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20-7 - Characterization of the structure and mode of action of *Os*JAC1, a rice protein involved in broadspectrum disease resistance

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The constitutive expression of *OsJAC1* in transgenic rice plants confers broadspectrum disease resistance against different pathogens (Weidenbach et al., 2016). *Os*JAC1 belongs to a class of chimeric proteins consisting of a dirigent and Jacalin-related lectin (JRL) domain which occurs exclusively in monocotyledonous plants. In dicots, like Arabidopsis, proteins exist with single dirigent or JRL domains (Esch & Schaffrath, 2017). Interestingly, artificially separated dirigent and JRL domains of *Os*JAC1 were shown to interact which each other and this is a prerequisite for its function in pathogen resistance. Investigation of respective single domain proteins of Arabidopsis failed to confirm interaction in a similar manner which prompted us to conclude that the *Os*JAC1-related resistance is unique for monocots (Esch et al., 2023).

Focusing on the mode of action of *Os*JAC1, we tried to identify putative interaction partners in cooperation with our partners at FZ Jülich. Thereby, we were able to determine the crystal structure for both artificially separated domains of *Os*JAC1. This enabled further prediction of binding sites and revealed galactose-containing saccharides as interaction partners for the dirigent domain and mannose-containing carbohydrates were found to bind to the JRL domain (Huwa et al., 2022). Further proof, that these carbohydrates are true interaction partners came from studies confirming an effect of binding to protein stability (Huwa et al., 2021). We also found that *Os*JAC1 seems to form dimers, which could explain why no crystal structure of the holoprotein of *Os*JAC1 could be obtained so far. As mentioned above not only carbohydrates could be interaction partners of *Os*JAC1 but also proteins. In this second line of experiments, two candidates obtained in a Y2H screen were tested for their role in plant defense.

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20-8 - Isolation of Magnaporthe oryzae infected barley cells using flow cytometry for transcriptome profiling

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After being attacked by a pathogen, plants rely on defense strategies of individual cells (Jones & Dangl, 2006). Conversly, this means that in order to be successfull, a pathogen must evade or suppress these defense reactions which can e.g. be achieved by secretion of so-called effectors (Kamoun, 2007). While defense suppression might result in colonization of one plant cell, neighboring cells may still resist invasion (Gjetting et al., 2007). In order to target the genetic and metabolic processes that differentiate susceptible from resistant plant cells, and the mechanisms by which a pathogen manipulates the host's immune system, an analysis of gene expression profiles of individual cells is mandatory.

Therefore, we intended to evolve a strategy which enables the enrichment of barley cell populations colonized by the fungus *Magnaporthe oryzae* and their separation from those that are not yet attacked by the pathogen using Fluorescence Activated Cell Sorting (FACS). To achieve this goal, we generated *M. oryzae* mutants that constitutively express a fluorophore. These mutants were inoculated on barley primary leaves, which subsequently were used for the generation of protoplasts. By establishing an advanced protocol, we were able to separate mesophyll from epidermal protoplasts and then used FACS to sort the latter based on the fluorescence signal emitted by the fungal hyphae. Our next step is to perform RNA-sequencing of the different cell populations. By comparing gene expression profiles, we aim to identify barley genes that are either exclusively or differentially expressed in infected cells compared to non-infected cells, as well as fungal genes that are expressed during plant colonization. Finally, potential candidate genes will be characterized by using various molecular tools.

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