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学位論文題目 Comparative Genomics and Evolutionary Studies of
Oryza Wild Species Using Expressed Sequences

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論文内容の要旨

The genus *Oryza* comprises 25 species in 10 different genome types (AA, BB, BBCC, CC, CCDD, EE, FF, GG, HHJJ and HHKK). The AA genome species contain two cultivated rice species and others are wild relatives of rice. Wild relatives of rice are important as a genetic resource to resolve the questions about domestication, speciation, polyploidy, evolution and to identify useful traits such as resistance to various stresses and vigorous growth characters those were missing from cultivated rice. *Oryza sativa*, one of the cultivated rice has been analyzed well, and the sequencing of the *O. sativa*, *japonica* and *indica* genomes has been completed. Molecular and genetic analyses of the wild relatives have recently stated to show phylogenetic relationships among them, but not many studies have been carried out to date. In this study, therefore, I examined the differences of gene sequences, and structure among three different genome types and the presence of the wild relatives of rice unique genes to gain an insight of the evolutionary state in the genus *Oryza*. I have examined genome differences for expressed sequences between AA and BB, and between AA and CC by comparison of cDNA sequences.

In chapter I, the divergence between *O. sativa* and *O. punctata* (BB genome) or *O. officinalis* (CC genome) was analyzed. cDNA libraries were constructed using mRNAs isolate from the shoots of *O. punctata* (BB) and the panicles of *O. officinalis* (CC), and 3087 ESTs containing 973 clones of *O. punctata* and 1425 clones of *O. officinalis*, were used for sequence comparison against *O. sativa* (AA), Nipponbare sequences. The results of BLASTN showed that 88-96% cDNA clones of the BB and CC genomes showed high similarity (90-100%) to the *O. sativa* sequence, and 8% of the *O. punctata* clones and 4% of the *O. officinalis* (CC) clones did not hit against the *O. sativa* cDNA and genome sequences. Substitution rate between *japonica* and *O. punctata*, and between *japonica* and *O. officinalis* were 31 and 36 bp/kb in the coding region and 44 and 44 bp/kb in the untranslated region, respectively. They were three or four times higher than those of *japonica* and *indica*. The number of nonsynonymous and synonymous substitutions per site (Ka and Ks) was calculated 0.0018 and 0.022 for Ka and 0.091 and 0.106 for Ks between *japonica* and *O. punctata* or *O. officinalis*, respectively. These results together with in/del comparison indicate that the genetic structure of BB and CC genomes are very close with each other and in/dels are accumulated in the untranslated region.

In chapter II, the phylogenic relationships of the species in the genus *Oryza* were examined using seven nuclear DNA sequences; *O. sativa* full length cDNA (AK058507, AK059353 and AK098919), RAFTIN 1 (AJ575667), MAP kinase 5 (AF479884), fructokinase 1 (AF429948), teosinte branched 1 (*tb1*) and tryptophan synthase (*orp*), and one intron sequence of *Adh1* 3rd intron. The results showed that the phylogenetic relationships among *O. brachyantha* (FF genome), AA genome species (*O. sativa*, *japonica*, *O. rufipogon* and *O. barthii*) and other *Oryza* species was supported by high bootstrap values, while in the *O. officinalis* complex being composed of BB, CC, CCDD, and EE genomes, pylogenetic tree was entangled among genes used for the analysis. Estimation of the divergence time base on a molecular clock approach was performed by assuming maize and rice diverged 50 million years ago [mya]. *O. brachyantha* (FF) vs. remaining *Oryza* species revealed to be at 11.7~21.6 (mya) (average 17.2 mya). The divergence time within the *O. officinalis* complex containing BB, CC, CCDD and EE genomes was estimated later than 9.5 mya (2.1~12.1 mya). AA genome was diverged from *O. officinalis* complex at 2.2~14.9 mya (average 8.8 mya) and speciation within the AA genome was estimated to occur at 0~5.6 mya. These values can explain well the phylogenetic relationships and the estimated divergence time of these species in relation to other evolutionary evidences. Synonymous (Ks) and non-synonymous (Ka) substitution rates showed that

RAFTIN 1 and *tb* showed higher K_a/K_s than other genes in all genome types. These genes might contribute to speciation, or phenotypic diversity.

In chapter III, 13 clones identified as unique to the wild relatives, but not to *japonica* rice, were analyzed. Full length cDNAs were obtained from four genes of BB or CC genome. These genes encoded a ribosomal protein, a cyclin, a cold response protein and an unknown protein. One of the BB genomes clones, the BBS13D01 encodes a cyclin D2/4 like protein and its expression in yeast cells complemented cyclin mutations. This indicates that the BBS13D01 encodes a functional cyclin unique to the wild relatives of rice. Another clone CCP17F01 from CC genome has domains conserved among cold response proteins, and its expression was induced by a cold treatment. This suggests that CCP1701 encodes a cold response protein and is involved in the cold response of the wild relatives. The results suggest that wild relatives of rice have various unique genes which may be related to the characteristic features of the wild relatives in the morphogenesis and stress resistance. Unique genes of the wild relatives were divided into two categories: One was unique sequence present only in the wild relatives of rice, and the other has homologous genes in *japonica*, but its expression might be controlled differently.

My approach to analyze molecular characteristics of the wild relatives genome species in rice revealed new information about nucleotide substitution rate, in/del characteristics, unique genes of the wild relatives, phylogenetic relationships and divergence time of the wild species of rice. *Oryza* is one of the most valuable species in the studies of domestication, speciation, polyploidization and evolutionary analysis, due to the accumulation of fundamental knowledge of genomics and a plenty of genetic resources. Further analysis of the wild relatives of rice should provide us a lot of fundamental and valuable resources to resolve questions for evolution and speciation, and for practical application of the genomes in the genus *Oryza*.

イネは人類にとってもっとも重要な穀物のひとつであり、その生物学的特性は以前から多くの研究がなされてきた。しかし、栽培イネ (*Oryza sativa*; AA型ゲノム) と24種にのぼる同属の野生イネとの遺伝学的比較は、それらの系統関係を含めてまだ十分に行なわれてはいなかった。

そこで佐野幸恵さんは、本研究において栽培イネのゲノム配列データと新しく得られた2種の野生イネの EST (Expressed Sequence Tag) データを用いた比較ゲノム解析および進化的解析を行なった。EST は、*Oryza punctata* (BB型ゲノム) と *Oryza officinalis* (CC型ゲノム) のそれぞれ茎頂分裂組織と穂形成組織で発現している mRNA 由来であり、前者から 973 クローン、後者から 1425 クローンの部分配列が決定された。佐野さんは、この配列生成プロジェクトに途中から参加し、配列データをまとめるのに大きな貢献をした。一方、栽培イネの塩基配列については、*japonica* 亜種と *indica* 亜種の公開されているゲノムデータおよび cDNA データを比較に用いた。

BLASTN プログラムを用いた塩基配列の相同性検索を行なったところ、90%以上の野生イネ配列は栽培イネ配列と高い相同性をしめした。これらが ortholog だと仮定して、*O. punctata* と *O. officinalis* について、EST の塩基配列総塩基数 41 万あまりを栽培イネの配列と比較したところ、塩基置換の相違度はタンパク質コード領域ではそれぞれ 3.1%と 3.6%、非コード領域では 4.4%と 4.4%だった。これらの値は *japonica* 亜種と *indica* 亜種間の違いの約 10 倍である。非コード領域における塩基の挿入欠失による相違度は塩基置換の場合の 1/6-1/3だった。一方、同義置換数と非同義置換数の比率は、野生イネと栽培イネのあいだで 0.2 前後であり、栽培イネ間の比率 (>0.3) よりも低かった。栽培化により淘汰上の制約が弱まった結果である可能性が考えられる。

野生イネの EST 配列には、栽培イネ配列と相同性を示さないものが 20 個あったので、サザンブロット解析と 5'RACE 法を用いて、それらから4種類の遺伝子について ORF の全領域の塩基配列を決定した。これら野生イネ特有の遺伝子はリボソームタンパク質の一種、寒冷反応タンパク質、cyclinD、および機能未知のタンパク質であった。遺伝子発現解析と分子系統解析の結果、前者2遺伝子については3'非翻訳領域で塩基置換が蓄積した結果の変化と推定されたが、cyclinD は栽培イネの祖先系統で遺伝子の脱落が生じ、機能未知のタンパク質遺伝子は遺伝子配列未知の他の生物から水平移動で飛び込んだものだと考えられた。

佐野さんはまた、9種類の遺伝子を選んで、今回 EST 解析に用いた野生イネ2種を含むイネ属8種の系統関係を、外群としてトウモロコシ・シロイヌナズナを用いて解析した。その結果、イネとトウモロコシが 5000 万年前に分岐したと仮定すると、まず FF 型ゲノム系統 (*O. brachyantha*) が約 1700 万年前に分かれ、次に DD 型と EE 型ゲノムの共通祖先系統が約 1000 万年前に分かれ、その後 *O. punctata* と *O. officinalis* がどちらもほ 900 万年前に分岐し、最後に、AA 型ゲノム3種 (*O. sativa*, *O. rufipogon*, *O. barthi*) の分岐が最近 100 万年ほどのあいだに生じたと推定された。

以上のように、佐野幸恵さんは、新たに決定された野生イネの EST 配列と栽培イネのゲノム配列を比較し、両者の遺伝的多様性とイネ属全体の系統関係を推定した。また野生イネ特有の遺伝子4種類について詳細に解析し、それらの進化的由来を推定した。この研究は、野生イネの遺伝学的研究として新しい知見を加えたものとして高く評価でき、佐野さんの論文は遺伝学専攻の博士論文としての条件を満たしていると、審査員全員が認めた。