

Summary of Doctoral Thesis

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Title: **Molecular diversity analysis of Ethiopian rice genetic resources**

(エチオピアイネ遺伝資源における遺伝的多様性解析に関わる研究)

Rice is an important staple crop with its origins in South and Southeast Asia (*Oryza sativa*), and West Africa (*Oryza glaberrima*) where it has a large gene pool consisting of wild relatives and landraces in addition to improved varieties. In Ethiopia, the crop is also considered as one of the most important food crops primarily due to its various uses; as food, beverage, livestock feed, source of income and employment. Despite a rapid expansion of cultivation, domestic production has not yet met the growing demand for rice. In order to accelerate rice breeding program, understanding of Ethiopian rice genetic resources are required. Genetic resources are quite important to improve cultivars for higher yield and diseases resistance. Since the first introduction, several rice germplasms have been introduced into Ethiopia. However, details of these genetic resources including native wild rice are not well studied to make use of them in breeding. In view of this, the current study focused on genetic analysis of cultivars and wild rice collected in Ethiopia. This study comprised four experiments: (1) analysis of genetic diversity among rice cultivars based on molecular markers and morpho-physiological characteristics, (2) assessment of phenotypic variation for blast resistance among cultivars using differential system, (3) study on genetic variation for agronomic traits of cultivars between two different sites in Ethiopia,

and (4) genetic diversity, maternal lineage and population structure analysis of wild rice from Ethiopia using chloroplast and nuclear DNA markers.

In the first experiment (Chapter 2), 79 rice accessions composed from 60 Ethiopian cultivars and 19 control accessions (12 Japonica and 7 Indica types) were analyzed using fifty SSR and four INDEL markers. They were also evaluated for phenol reaction, grain size, starch digestibility by alkaline solution, and apparent amylose content. A total of 351 alleles with a mean of 7.02 alleles per locus, ranging from 2 to 13 alleles per locus were observed. Genetic diversity ranged from 0.23 to 0.88, with mean of 0.65. Improved and landrace populations separately showed genetic diversity of 0.55 and 0.48, respectively. Accessions were classified into two major clusters corresponding to Japonica/Japonica-like varieties including NERICAs and Indica/Indica-like varieties. Phenol reaction and grain size were also corresponded to the classification. Chloroplast and nuclear INDEL markers identified true Japonica and Indica with their recombinant types. In alkali digestibility and apparent amylose content characterization, most Ethiopian accessions showed intermediate value for both characters.

The second experiment (Chapter 3) focused on blast inoculation experiment and a total of 92 accessions comprising 60 Ethiopian rice cultivars, 28 differential varieties and four control accessions were evaluated using 20 blast isolates. Results showed that accessions were grouped into two major clusters corresponding to resistant and susceptible groups. Except for few landraces, the majority of Ethiopian cultivars including NERICAs belonged to resistant group. But in terms of blast resistance frequency, about 78% of Ethiopian cultivars showed intermediate blast resistance frequency while 17% of them showed high blast resistance frequency and the rest 5% which included X-Jigna were with

low resistance frequency. Moreover, gene postulation in Ethiopian cultivars compared to differential varieties indicated the involvement of several resistance genes in high frequency including *Pit*, *Pik-p*, *Pish*, *Pib*, *Pik-s*, *Pik-m*, *Pi7(t)*, *Piz-t*, *Pi9(t)*, *Pi12(t)*, *Pi19(t)*, and *Pi20(t)*. More than 50% of Ethiopian rice cultivars have been postulated to have a combination of more than one gene for blast resistance.

In the third experiment (Chapter 4), a total of 60 Ethiopian rice cultivars were compared for agronomic traits between different environmental conditions at two sites, Fogera and Pawe, in Ethiopia. Highly significant differences were observed among cultivars for 90% of the traits. Most of the traits such as days to heading, days to maturity, panicle length, grain yield, thousand seed weight, biomass yield and harvest index showed high broad sense heritability at both sites and they also showed significant correlation coefficients with grain yield. Principal component analysis of the first four significant components contributed more than 81%, 74%, and 80% of total variation at Fogera, Pawe and combined data, respectively with highly contributing traits including days to heading, days to maturity, plant height, panicle length, fertility rate, grains per panicle, thousand seed weight, biomass yield, harvest index, and grain yield. Hierarchical cluster analysis classified cultivars into four clusters (I, II, III and IV). Cluster I (22) and II (20) comprised the largest number of cultivars. About 77% of cultivars in Cluster I consist of improved varieties including NERICAs. Cultivars in Cluster I were relatively early in days to heading and days to maturity while those in Cluster II were intermediate in days to heading and days to maturity with higher mean values for grain yield, and biomass yield. Clusters III and IV were dominated by landraces with few improved varieties and they showed late in days to heading and maturity. This information combined with results of

molecular analysis and blast inoculation of cultivars can accelerate our efforts of identifying potential plant materials for rice crossbreeding in Ethiopia.

The fourth experiment (Chapter 5) assessed genetic diversity, maternal lineage, and population structure of 163 wild rice accessions from Ethiopian compared to 52 control accessions comprising *O. barthii* (20), *O. longistaminata* (19) and *O. glaberrima* (13). Eight chloroplast INDELs (cpINDELs) in addition to 16 SSR markers were newly developed based on publicly available whole chloroplast genome data of *O. barthii* and *O. longistaminata*. Twenty plastid type combinations were detected. Four out of them, Types 1, 2, 3 and 6, were found among five populations in Ethiopia. Type 6 was specific to the north group (Amhara) and it was shared with control *O. longistaminata* population but three were unique to Ethiopia. Type 2 and 3 were unique to south group (Gambella). Type 1 was shared between north and south groups. Using 16 SSRs, total number of alleles amplified per locus ranged from 4 to 14 with mean value of 9.69, with 155 alleles in total. From five populations in Ethiopia, Fogera population showed the highest *He* (0.67), followed by Dera population (*He* = 0.62) while, Kera, Lare, and Abobo populations showed *He* of 0.57, 0.56, and 0.55, respectively. In fact, *He* of control *O. longistaminata* population (0.70) was the highest of all eight populations attributed to its diverse origin in Africa. Neighbor-joining method phylogenetic tree analysis classified accessions into five cluster groups, out of which Ethiopian wild rice accessions corresponded to only three clusters, III, IV and V with some admixtures. Population structure analysis at K=2 revealed that all populations from Ethiopia were clustered with control *O. longistaminata* while *O. bathii* and *O. glaberrima* belonged to another group. Structure analysis at K=5 showed that five natural populations of Ethiopia were classified into three subpopulations corresponding to phylogenetic tree

analysis. Phylogenetic tree analysis and structure analysis at $K=5$ suggested the presence of three groups of *O. longistaminata* natural populations in Ethiopia. These genetic resources would be valuable resources for future breeding program to supply disease resistance or abiotic stress tolerance genes.

In conclusion, this study provides valuable information to better understand the genetic characteristics of rice genetic resources from Ethiopia that can be used to improve elite rice cultivars but susceptible to different stresses.