学 位 論 文 要 旨

Physiological and genetic study on mechanisms of growth promotion and salt tolerance improvement in rice seedlings by inoculation with biofertilizer microorganism *Bacillus pumilus* TUAT1 strain

バイオ肥料微生物 *Bacillus pumilus* TUAT1 株を接種したイネ実生における生育促進と耐塩性向上機構に関する生理学的および遺伝学的研究

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Rice is most broadly expended staple food everywhere throughout the world, particularly in Asia. It is very sensitive to salt stress at seedling stage. Yield is drastically reduced under salt stress. Mitigation of salt stress at the seedling stage could minimize the yield loss at later stages as the rice plant is reported to be the most susceptible to salinity at vegetative period than the reproductive period.

Plant Growth Promoting Rhizobacteria (PGPR) have been indicated as efficient overcome the salt stress at seedling stage. PGPR used in this study was *Bacillus pumilus* TUAT1 strain (hereinafter referred to as “TUAT1” which used as an ingredient of commercialized biofertilizer “Kikuichi”. Previous reports in rice and several other plants had suggested that applying “Kikuichi” or TUAT1 spores at the nursery stage enhances the growth of seedlings as well as the yield. However, the mechanism by which inoculation with TUAT1 spores promotes the growth of rice seedlings has not been fully elucidated. In addition, it was not clarified whether inoculation with TUAT1 spores would increase the salt tolerance of rice seedlings. In this study, experiments were conducted to elucidate the mechanism by which TUAT1 spores inoculation promotes growth of rice seedlings, to confirm salt tolerance of the seedlings is increased, and to identify genes involved in varietal differences in the increasement of salt tolerance.

In Chapter 2, study was designed to track the colonization of TUAT1as well as determination the effects of inoculation with spores and vegetative cells of TUAT1on the seedlings of rice cultivar “Hitomebore”. Moreover, elucidation the possible involvement of nitric oxide (NO) production and expression of root development-related genes in growth-promoting effect of TUAT1 on rice seedlings. The results showed that inoculation of TUAT1 spores at a density of $1x10^7$ colony-forming units (CFU) per mL yielded a significant increase in all traits compared to the control. In contrast, inoculation with vegetative cells did not yield significant effects in several traits, including the numbers of crown roots and lateral roots. CFUs of TUAT1 increased with time and maintained to a higher number in the root zone than at the stem base of rice seedlings. TUAT1 inoculation with spores led to increased accumulation of *CRL5* transcripts and decreased accumulation of *WOX11* transcripts in the basal stem, where crown root initiation occurs, as well as involved to promoting the NO production. These results provide partial clarification of the mechanism of TUAT1’s growth promotion in rice.

In Chapter 3, hypothesis that inoculation of TUAT1 spores promotes the early growth of rice seedlings under salt stress condition by increasing salt tolerance was tested. “Hitomebore” was used to clarify the effect of TUAT1 inoculation on the growth of seedlings under different salt treatments from different growth stages. Four cultivars, “Kasalath”, “Nipponbare”, “Hitomebore”, and “Koshihikari”, were used to comprehensively investigate the differences in rice seedlings responsiveness to TUAT1 within and between cultivars under salt stress. The results clearly indicate the growth of seedlings significantly decreased with the increase of salinity levels. It was also confirmed that the two cultivars “Kasalath” and “Nipponbare” had low inoculation effect on increasing salt tolerance but other cultivars had it. In addition, the inoculation effect was also different depending on the concentration and exposure time of salt stress.

Chapter 4 was based on the hypothesis that the genetic variation is involved in varietal difference in the promoting effect of TUAT1 inoculation on salt tolerance, the seedlings of 70 rice cultivars were inoculated with TUAT1 under salt treatment. The growth and salt tolerance of rice seedlings showed a wide range of varietal differences in both inoculation and non-inoculation of TUAT1. Therefore, using these varietal differences, it was attempted to identify genes related to varietal differences in the inoculation effect of TUAT1 strain by GWAS. Although there was no significant SNPs obtained, the results obtained by GWAS suggested that genes containing the selected candidate SNPs play an important role in the enhancement of salinity tolerance by TUAT1 inoculation.