

Development of a Core Set of Korean Soybean Landraces [*Glycine max* (L.) Merr.]

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Abstract

A total of 2,765 accessions were used as the initial set having both seed coat color and 100-seed weight data. As a result of molecular profiling using six SSR markers followed by stratification based on their usages, 335 accessions (12.1%) were selected by clustering based on UPGMA. Since 75 out of 335 accessions were mixed in phenotypic traits as a result of characterization, 260 accessions were finally set as a core set. This core set revealed nearly the same diversity compared with the other results on morphological traits of Korean soybean landraces. In total, 115 alleles (19.2 alleles per locus) were detected in the initial set and 79 alleles (13.2 alleles per locus) were detected in the core set. All 30 major alleles were present in the initial set and in the core set as well. In allele coverage, the core set was 71.4% of the initial set. These comparisons of number of alleles, gene diversity and coverage indicated that the core set represented the entire set well.

Key words: *Glycine max*, Genetic diversity, SSR, Core set.

Introduction

With the rapid increase in the number of accessions conserved in crop germplasm collections, redundant resources have become an obstacle to the effective maintenance and utilization of the genetic resources. To solve this problem, Frankel (1984) proposed the concept of the core collection. The core collection could serve as a working collection that could be extensively examined and utilized, and the remnant accessions excluded from the core collection would be retained as the reserve collection. Thus, the establishment of core collections can be a helpful means to make better use of plant germplasm and to assist in managing the initial set.

Dozens of core collections have been successfully developed and most of the data used to analyze the genetic diversity of crops were morphological, agronomic, and eco-geographical traits (Dwivedi et al. 2005; Grenier et al. 2000a; Upadhyaya and

Ortiz 2001). However, only one selective sampling without considering the total traits did not affect the morpho-agronomic characters that were not previously considered in the sampling. In order to explain these results, it was suggested that molecular markers could help resolve such situation (Grenier et al. 2000b). Akkaya et al. (1992) were the first to analyze DNA length polymorphism in the soybean using three SSR markers. Subsequently, SSRs were used for identifying the genetic structure, analyzing diversity, grouping varieties, etc. (Brown-Guedira et al. 2000; Diwan and Cregan 1997; Kim et al. 2006; Li et al. 2001; Narvel et al. 2000; Song et al. 1999).

Soybean has been cultivated for various uses such as foods and medicines, and have adapted to different climates of East Asia including Korea (Lee 2003). Because soybean has been cultivated for a long time, wide variation has accumulated in Korean soybean landraces (Kwon et al. 1974; Song et al. 1991; Yoon et al. 2003). Abe et al. (2003) suggested that an involvement of Korean soybean accessions in both Japanese and Chinese germplasm pools were intermingled with the accessions from the Japanese and Chinese pools; alternatively, the Korean

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population might be ancestral to the two populations.

To prevent genetic erosion and to conserve such diverse genetic resources, The National Genebank of Rural Development Administration, Korea (RDA-Genebank) has tried to secure its genetic resources. As a result, a large collection of Korean soybean landraces, approximately 7,000 accessions, has been conserved in the RDA-Genebank and been distributed to the users. These accessions may include redundant and/or misidentified ones. These accessions, therefore, should be evaluated for various agronomic traits at a number of locations in replicated trials for effective management and practical use. However, this is a costly exercise due to the large size of the collection.

Therefore, the purpose of this study was (i) to use SSR markers to develop a core set of Korean soybean landraces from a total collection of 2,765 accessions as an initial set and (ii) to evaluate the core set.

Materials and Methods

Data set

Molecular profiling was carried out using six SSR markers (Table 1) to the soybean germplasm conserved in RDA-Genebank, including 3,248 Korean landrace accessions, 646 Chinese landrace accessions, 257 Japanese landrace accessions and 259 Korean wild soybean accessions (Yoon et al. 2007). Among the 3,248 Korean soybean landrace accessions collected from the 1960s to 1980s throughout South Korea, 2,765 accessions were used as an initial set since they are the only ones with the passport information on both 100-seed weight and seed coat color which is necessary for determining their use.

Table 1. Soybean SSR loci, linkage groups, and sequences of primers used for DNA profiling of soybean germplasm. Marker designations are from Cregan et al. (1999).

Locus	Linkage group	Forward primer	Reverse primer
Sat_074	F	GGGTGAGAAATACGCAACTTACA	GGGCATCAAATGATATTAATGTCTAA
Satt187	A2	GCGTTTAAATTATGATATAACCAA	GCGTTTTATCTCTTTCCACAAC
Satt197	B1	CACTGCTTTTTCCCTCTCT	AAGATACCCCCAACATTATTGTAA
Satt532	D1a+Q	GCCCAATATTATCATGCTTTATGT	GCGTGAAAAATCTTGAATCTGA
Satt141	D1b+W	CGGTGGTGGTGTGCATAATAA	CCGTCATAAAAAGTCCCTCAGAAT
Satt286	C2	GCGCGGTTAATTTATGCCGAAA	GCGTTTGGTCTAGAATAGTTCTCA

DNA extraction

Genomic DNA was extracted from fresh leaf tissues of seedlings (10-12-day old) for each accession. Sampled leaf tissues were ground in liquid nitrogen. DNA was extracted from

powder of frozen tissue using DNeasy Plant Mini Kits (Qiagen #69106). The precipitated DNA was dissolved in 10 mM TE buffer and stored at -20 °C until use. The final concentration was adjusted to 20 ng/ μ L for PCR reaction.

SSR genotyping

PCR amplification was performed on each of the soybean genomic DNA using primers for each SSR locus. Fifteen μ L of reaction mixtures contained 5.0 μ L of sterile water, 2.5 μ L (20 ng) of soybean genomic DNA, 4 μ L of dNTP mixture containing 10 mM of each nucleotide (final concentration of 0.2 mM each) (Promega, Madison, WI, USA), 1.5 μ L of 10X PCR buffer including 50mM of KCl, 0.5 μ L (0.15 μ M) of each forward and reverse primers, and 1 μ L of 1 unit of Taq DNA polymerase (Promega, Madison, WI, USA). All PCR reactions consisted of 1 cycle of 25 s with incubation at 94 °C, 32 cycles of 25 s for denaturation at 94 °C, 25 s for annealing at 46 °C, and 25 s for extension at 68 °C, and final extension at 72 °C for 15 min on PTC-100 thermocycler (MJ Research, Inc., Watertown, MA, USA). After PCR amplification, SSR alleles were resolved on an ABI-PRISM 3100 DNA sequencer (Applied Biosystems, CA, USA) using GENESCAN 3.7 software, and sized precisely against 6-carboxy-X-rhodamine (ROX) molecular size standards using GENOTYPER 3.7 software (Applied Biosystems, CA, USA).

Core set development

Yonezawa et al. (1995) recommended the stratified sampling for forming an effective representation of the genetic diversity of the whole collection in a core set. Burnham et al. (2002) assumed that a distinct genetic diversity did exist within South Korean soybean landraces but it was not dependent on the geographical relationship of the eight provinces. Soybean landraces grown by farmers in Korea were selected according to two distinctly different directions based on seed size for end use (Kwon et al. 1974; Song et al. 1991). Subsequently, 2,765 accessions were stratified to four groups on the basis of four end uses such as sauce soybean, sprouting soybean, soybean for cooking with rice, and others described by Hong et al. (1988) and Yoon (1998). Seed coat colors and 100-seed weights were used as the criteria for classifying Korean soybean landraces. Soybeans with yellow seed coat color and 100-seed weight greater than 15 g/100 seeds were selected for sauce soybean, while soybeans with 100-seed weight less than 15 g/100 seeds regardless of its seed coat color were selected for sprouting soybean. Soybean for cooking with rice was colored and larger than 30 g/100-seed weight. Soybeans with many different colors and having 15 g~30 g/100-seed weight were grouped into others.

Four data sets according to usage were grouped separately for clustering based on unweighted pair group method with arithmetic average (UPGMA). The presence (1) and absence (0) values of alleles for each SSR were recorded for all accessions. Genetic similarities were calculated based on the Dice coefficient (Nei and Li 1979). Based on the genetic similarity matrix, dendrograms for each usage group were constructed using NTSYS-pc (ver. 2.11a). From each dendrogram, the similarity coefficient cutoff value was set to have around 80 clusters which were decided for choosing approximately 10% or more of total accessions. One accession in each cluster was randomly selected.

Field data

Accessions were sown in single rows spaced 60 cm apart. Spacing between plants within rows was 15 cm. The evaluation was carried out in 2004 in Suwon, South Korea in non-heated, rain shield plastic house with drip irrigation, protection from weeds, pests and diseases, and no fertilization. Characterization data were recorded for nine phenotypic characteristics in accordance with the soybean descriptors of genetic resources (RDA 1986). Days to flowering were recorded as the mean emergence days to the date when 50% plants started to flower. Days to maturity were recorded as the mean days to the date when 95% pods matured and most of leaves had faded and fallen. After harvest, seed weight was recorded as 100-seed weight (g). Seed length (mm), seed width (mm), pod length (mm), and pod width (mm) were measured as a mean of 10 seeds. Flower color was recorded as white and purple. Hilum color was recorded as yellow, brown, and black. Seed coat color was recorded as yellow, brown, green, and black.

Diversity analysis

Basic statistics, including number of alleles, gene diversity (or expected heterozygosity), and number of effective alleles were calculated using PowerMarker v.3.0 (Liu and Muse 2005). The effective number of alleles per locus was obtained from the number of alleles whose frequency was higher than 5%.

Gene diversity (GD) for each SSR marker was calculated by Nei's Gene diversity (Nei 1973);

$$GD = 1 - \sum P_{ij}^2$$

where P_{ij} is the frequency of the j -th allele for the i -th marker. Coverage was used to evaluate a core set for its coverage of alleles (Kim et al. 2007);

$$\text{Coverage (\%)} = \frac{1}{m} \sum_{j=1}^m \frac{D_c}{D_e} \times 100$$

where D_c is the number of different alleles occupied in the core collection and D_e is the number of different alleles occupied in the entire accessions in each locus and m is the number of loci. If a core set shows 100% coverage of alleles without any deviations, this suggests the core set maintains all the diversity present in each class.

Results and Discussion

Core set development

Eighty-eight accessions (10.2%) were selected from a total of 865 accessions from sauce soybean group, 80 (8.3%) from 964 of sprouting soybean group, 87 (34.5%) from 252 of soybean for cooking with rice group, and 80 (11.7%) from 684 of others group, constituting a total of 335 accessions (12.1%). Similarity coefficient cutoff values for pre-selection of materials were 95.6, 94.7, 97.8, and 94.6 in sauce soybean group, sprouting soybean, soybean for cooking with rice, and others group, respectively (Table 2). Constitution ratio of cooking with rice group in selected accessions was relatively high (34.5%) compared to the other groups. This was attributed to the number of accessions consisting of each usage group which was artificially adjusted to a similar number to make a core set with approximately 10% of the initial set.

Seventy-five of the 335 accessions were mixed in flower color, leaf shape, pubescence color, or seed coat color based on morphological characterization. The mixed accessions, therefore, had to be discarded, and finally 260 accessions, 9.4% of the initial set, were set as a core set (Table 2).

Table 2. The number and percentage of accessions of the initial set and core set according to the usage groups of Korean soybean landrace used in this study.

Usage group ^a	No. of initial set	Pre-selected materials			Core set	
		No. of Accessions	Similarity cut-off value	Percentage (%) ^b	No. of Accessions	Percentage (%) ^b
SAU	865	88	95.6	10.2	66	7.6
SPR	964	80	94.7	8.3	61	6.3
COO	252	87	97.8	34.5	71	28.2
OTH	684	80	94.6	11.7	62	9.1
Total	2,765	335		12.1	260	9.4

^a Usage Group = SAU: Sauce soybean, SPR: Sprouting soybean, COO: Soybean for cooking with rice, OTH: Others.

^b Percentage of pre-selected materials to the number of the initial set.

^c Percentage of the accession number of the core set to the initial set.

Morphological diversity

This core set was characterized to have the color purple (83.5%) and white (16.5%). Song (1991) reported that there were more purple (78.3%) than white flowers (21.7%). Yoon (1998) also reported that the ratio of purple to white flowers was approximately 3 (71.3%) to 1 (28.7%). As to the seed coat color, yellow had the highest (37.7%), while brown had the lowest (8.5%). Song (1991) reported that seed coat colors were composed of yellow (39.5%) which was the highest and black (14.5%) which was the lowest. Hong et al. (1988) reported that hilum color of Korean soybean landrace accessions had more black than yellow. In this core set, black hilum was also the majority at 84.6% (Table 3). The slight ratio difference with the results of other reports in three morphological traits might be contributed by the process of artificial stratification.

Table 3. Frequency distribution for the qualitative characters of core set.

Character	Character state	Frequency	Percentage (%)
Flower color	White	43	16.5
	Purple	217	83.5
Seed coat color	Yellow	98	37.7
	Brown	22	8.5
	Green	55	21.2
	Black	85	32.7
Hilum color	Yellow	37	14.2
	Brown	3	1.2
	Black	220	84.6

In quantitative traits, mean values (coefficients of variability, CV) of days to flowering (days), days to maturity (days), pod length (mm), pod width (mm), seed length (mm), seed width (mm), and 100-seed weight were 53.68 (11.13), 129.92 (9.10), 43.28 (16.89), 10.56 (16.89), 8.74 (12.66), 7.58 (12.30), and 25.90 (34.94), respectively. Among them, 100-seed weight was the highest at 34.9% and days to maturity was the lowest at 9.10% in their CVs (Table 4). Song et al. (1991) and Kwon et al. (1974) reported that CVs of days to flowering, days to maturity, and 100-seed weight were 6.3, 5.6, 30.3, and 7.68, 5.89, 29.91, respectively. Yoon (1998) also reported those values were 11.6, 9.9, 42.6, respectively. CVs of this study were similar to values of Yoon (1998) but higher than those of Song et al. (1991) and Kwon et al. (1974). In this regard, it is supposed that the core set of this study covers variation of the initial set of Korean soybean landraces.

Genetic diversity

In the initial set, a total number of 115 alleles ranging from nine alleles (Satt187) to 43 alleles (Sat_074) with a mean allelic richness of 19.2 were recorded for the six loci. Among them, 30 alleles had a frequency higher than 5%. All these major alleles

Table 4. Phenotypic variation for the qualitative characters of core set.

Characters	Average	Maximum	Minimum	Range	SD ^a	CV (%) ^b
Days to flowering (days)	53.68	71.00	34.00	37.00	5.97	11.13
Days to maturity (days)	129.92	146.00	82.00	64.00	11.82	9.10
Pod length (mm)	43.28	60.65	26.64	34.02	7.31	16.89
Pod width (mm)	10.56	18.27	7.54	10.73	1.78	16.89
Seed length (mm)	8.74	11.57	5.98	5.59	1.11	12.66
Seed width (mm)	7.58	9.40	5.40	4.00	0.93	12.30
100-seed weight (g)	25.90	50.76	10.31	40.45	9.05	34.94

^a SD: Standard deviation.

^b CV (%): Coefficient of variability.

were also present in the core set. Out of 85 rare alleles with a frequency lower than 5% in the initial set, 49 (58.7%) alleles were present in the core set (Table 5).

Table 5. The number of alleles in the initial set and the core set.

	Initial Set	Core Set (%)
Total Number of alleles	115	79 (68.7)
Number of Major alleles	30	30 (100.0)
Number of Rare alleles	85	49 (58.7)

In comparison among usage groups, gene diversity indices of the core set were highest in sprouting soybean (0.808) and lowest in soybean for cooking with rice (0.698). Mean allelic richness of the core set was highest in others (12.2) and lowest in soybean for cooking with rice (9.8) (Table 6).

Table 6. Observed number of alleles, gene diversity in the initial set (IS) and in the core set (CC) by usage groups.

Group ^a	Sample Size		Observed number of alleles		Nei's (1973) gene diversity	
	IS	CC	IS	CC	IS	CC
SAU	684	62	13.5	11.3	0.692	0.781
SPR	964	61	16.3	10.5	0.666	0.808
COO	252	71	11.0	9.8	0.643	0.698
OTH	865	66	14.6	12.2	0.679	0.773

^a Group = SAU: Sauce soybean, SPR: Sprouting soybean, COO: Soybean for cooking with rice, OTH: Others.

Gene diversity indices were 0.699 and 0.778 for the initial set and the core set, respectively. Gene diversity values varied from 0.351 (Satt187) to 0.941 (Sat_074) in the initial set, and from 0.474 (Satt187) to 0.947 (Sat_074) in the core set (Table 7). The mean allelic richness of the core set (13.2) is a little higher than those of other reports (Abe et al. 2003; Fu et al. 2007; Wang et al. 2006). The allele coverage of the core set was 71.4% of the initial set. Brown (1989) assumed that over 70% of the alleles present in the whole collection would generally be retained in 10% of the total collection size based on the theory of selectively neutral alleles. In this study, 71.4% of the alleles of the initial set were present in the core set, accessions of 9.4% of the initial set were retained in the core set, and thirty major alleles of the

initial set were retained in the core set (Table 7). These results showed that this core set well represented the initial set on the basis of Brown's assumption.

Table 7. The number of alleles per locus, mean number of effective alleles, Nei's gene diversity and coverage in the initial set (IS) and in the core set (CC).

Marker	NA ^a		NE ^b		H ^c		Coverage ^d	
	IS	CS	IS	CS	IS	CS	IS	CS
Sat_074	43	27	17.0	13.9	0.941	0.947	100	62.8
Satt187	9	6	1.5	2.8	0.351	0.474	100	93.3
Satt197	16	13	3.9	5.7	0.745	0.838	100	66.7
Satt532	13	10	5.0	6.6	0.800	0.831	100	81.3
Satt141	15	14	2.6	5.9	0.617	0.778	100	47.4
Satt286	19	9	3.8	4.5	0.737	0.803	100	76.9
Mean	19.2	13.2	5.6	6.6	0.699	0.778	100	71.4

^a NA: Observed number of alleles.

^b NE: Mean number of Effective alleles.

^c H: Nei's (1973) gene diversity.

^d Allele coverage

This core set is supposed to provide an opportunity to evaluate agronomic traits, functional components, and resistance to abiotic and biotic stresses to identify diverse germplasm with beneficial traits for enhancing the genetic potential of soybean. It also can be used for more cost-effective molecular characterization studies.

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