Studies on genetic diversity and linkage map of soybean for applied breeding

（ダイズにおける遺伝的多様性及び分子連鎖地図に関する研究）

学位論文内容の要旨

Soybean [*Glycine max* (L.) Merrill] is originated in Eastern Asia, and one of the most important grain legumes in terms of its use as food, animal feed, and industrial material, with its seed being characterized by a high content and quality of protein and oil. Soybean was planted on more than 94 million hectares worldwide in 2007, yielding 237 million tons. More than 230 million tons (96%) of soybeans were commercially produced in the seven countries, the United States, Brazil, Argentina, China, India, Paraguay, and Canada, and amount of production is increasing from year to year. In contrast, Japan and Korea are leading consumers of soybean, and imported around 4 million 50 thousand tons and 1 million 260 thousand tons respectively from the main producing countries in 2007 (FAS, 2008). It is therefore imperative to develop new cultivars that would be expected to overcome the biological and abiological factors causing the low soybean productivity in order to achieve a supply for domestic human consumption at least. Breeding of soybean has improved seed quality in Japanese elite cultivars for specific food products such as tofu and natto. The introduction of new genetic resources into breeding programs may further improve agronomic traits and increase genetic variability and thereby lead to the development of high-yield cultivars. On the other hand, the introgression of desirable genes from such resources into breeding lines may result in detrimental changes in traits such as seed quality (Jacobsen and Schouten 2007). An accurate and well-saturated genetic linkage map is fundamental for modern plant breeding thorough identification of substantial or new agronomic trait loci including quantitative trait loci (QTL), and understanding genetic diversity and genome structures of genetic resources.

In the first main chapter, we have analyzed 377 SSR loci distributed throughout the soybean genome in a total of 87 cultivated or wild accessions including Japanese elite cultivars and irreplaceable genetic resources in order to estimate the genetic diversity and the genetic relationship among them.

The length polymorphisms of 377 simple sequence repeat (SSR) markers were analyzed in 87 cultivated or wild soybeans, including Japanese elite cultivars and irreplaceable genetic resources. The average number of SSR markers in the 20 linkage groups was 18.9, with a range of 13 to 25. All SSR markers showed polymorphism on agarose gel electrophoresis, which revealed a total of 1380 alleles for all accessions. The number of alleles at each locus ranged from 2 to 10, with an average of 3.7, and the polymorphic information content of each marker ranged from 0.02 to 0.86, with an average of 0.44. Cluster analysis separated those accessions into three clusters. Most Japanese cultivars were found in Cluster 1, which was further divided into three subclusters that corresponded to the geographic origin of the cultivars. Most foreign cultivars and all wild accessions were present in Clusters 2 and 3, respectively. The data on the polymorphism of these SSR loci should facilitate genetic mapping of agronomically important traits and the choice of appropriate markers for marker-assisted selection (MAS) in soybean breeding programs in Japan.

In the second main chapter, a high-density integrated linkage map of soybean was constructed on the basis of SSR markers. In addition, the polymorphisms in several genotypes were compared between public SSR markers, which developed from genomic DNA sequence by USDA-ARS, and EST-derived SSR markers, which developed from public database of soybean expressed sequence tags (EST) by Kazusa DNA Research Institute.

A well-saturated molecular linkage map is prerequisite for modern plant breeding. A number of molecular genetic maps have been developed in the soybean with several types of molecular markers. Compared to other DNA markers, SSR markers are single locus markers with high allelic variation, which are applicable widely to different genotypes. A total of 1,811 SSR and PCR based markers were mapped in one or more of three recombinant inbred populations: the USA cultivar 'Jack' × the Japanese cultivar 'Fukuyutaka', the Chinese cultivar 'Peking' × the Japanese cultivar 'Akita', and the Japanese cultivar 'Misuzudaizu' × the Chinese breeding line 'Mashidou Gong 503', and aligned the 20 consensus linkage groups. The total length of the integrated linkage map was 2,442.9 cM, and the average number of the molecular markers was 90.6 with range from 70 to 114 in each linkage group. The allelic diversity was investigated among 23 soybean cultivars/lines and a wild accession for the 1,238 marker loci. The number of alleles per locus ranged from 2 to 7 with an average 2.8. The high-density linkage map merged here will promote ongoing and oncoming genomic research such as QTL analysis and positional cloning in addition to MAS in soybean breeding.

学位論文審査の要旨

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学 位 論 文 題 名

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　本論文は４章１１３頁からなる英文論文であり、図７、表６、別表１を含む。

　ダイズは良質なタンパク質や脂質を豊富に含むことから食料、飼料ならびに工業原料として世界的に生産量が拡大している。2007 年には全世界で約 9400 万ヘクタールの耕地で約２億４千万トンのダイズが生産された。ダイズは中国を含む東アジア地域で栽培化され、多様な品種に分化したと考えられている。日本や韓国でも古くからダイズが栽培されてきたが、収量が低く、消費量のほとんどを輸入に依存している。このような状況から脱却し、自給率を高めるには、高収量かつ高品質な品種の育成が欠かせない。ところが、病害虫抵抗性や収量性に優れた遺伝資源は必ずしも、種子品質や栽培特性が優れているわけではない。そのため、これら重要な形質を導入する上で、形質を支配する遺伝子座の染色体上の位置情報に基づく選抜、すなわち DNA マーカー選抜育種は有力な手段となる。そこで、本研究では、日本の主要品種と米国を始めとする外国品種の遺伝的関係を DNA マーカー（SSR マーカー）によって明らかにするとともに、多型率が高く、比較的容易に解析できる SSR マーカーを主体にダイズの染色体数 20 に収束する高密度分子連鎖地図を作製した。

1. The length polymorphisms of 377 simple sequence repeat (SSR) markers were analyzed in 87 cultivated or wild soybeans, including Japanese elite cultivars and irreplaceable genetic resources. The average number of SSR markers in the 20 linkage groups was 18.9, with a range of 13 to 25. All SSR markers showed polymorphism on agarose gel electrophoresis, which revealed a total of 1380 alleles for all accessions. The number of alleles at each locus

ranged from 2 to 10, with an average of 3.7, and the polymorphic information content of each marker ranged from 0.02 to 0.86, with an average of 0.44. Cluster analysis separated those accessions into three clusters. Most Japanese cultivars were found in Cluster 1, which was further divided into three subclusters that corresponded to the geographic origin of the cultivars. Most foreign cultivars and all wild accessions were present in Clusters 2 and 3, respectively. The data on the polymorphism of these SSR loci should facilitate genetic mapping of agronomically important traits and the choice of appropriate markers for marker-assisted selection (MAS) in soybean breeding programs in Japan.

2. A well-saturated molecular linkage map is prerequisite for modern plant breeding. A number of molecular genetic maps have been developed in the soybean with several types of molecular markers. Compared to other DNA markers, SSR markers are single locus markers with high allelic variation, which are applicable widely to different genotypes. A total of 1,811 SSR and PCR based markers were mapped in one or more of three recombinant inbred populations: a USA cultivar 'Jack' × a Japanese cultivar 'Fukuyutaka', a Chinese cultivar 'Peking' × a Japanese cultivar 'Akita', and a Japanese cultivar 'Misuzudaizu' × a Chinese breeding line 'Moshidou Gong 503', and aligned the 20 consensus linkage groups. The total length of the integrated linkage map was 2,442.2 cM, and the average number of the molecular markers was 90.6 with range from 70 to 114 in each linkage group. The allelic diversity was investigated among 23 soybean cultivars/lines and a wild accession for the 1,238 marker loci. The number of alleles per locus ranged from 2 to 7 with an average 2.8. The high-density linkage map merged here will promote ongoing and oncoming genomic research such as QTL analysis and positional cloning as well as MAS in soybean breeding.

　本研究は、日本品種における遺伝的多様性拡大の可能性を示すとともに、DNA マーカー選抜育種を実施する上での研究基盤を打ち立てるものであり、学術的に高く評価できる。
　よって、審査員一同は、黄　太暎が博士（農学）の学位を受けるに十分な資格を有するものと認めた。