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論文題目	A study on the meristem fate regulation by TAW1 in rice panicle development（イネの穂の発生における TAW1による分裂組織の運命制御に関する研究）
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Abstract

Rice (*Oryza sativa*) is one of the most widely consumed staple foods in the world, making it one of the most important crops. Panicle formation is an important process in rice development which determines grain yield. In the rice development process, rice will grow starting from the vegetative phase until the transition to the reproductive phase, which changes the fate of the shoot apical meristem (SAM) to the inflorescence meristem (IM). In rice, the main axis of the inflorescence is known as the rachis. The IM forms main rachis, primary branch meristem (PBM), secondary branch meristem (SBM), and spikelet meristem (SM). PBM are the lateral branch meristems that attached directly to the main rachis. While, the lateral branch meristems that are attached to the PBM are termed SBM. During the rice IM development, the early IM acquires an indeterminate branch meristem (BM) identity and continuously produces PBM and SBM, until, eventually, the determination of its fate into the SM. Several genes involved in the control of rice panicle development have been reported. *TAWAWA1* (*TAW1*), encodes an *Arabidopsis* LSH1 and *Oryza* G1 (ALOG) transcription factor, is a regulator of rice panicle architecture through the suppression of BM phase transition to SM identity and extending IM activity. The *taw1-D2*, an overexpression *TAW1* mutant, showed an increase of secondary branches. However, the mechanism(s), in which *TAW1* regulates the meristem fate, is still unknown. Understanding the molecular basis of meristem fate determination during rice panicle development will greatly aid the development of high yield rice variety. Thus, the purpose of this study is to gain a better understanding of the molecular basis of meristem phase change by *TAW1*.

Results

Isolation of genes working downstream of *TAW1* in the control of panicle development

To identify the *TAW1* downstream target genes, chromatin immunoprecipitation sequencing (ChIP-seq) analysis was performed on *35S: TAW1-GFP*. The results of *35S: TAW1-GFP* ChIP-seq predicted a total of 2,770 target genes. GO analysis of these predicted target genes showed that their functions are related to developmental process involved in reproduction, reproductive structure development, system development, reproductive system development, flower development, shoot system development, and reproductive shoot system development. Among the *35S: TAW1-GFP* ChIP-seq predicted target genes is *FRIZZY PANICLE* (*FZP*). *FZP* is known for its antagonistic function with *TAW1* on the regulation of rice panicle development by promoting the transition of BM to SM identity. To further investigate the interaction between *TAW1* and *FZP*, transcriptional activity assay using *FZP* reporter constructs, which were designed according to the results of *35S: TAW1-GFP* ChIP-seq, was performed. The transcriptional activity assay showed that *TAW1* could activate the LUC activity of the *FZP* reporter constructs, even though there is still an unknown mechanism(s) to the interaction.

NARROW AND DWARF LEAF1 (*NDL1*) is also among the *35S: TAW1-GFP* ChIP-seq predicted target genes. To further analyze the interaction between *TAW1* and *NDL1*, transcriptional activity assay was performed and showed that *TAW1* could activate the LUC activity of the *NDL1* reporter construct, which was designed according to the results of *35S: TAW1-GFP* ChIP-seq. ChIP-qPCR also suggested that *TAW1* could bind to the region approximately 4.6 kb downstream of *NDL1* gene.

Analysis of genetic interaction between *TAW1* and *FZP*

To elucidate the genetic interaction between *TAW1* and *FZP*, *FZP*Ox is required. After the generation of the *FZP*Ox, the observation on the *FZP*Ox showed that the *FZP*Ox in wild-type background generated fewer tillers and comparable number of primary branches but reduced number of secondary branches, suggesting that the overexpression of *FZP* repressed axillary meristem formation in both the vegetative and reproductive phases. Next, the analysis on the genetic interaction between *TAW1* and *FZP* was conducted by crossing the homozygous *taw1-D2* with the homozygous *FZP*Ox. The *FZP*Ox *taw1-D2* showed a comparable number of primary branches compared to the wild-type, *taw1-D2*, and *FZP*Ox plants. In contrast, the *FZP*Ox *taw1-D2* showed an intermediate number of secondary branches phenotype, which is between the *FZP*Ox and *taw1-D2*, but comparable to the wild-type plants. To further analyze the genetic interaction between *TAW1* and *FZP*, the weak mutants of *FZP* were generated by using Target-AID, which is a fusion of CRISPR-Cas9 and activation induced cytidine deaminase, to change cytidine to thymine. Unfortunately, the homozygous *fzp-51 taw1-D2* and *fzp-71*, which were generated using the Target-AID, showed strong *fzp* phenotype with sequential rounds of branching instead of spikelet. However, the +/*fzp-51 taw1-D2* showed an even more increased number of secondary branches compared to the *taw1-D2*, even though the phenotype of +/*fzp-71*, which contain a more severe mutation compared to *fzp-51*, was comparable to the wild-type plants. These results showed that there is still an unknown mechanism(s) regarding the relationship between *TAW1* and *FZP*, including the possibility that *TAW1* also function through another gene. Nevertheless, even though the mechanism(s) is still unclear, this study also implied the possibility of *TAW1* to function through *FZP* on the regulation of rice IM fate.

***NDL1* is the ortholog of Arabidopsis *ENHANCER OF SHOOT REGENERATION1/DORNRÖSCHEN* and mediates leaf development and maintenance of the shoot apical meristem in rice**

Phylogenetic tree analysis showed that *NDL1* is the ortholog of Arabidopsis *ENHANCER OF SHOOT REGENERATION1* (*ESR1*)/*DORNRÖSCHEN* (*DRN*), which encodes an AP2-type transcription factor family protein. However, there is no information regarding *NDL1* in rice. Thus, the characterization of *NDL1* is needed. Here, the generation of *ndl1* mutant was performed using CRISPR-Cas9. The *ndl1-2* and *ndl1-3*, loss of function of *NDL1* mutants, were generated and showed bladeless leaves and SAMs that are flat, rather than dome-shaped, and which lack cell proliferation activity. The growth of the *ndl1-2* and *ndl1-3* were terminated soon after germination and could not survive until the reproductive phase, thus, making it impossible to analyze *NDL1* role during rice IM development. The flat SAM phenotype indicates that the *ndl1* mutant could not maintain its SAM. Thus, qPCR analysis was conducted to analyze the expression of *OSH1*, which is a SAM maintenance gene. Indeed, the expression of *OSH1* in the *ndl1-3* mutants were significantly lower compared to the wild-type plants. In Arabidopsis, *ESR1/DRN* is known for its function during the regeneration process. To elucidate whether *NDL1* has a similar function with *ESR1/DRN* in Arabidopsis, the callus growth and the shoot regeneration of *ndl1-3* calli were compared with the wild-type calli. As is the case with Arabidopsis *ESR1/DRN*, *NDL1* plays crucial roles in shoot regeneration, while, in contrast, the growth of *ndl1-3* calli was comparable with the wild-type calli. To get a better understanding regarding the cause of *ndl1* phenotype, *in situ* hybridization analysis was conducted on 5 days after germination and late vegetative samples in wild-type background. Interestingly, *NDL1* were not expressed in the SAM, but in the leaf primordia during the vegetative developmental stage. Thus, *in situ* hybridization analysis was further conducted on the samples from earlier developmental stage, which are 3-10 days after pollination (DAP). Similar to the previous *in situ* observation in the vegetative developmental stage, *NDL1* expression was also found in the leaf

primordia, but not in the SAM. To investigate whether *NDL1* is related to auxin, wild-type plants were treated with 10 μM NAA for 3 hours. The 10 μM NAA could induce *NDL1* expression. Furthermore, *OsYUC4* expression in the *ndl1-3* were diminished compared to the wild-type plants. These results suggest that *NDL1* cell autonomously regulates leaf development but non-cell autonomously regulates SAM maintenance in rice.

Analysis of the genetic interaction between *TAW1* and *NDL1*

To gain an insight regarding *NDL1* function during rice IM development, *in situ* hybridization analysis was conducted. The *in situ* hybridization results showed that *NDL1* is expressed in PBM and SBM, indicating that *NDL1* might play a role during these phases of rice IM development, on the other hand, *NDL1* was not observed during the transition phase from vegetative to reproductive phase and SM development. However, the generation of weak *NDL1* mutant is necessary to clarify the *NDL1* function during IM development, due to the terminated growth before reproductive phase phenotype of *ndl1* mutants. In contrast to *ndl1-2* and *ndl1-3* which contain mutations that abolish the AP2 domain, *ndl1-c3* contain a mutation after the AP2 domain, resulting in an intact AP2 domain. The *ndl1-c3 taw1-D2* could grow normally, even though the *ndl1-c3* in wild-type background also showed inhibited growth phenotype, similar to the other n*dl1* mutants. To confirm whether *taw1-D2* could rescue the other *ndl1* mutants, *ndl1-3* was crossed with *taw1-D2*. In contrast, *taw1-D2* could not rescue the inhibited growth phenotype of *ndl1-3*, indicating that the overexpression of *TAW1* in *taw1-D2* must function through the partially functional *NDL1* in *ndl1-c3* to rescue the inhibited growth phenotype. Next, the *ndl1-c3 taw1-D2* was grown to further analyze the function of *NDL1* during panicle development. The *ndl1-c3 taw1-D2* showed smaller panicle, with fewer primary branches and secondary branches, compared to the wild-type and *taw1-D2* plants, suggesting the importance of *NDL1* on rice panicle development. However, the generation and further study on weak *ndl1* mutant in wild-type background is necessary to clearly explain the function of *NDL1* during rice panicle development.

Conclusion

This study showed the possibilities of *TAW1* to function through *FZP* and introduced *NDL1*. *NDL1* is an ortholog of Arabidopsis *ESR1/DRN* but has not been reported in rice. In the vegetative phase, *NDL1* is proposed to cell autonomously regulates leaf development but non-cell autonomously regulates SAM maintenance in rice. While, during the IM development, *ndl1-c3 taw1-D2* showed that *NDL1* is important for rice panicle development, even though the generation of *NDL1* weak mutant in wild-type background is still needed to clarify *NDL1* function and its interaction with *TAW1*. Furthermore, this study also showed the possibility of *TAW1* to function through *FZP* on controlling the rice IM fate. Even though, there is still a possibility that *TAW1* also functions through other gene(s) and further study is still needed. This study showed that these three genes are important for rice panicle development. Interestingly, in Arabidopsis, *PUCHI*, the ortholog of *FZP*, and *ESR1/DRN* and *ESR2/DRNL*, the orthologs of *NDL1*, function redundantly to control the floral meristem identity. Thus, it is fascinating to elucidate the roles of these three genes and their interactions on the regulation of rice meristem fate.

論文審査結果の要旨

イネ、トウモロコシ、コムギなどイネ科植物にとって、いくつ種子をつけるかは繁殖を左右する重要な問題であるだけではなく、生産性という観点からも重要である。種子の数は基本的に花序に花が着くパターンによって決定される。花序に花が着くパターンは遺伝的に制御されている。植物はメリステム（未分化な細胞を含み、器官形成のもとになる）の幹細胞により枝分かれを続けるが、メリステムが花芽に運命づけられるとそれ以上の枝分かれは起こらず花が形成される。したがって、花芽運命決定のタイミングが花序の形態を決定する。

*TAWAWA1(TAW1)*は植物に固有の転写因子をコードする。イネ花序である「穂」に花が着くパターンの決定に関わっており、*TAW1* の発現が上昇すると花芽への運命決定が遅れ、その結果、花序の枝分かれが増加する。*TAW1* が花芽運命決定を抑制する機構や転写因子としての下流の標的遺伝子などは研究が進んでいなかったため、本研究では *TAW1* の下流遺伝子の単離、花芽運命抑制に関わる遺伝子の探索、そのメカニズムの解明を目的として、Chip シークエンス（Chip-seq）によるゲノムワイドな *TAW1* 結合領域の特定、花芽運命に関与すると考えられる候補遺伝子の特定、候補遺伝子の機能解析、候補遺伝子と *TAW1* との遺伝学的関係の解析を行った。

まず、Chip-seq 解析で見出された 3,012 の配列を解析し、*TAW1* の標的配列モチーフを同定し、下流候補遺伝子を特定した。下流候補遺伝子から *FRIZZY PANICLE1*（*FZP1*)および *NARROW AND DWARF LEAF1*（*NDL1*)に着目し、さらに解析を進めた。どちらも AP2 ドメインをもつ転写因子であり、*FZP1* はイネの花芽運命を決定し、*NDL1* はシロイヌナズナの再分化に必須である。Chip-qPCR 法やイネプロトプラストを用いた一過的発現解析により、*TAW1* がこれらの遺伝子を直接制御する可能性を示した。また、*TAW1* 発現が増加した *taw1-D2* 変異体と *FZP* 過剰発現体を用いて、*FZP* が *TAW1* の下流で働くという仮説を証明した。*NDL1* については、イネでの機能が解析されていなかったため、CRISPR 法により機能欠損変異体を作成し、*NDL1* が葉の分化を促進する同時にメリステムの維持に不可欠の遺伝子であることを明らかにした。

以上の研究成果は、イネ花序の形成という植物科学の重要な問題の解明に大きく貢献するものである。これを総合的な解析により独自の視点から解明したことは、自立して研究活動を行うに必要な高度な研究能力と学識を有することを示す。したがって、Andree S Kusnandar 提出の論文は，博士（生命科学）の博士論文として合格を認める。