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研究課題名（和文）Genome-wide scan for footprints of adaptation/selection and phylogeography structure in the two closely related species of Azuki bean

研究課題名（英文）Genome-wide scan for footprints of adaptation/selection and phylogeography structure in the two closely related species of Azuki bean

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研究成果の概要（和文）：本研究では、制限酵素部位関連DNA 配列を用いて i)アズキおよびその近縁野生種とノラアズキ個体群の進化経路を理解し、ii）局所適応の一因となるゲノム領域を同定することを目的としました。その結果、92の野生アズキ、ノラアズキおよび栽培品種のアズキアクセッションから、232570の高品質全ゲノムSNPsを同定しました。系統発生推論により3種類の異なるグループが検出され、これらのグループとアズキ種の個体群に特異的な割り当ては、種よりもむしろ地理的起源に影響されています。ノラアズキ個体群が隣接する野生個体群の近縁種であるというこの結果は、地理的に近い個体群同士が遺伝的近縁種であることを示しています。

研究成果の概要（英文）：We used restriction-site associated DNA sequencing (i) to understand evolutionary pathway of Azuki, it’s wild relatives and weedy populations, and (ii) to identify genomic regions contributing to local adaptation. We identified 232570 high-quality genome-wide SNPs from 92 wild, weedy, and cultivated Azuki accessions. Three distinct groups were detected by phylogeny inference, and the population-specific assignment of Azuki species are more influenced by geographical origin than species. Weedy populations are more closely related to nearby wild populations and the results indicate geographically closer populations were likely to be closely related genetically. Further investigation is in progress to test candidate genes that might have contributed to the adaptation in different habitats.

研究分野：Evolutionary biology, Agriculture

キーワード：Vigna　Leguminosae　SNPs　RAD-seq

１．研究開始当初の背景

The domesticated and progenitor forms can be directly compared and crossed experimentally, providing insights into the molecular and developmental impacts of selection during domestication. Theses features make many crops as model systems for understanding plant adaptation and diversification.

Azuki (*Vigna angularis* var. *angularis*, 2n = 2x = 22) is a traditional legume crop grown across East and northern South Asia. The crop belongs to the same biological species as its wild progenitor, are fully compatible with it and, in some cases, are sympatric with its relatives, which may be in the form of wild or weedy plants. Wild-weedy-domesticated Azuki, therefore provides a unique opportunity to study evolutionary and ecological adaptation to different habitats for several reasons. First, the presumed progenitor of Azuki bean, *V. angularis* var. *nipponensis*, grows in the habitats of relatively humid, mesic sites near streams or paddy levees and distributed from the Himalayan highlands of subtropical Asia to the temperate areas of East Asia. They are differing in such traits as habit, seed size and color. Second, a conspecific weed of cultivated Azuki, weedy Azuki found in rural, human-disturbed habitats only in Japan and Korea. Weedy populations have morphological characteristics typical of wild species (e.g., plant architecture) and of cultivated Azuki (e.g., pod dehiscent habit). On the other hand, *V. nepalensis* is a very similar species to Azuki, both morphologically and with respect to molecular markers, and its distribution is limited to the forest margin area of the Himalayan highlands which is boundary zone of the *V. angularis* var. *nipponensis* distribution. However, a small number of loci and low polymorphism in the earlier studies does not allow to detail understanding of phylogeography and evolutionary pattern of Azuki, its wild relatives and weedy populations.

Genome-wide surveys provide opportunity not only to understand the patterns of genetic diversity and domestication, but also to identify regions associated with adaptation. Reduced-complexity approaches in genome typing as double-digest restriction-site associated DNA sequencing (ddRAD-seq) are useful for simultaneously developing and genotyping DNA markers such as single nucleotide polymorphisms (SNPs) and have been indicated as the most promising methodology for genomic analysis at different taxonomic levels (i.e., population, species).

２．研究の目的

In this project, we used ddRAD-Seq in 92 cultivated, wild, and weedy Azuki. Using the identified genomic SNPs, we aimed to:
(1) Understand evolutionary pathway among domesticated weedy, and wild relatives of Azuki.

(2) Search for genetic regions that are under selection, in order to determine the genetic basis of adaptation.

３．研究の方法

Plant materials and DNA isolation

A total 66 accessions selected from cultivated, wild, and weedy Azuki and its wild relatives (*V. nepalensis*, *V. nakashimae*, *V. umbellata*, and *V. riukiuensis*) for nucleotide diversity of five nuclear genes.

For ddRAd-seq analysis, a total 92 cultivated, wild, and weedy Azuki, and *V. nepalensis* from wide geographical regions were used. DNA was extracted from seeds or leaves using DNeasy Plant Kit (QIAGEN).

(1) Nuclear Loci studied

The five genes were selected for inclusion in this study are α-amylase (EC.3.2.1.1) gene, ACC synthase (EC.4.4.1.14), Peroxidase (EC.1.11.1.7), Cysteine endopeptidases (EC.3.4.22) and ACC oxidase [EC.1.14.17. 4].

Nucleotide diversity (π, θ), number of variable sites (S), minimum number of recombination (Rm) events, Tajima's D, and Fu & Li's D* were estimated using DnaSP 4.0. (Rozas et al. 2003)

(2) RAD Data Analysis and SNP Identification

The Illumina sequence reads were quality-filtered by removing the adapter sequences and reads containing greater than 50% low-quality bases (quality value ≤5) SOAPnuke (v.1.5.0). All reads were assigned to the tested accessions with unambiguous barcodes and the recognition site. Because a reference Azuki genome sequence is currently available, high-quality reads were then aligned to Azuki reference genome and SNPs identified by SOAP2.20 (Li et al. 2008) with default parameters. Based on the alignment result, the RAD-based SNP calling was done by SOAPsnp (Li et al. 2009).

(3) Phylogenetic analysis

To construct the phylogenetic tree, the genetic distances between the different accessions were calculated based on the high-confidence SNPs extracted from the RAD data using TreeBest 1.9.2 (Vilella et al. 2008).

４．研究成果

(1) Nucleotide diversity and evidence of selection

We analyzed the patterns of nucleotide polymorphism of five nuclear genes in the wild and domesticated Azuki from different geographical regions. As a result, the four of the five loci surveyed appeared to be evolving primarily under purifying selection, while the remaining locus (α-Amylase gene) may have been the subject of positive selection. Reduction nucleotide diversity at four loci and the elevated

non-synonymous substitution rate in α-amylase gene support that either positive selection has made a significant impact on genomic polymorphism patterns in the domesticated Azuki, or founder effect due to domestication process.

(2) RAD Sequencing and SNP Genotype Calling

A total 92 Azuki accessions of *V. angularis* var. *angularis*, it's wild relatives (*V. angularis* var. *nipponenis*, *V. nepalensis*) and weedy type from the genus *Vigna* were used for the construction of RAD libraries on Illumina Hiseq 4000 platform. After trimming the barcodes, quality filtering and cleaning of the raw reads, a total of 597.22 million of high-quality (average data size: 878.45 Mb) clean reads were generated. The sequence reads were aligned to the Azuki reference genome using SOAP2. The mapping rate in different accessions varied from 40% to 60%, averaging a 54.95% coverage. SNP calling and genotyping was conducted using SOAPsnp. A total of 232570 SNPs were adequate for genotyping.

(3) Genetic relationship among wild, weedy and cultivated Azuki

To examine the genetic relationships among wild, weedy and cultivated Azuki, a neighbor-joining phylogenetic analysis was conducted using 232570 genomic SNPs. Based on the genetic distances of the genotyped SNPs, the 92 accessions were clustered into three major groups (WH, CH, WWCA, Figure 1). The WH group includes wild Azuki (*V. angularis* var. *nipponenis* and *V. nepalensis*) from Himalayan regions. The CH group contained cultivated Azuki from Himalaya regions. The WWCA group was composed cultivated, wild and weedy Azuki from Southeast Asian countries. The cultivated and wild Azuki from Himalayan regions were separated from other Southeast Asian countries, suggesting that domestication event promoted genetic differentiation within the Azuki gene pool.

Further, in cultivated Azuki, accessions from Japan and China clustered in two separate subgroups within the WWCA group. This evidence indicates the population-specific assignment of Azuki accessions are more influenced by geographical origin.

Weedy Azuki populations are more closely related to nearby wild populations and the results indicate geographically closer populations were likely to be closely related genetically.

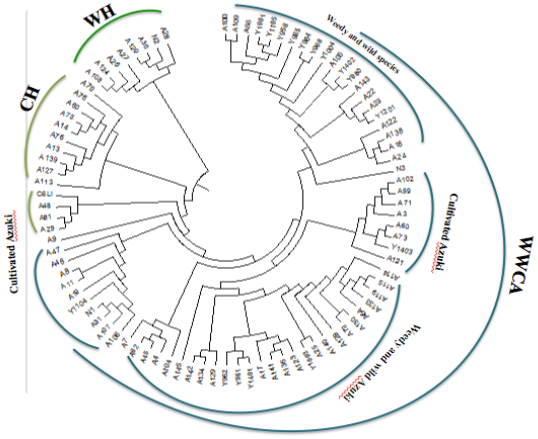

Figure 1. Genetic relationships in 92 Azuki

accessions. Neighbor joining tree showing relationships of wild, weedy, and cultivated Azuki. WH (wild Azuki) and CH (cultivated Azuki) from Himalayan regions. WWCA: wild, weedy, and cultivated Azuki from Southeast countries.

(4) Implications for conservation of wild relatives and targeting adaptive traits

Our analyses revealed a high degree of genetic divergence among populations of wild and cultivated Azuki from Himalayan regions. The two major genotypic groups that we detected in the sampled area can be used as a starting point for germplasm conservation. We expect that more extensive sampling of wild Azuki in Korea, China, and Himalayan regions would reveal many additional target populations for germplasm conservation. Furthermore, current genomic SNPs could help us to identify potential natural targets for adaptive trait in diverse climatic regions. Further investigation is in progress to test candidate genes that might have contributed to the adaptation in different habitats.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔学会発表〕（計 3　件）

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