論 文 の 内 容 の 要 旨

応用生命工学専攻
平成 26 年度博士課程進学
氏　　名　Valea　Ioana
ワレアイワナ
指導教員名　野尻　秀昭

論文題目

Study on mediating factors of early wound responses via jasmonic acid signalling in rice

(イネにおいてジャスモン酸を介した傷害ストレス初期応答を担う因子に関する研究)

Introduction

In the course of life, the rice plant has to adapt and become able to resist numerous biotic and abiotic threatening factors, for example surviving a pest period or adapting to incurring mechanical damages due to either insect damage or strong environmental conditions. Plants in general have shown advanced adaptability and evolved defence mechanisms to a myriad of threatening factors. Rice, as one of the main food sources in Asia and a main crop worldwide, is our plant system of choice to investigate the adaptability and response mechanisms to environmental changes or herbivory resulting in wounding of the rice plant.

One of the defence mechanisms known to be activated is the jasmonic acid pathway. After exposure to biotic or abiotic stresses jasmonic acid (JA) synthesis occurs in rice cells leading to the accumulation and perception of jasmonyl-isoleucine (JA-Ile). This active signalling molecule after recognition by its receptor F-box protein OsCOI1 triggers the degradation of the pathway inhibitory factor OsJAZ and with it the release of activity of OsMYC2, the main factor known to activate this system. OsMYC2 is always in position waiting for the repression to be lifted in order to being able to carry on with its activity of initiating the JA response as a defence form against the initial stress factor. When this defence become unnecessary, OsMYC2 activity is again inhibited by direct binding of OsJAZ. These three factors, a bHLH transcription factor, F-box protein, and a TIFY motif containing

protein, respectively, are until now the key players of the JA response. In rice there are 15 different OsJAZs known at the moment, characterized by the presence of a TIFY motif for their dimerization as well as a Jas motif responsible for the interaction with COI1 and MYC.

In this study we focused on the activity of two key players, OsMYC2 and OsJAZ, in the context of early wounding response, which in nature could be a daily occurrence in the life of rice plants. At the same time, we brought another factor in this system named RERJ1 (rice early responsive to jasmonates 1). This bHLH transcription factor has been previously identified in the differential screening of a cDNA library constructed from suspension-cultured rice cells treated with jasmonic acid (JA). Within 30 minutes after JA treatment and wounding RERJ1 transcript level has been shown to reach its peak showing an 800-fold induction compared to the non-treated control leaves.

Of my focus is exposing the early occurrences after wounding in the rice plant with special interest in the JA signalling factors OsMYC2 and OsJAZ, as well as RERJ1, which as a bHLH factor with previously demonstrated transactivation activity, must be involved in the signalling. For achieving my goals, I examined these three factors on the transcript and protein level, exploring their early wound and JA treatment response in transcript levels, as well as their potential to protein-protein interactions, and the RERJ1 regulon harnessing JA-mediated stress responses in rice.

RERJ1 directly regulates the rice linalool synthase

In previous studies, the involvement of RERJ1 in wounding had been established, however no direct evidence of the mechanism has been provided. Here, by using RERJ1 overexpressing lines, we were able to find an *in vivo* target of RERJ1.

One of the interesting aspects of this chapter is the usage of a novel human dopamine receptor derived 10 amino-acids long tag shortly called AGIA. Transgenic lines overexpressing the N-terminal AGIA fusion to RERJ1 were generated and confirmed the overexpression of AGIA-RERJ1 transgene on transcript and protein level. These lines were used for subsequent characterizations through Chromatin Immunoprecipitation (ChIP) analyses with both RERJ1 and AGIA antibodies and microarray analysis to survey a potential candidate of the RERJ1 regulon. In previous studies linking RERJ1 to herbivory, one particular gene has shown to be RERJ1 dependent, namely *OsLIS*, encoding the linalool synthase involved in the production of linalool, a monoterpene previously directly linked to defence against herbivory. ChIP-qPCR analysis conducted in this study prove that RERJ1 directly binds to the promoter region of 135-500-bp upstream of the *OsLIS* gene. This result provides direct evidence of the involvement of RERJ1 in the defence mechanism to herbivory and completes the previous studies regarding the *OsLIS* gene belonging to the regulon of RERJ1.

Two colour microarray analysis with AGIA-RERJ1 overexpressing plants provided insight into the transcriptome changes caused by constitutive expression of RERJ1, showing slightly increased RERJ1 and OsMYC2 transcript levels compared to the vector control, as well as a wide range of changes in the transcript levels of distinctive *OsJAZ* family genes.
The significance of this finding might possibly be linked to the overexpression level of RERJ1 and the activation of its downstream response genes, such as *OsLIS* and some other JA-related stress response genes including the OsJAZ repressor, a core component of the JA signalling.

Early transcripts analysis shows differently regulated OsJAZ dependent on RERJ1

Beside overexpression of RERJ1, loss-of-function approach was taken to investigate the involvement of RERJ1 in the JA signalling. To this purpose, two different plant materials, the *rerj1-Tos17* mutant and the *osmyc2-RNAi* knock-down lines, were used for the characterization of common signalling factors involved both in wounding and in the JA response, in which the transcript levels of our genes of interests, RERJ1, OsMYC2, and OsJAZ, were analysed.

Firstly, *osmyc2-RNAi* lines have shown that RERJ1 seems to be at least partly dependent on the activity of OsMYC2, mostly after JA treatment and less after wounding, suggesting OsMYC2 is indispensable for the RERJ1 dependent JA response, an already well-established fact. However, it seems to be only partly involved in RERJ1-dependent wounding response, despite showing a certain sensitivity of RERJ1 expression to this kind of stress as well. We hypothesise that there are two different response ways to wounding, one RERJ1 dependent without OsMYC2 being involved, and another OsMYC2 dependent with RERJ1 involvement.

A look at the OsMYC2 expression level in the *rerj1-Tos17* knock-down lines showed a RERJ1-dependent slight decrease of the OsMYC2 expression, and this constant small change might seem to signify that RERJ1 is responsible for providing input for OsMYC2 expression at some point along the signalling pathway.

The most probable way for RERJ1 to provide input for the JA signalling and link this system to other pathways involved in defence might be over OsJAZ. Although the temporal transcript pattern of every OsJAZ in response to wounding and JA treatment is similar, it can clearly be distinguished between OsJAZ affected by RERJ1 dependent wounding (for example OsJAZ9 and 11), and those unaffected by it (for example OsJAZ3 and 13). Each OsJAZ has its own function, not yet elucidated for every single one. In this study we could link OsJAZ5, 8, 11 and 12 to RERJ1-dependent early wound responses. Connected to both early responses after wounding and JA treatment, expression of OsJAZ9 and OsJAZ11 seem to be highly dependent on RERJ1. By analysing the transcriptional context, *OsJAZ* genes involved in wounding in a RERJ1-dependent manner were able to be categorised, and thus the transcriptional connection between RERJ1 and OsMYC2 was also partly elucidated.

RERJ1 and OsMYC2/OsJAZ directly interact on protein level

Considering possibilities for information input by RERJ1 into the JA signalling, a look at direct protein interactions provided more insight. Amplified Luminescence Proximity Homogeneous Assay Screen (AlphaScreen) was used for investigation of interaction of the target proteins synthesized by Wheat Germ Protein Expression System. The analysis on AlphaScreen provided evidence that RERJ1 has the ability to interact with most OsJAZ and OsMYC2 *in vitro*. These results are also backed up by yeast two-hybrid data identifying 10 out of 15 OsJAZs as possible interactors in the yeast system. OsMYC2 has been shown as a potential weak RERJ1 interactor *in vivo* in Bimolecular Fluorescence Complementation (BiFC) after transient expression in onion epidermis cells.

Considering that weak interactions between RERJ1 and OsMYC2, possibly meaning

that the affinity between these two proteins is not very high and they are occasionally connected by the ability of bHLH proteins to form hetero-dimers. At the same time these findings might signify that both are part of a bigger signalling complex, probably with OsJAZ acting as a possible bridge. All these interactions show the involvement of RERJ1 in the well described feedback loop between JAZ and MYC2.

Conclusion and future prospects

All these findings brought together show that different OsJAZ are responsible for different response connections. A most probable mechanism is RERJ1 providing input in the early stages of the wounding response over OsJAZ 9, 11 and possibly OsMYC2 direct interaction, leading to fast decisions of the rice plant as to signal perception and response pathway activation, eventually leading to the actual defence response involving genes such as *OsLIS* or other factors found responsive in the microarray analysis of RERJ1 overexpressing plants. For further describing the global RERJ1 regulon, other promising E-box *cis*-elements occupied by RERJ1 in the promoters of RERJ1 dependent OsJAZ genes and other candidates of RERJ1-regulon will be revealed by means of ChIP-qPCR to extend our understanding of RERJ1 involvement in the JA signalling system.