

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

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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.

In this study, 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, and developed high-density integrated linkage map based on SSR markers, significantly improved the capacity for structural and functional analyses of the soybean genome with a sufficient number of SSR markers to facilitate correct mapping of QTL, MAS, map-based gene cloning. Our data thus provide valuable information for selecting suitable SSR markers at target regions and for linkage analysis dependent on crossing combination.

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