Genetic Diversity of Soybean Cultivars from China, Japan, North America, and North American Ancestral Lines Determined by Amplified Fragment Length Polymorphism

George N. Ude, William J. Kenworthy,* Jose M. Costa, Perry B. Cregan, and Jennie Alvernaz

ABSTRACT

Asian soybean [*Glycine max* (L.) Merr.] improvement programs have been conducted for many years almost completely independent of U.S. breeding programs. Productive, modern Asian cultivars may be a promising source of new yield genes for U.S. breeding programs. However, this hypothesis has not been tested. The objectives of this study were to determine the level of genetic diversity within and between Asian and North American soybean cultivars (NASC) by amplified fragment length polymorphism (AFLP) analysis and to identify Asian cultivars with significant genetic difference from NASC. The genetic diversity and relationships were assessed among 35 North American soybean ancestors (NASA), 66 high yielding NASC, 59 modern Chinese cultivars, and 30 modern Japanese cultivars. Five AFLP primer-pairs produced 90 polymorphic (27%) and 242 monomorphic AFLP fragments. Polymorphic information content (PIC) scores ranged from zero to 0.50. Only 53 of the 332 AFLP fragments provided PIC scores ≥0.30. Genetic distance (GD) between pairs of genotypes was calculated on the basis of the similarity indices determined by the 332 AFLP fragments. Within each of the cultivar groups, the average GD between pairs of genotypes was 6.3% among the Japanese cultivars, 7.1% among the NASC, 7.3% among the NASA, and 7.5% among the Chinese cultivars. The average GD between the NASC and the Chinese cultivars was 8.5% and between the NASC and the Japanese cultivars was 8.9%. Although these distances were not significantly different, they were greater than the average GD between all pairs of NASC (7.1%). Clustering and principal coordinate analysis using all 332 fragments showed a separation of the cultivars into three major groups according to their geographic origin. North American soybean ancestors overlapped with all three cultivar groups. The Japanese cultivars were more removed from NASA and NASC than the Chinese cultivars and may constitute a genetically distinct source of useful genes for yield improvement of NASC.

Soybean is one of the world's most important oil and protein crops. By selection and hybridization, breeders in the USA have increased soybean yield by at least 20% (Fehr, 1984). More than 300 publicly developed cultivars have been released in North America in the past 50 yr (Thompson and Nelson, 1998b). However, it has been observed that the use of only a few plant introductions and intensive plant breeding have narrowed the genetic diversity among North American elite soybean cultivars (Gizlice et al., 1994; Sneller, 1994).

The genetic similarity among NASC has reached a level that could limit continued breeding success. Introduction of new sources of germplasm into the breeding pool may provide the genetic variability to permit continued progress in developing high yielding cultivars. Though plant introductions (PIs) provide genetic variability, they are less frequently used as sources of new yield genes than current cultivars and elite lines because they often yield less. Populations developed from crossing cultivars with PIs, which have been selected for good phenotypic traits, generally have a lower mean yield and lower frequency of desirable lines than those populations developed from crossing elite parents (Vello et al., 1984; Ininda et al., 1996). Recent studies have used molecular markers to help identify genetically diverse PIs to use in crosses in cultivar improvement programs (Thompson and Nelson, 1998a,b; Thompson et al., 1998; Narvel et al., 2000). These studies have had more success than conventional selection programs in producing productive lines from PI crosses with elite genotypes. Modern Asian cultivars, which share no ancestors with NASC, represent a potential reservoir of new alleles available for improving U.S. soybean yield, and is a different approach than using other germplasm in crosses with NASC.

Acquisition of soybean germplasm from Asia has increased over time, though not all the introduced cultivars or germplasm have been assessed for their usefulness in soybean improvement. There is a need for extensive evaluation of new germplasm from Asia to determine its genetic diversity and to identify Asian lines to serve as sources of unique genes for U.S. soybean yield improvement.

Conventional molecular marker analysis using restriction fragment length polymorphism (RFLP) (Apuya et al., 1988), ribosomal DNA (Doyle and Beachy, 1985), and random amplified polymorphic DNAs (RAPDs) (Williams et al., 1990) have identified only low levels of genetic diversity in cultivated soybean. Microsatellite markers can detect higher levels of genetic diversity among soybean cultivars but this marker system requires the synthesis of primers and construction of genomic libraries (Maughan et al., 1996). AFLP is a PCR-based, molecular technique that detects high numbers of polymorphic bands (Powell et al., 1996). AFLPs are detected frequently in soybean, are inherited in a stable Mendelian fashion, and exhibit high levels of diversity (Maughan et al., 1996). The objectives of this study were to determine the level of genetic diversity within and between Asian and NASC by AFLP analysis and to

G.N. Ude, W.J. Kenworthy, and J.M. Costa, Dep. of Natural Resource Sciences and Landscape Architecture, Univ. of Maryland, College Park, MD 20742; P.B. Cregan, USDA-ARS, Soybean Genomics and Improvement Lab., Beltsville, MD 20705; J. Alvernaz, Dep. of Crop and Soil Sciences, Univ. of Georgia, Athens, GA 30602. This research was supported in part by a grant from the United Soybean Board. The research was part of a dissertation submitted by the senior author in partial fulfillment of the Ph.D. degree. Received 5 Feb. 2002. *Corresponding author (wk7@umail.umd.edu).

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677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: AFLP, amplified fragment length polymorphism; GD, genetic distance; NASA, North American soybean ancestors; NASC, North American soybean cultivars; PIC, polymorphic information content; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism.

identify Asian cultivars with significant genetic difference from NASC.

MATERIALS AND METHODS

A sample of 59 Chinese and 30 Japanese cultivars that have no known NASC in their ancestry were compared with 66 high yielding NASC and 35 NASA genotypes to estimate the level of genetic variability between and within the groups. Twenty micrograms each of the 190 soybean DNA samples were extracted according to the procedure of Keim et al. (1988). Some of the soybean genotypes (Table 1) used in this study were also evaluated in field plots as part of a cooperative effort by USDA-ARS, North Carolina State University, Pioneer Hi-Bred International, Asgrow Seed Company, and the Universities of Arkansas, Georgia, Illinois, Maryland, and Minnesota (Manjarrez-Sandoval et al., 1997).

The AFLP procedure was performed according to Lin et al. (1996) with the AFLP primer starter kit and the core reagent kit supplied by Life Technologies Inc., Gaithersburg, MD. Primary template DNA was prepared by completing a restriction enzyme digest followed by an adaptor ligation. Five hundred nanograms of DNA from each of the 190 genotypes was digested with 2 μL of *Eco*RI/*Mse*I (1.25 units of *Eco*RI/μL and 1.25 units of *Mse*I/μL) at 37°C for 2 h, and then heated to 70°C for 10 min to inactivate the enzymes. In addition to the DNA and enzymes, the following were added to a 1.5-mL microcentrifuge tube: 5 μL of 5× reaction buffer and AFLP-grade water to a final volume of 25 μL. The DNA fragments were ligated to *Eco*RI and *Mse*I adapters provided in the kit. The ligation mixture (containing fragments with adapters at both ends) was diluted 10-fold with sterile distilled water and held at −20°C in a freezer until used.

The 10-fold diluted ligation mixture was preamplified by 20 PCR cycles. The PCR reaction was performed in a thermal cycler with the following temperature profile: 94°C for 30 s, 56°C for 60 s, and 72°C for 60 s using *Eco*RI+A(5′GACTGC GTACCAATTC+A3′) and *Mse*I+C(5′GATGAGTCCT GAGTAA+C3′) primers (provided in the kit) described by Vos et al. (1995). Five primer combinations, E-ACT/M-CAT, E-ACC/M-CAA, E-AAG/M-CTT, E-ACA/M-CAC, and E-AGC/M-CTC, were chosen from a pool of primer combinations that produced seven or more polymorphic bands among the parents of the cross PI290136 × BARC-2 (*Rj4*) (Ude et al., 1999) and were used for fingerprinting the 190 genotypes in this study.

One hundred-twenty microliters of distilled water was added to 5 μL of each of the preamplified DNA to make a 1:24 dilution from which 5 μL was used for selective amplification. Selective amplification was conducted in 5 μL-aliquots of the diluted preamplified fragments with ^{32}P-ATP labeled *Eco*RI+3 primer with an unlabeled *Mse*I+3 primer. Amplification was done by PCR with one temperature cycle at 94°C for 30 s, 65°C for 30 s, and 72°C for 60 s, followed by lowering the annealing temperature each cycle 0.7°C for 12 cycles. At the end of the 12 cycles, the reaction was programmed to amplify for 23 cycles at 94°C for 30 s, 56°C for 30 s, and 72°C for 60 s. The reaction products were loaded on a 5% (w/v) polyacrylamide DNA sequencing gel containing 7.5 *M* urea. Ten base pair (bp) DNA ladder (Cat. No. 10821-015) purchased from Life Technologies Inc., Gaithersburg, MD, was used as a molecular weight standard in every gel. Autoradiography was performed by exposing Kodak Bio-Max MR-2 film (Eastman Kodak Co., Rochester, NY) to the dried gel at room temperature for 48 h.

The gel autoradiographs were scored visually for polymorphism. A band was considered polymorphic if it was present in at least one soybean genotype and absent in others. A matrix was generated in which each band was scored as "1" if present and as "0" if absent for each genotype. Polymorphic information content (*PIC*); the fraction of polymorphic loci (β); the arithmetic mean heterozygosity (H_{av}); the effective multiplex ratio (*ER*); the marker index (*MI*), and the average expected heterozygosity for polymorphic markers [$H_{av(p)}$] for each of the primer combinations were estimated according to Powell et al. (1996).

The sum of polymorphic heterozygosity (ΣH_p) is the sum of the polymorphic information content for all loci for each primer pair ($\Sigma H_p = \Sigma PIC$) and the fraction of polymorphic loci (β) is the number of polymorphic loci (n_p) divided by the sum of polymorphic (n_p) and nonpolymorphic loci (n_{np}) [$\beta = n_p/(n_p + n_{np})$]. The arithmetic mean heterozygosity (H_{av}) is the product of the fraction of polymorphic loci (β) and the polymorphic heterozygosity (H_p) divided by the number of polymorphic loci (n_p) ($H_{av} = \beta \Sigma H_p/n_p$).

The effective multiplex ratio (E) is defined as the product of the total number of loci per primer (n) and the fraction of polymorphic loci (β) ($E = n\beta$). The marker index (*MI*) is the product of the total number of loci per primer pair (n) and the arithmetic mean heterozygosity (H_{av}) ($MI = nH_{av}$). The marker index (*MI*) can also be defined as the product of effective multiplex ratio (E) and the average expected heterozygosity [$H_{av(p)}$] for the polymorphic markers [$MI = EH_{av(p)}$ where $H_{av(p)} = MI/(n \times \beta)$ and $H_{av(p)} = MI/E$].

Genetic similarities between pairs of genotypes were estimated with 332 monomorphic and polymorphic bands by means of simple matching coefficients (Powell et al., 1996) in the NTSYS-pc software package version 2.02f (Rohlf, 1998). Genetic distances were calculated by subtracting the similarity indices from 1 and multiplying the result by 100. Student's t tests ($P = 0.05$) were used to compare the average genetic distances within and among the groups of soybeans studied. A dendrogram based on the similarity coefficient matrix and unweighted pair group method of the arithmetic average clustering was produced. Principal coordinate analysis was also done to show multiple dimensions of the distribution of the genotypes in a scatter-plot (Keim et al., 1992). Genetic distances calculated with monomorphic and polymorphic markers are about one third that calculated with only polymorphic bands (Becker et al., 1995).

RESULTS AND DISCUSSION

Primer Utility

The AFLP primer pairs used in this study were selected on the basis of our previous soybean studies (Ude et al., 1999). The five primer pairs revealed a total of 332 different bands that were of sufficient intensity to score (Table 2). The band sizes ranged from 50 to 500 bp but only 90 (27%) were polymorphic. The PIC scores ranged from zero for nonpolymorphic loci to 0.50 (Table 2). Average PIC score for the 332 AFLP bands was 0.10. Fifty-three polymorphic bands showed PIC scores ≥0.30 indicating that only 16% of the 332 AFLP bands contributed significantly to the genetic discrimination of the 190 soybean genotypes studied. A PIC score ≥0.30 has been used previously by Keim et al. (1992) and Lorenzen et al. (1995) with RFLP probes and by Thompson and Nelson (1998b) with RAPD fragments to determine usefulness in other soybean germplasm diversity studies.

Table 1. Name, code, PI number, maturity group, country of origin, average genetic distance (AGD), classification, and clusters based on the UPGMA clustering of the 190 soybean lines studied.

Code	Name	PI no.	MG	Country	AGD†	Classification	Cluster
A1		FC31745	VI	Unknown	8.6	North American ancestor	a
A2		PI71506	IV	China	8.0	North American ancestor	a
A3		PI88788	III	China	9.1	North American ancestor	d
A4	A.K. (Harrow)	PI548298	III	China	7.5	North American ancestor	a
A5	Anderson	FC33243	IV	Unknown	7.3	North American ancestor	b
A6	Arksoy	PI548438	VI	Korea, North	7.9	North American ancestor	a
A7	Bansei	PI548302	II	Japan	7.9	North American ancestor	a
A8	Bilomi No. 3	PI240664	X	Philippines	8.0	North American ancestor	a
A9	Capital	PI548311	O	Canada	7.1	North American ancestor	a
A10	CNS	PI548445	VII	China	7.8	North American ancestor	a
A11	Dunfield	PI548318	III	China	8.4	North American ancestor	a
A12	Fiskeby 840-7-3	PI438477	O	Sweden	7.8	North American ancestor	a
A13	Fiskeby III	PI438471	O	Sweden	8.5	North American ancestor	a
A14	Fiskeby V	PI360955A	O	Sweden	7.3	North American ancestor	a
A15	Flambeau	PI548325	O	Russian Federation	7.1	North American ancestor	b
A16	Haberlandt	PI548456	VI	Korea, North	8.7	North American ancestor	a
A17	Illini	PI548348	III	China	7.4	North American ancestor	a
A18	Improved Pelican	PI548461	VIII	USA	8.0	North American ancestor	a
A19	Jackson	PI548657	VII	USA	7.0	North American ancestor	a
A20	Jogun	PI548352	III	Korea, North	7.6	North American ancestor	a
A21	Kanro	PI548356	II	Korea, North	8.0	North American ancestor	a
A22	Korean	PI548360	II	Korea, North	8.7	North American ancestor	d
A23	Lincoln	PI548362	III	USA	7.0	North American ancestor	b
A24	Mandarin (Ottawa)	PI548379	O	China	7.1	North American ancestor	a
A25	Manitoba Brown	PI153217	O	Unknown	8.0	North American ancestor	a
A26	Mejiro	PI080837	IV	Japan	8.2	North American ancestor	a
A27	Mukden	PI548391	II	China	7.8	North American ancestor	a
A28	Ogden	PI548477	VI	USA	8.4	North American ancestor	a
A29	Peking	PI438497	III	China	10.3	North American ancestor	d
A30	Perry	PI548603	IV	USA	6.8	North American ancestor	a
A31	Ralsoy	PI548484	VI	Korea, North	8.2	North American ancestor	a
A32	Richland	PI548406	II	China	8.0	North American ancestor	a
A33	Roanoke	PI548485	VII	Unknown	7.9	North American ancestor	a
A34	S-100	PI548488	V	Unknown	7.4	North American ancestor	a
A35	Strain No. 18	PI180501	O	Germany	7.3	North American ancestor	a
U1	Agassiz	PI562372	O	USA	7.3	North American cultivar	a
Us2	Bay	PI553043	V	USA	7.4	North American cultivar	b
Us3	Benning	PI595645	VI	USA	7.3	North American cultivar	b
Us4	Braxton	PI548659	VII	USA	7.3	North American cultivar	b
Us5	Brim	PI548986	VI	USA	6.0	North American cultivar	b
U6	BSR 201	PI548521	II	USA	7.4	North American cultivar	b
U7	Burlison	PI533655	II	USA	7.9	North American cultivar	b
U8	Century	PI548512	II	USA	7.1	North American cultivar	b
U9	Cisne	PI593256	IV	USA	5.3	North American cultivar	b
U10	CN290	PI518677	II	USA	7.6	North American cultivar	b
U11	Conrad	PI525453	II	USA	6.6	North American cultivar	b
Us12	Cook	PI553045	VIII	USA	7.0	North American cultivar	b
U13	Dassel	PI508083	O	USA	7.8	North American cultivar	a
U14	Dawson	PI542403	O	USA	6.7	North American cultivar	a
Us15	Dillon	PI592756	VI	USA	6.1	North American cultivar	b
U16	Evans	PI548560	O	USA	7.2	North American cultivar	a
Us17	Gail	PI548978	VI	USA	8.2	North American cultivar	b
Us18	Gasoy 17	PI553046	VII	USA	7.5	North American cultivar	b
U19	Glacier	PI592523	O	USA	8.0	North American cultivar	a
U20	Glenwood	PI513382	O	USA	7.9	North American cultivar	a
Us21	Gordon	PI553047	VII	USA	8.0	North American cultivar	b
Us22	Graham	PI594922	V	USA	6.8	North American cultivar	b
U23	Hack	PI548569	II	USA	7.0	North American cultivar	b
U24	Harlon	PI548571	I	Canada	7.9	North American cultivar	d
Us25	Haskell	PI572238	VII	USA	8.0	North American cultivar	b
U26	Hoyt	PI540552	II	USA	6.0	North American cultivar	b
Us27	Hutcheson	PI518664	V	USA	5.8	North American cultivar	b
U28	IA2021	x	II	USA	6.7	North American cultivar	b
U29	Iroquois	PI593259	III	USA	6.7	North American cultivar	b
Us30	Johnston	PI508267	VIII	USA	7.7	North American cultivar	b
Us31	Kershaw	PI548985	VI	USA	6.9	North American cultivar	b
U32	KS4694	PI586981	IV	USA	6.4	North American cultivar	b
U33	Lambert	PI562373	O	USA	6.7	North American cultivar	a
U34	Lawrence	PI518673	IV	USA	8.0	North American cultivar	b
Us35	Lloyd	PI533602	VI	USA	7.1	North American cultivar	b
U36	Logan	PI548591	III	USA	6.6	North American cultivar	b
U37	Macon	PI593258	III	USA	6.8	North American cultivar	b
U38	Manokin	PI559932	IV	USA	8.8	North American cultivar	d
U39	Maple Donovan	PI548642	O	Canada	6.9	North American cultivar	a
U40	Maple Glen	PI548643	O	Canada	7.4	North American cultivar	a
U41	Maple Isle	PI548595	O	Canada	6.2	North American cultivar	a

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Table 1. Continued.

Code	Name	PI no.	MG	Country	AGD†	Classification	Cluster
U42	Maple Presto	PI548594	O	Canada	7.0	North American cultivar	a
U43	Maple Ridge	PI548596	O	Canada	8.4	North American cultivar	a
U44	McCall	PI548582	O	USA	7.7	North American cultivar	a
Us45	Narow	PI553052	V	USA	6.1	North American cultivar	b
U46	OAC Aries	PI548637	O	Canada	8.1	North American cultivar	a
U47	OAC Libra	PI548638	O	Canada	7.8	North American cultivar	a
U48	OAC Musca	PI548644	O	Canada	6.6	North American cultivar	b
U49	OAC Pisces	PI548639	O	Canada	6.8	North American cultivar	b
U50	Ozzie	PI542404	O	USA	7.2	North American cultivar	b
U51	Parker	PI562374	I	USA	6.7	North American cultivar	b
U52	Pennyrile	PI515961	IV	USA	7.0	North American cultivar	b
Us53	Perrin	PI536637	VIII	USA	7.3	North American cultivar	b
Us54	Pershing	PI548604	IV	USA	6.6	North American cultivar	b
U55	Preston	PI548520	II	USA	7.6	North American cultivar	b
U56	Ripley	PI536636	IV	USA	6.4	North American cultivar	b
U57	Savoy	PI597381	II	USA	6.9	North American cultivar	b
U58	Sibley	PI508084	I	USA	6.4	North American cultivar	b
U59	Sprite	PI536635	III	USA	7.2	North American cultivar	b
U60	Sturdy	PI542768	I	USA	6.1	North American cultivar	b
Us61	Thomas	PI522236	VII	USA	7.3	North American cultivar	b
U62	TN 4-86	PI518668	IV	USA	6.8	North American cultivar	b
Us63	Toano	PI508268	V	USA	7.2	North American cultivar	b
U64	Weber	PI548524	I	USA	7.3	North American cultivar	b
Us65	Young	PI508266	VI	USA	6.2	North American cultivar	b
U66	Zane	PI548634	III	USA	5.8	North American cultivar	b
C1	7605	PI592942	III	China	8.6	Chinese cultivar	c
C2	Bai nong 1 hao	PI592925	O	China	7.8	Chinese cultivar	d
C3	Chen dou 4 hao	PI592927	II	China	7.9	Chinese cultivar	d
C4	Dan dou 5 hao	PI503334	III	China	9.2	Chinese cultivar	d
C5	De dou 1 hao	PI467317	I	China	9.9	Chinese cultivar	d
C6	Dong nong 37	PI503336	O	China	8.4	Chinese cultivar	d
C7	Dong nong 42	PI592917	O	China	8.6	Chinese cultivar	d
C8	Feng shou 21	PI592916	O	China	9.9	Chinese cultivar	d
C9	Fu dou 1 hao	PI592935	III	China	8.9	Chinese cultivar	d
C10	Gong dou 4 hao	PI592928	II	China	9.1	Chinese cultivar	d
C11	Guan dou 1 hao	PI592929	IV	China	7.8	Chinese cultivar	c
C12	He feng 30	PI592918	O	China	8.3	Chinese cultivar	d
C13	He feng 31	PI592919	O	China	7.4	Chinese cultivar	d
C14	He feng 33	PI592920	O	China	8.0	Chinese cultivar	d
C15	Hei he 9 hao	PI592915	O	China	8.3	Chinese cultivar	d
C16	Hei nong 29	PI518706A	O	China	8.1	Chinese cultivar	d
C17	Hei nong 37	PI592921	I	China	8.8	Chinese cultivar	d
C18	Hong feng 3	PI549076A	O	China	7.9	Chinese cultivar	d
C19	Hong feng 3 hao	PI592922	O	China	7.7	Chinese cultivar	d
C20	Huai dou 1 hao	PI532459	IV	China	9.0	Chinese cultivar	c
C21	Ji dou 4 hao	PI592946	IV	China	7.9	Chinese cultivar	d
C22	Ji dou 7 hao	PI592936	II	China	9.6	Chinese cultivar	d
C23	Jilin 18	PI518709	I	China	8.9	Chinese cultivar	d
C24	Jilin 20	PI518710	I	China	9.7	Chinese cultivar	d
C25	Jilin 21	PI518711	II	China	9.6	Chinese cultivar	d
C26	Jin dou 14	PI592937	IV	China	10.4	Chinese cultivar	d
C27	Jin dou 15	PI592938	II	China	10.1	Chinese cultivar	d
C28	Jin dou 16	PI592939	IV	China	6.7	Chinese cultivar	a
C29	Jin dou 17	PI592940	IV	China	6.2	Chinese cultivar	a
C30	Jin yi 10 hao	PI592948	III	China	8.7	Chinese cultivar	d
C31	Jin yi 9 hao	PI592947	IV	China	7.7	Chinese cultivar	d
C32	Jiu nong No. 13	PI467323A	O	China	8.0	Chinese cultivar	d
C33	Jiu feng 1 hao	PI549077	O	China	7.6	Chinese cultivar	d
C34	Jiu feng 2 hao	PI549078	O	China	8.5	Chinese cultivar	b
C35	Kai yu 8 hao	PI518712	II	China	8.6	Chinese cultivar	d
C36	Ken nong 2 hao	PI592923	O	China	8.0	Chinese cultivar	d
C37	Ken nong 4 hao	PI592924	O	China	8.0	Chinese cultivar	d
C38	Liao dou 10 hao	PI592941	II	China	7.3	Chinese cultivar	d
C39	Liao nong 2 hao	PI518714	II	China	9.0	Chinese cultivar	d
C40	Lu dou 7 hao	PI518719	IV	China	8.0	Chinese cultivar	c
C41	Lu dou 4 hao	PI518718A	II	China	8.2	Chinese cultivar	b
C42	Lu dou 6 hao	PI592943	O	China	9.0	Chinese cultivar	d
C43	Nen feng 10 hao	PI511867	O	China	10.0	Chinese cultivar	d
C44	Nen feng 9 hao	PI511866	O	China	9.0	Chinese cultivar	d
C45	Nin zhen 1 hao	PI592954	II	China	7.8	Chinese cultivar	d
C46	Tie feng 22	PI592944	II	China	8.8	Chinese cultivar	d
C47	Tong nong 8 hao	PI592926	I	China	8.4	Chinese cultivar	d
C48	Tong nong 9 hao	PI503340	II	China	8.4	Chinese cultivar	d
C49	Xiang chun dou 12	PI592930	II	China	9.5	Chinese cultivar	d
C50	Yan huang 4 hao	PI592931	O	China	8.0	Chinese cultivar	d
C51	Yu dou 11	PI592950	IV	China	8.4	Chinese cultivar	c
C52	Yu dou 8 hao	PI592949	IV	China	7.1	Chinese cultivar	c

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Table 1. Continued.

Code	Name	PI no.	MG	Country	AGD†	Classification	Cluster
C53	Zao shu 14	PI592933	II	China	9.0	Chinese cultivar	b
C54	Zao shu 9 hao	PI592932	II	China	8.6	Chinese cultivar	d
C55	Zhe chun 2 hao	PI592934	II	China	8.4	Chinese cultivar	d
C56	Zheng 133	PI592951	IV	China	7.6	Chinese cultivar	a
C57	Zheng 77249	PI592952	III	China	8.5	Chinese cultivar	c
C58	Zhong dou 19	PI592953	IV	China	9.1	Chinese cultivar	c
C59	Zhong huang 1 hao	PI592945	III	China	9.5	Chinese cultivar	d
J1	Akishirome	PI506514	VI	Japan	8.8	Japanese cultivar	c
J2	Enrei	PI385942	IV	Japan	10.8	Japanese cultivar	c
J3	Fukunagaha	PI594166	III	Japan	7.8	Japanese cultivar	c
J4	Fukushirome	PI594167	III	Japan	8.3	Japanese cultivar	a
J5	Fukuyutaka	PI506675	VI	Japan	8.9	Japanese cultivar	c
J6	Gogaku	PI594172A	VII	Japan	9.0	Japanese cultivar	c
J7	Himeshirazu	PI594177	VIII	Japan	8.9	Japanese cultivar	c
J8	Himeyutaka	PI594178	I	Japan	9.5	Japanese cultivar	c
J9	Hourei	PI561394	IV	Japan	8.7	Japanese cultivar	c
J10	Hyuuga	PI506764	VII	Japan	8.2	Japanese cultivar	c
J11	Karumai	PI594193	III	Japan	8.3	Japanese cultivar	c
J12	Kitahomare	PI506895	I	Japan	7.9	Japanese cultivar	c
J13	Kitakomachi	PI594198	I	Japan	11.2	Japanese cultivar	d
J14	Misuzu daizu	PI423912	V	Japan	8.5	Japanese cultivar	c
J15	Mutsu shiratama	PI417171	IV	Japan	7.9	Japanese cultivar	c
J16	Nakasennari	PI561388	V	Japan	9.0	Japanese cultivar	c
J17	Nasu shirome	PI423914A	IV	Japan	8.4	Japanese cultivar	c
J18	Otsuru	PI594250	IV	Japan	9.7	Japanese cultivar	c
J19	Shirosennari	PI423893	IV	Japan	10.5	Japanese cultivar	c
J20	Suzuhime	PI594282	III	Japan	8.7	Japanese cultivar	c
J21	Suzukari	PI594283	IV	Japan	9.0	Japanese cultivar	c
J22	Suzumaru	PI593972	I	Japan	8.6	Japanese cultivar	a
J23	Tachikogane	PI594286	IV	Japan	8.7	Japanese cultivar	c
J24	Tachinagaha	PI561396	V	Japan	8.0	Japanese cultivar	c
J25	Tachiyukuta	PI594289	IV	Japan	8.4	Japanese cultivar	c
J26	Tanrei	PI594295	IV	Japan	8.7	Japanese cultivar	c
J27	Tokachikuro	PI594296	I	Japan	8.9	Japanese cultivar	c
J28	Toyokomachi	PI593973	I	Japan	8.0	Japanese cultivar	c
J29	Toyomusume	PI594301	I	Japan	8.3	Japanese cultivar	c
J30	Yuuhime	PI594319	I	Japan	10.5	Japanese cultivar	d

† AGD—Average genetic distance between this accession and the 66 North Amerian cultivars (NASC).

The average expected heterozygosity estimate for polymorphic markers [$Hav_{(p)}$] for each primer pair ranged from 0.15 to 0.37 with an average of 0.27 per primer (Table 2). The overall average expected heterozygosity estimate [$Hav_{(p)}$] for the 90 polymorphic AFLP markers was 0.30. The values of the average expected heterozygosity [*Hav*(p)] for the markers are in agreement with those previously reported in soybean for AFLPs (Powell et al., 1996), RFLPs (Keim et al., 1992), and RAPDs (Thompson and Nelson, 1998a). The primers E-ACC/M-CAA and E-ACT/M-CAT showed the highest average expected heterozygosity and produced the most informative DNA fragments for distinguishing among the genotypes. On the basis of this criterion, the primers E-AGC/M-CTC [$Hav_{(p)}$ = 0.27] and E-AAG/M-CTT [$Hav_{(p)}$ = 0.26] showed less discriminatory power than E-ACC/M-CAA and E-ACT/M-CAT, although they were better than E-ACA/M-CAC [$Hav_{(p)}$ = 0.15]. Multiplex ratio, which is the number of different genetic loci that may be scored in a gel using a primer combination, ranged between 46 and 83. Effective multiplex ratio, which is the number of polymorphic loci per primer combination, ranged from 7 to 34 (Table 2).

Marker index (MI) is the statistic used to calculate the overall utility of a marker system and is the product of expected heterozygosity and multiplex ratio. The primers E-AGC/M-CTC and E-ACA/M-CAC had low marker indices (2.16 and 1.11, respectively), while the other primers [E-AAG/M-CTT (9.14), E-ACC/M-CAA (8.24), and E-ACT/M-CAT (6.12)] showed high MI values. Just as the $Hav_{(p)}$ analysis indicated, these primers were more useful than E-AGC/M-CTC and E-ACA/

Table 2. Total number of bands, proportion of polymorphic bands, average expected heterozygosity for polymorphic markers, polymorphic information content, the effective multiplex ratio, and the marker index, for each primer pair used in the analysis of the 190 soybean lines.

Primer pairs	Total number of bands	Proportion of polymorphic bands	$Hav_{(p)}$†	Standard deviation of $Hav_{(p)}$	PIC‡	Effective multiplex ratio	Marker index (*MI*)	Standard deviation of *MI*
E-ACT/M-CAT	55	0.35	0.32	0.02	0.11	19	6.12	0.38
E-ACC/M-CAA	83	0.27	0.37	0.05	0.10	22	8.24	4.12
E-AAG/M-CTT	83	0.41	0.26	0.06	0.11	34	9.14	4.57
E-AGC/M-CTC	46	0.17	0.27	0.02	0.05	8	2.16	1.08
E-ACA/M-CAC	65	0.11	0.15	0.01	0.02	7	1.11	0.55
LSD0.05			0.13				5.78	

† $Hav_{(p)}$ = average expected heterozygosity for polymorphic markers.
‡ PIC = polymorphic information content calculated for both monomorphic and polymorphic markers.

Table 3. Mean, standard deviation, and range (in parenthesis) of the genetic distances (%) between all pairings of the North American soybean ancestors (NASA), North American soybean cultivars (NASC), Chinese cultivars, and Japanese cultivars.

	NASA	NASC	Chinese cultivars	Japanese cultivars
NASA	**7.3 ± 1.5 (1.2–12.1)**	**7.8 ± 1.6 (3.6–12.7)**	**8.4 ± 1.4 (3.6–13.0)**	**8.7 ± 1.6 (4.2–14.6)**
NASC	–	**7.1 ± 1.6 (0.9–11.6)**	**8.5 ± 1.5 (3.6–13.9)**	**8.9 ± 1.5 (4.8–14.5)**
Chinese Cultivars	–	–	**7.5 ± 1.7 (1.2–13.0)**	**8.9 ± 1.8 (3.4–15.0)**
Japanese cultivars	–	–	–	**6.3 ± 2.2 (2.1–13.5)**

M-CAC in discriminating between the soybean genotypes in this study. Average MI for the five-primer pairs used in this study was 5.35, and it was similar to the MI reported by Powell et al. (1996) for the AFLP marker system.

Genetic Relationships

Average genetic distance among the 190 soybean genotypes was 8.1%, and the range of genetic distance (GD) was 0.9 to 15.0% (Table 3). There were no significant differences between the genetic distance means of any of the four genotype groupings. The average GD was lowest among Japanese cultivars (6.3%) whereas the Chinese cultivars had the highest average GD estimate (7.5%). The average GD of NASA and NASC were also not significantly different. The average GD for all possible pairings of the 66 NASC with Chinese cultivars and with Japanese cultivars were 8.5 (GD range 3.6 to 13.9) and 8.9% (range 4.8 to 14.5), respectively. The most diverse cross between a NASC and an Asian cultivar would have a genetic distance of 14.5%.

Average genetic distance within germplasm groups of 36, 31, 32, and 26% has been estimated for these same NASA, NASC, Chinese, and Japanese cultivars, respectively, on the basis of 121 RFLP probes (Alvernaz et al., 1998). The average GD (with RFLP data) for all possible pairings of the 66 North American with all Asian cultivars from the RFLP study was 35% for Chinese and 37% for Japanese cultivars (Alvernaz et al., 1998). These RFLP results are similar to the AFLP data, which were produced with only five primer combinations. The difference in the magnitude of these two sets of genetic distances exists because of the use of only polymorphic markers in the RFLP analysis, whereas polymorphic and monomorphic markers were used in the AFLP analysis.

The UPGMA-derived dendrogram assigned the 190 genotypes into four major clusters (Fig. 1) designated as ‘a’, ‘b’, ‘c’, and ‘d’. The NASA were primarily in cluster ‘a’, the NASC in cluster ‘b’, the Japanese cultivars in cluster ‘c’, and the Chinese cultivars in cluster ‘d’ (Fig. 1). In general, 85% of the 190 soybean lines clustered between 90 and 95% similarity distance.

Although the major clusters were related to the geographic origin of the genotypes, smaller clusters of cultivars and genotypes with known pedigree relationships were evident. Cluster ‘a’ included 29 NASA (83% of all the NASA), 15 NASC, three Chinese cultivars, and two Japanese cultivars. Five subgroups were observed among the genotypes in cluster ‘a’. Ogden, Roanoke, and Jackson, which account for 24% of the genetic base of cultivars developed in the southern USA, were placed in the same subgroup. A similar cluster of Ogden, Roanoke, and Jackson was also identified by Kisha et al. (1998) and Brown-Guedira et al. (2000). The third subgroup showed that A.K. (Harrow) and Illini were very similar and both were closer to S-100 than to any other accessions in the study. Previous researchers (Kisha et al., 1998; Thompson et al., 1998; Brown-Guedira et al., 2000) had placed Lincoln in the A.K. (Harrow) cluster with Illini and S-100, but the present study distinguished it from that group and clustered it with other ancestors, Anderson and Flambeau (Fig. 1). S-100 is thought to be a selection from Illini or a progeny of Illini (Thompson et al., 1998). Gizlice et al. (1994) suggested that A.K. (Harrow) and Illini may be identical. These two genotypes were among the most similar in our analysis (Fig. 1).

The soybean ancestors Arksoy, Ralsoy, Mejiro, and Mukden were grouped and they clustered with seven NASC in subgroup 4 of cluster ‘a’. Arksoy and Ralsoy were always grouped together in studies by Brown-Guedira et al. (2000) and give an additional example of how these groupings agree with previous studies.

Mandarin (Ottawa) was the only NASA in subgroup 5 of cluster ‘a’. Also contained in subgroup 5 were eight Canadian cultivars, three Chinese cultivars, and two Japanese cultivars. The grouping of Mandarin (Ottawa) with other cultivars from North America, China, and Japan suggests that it has a broad genetic base that has some similarity to elite cultivars from both Asian regions. It has been reported that Mandarin (Ottawa), which was originally introduced from China, is a major ancestor of the Canadian and North American cultivars having contributed between 18 and 55% of their genomes (Lohnes and Bernard, 1991; Kisha et al., 1998).

Cluster ‘b’ consisted of four subgroups made of three NASA, 49 North American, and three Chinese cultivars. In this cluster, the NASC Hutcheson (Buss et al., 1988) and Narow (Caviness et al., 1985) grouped very closely. Although they are both in maturity group V and they have several common ancestors in their pedigree, this level of similarity was unexpected. Carter et al. (1993) reported a genetic similarity estimate of 0.529 between Hutcheson and Narow where 1.0 is defined as genetic identity. Since these cultivars also differ for several morphological traits, it seems unlikely that the observed level of similarity is correct and a more likely explanation is that the original DNA samples were mislabeled.

Cluster ‘c’ was composed of eight Chinese and 26 Japanese cultivars. This suggests that the Asian cultivars in cluster ‘c’ were derived from soybean ancestors that are very different from the ancestors of the NASC. A recent report by Cui et al. (2000) identified a very minor NASA (Mamotan) in the pedigrees of three of the Chi-

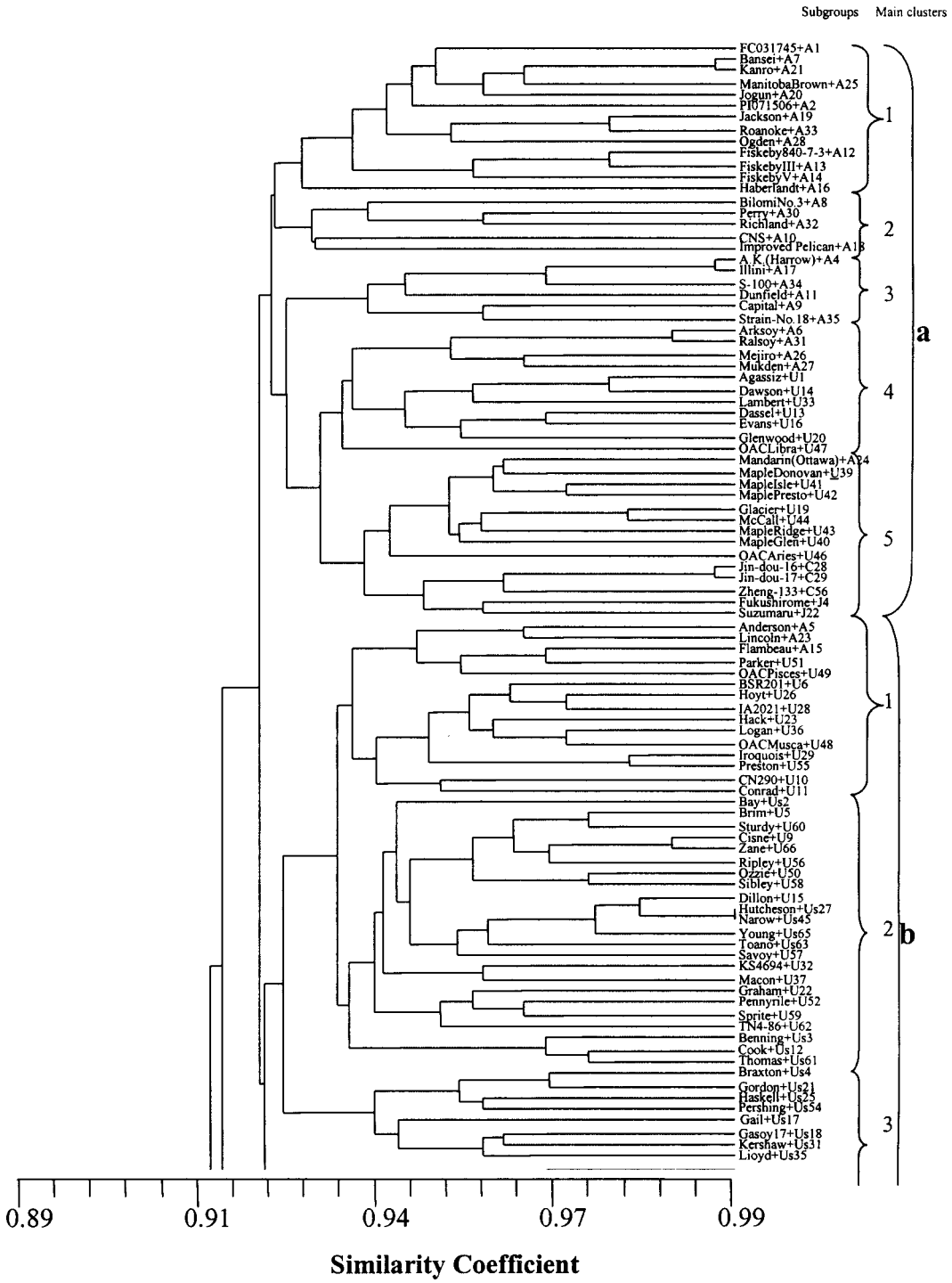

Fig. 1. Continued on next page.

nese cultivars in subgroup 1 of cluster 'c' (Yu dou 11, Zheng 77249, and Zhong dou 19), but essentially this cluster has no NASC or NASA.

The fourth cluster 'd' consisted of three NASA (Peking, PI88788, and Korean), two NASC, 45 Chinese, and two Japanese cultivars. The three soybean ancestors Peking, PI88788, (both collected from China), and Korean (collected from North Korea) were very different from the other NASA. Gizlice et al. (1993) also observed Peking to be very different from other NASA based on the 10 metric traits they measured on plants grown in a phytotron. In general, PI88788 and Peking [two sources of soybean cyst nematode (*Heterodera glycines* Ichinohe) resistance genes], Manokin, Jin dou 14, Kitakomachi, and Yuuhime constituted the most divergent group from all the soybean accessions used in our study.

It is not clear on the basis of their pedigrees why Manokin (Kenworthy et al., 1996) and Harlon would be in cluster 'd'. Manokin (maturity group IV) was the most genetically different within the NASC, with an

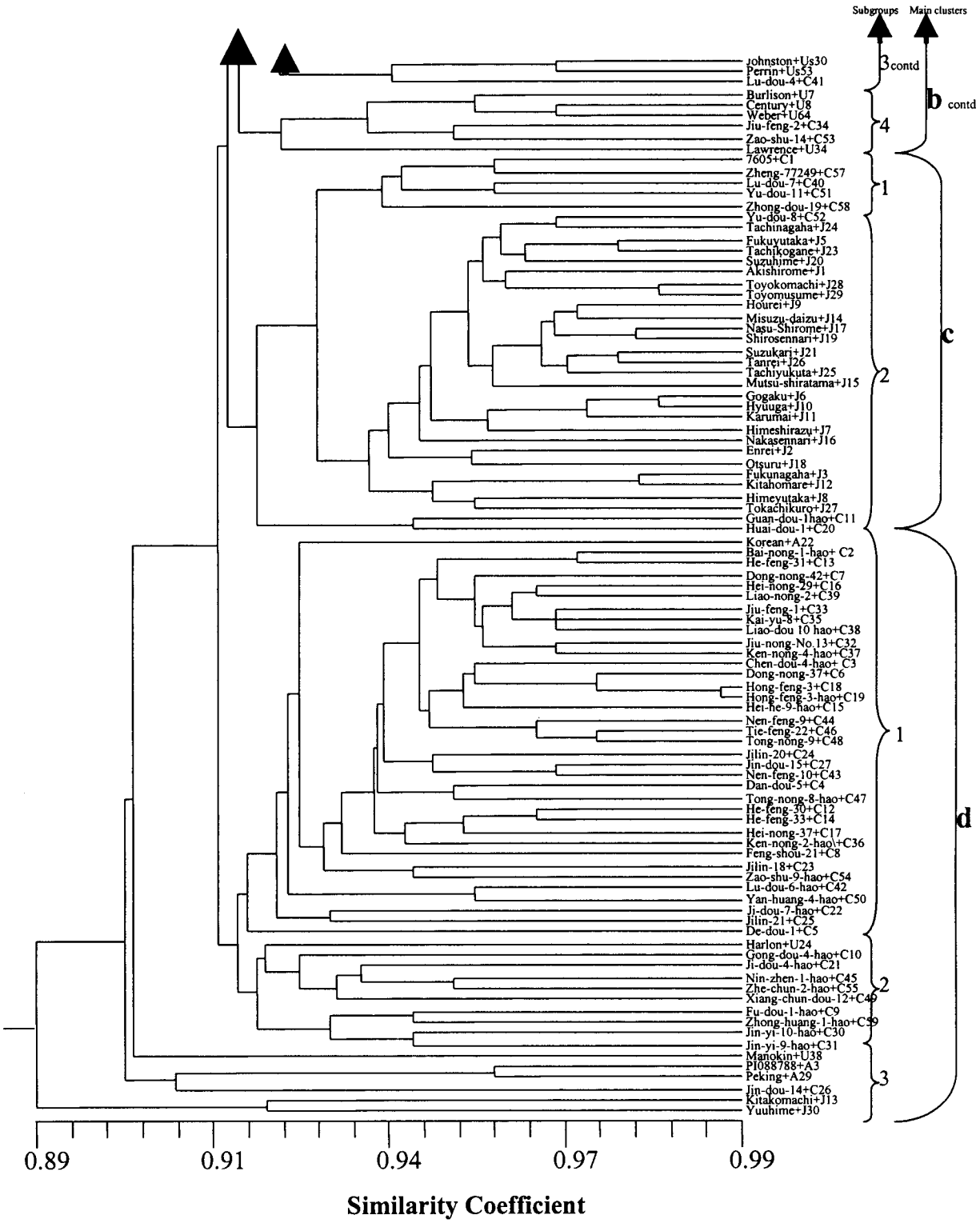

Fig. 1. Dendogram of 190 soybean lines produced by UPGMA clustering method based on the genetic similarity matrix derived from 332 AFLP markers. The letter and the numbers after the plus sign (+) in the cultivar names are codes from Table 1. A- = North American soybean ancestor; U- = North American soybean cultivar; Us- = Southern USA cultivar; C- = Chinese cultivar; J- = Japanese cultivar.

average GD of 8.8% from other NASC. A previous pedigree analysis by Sneller (1994) did not identify Manokin as having a unique coefficient of parentage. Manokin was derived from a cross of parents representing northern U.S. by southern U.S. germplasm. Manokin has cyst nematode resistance that was derived from Peking, an ancestor also in this cluster. Pedigree information (Lohnes and Bernard, 1991) on Harlon (maturity group I) indicates that it was selected from the cross of 'Blackhawk' × 'Harosoy 63' and has four ancestors from

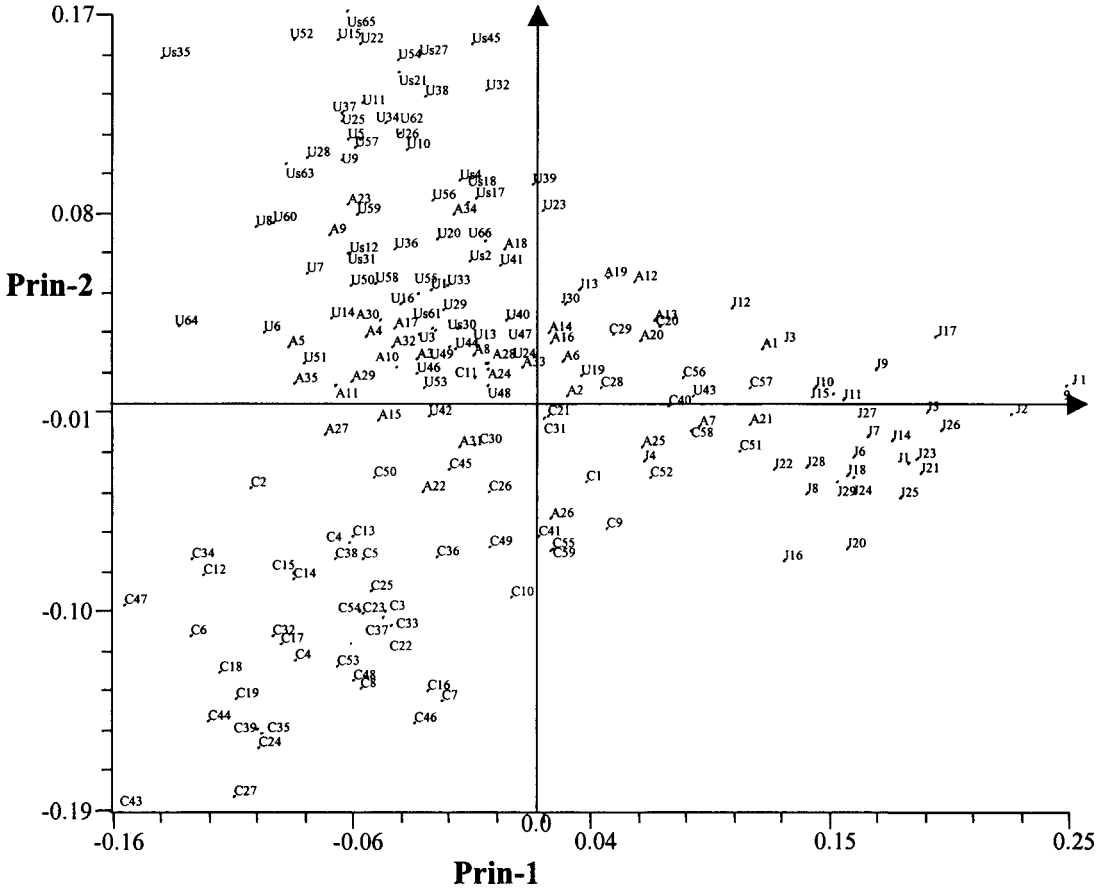

Fig. 2. Principal coordinate graph of the 190 soybean lines composed of the first and second principal coordinates derived from the analysis of 332 AFLP markers. Soybean lines in the scatter are identified by codes from Table 1. A- = NASA; U- = North American soybean cultivar (NASC); Us- = Southern USA cultivar; C- = Chinese cultivar; and J- = Japanese cultivar.

China-Mandarin (Ottawa), Mukden, Richland, and A.K. (Harrow) which contributed 37, 25, 25, and 13% of its genome, respectively. However, Harlon's pedigree would be similar to other NASC grown in the northern USA and has no obvious uniqueness.

Principal coordinate analysis (PCO) was used to identify multidimensional relationships that describe portions of the genetic variance in a data set. The first two principal coordinates of the AFLP data explained 15.4% of the total variance (Fig. 2). Principal coordinate analysis separated the germplasm into four broad groups corresponding to the UPGMA clusters ('a', 'b', 'c', and 'd') on the basis of the geographical origin of the accessions. The PCO scatter plot, however, showed overlap between accessions from different geographic origins. The NASA lines occupied a central position among North American, Chinese, and Japanese cultivars and overlapped each of them (Fig. 2). With the exception of Kitakomachi and Yuuhime, the rest of the Japanese cultivars were well separated from the North American cultivars and ancestors. The Chinese cultivars were widely scattered in all clusters and they also appeared as a bridge between the North American accessions (cultivar and ancestor) and the Japanese cultivars.

Breeding Implications

The AFLP genetic distance clearly formed a distinct grouping of cultivars on the basis of their origin. Even though NASC were derived from Chinese and Japanese introductions, subsequent breeding efforts have resulted in the development of rather distinct gene pools in each country. The Japanese cultivars in this study had the lowest average GD of the three groups of cultivars. They were also the most genetically different from NASC indicating separate ancestors for the elite cultivars in the two regions. Although a few Japanese cultivars, Yuuhime, Kitakomachi, Fukunagaha, and Kitahomare, showed close relationship to some NASA, the remaining 26 Japanese cultivars were very genetically different from both NASC and NASA. This suggests that some Japanese elite cultivars may serve as sources of exotic genes for the genetic improvement of North American soybean cultivars.

Agronomic data and yield for all of the Asian cultivars in this study are available from the Soybean Asian Variety Evaluation Project (Project SAVE) report by Manjarrez-Sandoval et al. (1997). Seven of the Asian cultivars yielded at least 80% of the NASC checks of similar maturity in at least 1 yr of the 2-yr SAVE project. Those cultivars and their yield as a percentage of the NASC checks (2-yr average) are Akishirome (76%), Hyuuga (83%), Misuzu Daizu (83%), Nakasennari (87%), Nasu Shirome (68%), Otsuru (75%), and Tachinagaha (70%) (Manjarrez-Sandoval et al., 1997).

The U.S. soybean breeders have been slow to utilize diverse genetic material in cultivar improvement pro-

grams. Gizlice et al. (1993) found that many recent U.S. cultivars were as closely related as half-sibs. Asian breeders have utilized a different strategy in their breeding programs. Cui et al. (2000) reported that Chinese breeders avoid mating related parents, and continue to introduce new germplasm in cultivar development programs. Chinese breeders have successfully introgressed U.S. germplasm into Chinese cultivars, but U.S. germplasm contributes only about 7% of the total genetic base of Chinese cultivars. Similarly, Zhou et al. (2000) reported that U.S. and Chinese cultivars have been utilized by Japanese breeders in their cultivar development programs. Intermating cultivars from these three major gene pools should provide new genetic recombinations to exploit in cultivar development programs. The information presented here should assist breeders in the selection of sources of new genes for the yield improvement of NASC.

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