ESP1/eRF1 involves in the translation termination of specific cysteine-poor prolamines in rice endosperm

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Title：ESP1/eRF1 involves in the translation termination of specific cysteine-poor
　　　prolamines in rice endosperm
　　　(ESP1/eRF1 はイネ胚乳における特定プロラミン分子の翻訳終結に関与する)
Category：Kou

Thesis Summary

Background and objectives

　　　Cereal seeds store the storage proteins for the nitrogen source for the germination. Prolamines, kinds of storage proteins, is soluble in alcohol solution and are classified into cysteine-poor (CysP) and cysteine-rich (CysR) forms. The factors influencing the gene expression, the synthesis and the accumulation of the prolamines are reported. In rice, the causative gene of the *endosperm storage protein 1* (*esp1*) mutation, reducing the amount of CysP prolamines, is encodes a eukaryotic peptide chain release factor 1 (eRF1), which participates in the recognition of the mRNA stop codon and termination in the translation process. The genomic DNA sequences of *eRF1* gene in the *esp1* lines, CM21 and EM711, indicated the nucleotide substitution with amino acid substitution. Therefor it named as ESP1/eRF1. Since the sequence of the stop codon of mRNA corresponding to the CysP prolamine reducing in *esp1* mutant is UAA, it was hypothesized that the ESP1/eRF1 recognized specially the UAA stop codon. This research aims to clarify the function of ESP1/eRF1 for the specific stop-codon recognition.

Results

The expression of ESP1/eRF1

　　　The expression analysis of *ESP1/eRF1* gene in different tissues, including leaves and developing seeds using RT-qPCR analysis, demonstrated that *ESP1/eRF1* was abundantly expressed in both of the wild type and *esp1* mutant leaves. The microarray analysis of the developing endosperm showed that lower level of transcript of *ESP1/eRF1* was observed in the wild types at 7, 14, and 21 DAFs (day after flowering) compare to *esp1* mutants. The immunoblot analysis showed that the amount of ESP1/eRF1 protein in mature seed relatively reduced in *esp1* lines, compared to wild types. These results indicate that the *esp1* mutation used in materials doesn't influence to the transcript and protein levels of ESP1/eRF1.

Genetic analysis of the ESP1/eRF1 stop codon recognition

　　　In order to provide the function of the ESP1/eRF1 for the specific stop codon recognition, *esp1* mutant and the wild type were transformed with the *luciferase* (*LUC*) gene constructs modified with UAA, UAG, and UGA as stop codon. Their expression level was analyzed by the RT-qPCR. In the leaf and seeds of *esp1* mutant transformants, the relative expression of *LUC* genes with stop codons UAA and UAG decreased significantly compared with the levels of wild-type transformants. These results suggested that ESP1/eRF1 recognizes specifically UAA and UGA stop codons.

Gene expression profiling in *esp1 mutation*

In order to elucidate the function of ESP1/eRF1 in the translation termination of the proteins besides CysP prolamines, the gene expression profiling in *esp1* mutation was investigated by the DNA microarray analysis. The significant difference among the stop codon usage was compared with those in the whole rice genome by chi-squared test. The results suggested ESP1/eRF1 contributes in the translation termination of proteins besides CysP prolamines.

Conclusion

It is reported that eRF1 in the eukaryotes recognizes all three stop codons, whereas RF1 and RF2 in prokaryotes recognize UAA/UAG and UAA/UGA, respectively. The results of recognition and microarray assays indicate that ESP1/eRF1 specifically recognizes UAA and/or UAG as stop codons, but not UGA. In conclusion, these findings regarding ESP1/eRF1 contribute to advancing the current understanding of prolamine translation mechanisms in rice.