

学 位 論 文 の 要 約

三 重 大 学

所 属	三重大学大学院生物資源学研究科 資源循環学専攻	氏 名	MARUBODEE RUSAMA (マルボディー・ルサマ)
<p>学位論文の題名 : Detection of Azuki Bean Wild Relatives Having Different Salt Tolerance Mechanism and Construction of Molecular Linkage Map of Tuber Cowpea</p> <p>学位論文の要約 In this study, it is aim to contribute agricultural production where saline soil problem spreading by distribute salt tolerant crop as well as consider in proper salt tolerant evaluation in cowpea and new varieties. It causes to construct the fine molecular linkage map for towards development. From many reported of tolerance to abiotic stress of <i>V. vexillata</i>, we try to examine <i>Vigna</i> crops on more humidity condition in the first of the research. Three kind of different species <i>Vigna</i> crop: cowpea, mungbean and azuki bean was examined the optimal condition of hydroponic culture. The plant growth was investigated from the effect of aeration and non-aeration conditions. As a results, the better growth characteristics of cowpea and mungbean were observed in non-aeration condition, but not significant different from aeration condition. In contrast with the legumes crops such as soybean that requires the aeration condition in general. From the results indicated that the root system of cowpea was high environmental adaptability in abiotic stress.</p> <p>Next, 74 accessions of azuki bean and 145 accessions of cross-compatible 7 wild relatives' species were screened in order to examine the salt tolerance mechanism of <i>Vigna</i> genus. The primary screening was carried out in soil culture condition and the secondary screening was carried out in hydroponic culture condition, As a result of Hinaazuki ('Tojinbaka') of <i>V. riukiensis</i> and Himetsuruazuki ('Ukushima') of <i>V. nakashimae</i> were identified as the valuable source of salt tolerance of azuki bean. These two strains had different salt tolerance mechanism, where 'Ukushima' prevented Na⁺ accumulation in leaves, while 'Tojinbaka' accumulated Na⁺ throughout the plant. In addition, 'Ukushima' and especially 'Tojinbaka' showed even better growth in a salt-damaged field in Fukushima, Japan where soybean cultivar 'Tachinagaha' could not survive. Our results will facilitate developing salt tolerant azuki bean cultivar by introducing two different mechanisms of salt tolerance.</p> <p>Subsequently, Akasasage (<i>V. vexillata</i>) was selected for studying genetics performance of salt tolerance. An F₂ population consist of 300 individuals was developed between salt tolerant 'V1' and sensitive 'V5' accessions to evaluate salt tolerance screening by trial test conduction; (1) wilting score of shoot ('Shoot'): plant developed from the top part cut roots off of F₂ population under 150 mM NaCl hydroponic pool, (2) wilting score of leaf ('Leaf'): plant developed from a single leaf without stem and root of F₂ population under 200 mM NaCl hydroponic pool, and (3) wilting score of seedling ('Seedling') of F₃ population in soil submerged with 250 mM NaCl solution. Leaf wilting score of 5 ranks ranged from 1 (no damage) to 9 (complete wilt) were visually determined for segregating F₂ individuals or F_{2,3} lines and the average values were used as phenotypic data.</p> <p>As a next step, 1,336 SSR (Simple sequence repeat) markers developed from azuki, cowpea and common bean were screened in order to carry out salt tolerance gene analysis from the hybrid population. Relatively good transferability was observed where 874 SSR markers (65.4% on average) could amplify DNA fragments. However, only 84 SSR markers (6.2%) showed polymorphism between parents, suggesting very close genetic relationship. A total of 82 polymorphic SSR loci could be assigned to 11 LGs covering a total length of 510.5 cM at an average marker distance of 7.2 cM (1.4 – 15.7 cM). To develop higher density molecular linkage map, RAD (Restriction site associated DNA) markers were developed and a combined SSR and RAD markers linkage map was constructed. A total of 559 (84 SSR and 475 RAD) markers loci could be assigned to 11 linkage groups spanning 973.9 cM in length with a mean distance of 1.8 cM (1.4 – 2.4 cM). Then a quantitative trait locus (QTL) of salt tolerance was analyzed on the first SSR linkage map and the first SSR and RAD marker-based <i>V. vexillata</i> linkage map with a phenotypic screening data. Two major QTLs were detected on the same LG2 and LG9 from both of two maps with the high phenotypic variation explained (PVE) value was 35.54% on LG2 and 14.42% on LG9 on The SSR linkage map and 37.24% on LG2 and 11.99% on LG9 on the SSR-RAD linkage map, but other one QTL on LG11 was only detected from the SSR-RAD linkage map with PVE value was 12.95%. We could narrow down the QTL position from the neighboring markers from the SSR linkage map to the SSR-RAD linkage map.</p>			