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Identification and functional analysis of novel regulatory components of the potato NLR immune receptors Rx1 and Gpa2

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Following the recognition of pathogen-derived avirulence proteins, the mechanism by which plant nucleotide-binding and leucine-rich repeat (NLR) immune receptors trigger defense remains vague. However, the modular architecture of these receptors suggests that they may engage in a network of interactions with other host factors, presumably for defense signaling. Identifying interacting partners of plant NLRs is therefore key to advance our understanding of how they function. Here, we aim to identify and characterize novel regulatory components of the potato NLRs Rx1 and Gpa2. Both NLRs are characterized by an archetypical N-terminal coiled-coil (CC) domain and share high sequence conservation. Nonetheless, they mediate distinct responses against two unrelated pathogens, providing a unique platform for research. Rx1 confers rapid, extreme resistance against Potato Virus X (PVX) whilst Gpa2 triggers mild resistance against the potato cyst nematode Globodera pallida. Existing models predict the N-terminal domain to act as a platform for downstream interactions. We therefore used the CC domains of Rx1 or Gpa2 as baits in a Co-IP/MS analysis to co-purify putative interactors from Nicotiana benthamiana. Five hits (designated Rp01-Rp05) were further prioritized as candidate Rx1/Gpa2 interacting proteins. Similar pull-down experiments corroborated complex formation with the full-length immune receptors in planta. A combination of reverse genetics and advanced microspectrocopic studies was then used to resolve the functional relevance of the interactions detected. Interestingly, we could demonstrate that co-expression of Rp05 alters the subcellular distribution of the Rx1-CC domain, hinting that Rp05 may be involved in Rx1-functioning. It is also worth noting that transient overexpression of Rp05 enhanced resistance against PVX independently of Rx1, pointing to its importance as a component in immune signaling. We currently focus on substantiating this model by investigating the broader role of Rp05 in defense against other pathosystems.