学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Molecular biological studies on *Arabidopsis* DEAD-box RNA helicase 7
(シロイヌナズナ DEAD-box RNA helicase 7 の機能に関する分子生物学的研究)

DEAD-box proteins comprise the largest family of RNA helicases and participate in a variety of RNA-associated events from transcription to RNA decay. To date, a number of DEAD-box proteins from *Arabidopsis* have been functionally characterized. They are involved in plant growth, development and stress response through specific RNA processing events. However, the function of many members remains uninvestigated. In this study, I isolated AtRH7 as a common interactor of AtCSP2 and AtCSP3, and analyzed the physiological and molecular role of *AtRH7* in plant morphogenesis under both standard and cold conditions. In addition, the functions of *AtRH7* in abiotic stress response were also elucidated.

1. AtCSP2 negatively regulates plant salt tolerance

Plant cold shock domain (CSD) proteins are RNA chaperones that destabilize RNA secondary structures. In the previous studies, one of the *Arabidopsis* CSD proteins, AtCSP2, was considered to be a negative regulator of cold acclimation. In this study, I examined whether AtCSP2 regulates salt stress tolerance, and screened its interactors through a yeast two-hybrid screening. The results indicated that, in addition to cold, AtCSP2 is negative regulator of plant salt tolerance; interactome analysis revealed that it might participate multiple RNA metabolisms. Among the interactors of AtCSP2, AtRH7, which also can interact with AtCSP3, was chosen for further studies.

2. AtRH7 regulates plant growth and development under low temperature.

In this study, we performed biochemical and genetic analyses of AtRH7. The recombinant AtRH7 showed ATPase activity that was stimulated by addition of RNA, indicating AtRH7 is a functional RNA helicase. AtRH7 is a homolog of CsdA from *E.coli*,. Heterologous expression of *AtRH7* complemented the cold-sensitive phenotype of *csdA* mutant. Knockout mutant lines displayed several morphological alterations that resemble ribosome-related mutants of *Arabidopsis*. In addition, some aberrant floral developments were also observed in *rh7* mutants. When the mutants were germinated at low temperature (12°C), the radicle emergence and first leaf development were severely delayed. After exposing seedlings to a long period of cold, the mutants developed aberrant, fewer, and smaller leaves. RNA blots and circular RT-PCR revealed that 35S and 18S rRNA precursors were accumulated to higher levels in the mutants than in WT under both normal and cold conditions, suggesting the mutants

are partially impaired in pre-rRNA processing. Taken together, the results suggest that AtRH7 affects rRNA biogenesis and plays an important role in plant growth under cold.

3. AtRH7 is involved in plant abiotic stress tolerance

As AtCSP2 regulates stress tolerance, AtRH7 may also be involved in response to abiotic stress. Interestingly, the knockout mutants of *AtRH7* exhibited higher freezing tolerance than WT when cold acclimated. The increased freezing tolerance was independent from CBF pathway, but was associated with higher soluble sugar levels during cold acclimation. Besides freezing, the *rh7* mutants also displayed enhanced tolerance against salt and drought. Transcriptome analysis revealed that many stress responsive genes were up regulated in *rh7* mutant. Together, our data demonstrated AtRH7 is a negative regulator of multiple abiotic stresses in *Arabidopsis*.

Conclusion: AtCSP2 negatively regulates salt tolerance and physically interacts with AtRH7. AtRH7 is involved in rRNA processing and affect growth and development under both normal and cold conditions. AtRH7 negatively regulate freezing, salt and drought tolerance.