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学位論文題目	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かかわる研究）
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論文の内容の要旨

Currently, rice is regarded as one of the most important food crops in Ethiopia primarily due to its multitude of uses. However, domestic production has not yet met the growing demand for rice and hence filled by huge annual import. In response, efforts are underway to curb the situation, one of which is improving productivity through use of rice genetic resources in breeding. Since the first introduction, several rice germplasms have been introduced into Ethiopia. Details of these genetic resources including native wild rice are not well studied to make use of them to address currently pressing challenges such as low productivity and blast outbreak. In view of this, four research experiments including two molecular based experiments, one green house and another one field experiment were carried out from 2017 to 2019. The objectives of the study were to investigate genetic diversity and characterize Ethiopian rice cultivars based on molecular markers and morpho-physiological characters, assess phenotypic variation for blast resistance in Ethiopian rice cultivars using differential system, study genetic variation in Ethiopian rice cultivars based agronomic traits and elucidate maternal lineage, genetic diversity and population structure of wild rice from Ethiopia using chloroplast and nuclear DNA markers.

The first experiment evaluated 79 rice accessions using fifty SSR and four INDEL markers. They were also evaluated for phenol reaction. Sixty Ethiopian accessions out of 79 were characterized for alkali digestibility 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 as expected heterozygosity 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 which indicated relatively their high genetic diversity. Accessions were classified into two major clusters corresponding to Japonica and Indica.

Phenol reaction also classified accessions into Japonica and Indica groups. However, examination using nuclear INDEL markers revealed that most accessions are recombinant types with only few identified as Japonica and Indica. Population structure analysis also classified accessions into three subgroups corresponding Japonica, Indica and recombinant groups. In alkali digestibility and apparent amylose content characterization, most Ethiopian accessions showed intermediate value for both characters while some others showed high or low estimated values. In this experiment, relatively high genetic diversity coupled with clustering information of accessions is pertinent for selection of counter parts in crossbreeding efforts.

Blast inoculation experiment using 92 accessions comprising 60 Ethiopian rice accessions, 28 differential varieties and four control accessions against 20 blast isolates in the second experiment revealed that nearly 65% of total accessions showed resistance reaction to blast isolates. The result also showed that most Ethiopian accessions were resistant to blast isolates of Japan but less resistant to other blast isolates. About 78% of Ethiopian accessions intermediate blast resistance frequency (50-85%) while 17% of them showed high blast resistance frequency (86-100%) and the rest 5% which included X-Jigna showed low resistance frequency (0-49%). Including upland NERICAs popular improved varieties and some landraces were highly resistant to blast isolates used. Moreover, resistance gene postulation in Ethiopian accessions 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 accessions were postulated to have a combination of more than one gene for blast resistance in their genetic background. Thus, accessions with better blast resistance can be used to improve elite rice cultivars with good agronomic characteristics.

The third experiment evaluated a total of 60 Ethiopian rice cultivars for agronomic traits variation under lowland rain fed condition at two sites in Ethiopia. Highly significant differences were observed among accessions for 90% of the traits at both Fogera and Pawe, and on combined data as well. 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 the important traits 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 accessions. About 77% of accessions in Cluster I consist of improved varieties including NERICAs. Accessions in Cluster I were relatively early in days to heading and days to maturity with intermediate mean values for plant height, panicle length, grain yield, and biomass yield while accessions 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 accessions and they showed late in days to heading and maturity. This information combined with molecular analysis and blast inoculation result of cultivars can accelerate our efforts of identifying potential plant materials for rice crossbreeding in Ethiopia.

The fourth experiment focused assessing maternal lineage, genetic diversity and population structure in 163 wild rice accessions from Ethiopian and 52 control accessions representing *O. barthii* (20), *O. longistaminata* (19) and *O. glaberrima* (13) using eight chloroplast INDELs (cpINDELs) and sixteen SSR markers. Twenty plastid type combinations were detected out of which four plastid types, Types 1, 2, 3 and 6, were found among five populations from 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. While 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. Mean observed (H_o) and expected (H_e) heterozygosity was 0.24 and 0.73, respectively. From five populations in Ethiopia, Fogera population showed the highest H_e (0.67), followed by Dera population ($H_e = 0.62$) while, Kera, Lare, and Abobo populations showed H_e of 0.57, 0.56, and 0.55, respectively indicating all five populations exhibited relatively high genetic diversity. In fact, H_e of control *O. longistaminata* population (0.70) was the highest of all eight populations which could be attributed to its diverse origin in Africa. NJ 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. barthii* and *O. glaberrima* belonged to another group which confirmed wild rice type that predominated five populations in Ethiopia. However, further structure analysis at $K=5$ showed that five natural populations were classified into three subpopulations with some admixtures corresponding to phylogenetic tree analysis. Results of phylogenetic tree analysis and model based clustering at $K=5$ suggested the presence of three groups of *O. longistaminata* natural populations in Ethiopia. Thus, this valuable resources need to be conserved to maintain its high genetic diversity for future use in breeding. In conclusion, all experiments in this study provide 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.

エチオピアにおいて、イネは多様な用途があるため重要な食料作物としてみなされている。しかしながら、国内の生産は成長する需要に追いついておらず、多大な輸入日本晴より満たされている。それに対して、その状況を打開するために努力がなされている。その1つがイネ遺伝資源の育種的利用による生産性の改良である。最初の導入以来、いくつかの複数の導入が行われてきた。これらの遺伝資源の解析はなされておらず、また野生イネについても解析されていない。

この博士論文においては 2017-2019 年において 4 つの研究, 1) 遺伝的多様性を調査しエチオピアのイネ栽培品種を分子的なマーカーや形態-生理的形質で多様性を評価すること, 2) いもち病の抵抗性についての表現型変異を明らかにすること, 3) 農業形質における多様性を理解すること, 4) 野生イネの集団構造を明らかにすることを行った.

1 つめの遺伝的多様性解析では 79 品種を 50SSR 座と 4 つの INDEL により解析した. また, 籾のフェノール反応, 種子サイズ, デンプンのアルカリ崩壊性, アミロース含量についても調査を行った. SSR 解析では 1 座あたりに 2-13 アレルを見出して, 全 351 アレル, 平均 7.02 アレルを見出した. これらの品種は, 日本型/日本型的改良品種 (NERICA を含む) とインド型/インド型的改良品種に大きく分けられた. フェノール反応と種子サイズもこの分類を支持した. インド型と日本型を識別する INDEL マーカーは, それぞれの品種群とともに組換え型を識別することができた.

2 つめのいもち病の抵抗性解析では, 20 レースを用いたいもちレース接種ではエチオピアの 60 品種とともに 28 の異なる耐性遺伝子を個別に有する識別品種とともに対照 4 系統を利用した. この結果は, 多くのエチオピアのイネ系統が抵抗性であることを示した. 耐冷性を示す在来種, X-Jigna を含む 5% が罹病性を示した. 識別系統の反応を利用して, 高い抵抗性系統は *Pit*, *Pik-p*, *Pish*, *Pib*, *Pik-s*, *Pik-m*, *Pi7* (t), *Piz-t*, *Pi9* (t), *Pi12* (t), *Pi19* (t), and *Pi20* (t) 抵抗性遺伝子を有することが明らかとなった.

3 つめの実験では, 60 品種を供試してエチオピアにおける対照的な 2 つの異なる地域, Fogera と Pawa で栽培形質の比較が行われた. 90%以上の形質において地域間における異なる反応が認められた. それらの形質は, 出穂日, 穂長, 収量, 100 粒重, バイオマス, そして収穫指数は高い広義の遺伝性を示した階級的クラスター解析においては, これらの品種は 4 つに分類された. I ならびに II はそれぞれ 22 ならびに 20 品種と多くの品種を内包した. クラスター I の 77% は, NERICA 品種を含んでいた. 比較的早生であり, 草丈や穂長, 収量, バイオマスでは中間的な数値を示した.

論文審査の結果の要旨

エチオピアにおいて, イネは多様な用途があるため重要な食料作物としてみなされている. しかしながら, 国内の生産は成長する需要に追いついておらず, 多大な輸入日本晴より満たされている. それに対して, その状況を打開するため既に輸入されているイネ遺伝資源の遺伝的評価, 栽培地域で問題が顕在化しつつあるいもち病抵抗性についての育種・遺伝資源の評価, ならびに 2 カ所の稲作地帯における栽培特性の評価を行った. この評価から今後のイネ交雑育種の計画を進めることを目的として実験を行った. DNA マーカーでの遺伝的多様性調査では, イネ遺伝資源は大きく 2 つのクラスター I ならびに II にわけられ, それぞれ日本型並びにインド型に対応することが明らかとなった. 日本型はやや低めのアミロース含量とやや丸型, インド型は高いアミロース含量と細粒形が特徴として認められた. いもち病に対する反応は異なる申請抵抗性遺伝子を 1 つずつ有するディエレンシャル系統と比較することで検証された. 改良品種は複数の抵抗性遺伝子を有しているものの, 農家に好まれる日本型在来種は少数の抵抗性しか有さないことが明らかとなった. そのため, 日本型どうしでの改良品種と多収かつ低温抵抗性がみられる日本型在来種の組み合わせ, エチオピアの温暖地に適する改良型のインド型品種間やインド型在来種の組み合わせが交雑として有望であることが示唆された. 栽培特性が

らは稲作の主産地である Fogera においては降水量との関係から早生型が最も高い収量を示す相関が検出された。さらに日本型の改良品種は早生、同在来種は幅広い出穂特性が見られた。さらに、早生タイプが高いバイオマスを示すことがわかり、穀粒以外のわらの家畜飼料への利用が期待される品種群であることがわかった。以上から、今後の交雑育種における抵抗性とともにも農業形質の育種目標を設定することができた。さらに、将来的な育種資源としての野生イネである *O. longistaminata* が広範囲に生息しているため、その遺伝的多様性の評価を行った。細胞質の多様性は既に報告されている完全長葉緑体ゲノム情報をもとに新たに開発して評価した。その結果、アフリカ全体に散在する同種の分布の外縁部であるエチオピアにはこれまで遺伝資源の集では認められていない特殊なタイプが生息しており、北部と南部で異なることが明らかとなった。このことから今後の収集計画を立てる有益な情報を得たと結論した。よって本審査委員会は、「岩手大学大学院連合農学研究科博士学位論文審査基準」に則り審査した結果、本論文を博士（農学）の学位論文として十分価値のあるものと認めた。

学位論文の基礎となる学術論文

Genetic study of diversity and blast resistance in Ethiopian rice cultivars adapted to different ecosystems. 発行予定の時期及び誌名 **Breed. Science.** Lakew Tadesse, Yoshimichi Fukuta, Ryuji Ishikawa Accept In press.