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Analysis of Genetic Variation in Soybean Flowering Time Based on Agronomic Performance and SSR Markers

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ABSTRACT
The aim of this study is to evaluate the genetic variation in flowering time among soybean cultivar groups based on flowering character and simple sequence repeats marker, analyze linkage disequilibrium, and identify marker-trait association.

KEYWORDS
Stem cutting; Spacing; Tossa jute; Seed yield.

1. Introduction

Soybean cultivars have been originated from different regions and production areas based on their responsiveness to environment during the growing season. Soybeans are classified as short-day plants [9] and are assigned to maturity groups according to the time required to maturity at specified latitudes. Days to flowering in soybean not only depend on cultivars but also on environmental conditions [13]. Photoperiod influences the development of soybeans from time of emergence to anthesis [3], [10]. Day length and temperature are considered two important factors regulating flowering time in soybean cultivars.

In order to evaluate soybean genetic resources at the DNA level, utilization of soybean simple sequence repeats (SSRs) marker to classify cultivars and investigation of genetic diversity is necessitated. Simple sequence repeat (SSR) marker belong to the family of repetitive non-coding DNA sequence. Microsatellite marker has been applied to many studies, such as variety identification, genetic patterns, phylogenesis, and so on. High rates of polymorphism ensure that SSR markers are efficient for germplasm evaluation and in marker-assisted breeding programmes.

2. Materials and Methods

2.1 General Environmental Conditions

All cultivar groups were grown under field conditions. The first experiment was conducted in Suwon, Korea at 127o02′ E longitude and

37o26′ N latitude. The second experiment was planted in Can Tho city, Vietnam at 105O54′E longitude and 10O9′N latitude.

The mean temperature in Vietnam was higher and less fluctuant than the mean temperature in Korea. The lowest temperature about 26oC falled in January, it then gradually increased and reached the highest in April about 29oC in Can Tho, Vietnam. The temperature influenced the growth and development of soybean crop. Seeds germinated rapidly, and soybean plant developed well under high temperature.

In Korea, the mean temperature varied from 14oC (in October) to 27oC (in August). The germination of seed was a little slow due to low temperature at the end of May. Then, the mean temperature gradually increased to 27oC in August. All soybean cultivars grew and developed well. However, the mean temperature declined rapidly about 14oC to 10oC at the end of season, this caused some soybean cultivars incompletely matured.

The photoperiod at Can Tho, Vietnam including the civil twilight ranged from 11:50 hours to 12:05 hours during soybean production season from January to April. This condition made soybean cultivars flower early about one month after sowing. Meanwhile, the photoperiod at Suwon, Korea was from 11 hours to 14:50 hours. The longest photoperiod was about 14:50 hours in June. Soybean plants responded to long-day condition, so the floweringtime of soybean cultivars was delayed more than one month as compared with Vietnamese condition. In general, temperature and photoperiod affect during soybean growth and development. All stages of soybean plant are almost influenced by temperature, but the soybean plant does not appear to be sensitive to photoperiod at all time during its development [1].

2.2 Soybean Plant Material

A total of 108 soybean cultivars collected from different geographic regions were used in this study. They were classified into two groups, one from low latitude region (Vietnamese cultivars) and the other from high latitude regions (Chinese, Japanese, and Korean cultivars).

2.3 DNA Extraction

Genomic DNAs were isolated from frozen fresh leaf tissue of ten seedlings (10-12 days old) for each cultivar. Sampled leaf tissues were ground in liquid nitrogen and stored at –70oC. DNA was extracted according to the protocol described by Shure et al. (1983) with some modifications. The precipitated DNA was dissolved in 10mM TE buffer and stored at –20oC until use. The final DNA concentration was adjusted to 20ng/ml for PCR reaction.

2.4 PCR Amplification of SSR loci

PCR amplification was performed on each of the 108 soybean genotypes, using primers for each SSR locus. Reaction mixtures contained 20ng of soybean genomic DNA, 5㎛M 3′ and 5′ end primers, 2.5M dNTPs, 10X PCR buffer containing 2mM MgCl2, and 2 unit of Taq DNA polymerase, in a total volume of 8㎛L. All PCR reactions consisted of 1 cycle of 12 sec with incubation at 94oC, 32 cycles of 25 sec of denaturation at 94oC, 25 sec of annealing at 46oC and 25 sec of extension at 68oC, and final step for ever at 4oC, on MJ Research model PTC 225 Peltier thermocycler (MJ Research, Watertown, Mass.).

2.5 Microsatellite Allele Sizing

One-hundred and twenty six SSR loci with tri-nucleotide repeats (att) and di-nucleotide repeats (ct_) or (at_) motifs described by Tasma and Shoemaker (2003) [27] were tested for genotyping assays. The sequences of the Forward and Reverse primers are available at the soybean website USDA-ARS Soybean Genome Database (http://129.186.26.94/SSR.html).

PCR-amplified fragments from differentially labeled SSR primers, and with non-overlaping fragment sizes were simultaneously analyzed in the same gel lane and separate on an ABI Prism 377 DNA sequencer in National Instrumentation Center for Environmental Management (NICEM), Seoul

National University. Gene Scan® software and Genotyper® software (Applied Biosystems, Perkin Elmer) were used for automated data collection and to determine the allele sizes in base pairs (bp), based on the internal standard.

3. Data Analysis

To understand the genetic variation of flowering time, days to flowering (R1 stage), and maturity (R8 stage) were evaluated. In addition, the length from R1 to R8 stage was calculated at two differental experimental sites. Genetic diversity was measured by evaluating the differences in allele number per locus, allele frequency, allele sizes, polymorphic information content, and genetic diversity (GD). To measure the informativeness of each SSR locus, the PIC's values, was calculated using the following formula [25]:

PIC = 1 – pij2

Where pij is the frequency of the jth allele for ith locus summed across all alleles in the locus.

To evaluate molecular variance within and among populations, GenAlEx software [20] was used. In addition, TASSEL software [5] was applied to analyze linkage disequilibrium. Strucutre version 2.0 software [21] was used for analyzing population structure.

4. Results

Agronomic Performances

Evaluation of quantitative characteristics revealed that considerable variation was found among soybean cultivars within and among groups. Days to flowering (R1 stage) varied from cultivar to another (Table 1).

Four groups had flowering-time from twenty-eight to thirty-five days, when they were grown in low latitude region. Days to flowering varied from 28 days for Japanese and Korean cultivar groups to 35 days for Vietnamese cultivar group. This period varied from fifty to ninety days after sowing when they were grown in high latitude region (Table 1).

Table 1. This period varied from fifty to ninety days after sowing when they were grown in high latitude region

Agronomic	Parameter	Chinese group			Japanese group			Vietnamese group		
Performance	Latitude	10°N*	37°2N**	Differ***	10°N*	37°2N**	Differ***	10°N*	37°2N**	Differ***
Days to Flowering	Mean	29±4.32	50±3.92	21±4.40	28±0.82	51±5.95	23±6.87	35±4.9	91±11.89	56±12.3
(R1 Stage)	CV%	14.81	7.82	16.56	2.97	11.72	13.75	13.97	13.14	14.83
Maturity	Mean	84±2.69	129±5.3	45±6.88	80±5.39	137±9.61	57±10.5	84±7.5	159±12.1	75±12.2
(R8 Stage)	CV%	3.22	3.94	8.14	6.72	7.03	12.71	8.88	7.61	7.65
Length of	Mean	55±5.08	79±6.1	24±6.96	53±5.1	86±8.9	33±10.6	49±5.1	68±10.45	19±13.6
R1- R8	CV%	9.32	7.67	10.8	9.67	10.36	13.41	10.24	15.35	18.61

Remarks:
10°N*: cultivars grown in Vietnam.

37°2N**: cultivars grown in Korea.
Differ**: Difference days to flower between Korea and Vietnam.

Table 2. Analysis of molecular variance.

Source of variance	df	SS	MS	Est. Var.	%	Value	*P < 0.01*
Among pop.	3	323813.8	107937.9	1727.6	10	7.253***	3.88
Within pop.	212	3154873.0	14881.5	14881.5	90		

Table 3. Amplified band number through 126 loci in four cultivar groups.

Linkage groups	Loci	Distance (cM)	r^2
A2	Satt177	36.770	0.256
	Satt315	45.291	0.321
	Satt187	54.917	0.244
	Satt341	77.695	0.175
	Satt327	109.828	0.307
	Satt470	116.731	0.263
	Satt409	145.565	0.088
	Satt429	162.033	
C1	Satt190	73.324	0.360
	Satt399	76.228	0.201
	Satt294	78.645	0.156
	Sat_042	82.506	0.111
	Satt524	120.115	0.156
	Satt180	127.774	
D2	Satt135	26.049	0.231
	Satt014	29.562	0.251
	Satt498	32.144	0.187
	Satt372	39.345	0.245
	Satt154	57.070	0.107
	Satt208	67.910	0.226
	Satt397	69.296	0.217
	Satt389	79.231	0.231
	Satt226	85.148	0.136
	Satt386	124.998	
L	Satt495	0.000	0.236
	Satt388	23.547	0.207
	Satt143	30.187	0.234
	Satt398	30.581	0.184
	Satt006	92.001	0.245
	Satt513	106.366	0.201
	Satt373	107.240	

Of 20 observed linkage groups, four linkage groups were chosen to analyze LD with 7-10 loci for each linkage group. The higher r2 values, the more linkage disequilibrium (Table 4). r2 value depends on locus distance, linkage group, and number of observed loci. The low r2 value between two loci indicates weak linkage between these loci or between locus and interested gene. This is very important when using marker to detect interested gene for improving certain trait.

5. Cluster Analysis

Two types of clustering methods were used. One was distance-based method, and the other was model-based method [21]. The model-based method with Baysesian approach provides a coherent framework for incorporating the inherent uncertainty of parameter estimates into the inference procedure and for evaluating

the strength of evidence for the inferred clustering. This approach used model of Markov chain Monte Carlo (MCMC) to compute exactly the distribution of parameter estimates.

Nei's genetic distance of soybean cultivar groups was presented in Figure 1. Vietnamese and Chinese goups were far in distance, meanwhile Japanese and Chinese groups were near in distance.

Model-based method by Bayesian clustering approach gave the same result of distance-based method. Figure 1 illustrated for these results. The genetic relation of four cultivar groups explained that Vietnamese and Chinese genotype groups showed far distance, as well as Korean and Chinese genotype groups. 18 Chinese genotypes were similar to Japanese genotypes. Chinese and Japanese cultivar group could be mixed. Meanwhile, Korean and Vietnamese cultivar groups were separated. In breeding, the genetic advance only gained when two parents used for crossing were far genetic distance.

6. Discussion

Flowering times of field-grown soybean are complicated process including the interaction between genotype and environmental factor (i.e temperature, photoperiod), and the impact of internal clues to physiological process [1].

Significant differences among soybean cultivar groups indicating that there is genetic variation of this trait in soybean. Days to flowering is not only influenced by environmental factors (photoperiod and temperature) but also by genetic factor. Our results showed that cultivar group originated from low latitude was more photo-sensitive than the others from high latitude. The country or latitude of origin of an accession has limited use in predicting adaptation to a specific environment as might be expected if photoperiod responses were all important [6], and the effects of temperature on photoperiod insensitive genotypes can be so pronounced that their supposedly broad-adaptation to a wide range of latitudes is open to question [26]. In our study, Vietnamese soybean cultivars belonged to photoperiodsensitive groups as they prolonged flowering time in temperate condition. Meanwhile, cultivar groups of Korean, Japanese, and Chinese were less photoperiod-sensitive.

SSRs marker are advantageous to applied plant breeding because they are co-dominant, easily assayed and detected high level of polymorphism [17]. SSRs marker detected genetic variation of flowering time, primers related to candidate genes controlling flowering time generated high polymorphism loci. These polymorphism of SSR loci detected in this study was consistent with previous studies by Akkaya (1992) [2], Morgante and Olivieri (1994) [18], Maughan et al. (1995) [16], Doldi et al. (1997) [8], Diwan and Cregan (1997) [7], and Narvel et al. (2000) [19].

The obtained genetic diversity (GD) was in a good agreement with the data of Rongwen et al. (1995) [23], who found a mean value of 0.74 in a group of 96 soybean genotypes. It is in line with the results of Diwan and Cregan (1997) [7], who found mean GD values close to 0.69 in a group of 36 commercial soybean lines, and in agreement with the data of Narvel et al. (2000) [19], who detected a mean value of 0.50 0.02 in a group of 39 elite cultivars.

Distance-based methods are usually easy to apply and are often visually appealing. In the genetics literature, it has been common to adapt distancebased phylogenetic algorithms, such as neighbor-joining to clustering multilocus genotype data (Bowcock et al. 1994) [4]. Model-based method with Bayesian clustering approach can be applied to various types of markers like RFLPs, SSRs, SNPs…. But it assumes that the markers are unlinked and at linkage equilibrium with one another within populations. It also assumes Hardy-Weinberg equilibrium within populations (Pritchard et al., 2000) [21]. In our study, two methods gave the same result. In addition, model-based method provided information to explain the population genetic structure, this can help us well understand evolution and origin of cultivars as well as species.

In conclusion, understanding the geographical distribution and genetic variation of cultivars will provide useful information and abundant source genes for soybean breeding program to make new cultivars adapting to a wide range of ecological regions

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