学位論文要旨

Studies on longevity in rice seeds focusing on the stability of embryonic RNAs
胚の RNA の安定性に視点を置いたイネの種子寿命に関する研究

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Seed longevity is defined as the ability of a seed to remain viable during storage. Over time, seed viability gradually decreases because of aging or deterioration events, which eventually result in poor seedling establishment. Seeds with long longevity are intact and produce vigorous seedlings, which ultimately ensures a high yield. Therefore, the seed longevity is an important trait not only for the seed industry but also for seed storage and conservation of genetic resources. This agronomic trait can be improved by breeding methods, because the seed longevity is partially affected by genetic factors. In order to create the excellent cultivars with long seed longevity by breeding, it is important (i) to understand the mechanism keeping seed germinability for long term; (ii) to find out the useful genetic resource(s) as prospective breeding materials; and (iii) to identify the gene(s) involved in the seed longevity.

In various crops, it has been revealed that the RNA stability in the embryos of the dry seeds is related to seed longevity. However, the mechanism of decline in germinability mediated by degradation of embryonic RNAs remains unidentified. The molecular mechanisms of various phenomena in plant cells have been elucidated using model plants. In rice, it has been reported that mature dry seeds contain a great number of stored mRNAs (long-lived mRNAs) that accumulate during seed maturation and protein synthesis during the initial phase of germination occurs from these stored mRNAs. In addition, the RNA-binding proteins, which are known to govern many aspects of RNA metabolism including stability and decay of RNAs were detected by applying proteomic methods in rice seeds. Therefore, rice seeds are useful material for elucidation of the mechanism of decline in germinability mediated by degradation of embryonic RNAs. Moreover, the core collection, which is useful to obtain the excellent rice cultivar(s)

having long seed longevity as potential breeding materials, and the chromosome segment substitution lines (CSSLs), which are a powerful genetic resource for surveying the genetic potential(s) of donor germplasm, have been established in rice. By using rice as an experimental material, the seed longevity was analyzed in this study. The results of the studies are as follows.

(i) To clarify the relationship between seed longevity and RNA integrity in embryos, germinability and stability of embryonic RNAs were analyzed using the seeds of *japonica* rice cultivars subjected to controlled deterioration treatment (CDT) at high temperature and high relative humidity or long periods of storage. Degradation of RNA from embryos of a *japonica* rice cultivar "Nipponbare" was induced by CDT before the decline of the germination rate and a positive relationship between seed germinability and integrity of embryonic RNAs was observed. Moreover, this relationship was confirmed in the experiments using old seeds from the "Nipponbare", "Sasanishiki" and "Koshihikari" rice cultivars. In addition, the RNA integrity number (RIN), calculated using electrophoresis data, had a positive correlation with germinability ($R^2 = 0.75$). From these results, it was concluded that the stability of embryonic RNAs required for germination is involved in maintaining seed longevity over time and RIN values can serve as a standard index to evaluate germinability in rice.

(ii) To find out the useful genetic resource(s) as prospective breeding materials, the seed longevity of the cultivars in the NIAS world Rice Core collections (RDRS) was analyzed. Among this collection, the seeds of three cultivars, such as "Vandaran (*indica*)", "Tupa 729 (*japonica*)" and "Badari Dhan (*indica*)", were constantly having long longevity under CDT and long periods of storage in comparison with other varieties. Therefore, it was concluded that these cultivars are useful for improvement in seed longevity as potential breeding materials. In addition, the positive correlation between the seed longevity and the integrity of embryonic RNAs was confirmed in this experiment using the seeds of RDRS.

(iii) Because it has been shown that the seed longevity of "Habataki" is higher than that of "Sasanishiki", the analyses were carried out using chromosome segment substitution lines (CSSLs) derived from "Sasanishiki" and "Habataki" as background and donor, respectively. Among all the 39 CSSLs, the line "SHSL06 " exhibited the highest germination rate (%) and the high stability in embryonic RNAs under CDT, indicating that this line might have useful gene(s) from "Habataki". Based on the data of genotyping of CSSLs and progenies from "SHSL06" and "Sasanishiki", the gene(s) responsible for the seed longevity of "Habataki" is located on chromosome 2 at the position between 32.8 – 42.1 cM regions. Therefore, it has been suggested that the gene product(s) on chromosome 2 in "SHSL06" is/are involved in keeping the integrity of embryonic RNAs under ageing condition.

Overall, this study provides useful information for breeding of the cultivars with long seed longevity in rice.