論　文　の　内　容　の　要　旨

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論文題目　　Control of tiller outgrowth in rice
（イネ腋芽の伸長制御機構）

The process of development continues throughout the plant lifetime. Unlike animals in which the organs are all produced during embryogenesis, in plants new organs are produced from the meristems post-embryonically. The shoot apical meristem (SAM) gives rise to the overground tissues such as leaves and flowers while the root apical meristem (RAM) gives rise to the underground tissues such as the root system. In addition to the continuing organ formation at the periphery of SAM, meristems are also continuously generated. The newly generated meristem at the axil of leaf is called axillary meristem (AM). AMs produce a few leaves and become axillary buds. Axillary buds may continue to grow and form branches or become dormant and arrest their growth. The phytohormones, auxin and cytokinin, are thought to play major roles in the control of axillary bud growth. Moreover, strigolactone (SL) was recently discovered as a novel phytohormone which suppresses shoot branching.

In rice, axillary buds grow as tillers that bear the panicle. The tiller number is one of the most important traits for rice production. It has been demonstrated that the genetic framework of SL biosynthesis, signaling and function are well conserved in rice. Furthermore, several key genes in the control of tiller growth have been identified through genetic analysis and QTL analysis. However, in spite of these recent extensive progresses, our understanding of the mechanisms controlling tiller

outgrowth is still unsatisfactory. In this study, I first performed detailed analysis of rice tiller bud development and transcriptome analysis of tiller buds. Then I analyzed the interaction between *SPL14*, a major regulator of tiller growth, and the SL pathway.

Observation of tiller bud development

The pattern of rice tiller growth is determined by both endogenous and environmental factors. Some of tiller buds become dormant and stay as tiller buds until the condition becomes suitable for their growth. To understand the mechanisms that confer the dormancy to the tiller buds, first, I set up the growth condition in which tiller growth can be reproducibly observed. The second bud (the bud at the axil of the second leaf) was chosen for the analysis. The hydroponic culture system was used and the culture condition in which the second bud becomes dormant was established. In this culture system, the second bud of *d10-2*, a SL deficient mutant, continues to grow without entering into the dormant state. To determine the timing when the dormancy takes place, bud growth pattern was compared between WT and *d10-2*. In this study, new counting system was used. When the third leaf fully expanded is 3.0L stage which is equals to P (Plastochron) 5.5 in plastochron counting system, and when the fourth leaf half expanded is 3.5L equals to P6.0. At 3.5L stage, the growth of the second bud almost stopped in WT. This indicated that the transition of the bud phase from active to dormant takes place by 3.5L stage. The growth phase of the tiller buds changed between 3.0L and 3.5L stages. RNA *in situ* hybridization of a cell cycle marker gene *Histone H4* showed that, in the second bud, cell division almost stopped by 3.5L stage in both meristem and leaf primordia of WT. The arrest of cell cycle was specifically observed in the tiller bud and *Histone H4* expression level was not significantly changed in the SAM. The expression pattern of *OSH1*, a marker of the meristematic cells, was also analyzed. Although *OSH1* signal was detected in the dormant buds at 3.5L stage, the signal was weaker compared to that in active buds and gradually disappeared. This indicated that the dormant bud probably remains the meristematic identity for a period. In conclusion, the second bud of WT at 3.5L stage is dormant in my experiment condition which is dependent on the endogenous SL.

Transcriptome analysis of the phase shift to dormant state in tiller buds

In order to find out the factors that trigger the dormancy in tiller buds, microarray analysis was carried

out. Changes of gene expression profiles were compared between active and dormant buds of WT plants sampled at 9 day (3.0L) and 11 day (3.5L), respectively. The buds were also sampled at 10 day and analyzed as transition stage buds. Buds were sampled from *d10-2* plants in which buds are maintained at the active state. To analyze gene expression profiles specifically related to bud dormancy, tiller buds including the axillary meristem and youngest two leaves were sampled using laser capture microdissection technique and used for RNA isolation. The overall gene expression levels were higher in dormant buds compared to active buds. In total, 1,718 genes were up-regulated (fold change more than 2) while 829 genes were down-regulated (fold change more than 2) in dormant buds. This implied that an active mechanism probably operates to cause the phase transition from active to dormant state in the tiller buds. An enrichment of ribosomal protein genes was a prominent feature in down-regulated genes. All four homologs of dormancy-associated genes were up-regulated in dormant buds, supporting the relevance of our experimental system to analyze bud dormancy. Expression levels of cell cycle related genes were also changed. Expression of *EL2* and *EL2-like*, putative plant specific cell cycle inhibitors, were increased in dormant buds. It was well known that Abscisic Acid (ABA) is involved in seed dormancy and seasonal bud dormancy of perennial trees. In my microarray data, extensive increase of ABA inducible genes was observed. Moreover, genes involved in ABA biosynthesis and catalysis were also increased. In the contrast to the extensive changes of gene expression levels in WT bud, much less changes were observed in *d10-2*. All these results indicate that ABA may be involved in the control of bud dormancy and the changes are dependent on SL.

Genetic analysis of *SPL14* and SL

SPL14, a member of the *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* genes, is known to suppress outgrowth of tiller buds and to regulate panicle development in rice. The mechanism of *SPL14* on tiller outgrowth is unclear. First, the function site of *SPL14* was examined. I showed that *SPL14* mRNA accumulated in leaf primordia during the vegetative phase and in the primordia of bracts, or modified leaves, in the panicles, but not in the meristems. Next, genetic interaction between *SPL14* and SL was analyzed. *SPL14* is a target of *miR156* and accumulation of *SPL14* transcripts is negatively regulated by *miR156*. The enhancement of the expression level of *SPL14* by the introduction of the *mSPL14* gene, in which the *miR156* cleavage site is mutated, resulted in a decrease in tiller number in

both WT and in *d10-2*, a SL-deficient mutant. Expression of *miR156* by a constitutive CaMV 35S promoter dramatically increased tiller number. The enhanced tiller growth was suppressed by an application of SL. There analysis suggested that *SPL14* and SL function in parallel pathways to suppress tiller growth. SL exuded from roots trigger germination of root parasitic plants which causes severe damage to crop productivity. SL-deficient mutants, however, exhibit an excess branching phenotype which is usually undesirable for productivity. Introduction of the *mSPL14* gene in the *d10-2* mutant suppressed over-branching defects in *d10-2* in an expression dependent manner. This indicated that *SPL14* can be used to manipulate the branching patterns of SL-deficient mutants. We also confirmed that this strategy is applicable to Arabidopsis. A greater understanding of the *SPL14* and SL pathways and their interactions may help in the production of root parasite-resistant crops.

In conclusion, the analysis of rice tiller bud development discovered the time when the tiller bud stops growing and becomes dormant. The transcriptome analysis of tiller bud at active and dormant phase revealed the basic information of buds and suggested that cell cycle genes and ABA were involved in the control of bud dormancy. The investigation of *SPL14* and SL pathway showed that the regulation of plastochron is important for *SPL14* on controlling tiller outgrowth which is independent of SL.