

令和 2 年 9 月 7 日現在

機関番号：17102

研究種目：基盤研究(C) (一般)

研究期間：2016～2019

課題番号：16K07234

研究課題名(和文) An innovative approach of using wild relatives genomics for the climate-resilient crop development and conservation

研究課題名(英文) An innovative approach of using wild relatives genomics for the climate-resilient crop development and conservation

研究代表者

Firouzeh Javadi (Javadi, Firouzeh)

九州大学・持続可能な社会のための決断科学センター・助教

研究者番号：70640910

交付決定額(研究期間全体)：(直接経費) 3,800,000円

研究成果の概要(和文)：本プロジェクトでは、経済的に重要なひよこ豆の野生近縁種における進化パターンおよび適応のゲノムベースを検討した。19のひよこ豆属の71のアクセッションから16,595のSNPを得て検討した結果、系統発生の頑健な枠組みが得られ、アフリカの種が早期に分化したことが示されたことで、種分化プロセスと生態学的多様性に関する洞察が得られた。RAD-seq法により、固有種では遺伝学的多様性が高く、また危機に瀕している種では遺伝的多様性が低いことが示され、稀少種を絶滅から保護する取組みに有望な所見が得られた。多様な生息環境への適応に寄与する候補遺伝子の研究を継続中である。

研究成果の学術的意義や社会的意義

This project had a strong food security trust. Economically important chickpea suffers from a narrow genetic diversity. The results will add considerable resources to develop crop adapted to climate and toolkit for conservation. Research results are publicly available in database and articles.

研究成果の概要(英文)：Using genome-wide RAD-seq approach, this project aimed to investigate evolutionary patterns and genomic basis of adaptation in wild relatives of economically important chickpea (*Cicer arietinum* L.). We obtained 16,595 SNPs from 71 accessions of 19 *Cicer* species. The results provide a robust phylogenetic framework and indicates the early divergence of African species, which may shed light on the speciation process and ecological divergence of *Cicer* species. The RAD-seq data found high genetic diversity of endemic species and low genetic diversity of endangered species which holds promise for conservation efforts to save the rare and endangered species from extinction. Investigation of candidate genes that might have contributed to the adaptation in different habitats is ongoing.

研究分野：Plant Genetic Resources

キーワード：Cicer Endemic Endangered RAD-seq crop wild relatives

科研費による研究は、研究者の自覚と責任において実施するものです。そのため、研究の実施や研究成果の公表等については、国の要請等に基づくものではなく、その研究成果に関する見解や責任は、研究者個人に帰属されます。

1. 研究開始当初の背景

Climate change is likely to be one of the most important factors affecting our future food security. How can we ensure the food and biodiversity security? The greatest source of untapped diversity, especially for the adaptive characteristics needed to confront the challenges of a changing climate, are the wild relatives of our domesticated food crops. However, there is the lack of sufficient knowledge of these crop wild relatives genome. They are also threatened in their natural environment, missing in crop collections and therefore not yet available for use.

Chickpea (*Cicer arietinum* L.) is the world's second most important legume after beans. It is only domesticated species in the genus *Cicer* which suffers from a narrow genetic diversity. The genus *Cicer* comprises 42 wild species which are distributed throughout the temperate zones of the Northern Hemisphere and adapted to diverse environmental conditions and resistance to various biotic stresses (e.g., *C. judaicum*). Currently, only two immediate wild relatives of chickpea (*C. reticulatum* and *C. echinospermum*) are the main focus of new variation. Although introgression is possible from distant wild relatives, their genomics has received limited attention or neglected. Several wild relatives of chickpea are increasingly at risk (e.g., *C. bijigum*, *C. canariense*, *C. kermanense*) from multiple climate change threats and remain largely understudied.

Genome-wide surveys provide opportunity not only to understand the patterns of genetic diversity, but also to identify regions associated with adaptation and conservation program. 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.

2. 研究の目的

Using ddRAD-seq, this study aimed to (1) understand evolutionary patterns of wild relatives of chickpea especially rare and threatened species and (2) investigate the genomic basis of adaptation. This knowledge will permit selection of novel sources candidates for the climate-resilient crop development and conservation.

様式 C-19、F-19-1、Z-19 (共通)

3. 研究の方法

(1) Plant materials and DNA isolation

A total 71 accessions from 19 species were sampled. This sample includes widespread, endangered and rare species (e.g., *C. canariense*, *C. bijugum*, *C. kermanense*) in Africa, Southwest and Central Asian, and Mediterranean region. DNA was extracted from seeds or leaves using DNeasy Plant Kit (QIAGEN).

(2) RAD Data Analysis and Single-nucleotide polymorphism (SNPs) discovery

RAD-seq uses restriction enzymes to break down the genome to easily sequenced fragments that can be reconstructed into thousands of DNA loci for estimating robust phylogenetic trees and genomic analyses. The Illumina sequence reads were quality-filtered by removing the adapter sequences using Trimmomatic version 0.39. Quality of the bases was assessed for each sample both before and after trimming using the program FastQC. Stacks version 1.44 was then used to obtain SNPs de novo.

(3) Phylogenetic and Network analysis

In order to determine evolutionary relationships among *Cicer* species, maximum likelihood (ML) phylogenies were estimated using IQ-TREE version 1.6.12 with 1000 ultrafast bootstraps. Network Analysis was also performed using SplitsTree5 5.0.0 alpha.

(4) Sequence divergence analysis

The pairwise genetic distance between concatenated ddRAD sequences from different individuals estimated using the maximum composite likelihood approach implemented in MEGA version 10.1.18.

4. 研究成果

(1) RAD Sequencing and SNP Genotype Calling

A total of 71 accessions from 19 *Cicer* species were used for the construction of RAD libraries on Illumina HiSeq 4000 platform. Illumina Sequencing yielded an average of 5,674,031 reads per sample (1,592,678–8,898,068). After de-multiplexing and quality filtering, an average of 4,293,187 reads per sample (1,201,088 – 6,943,166) were left. The resulting matrix of 16,595 single nucleotide polymorphisms (SNPs) for 71 accessions was used to construct an unrooted network and a maximum likelihood phylogeny and genomic analyses.

(2) Phylogenetic reconstruction

De novo assembly of ddRAD-seq data yielded a fully resolved and nearly fully supported phylogenetic relationships among species and individuals of *Cicer*. Both phylogenetic network and ML phylogenetic tree yielded similar topologies that included six clades (AF, ME, AGM, CH, CWA, CW; Figure 1). African clade (AF) represents the earliest-diverging extant lineage and is supported by the morphology of the species; indeed, they are only *Cicer* species with a climbing growth habit, elongate pods, and globular seed. The three observed clades (SW, SWC, CH) contain species with adaptations to high altitudes in Central and Southwest of Asia (1500-4500m) and correlation their phenotypic variation (e.g., small leaflets and spiny tendril) to habitats can aid in understanding the evolutionary response to aridity. Monophyletic trifoliolate annual species, *C. chorassanicum* clade (CH clade) derived early during the diversification of the Southwest and Central Asian perennial species. This may point to an earlier switch to the annual life form in Southwest Asia where aridity was more pronounced. The two perennial species of Aegean-Mediterranean (AGM) clade having a distribution in a relatively warm, humid forest zone at lower altitudes (500-1700m) in Eastern Europe and Anatolia tend to have large flowers and leaflets. As the dry areas of Central and Southwest Asia are the diversity centers of the genus *Cicer*, both phylogenetic niche conservatism and adaptation probably play roles in evolution and diversification of *Cicer* species.

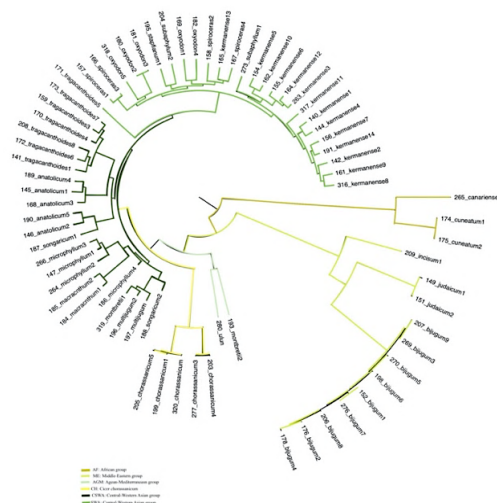


Figure 1: Maximum likelihood phylogenetic tree of 71 accessions from 19 *Cicer* species.

(3) Genetic diversity in endemic and threatened *Cicer* species.

RAD-data showed high genetic diversity within some of endemic species such as *C. kermanense* and low genetic diversity in threatened species such as *C. bijugum*. *C. kermanense* consists two subgroups and distributed in rubble habitat in SW Iran. The major risk to *C. kermanense* maintenance is its rarity, suggesting the necessity of a preservation program. Seeds of *Cicer* species lack any obvious adaptation for long-distance dispersal and barochory is likely the major dispersal mode. However, seed dispersal by grazing mammals that consume the seeds along with the foliage cannot be ruled out. The low dispersal rates that probably could not keep pace with fast rates of environmental change, make the long-term survival of rare endemic species in its natural habitat especially uncertain. The low genetic diversity of rare species holds promise for conservation and restoration efforts to save endangered species from extinction.

5. 主な発表論文等

〔雑誌論文〕 計2件（うち査読付論文 1件/うち国際共著 2件/うちオープンアクセス 0件）

1. 著者名 Allkin R, Borges ML, Bruneau A, Egan AN, Estrella M, Javadi F. et al.	4. 巻 Special issue
2. 論文標題 Towards a new legume systematics portal	5. 発行年 2019年
3. 雑誌名 Australian Systematic Botany	6. 最初と最後の頁 495-518
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.1071/SB19025	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

1. 著者名 Azani et al.	4. 巻 66 (1)
2. 論文標題 A new subfamily classification of the Leguminosae based on a taxonomically comprehensive phylogeny	5. 発行年 2017年
3. 雑誌名 Taxon	6. 最初と最後の頁 44-77
掲載論文のDOI（デジタルオブジェクト識別子） https://doi.org/10.12705/661.3	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕 計4件（うち招待講演 0件/うち国際学会 2件）

1. 発表者名 Firouzeh Javadi
2. 発表標題 Ecological and evolutionary genetics of Cicer
3. 学会等名 Biology 20 (国際学会)
4. 発表年 2020年

1. 発表者名 Javadi F
2. 発表標題 Genomic insights into the distant wild relatives of chickpea
3. 学会等名 9th International Conference on Legume Genomics and genetics (国際学会)
4. 発表年 2019年

1. 発表者名 Firouzeh Javadi
2. 発表標題 Ecological and evolutionary genetics of wild Cicer species
3. 学会等名 the 8th International Conference on Legume Genetics and Genomics (ICLGG)
4. 発表年 2017年

1. 発表者名 Firouzeh Javadi and Takahiro Murakami
2. 発表標題 How education can help to reduce food waste and sustainable development in Japan
3. 学会等名 7th International Conference on Sustainability Science
4. 発表年 2017年

〔図書〕 計0件

〔産業財産権〕

〔その他〕

-

6. 研究組織

	氏名 (ローマ字氏名) (研究者番号)	所属研究機関・部局・職 (機関番号)	備考