest that the limiting role of MPK4 could be in guard cells.[4] This finding was correlated with the observation that MPK4 protein is highly expressed in guard cells. However, our investigation revealed no significant differences between WT and CA-MPK4 lines at the stomatal density level or in the stress-induced closure of stomatal ostioles. These last assays are obviously difficult to perform: as the stomates are removed from their tissue-specific environment, they need to be incubated in buffers which are well defined and clamped but obviously not resuming the complexity of the apoplast medium. Therefore, scientists are never sure to have the right conditions to observe a genetic effect on the stomatal movement. Alternatively, the difference obtained between spraying and injection may not be linked to a stomatal misfunction but to another role in an early step of plant immunity in mesophyll cells. As bacteria enter progressively into the apoplast upon spray inoculation, mesophyll cells will progressively turn on intracellular defense responses, a step that may be delayed in CA-MPK4 lines. In contrast, when bacteria are injected into leaves, the apoplast is rapidly overloaded with bacteria and the overwhelmed mesophyll cells have no time to adapt. Potential phenotypes in this early interaction step may be masked. This hypothesis implies that MPK4 is a negative regulator of early plant defense processes, which would be in agreement with our general knowledge on MPK4.

The study of CA-MPK4 lines generated another interesting piece of information on a potential function of MPK4 in ETI signaling. Compared with virulent *Pseudomonas syringae DC3000*, *P. syringae* strains expressing avirulent genes do not reach high proliferation levels as plants detect effectors through two distinct subfamilies of NBR-LRR receptors (**Fig. 1**). Certain effectors such as avrRps4 are detected by TIR-NBS-LRR type receptors whereas others like avrRpm1 and avrRpt2 are recognized by CC-NBS-LRRs. The enhanced bacterial titers in the CA-MPK4 lines challenged with *P. syringae* expressing avrRps4 suggested that MPK4 might influence the RPS4-dependent ETI pathway. Interestingly, such a phenotype was not observed with *Pseudomonas* expressing avrRpm1 and avrRpt2 that are recognized by CC-NBS-LRRs. These results suggest that MPK4 specifically affects TIR-NBS-LRR- but not CC-NBS-LRR-mediated defense pathways. In recent genetic screens, it was found that the programmed cell death regulated by the MPK4 pathway depends on the MAPKKK MEKK2/SUMM1[5] and the NBS-LRR protein SUMM2[6] and that both of these genes act downstream of MPK4. In summary, these results provide a clear mechanism how programmed cell death and innate immunity are regulated by the MPK4 pathway. This insight should help us to engineer crops with enhanced pathogen resistance and reduced pesticide requirements.

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