Illinois Bundleflower Genetic Diversity Determined by AFLP Analysis

L. R. DeHaan,* N. J. Ehlke, C. C. Sheaffer, G. J. Muehlbauer, and D. L. Wyse

ABSTRACT

Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMillan] is an herbaceous perennial legume native to North America. Useful as a N_2–fixing plant in warm-season grass pastures, Illinois bundleflower is also a promising perennial grain crop. Knowledge of the distribution of genetic variation in Illinois bundleflower would increase efficiency of germplasm preservation and expedite plant breeding progress. The objective of this experiment was to determine the distribution of genetic variation within and between Illinois bundleflower accessions by amplified fragment length polymorphism (AFLP) markers. Semi-automated fluorescence-based AFLP analysis was performed on three individuals from each of 50 accessions. We identified 222 markers, 159 of which were polymorphic. Within-accession diversity was low (H_S = 0.013) compared with total gene diversity (H_T = 0.086). Analysis of molecular variance (AMOVA) showed that 83% of the molecular variance was explained by two major clusters, supporting a previous phenotypic study that suggested the existence of two distinct races of Illinois bundleflower. The less commonly occurring of the two races contained greater genetic diversity and originated from a localized region in the south central USA. The collection of more accessions from throughout the USA and focused on the Southeast would likely increase the genetic variation available to plant breeders.

Illinois bundleflower is an herbaceous warm-season N_2–fixing perennial legume native to North America and useful in warm-season grass pastures. Illinois bundleflower is an excellent candidate for use as a perennial grain crop because accessions collected from the wild can produce in excess of 3000 kg ha^{-1} of seed (Adjei and Pitman, 1993) and the crude protein content of the seed is about 380 g kg^{-1} (Kulakow et al., 1990).

The potential of Illinois bundleflower as a forage crop has been illustrated in several experiments. 'Sabine' Illinois bundleflower (Muncrief and Heizer, 1985) was readily established in a Texas kleingrass (*Panicum coloratum* L.) pasture (Dovel et al., 1990). In the second, third, and fourth years of the study, kleingrass plots interseeded with Illinois bundleflower yielded 43, 47, and 45% more forage dry matter than noninterseeded plots in the respective years. Comparison tests have been made between warm-season grasses interseeded with Illinois bundleflower and warm-season grass monocultures (Posler et al., 1993). In the study, mixtures including Illinois bundleflower generally had forage yield and crude protein content more than double that of grass alone.

Kulakow (1999) conducted an extensive evaluation of phenotypic variation in 141 Illinois bundleflower accessions collected primarily from the Great Plains. Substantial variation was observed among accessions for traits including plant height, seed yield, seed size, survival, shatter resistance, and maturity. On the basis of phenotype, this study showed that there are at least two distinct races of Illinois bundleflower. Although variation within accession was not documented, accessions appeared uniform. Kulakow (1999) stated that molecular markers would be helpful for estimating within- versus between-population genetic diversity.

The AFLP technique for DNA fingerprinting enables visualization of restriction fragments without knowledge of nucleotide sequence (Vos et al., 1995). Fluorescence labeling and semi-automated detection of fragments have increased the speed and accuracy of the AFLP technique (Huang and Sun, 1999). Because fluorescence AFLP allows researchers to obtain genetic fingerprints rapidly without prior sequence information, the technique has been widely used in plant species with which little or no molecular research has been done. Fluorescence AFLP has been used to analyze genetic diversity in hops (*Humulus lupulus* L.), bermudagrass (*Cynodon* spp.), *Onopordum* thistles, ryegrasses (*Lolium* spp.), azaleas (*Rhododendron simsii* Planch.), and mulberry (*Morus* spp.) (Hartl and Seefelder, 1998; Zhang et al., 1999; O'Hanlon et al., 1999; De Riek et al., 1999; Roldán-Ruiz et al., 2000; and Sharma et al., 2000).

Although phenotypic diversity of Illinois bundleflower has been studied, the genetic diversity within and between Illinois bundleflower accessions is unknown. Knowledge of genetic diversity within and among accessions would enable plant breeders to choose parental sources that will generate diverse populations for selection. Knowledge of the distribution of genetic diversity within the species would also allow for an optimal germplasm preservation strategy, with future sampling focused on regions of greatest diversity. Our objective was to determine the genetic variation within and between Illinois bundleflower accessions with AFLP molecular markers.

MATERIALS AND METHODS

Plant Materials and DNA Extraction

Seed of 50 Illinois bundleflower accessions was obtained from throughout the central USA (Fig. 1). Fifteen accessions were from the University of Minnesota Native Perennial Legume Collection, 25 accessions were from The Land Institute, Salina, KS, and 10 accessions were from the National Plant Germplasm System (USDA, ARS, 2001). For purposes of this study, the accessions were assigned numbers from 1 to 50 (Table 1).

Plants of Accessions 1 through 20 were grown in a regularly irrigated field environment for a separate study. Tissue from

L.R. DeHaan, The Land Institute, 2440 E. Water Well Rd., Salina, KS 67401; N.J. Ehlke, C.C. Sheaffer, G.J. Muehlbauer, and D.L. Wyse, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108. Contribution of the Minnesota Agric. Exp. Stn. Received 8 Oct. 2001. *Corresponding Author (dehaan@landinstitute.org).

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Abbreviations: AFLP, amplified fragment length polymorphism; AMOVA, analysis of molecular variance; bp, base pair; H_S, within-accession diversity; H_T, total gene diversity; RAPD, random amplified polymorphic DNA; UPGMA, unweighted pair-group method, arithmetic average.

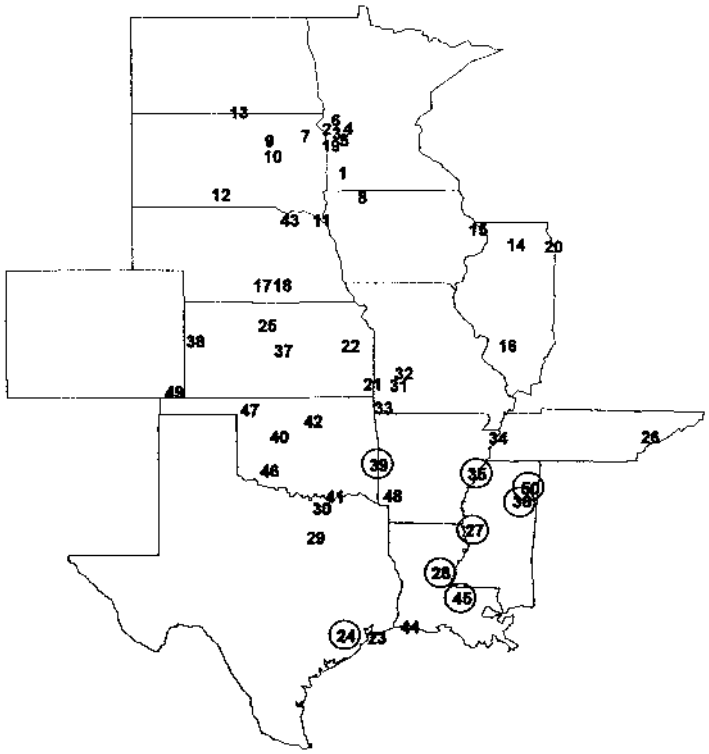

Fig. 1. Geographic origin of the Illinois bundleflower accessions used for AFLP analysis. Circled accession numbers belong to Cluster 2 and noncircled accession numbers belong to Cluster 1, as determined by the unweighted pair-group method, arithmetic average (UPGMA) clustering procedure applied to 159 polymorphic AFLP markers.

these accessions was collected on July 11 from randomly selected plants in the third growing season after establishment. Plants of Accessions 21 through 50 were grown in the greenhouse and tissue was harvested from these plants about 3 wk after planting.

About 0.1 g of tissue was collected from developing leaves of three plants from every accession. The tissue was sampled and immediately frozen in liquid N_2. The samples were ground in liquid N_2 with a glass pestle. Total genomic DNA was extracted by means of the Dneasy Plant Mini Kit[1] (Qiagen Inc., Valencia, CA) because the kit provided a clean extraction despite the fact that many samples were taken from mature field-grown plants. The DNA was concentrated to a final volume of about 10 μL with a DNA SpeedVac DNA 100 (Savant Instruments, Holbrook, NY) and quantified with an Ultrospec 3000 UV/Visible Spectrophotometer (Pharmacia Biotech Ltd., Cambridge, England).

The AFLP fingerprinting was performed with the AFLP Plant Mapping Kit (PE Applied Biosystems, Foster City, CA). The enzymes *Eco*RI and *Mse*I (New England Biolabs, Inc., Beverly, MA) were used to digest 0.5 μg genomic DNA from each sample. Preselective amplification was performed with *Eco*RI-A and *Mse*I-C primers. Selective amplification was performed with two fluorescently labeled primer sets: *Eco*RI-AA-Joe/*Mse*I-CTA and *Eco*RI-AA-Joe/*Mse*I-CAT. All amplifications were performed with a GeneAmp PCR System 9700 (PE Applied Biosystems). The selective amplification step was performed on two subsamples of each amplification product to replicate a portion of the laboratory procedure.

The AFLP products were separated with an automated DNA sequencer (ABI Prism 377, PE Applied Biosystems). The GeneScan-500 ROX (PE Applied Biosystems) internal size standard was used to accurately size the amplified fragments. The electropherograms generated by the sequencer were interpreted with GeneScan software (PE Applied Biosystems). Genotyper software (PE Applied Biosystems) was then used to create a list of fragments detected in each lane by fragment size. Fragments sized from 50 to 500 base pairs (bp) with a peak height >50 in the electropherogram were retained for subsequent analysis. Peakmatcher software (DeHaan et al., 2002) was used to convert the list of fragments detected in each sample into a binary (1/0) table for the presence or absence of each fragment in each sample. Peakmatcher was set to retain markers with repeatability >90%.

A separate matrix of Nei's unbiased genetic distances (Nei, 1978) was calculated by POPGENE (Yeh and Boyle, 1997) using markers derived from each of the two AFLP primer combinations. The correlation between the two distance matrices was determined with the MXCOMP function of NTSYSpc (Rohlf, 2000), and the significance of the correlation was tested with a Mantel test of 1000 random permutations.

POPGENE software was used to calculate Nei's unbiased genetic distance between accessions with all markers, including monomorphic markers. Nei's unbiased genetic distance is an accurate estimate of the number of gene differences per locus when populations are small (Nei, 1978). The matrix of unbiased genetic distances between accessions was analyzed by NTSYSpc. Dendrograms were generated by the unweighted pair-group method, arithmetic average (UPGMA), and the ball-cluster method of Jardine et al. (1969). Ordination analysis via nonmetric multidimensional scaling was used to represent the relationships between the accessions in two dimensions by means of the MDSCALE module of NTSYSpc. To evaluate goodness of fit of the UPGMA dendrogram and ordination analysis to the original distance matrix, the cophe-

[1] Names are necessary to report factually on available data; however, the Univ. of Minnesota neither guarantees nor warrants the standard of the product, and the use of the name by the Univ. of Minnesota implies no approval of the product to the exclusion of others that may be suitable.

Table 1. Illinois bundleflower accession information and cluster grouping.

Accession number	Accession indentifier†	North latitude	West longitude	County	State	AFLP cluster	Phenotypic cluster‡
1	PNL532	44°15′00″	95°52′48″	Lyon	MN	1	
2	PNL533	45°35′24″	96°29′24″	Traverse	MN	1	
3	PNL534	45°32′24″	96°05′24″	Stevens	MN	1	
4	PNL535	45°30′00″	96°00′00″	Stevens	MN	1	
5	PNL536	45°23′24″	96°08′24″	Big Stone	MN	1	
6	PNL537	45°49′48″	96°07′12″	Grant	MN	1	
7	PNL538	45°24′36″	97°19′48″	Day	SD	1	
8	PNL539	43°29′24″	95°06′00″	Dickenson	IA	1	
9	PNL540	45°16′12″	98°45′00″	Edmonds	SD	1	
10	PNL541	44°46′12″	98°42′00″	Spink	SD	1	
11	PNL542	42°42′36″	96°48′00″	Union	SD	1	
12	PNL543	43°33′36″	100°43′48″	Mellette	SD	1	
13	PNL550	46°01′48″	100°04′48″	Emmons	ND	1	
14	PNL544	42°00′00″	89°12′00″	Ogle	IL	1	
15	PNL545	42°28′12″	90°39′36″	Dubuque	IA	1	
16	LI1046	38°48′36″	89°33′00″	Bond	IL	1	1
17	LI1098	40°42′36″	99°07′48″	Buffalo	NE	1	1
18	LI1132	40°43′48″	98°49′48″	Buffalo	NE	1	1
19	LI1134	45°19′12″	96°27′00″	Big Stone	MN	1	3
20	LI1062	41°52′48″	87°36′36″	Cook	IL	1	2
21	LI1121	37°36′36″	94°50′24″	Crawford	KS	1	1
22	LI1128	38°48′36″	95°41′24″	Shawnee	KS	1	1
23	LI1065	29°30′00″	94°40′12″	Galveston	TX	1	1
24	LI1094	29°39′36″	95°56′24″	Fort Bend	TX	2	4
25	LI1041	39°28′12″	98°58′12″	Osborne	KS	1	2
26	LI391	35°57′36″	83°55′48″	Knox	TN	1	4
27	PI543908	32°58′48″	90°51′36″	Sharkey	MS	2	
28	PI543914	31°39′00″	92°10′48″	La Salle	LA	2	
29	LI1097	32°45′00″	97°05′24″	Tarrant	TX	1	1
30	LI1103	33°40′12″	96°51′00″	Grayson	TX	1	3
31	LI1106	37°36′00″	93°52′12″	Cedar	MO	1	2
32	LI1108	37°40′48″	93°48′36″	Cedar	MO	1	2
33	LI1109	36°52′48″	94°22′48″	Newton	MO	1	1
34	LI1142	35°55′12″	89°55′12″	Mississippi	AR	1	2
35	LI1143	34°46′12″	90°45′00″	Lee	AR	2	4
36	LI1144	33°52′48″	89°00′00″	Chickasaw	MS	2	4
37	LI1040	38°39′00″	98°19′12″	Ellsworth	KS	1	2
38	LI1038	38°57′36″	101°45′36″	Wallace	KS	1	2
39	LI1131	35°03′00″	94°36′36″	Le Flore	OK	2	4
40	LI1084	35°57′00″	98°30′00″	Blaine	OK	1	
41	LI1104	33°58′48″	96°22′12″	Bryan	OK	1	1
42	LI1137	36°27′00″	97°09′36″	Noble	OK	1	1
43	PI215203	42°45′36″	98°03′00″	Knox	NE	1	
44	PI543897	29°57′00″	93°23′24″	Cameron	LA	1	
45	PI543898	30°51′00″	91°20′24″	West Feliciana	LA	2	
46	PI421093	34°52′12″	98°52′48″	Kiowa	OK	1	
47	PI436881	36°47′24″	99°39′00″	Harper	OK	1	
48	PI543900	34°03′36″	94°03′00″	Howard	AR	1	
49	PI900298	37°21′36″	102°34′12″	Baca	CO	1	
50	PI543902	34°18′00″	88°40′48″	Lee	MS	2	

† Accession origin: PNL = University of Minnesota Native Perennial Legume Collection, LI = The Land Institute, Salina, KS, and PI = The USDA ARS National Plant Germplasm System.
‡ Phenotypic clustering according to Kulakow (1999).

netic correlation was calculated by means of the MXCOMP function of NTSYSpc. Within-accession diversity (H_S) and total gene diversity (H_T) (Nei, 1973) were calculated within the species and within two major groups by POPGENE software. The calculations were performed with all markers, both monomorphic and polymorphic.

The partitioning of molecular variance within and among groups and accessions was calculated by the AMOVA technique (Excoffier et al., 1992) in ARLEQUIN software (Schneider et al., 2001). Three models were used: all accessions within two clusters; accessions within Cluster 1; and accessions within Cluster 2. All significance tests were calculated by performing 1023 permutations.

RESULTS AND DISCUSSION

Two AFLP primer combinations amplified a total of 222 unique fragments (Table 2). Of these, 159 fragments were polymorphic (72%). The average repeatability of

Table 2. Illinois bundleflower AFLP markers obtained from two primer combinations.

Primer pair†	Number of markers		Percent repeatable‡	Number of markers per plant		
	Total	Polymorphic		Maximum	Minimum	Average
AA-Joe, CAT	130	93	98.3	89	56	80.3
AA-Joe, CTA	92	66	98.3	58	41	49.7
Both pairs	222	159	98.3	143	103	130.0

† AA-Joe,CAT = *Eco*RI-AA-Joe/*Mse*I-CAT; AA-Joe, CTA = *Eco*RI-AA-Joe/*Mse*I-CTA.
‡ Percent repeatability between two selective amplifications of the same preselective amplification product.

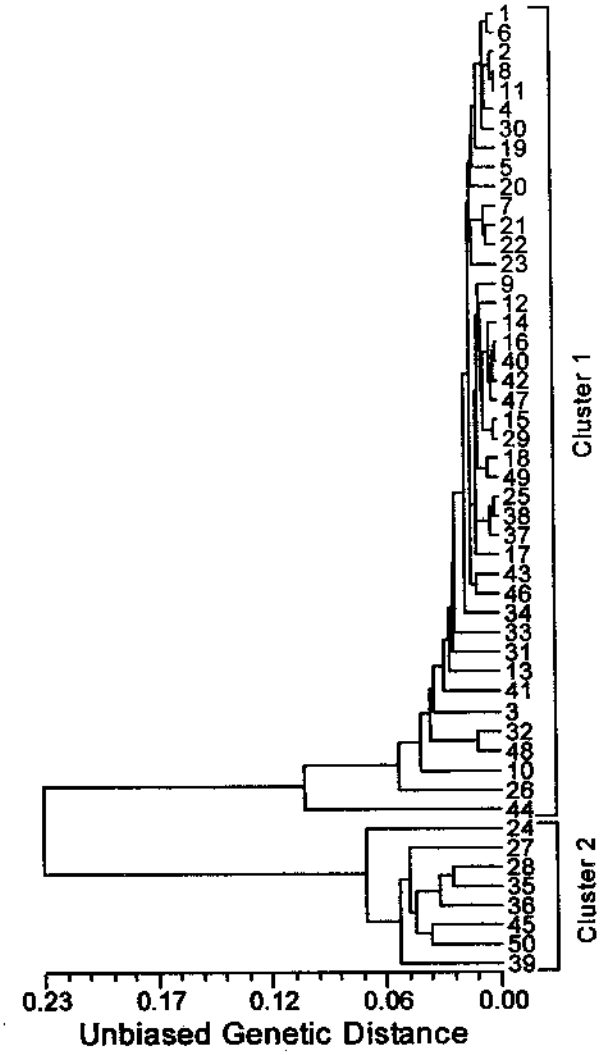

Fig. 2. Illinois bundleflower accessions clustered by the unweighted pair-group method, arithmetic average (UPGMA) procedure applied to 159 polymorphic AFLP markers.

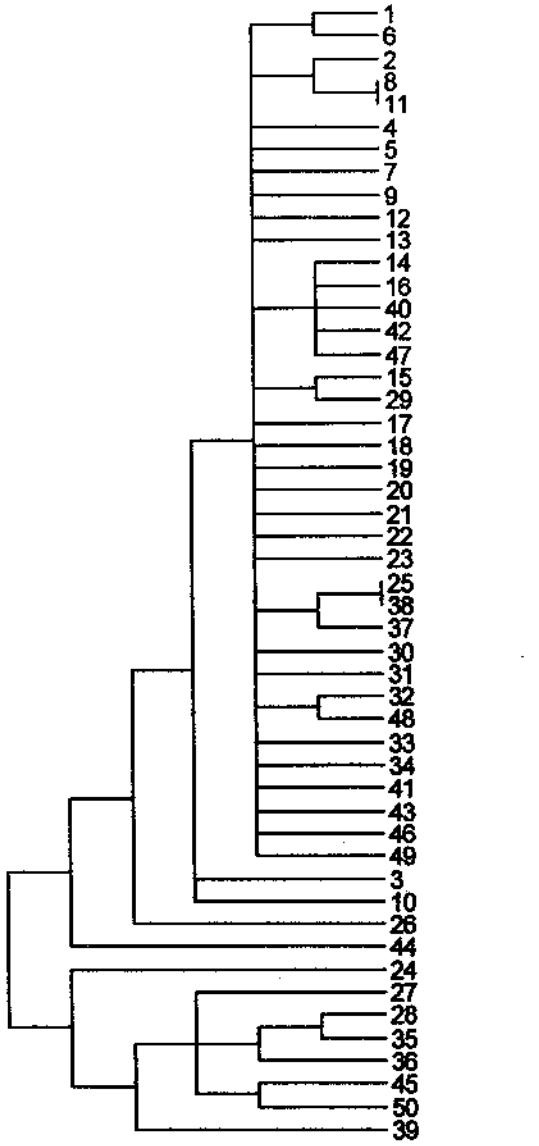

Fig. 3. Ball cluster dendrogram of Illinois bundleflower accessions based on 159 polymorphic AFLP markers.

the AFLP fragments across two replications was 98%. The primer pair AA-Joe, CAT amplified 130 fragments and the primer pair AA-Joe, CTA amplified 92 fragments. The unbiased genetic distance matrices calculated using each primer pair were similar (r = 0.965, P = 0.001) indicating that the information generated by the two primer pairs was consistent.

The present study is the first to use Peakmatcher software (DeHaan et al., 2002) to automate the gel scoring process. We verified the results produced with Peakmatcher by crosschecking a subset of the data with visual scoring using Genographer (Benham et al., 1999). The results of the two methods were consistent, and Peakmatcher enabled us to score a total of 600 lanes containing on average 49 fragments in about 6 h.

Cluster and Ordination Analysis

The dendrogram calculated from Nei's unbiased genetic distances between populations by the UPGMA method revealed two distinct clusters (Fig. 2). The unbiased genetic distance between the two clusters is 0.23. Cluster 1 comprises 42 accessions originating from throughout the central USA (Fig. 1). Cluster 2 comprises eight accessions originating from a localized region east of 96° W and south of 35°06′ N (Table 1).

The UPGMA-generated dendrogram (Fig. 2) is in close agreement with the matrix of unbiased genetic distances (cophenetic correlation = 0.98). The high cophenetic correlation is due primarily to the existence of two very distinct clusters. The UPGMA method will produce a hierarchical clustering even if there is little evidence for clustering in the original data. Therefore, we generated a dendrogram of ball clusters (Fig. 3). A ball cluster dendrogram has the advantage of only producing robust clusters, but it has the disadvantage of losing a readily interpreted scale. Each accession within a ball cluster is more similar to every other accession within the cluster than it is to any accession outside the cluster (Jardine et al., 1969).

The ball cluster dendrogram (Fig. 3) reveals little

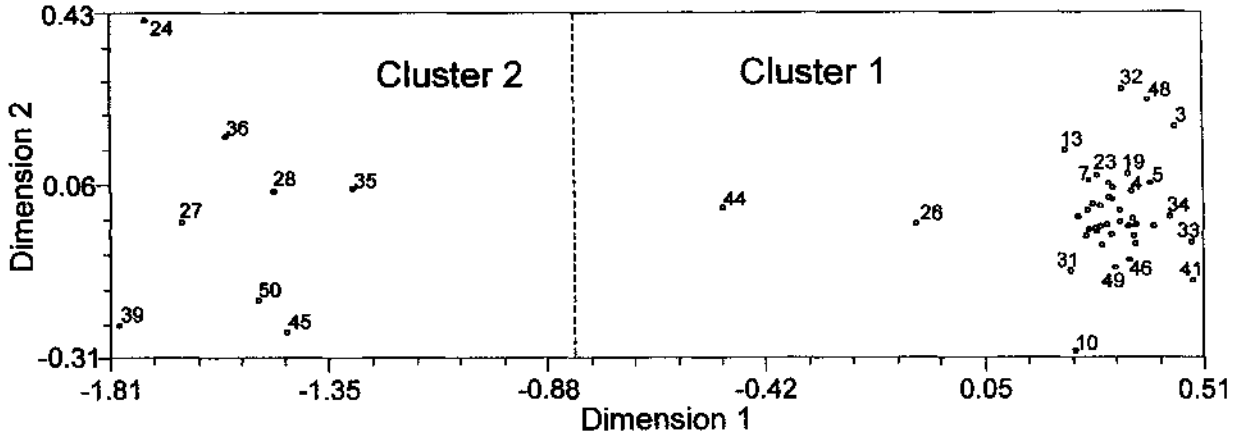

Fig. 4. Illinois bundleflower accessions plotted from nonmetric multidimensional scaling analysis of 159 polymorphic AFLP markers. The dashed line separates the two major clusters from unweighted pair-group method, arithmetic average (UPGMA) analysis (Fig. 2). The tightly grouped unlabeled points represent Accessions 1, 2, 6, 8, 9, 11, 12, 14, 15, 16, 17, 18, 20, 21, 22, 25, 29, 30, 37, 38, 40, 42, 43, and 47.

clustering among the majority of the accessions. The clustering that is present is generally in agreement with geographic origin. For instance, Accessions 25, 37, and 38, which originated from Kansas, all cluster together. Exceptions are Accessions 14 and 16 from Illinois that clustered with Accessions 40, 42, and 47 from Oklahoma, and Accession 15 from Iowa that clustered with Accession 29 from Texas. All accessions obtained from latitude greater than 36° N were placed in the same third-level ball cluster, except Accessions 3 from Minnesota and 10 from South Dakota. Therefore, these two accessions may be important sources of genetic variation within northern germplasm.

Ordination analysis by nonmetric multidimensional scaling (Fig. 4) provided an excellent summary of the data in two dimensions (cophenetic correlation = 1.00). Ordination analysis represents large distances most accurately, while cluster analysis (Fig. 2 and Fig. 3) represents the fine structure of the data most accurately (Sneath and Sokal, 1973). In the ordination analysis, Accessions 44 and 26 are placed on the horizontal axis between the centers of the two clusters. Therefore, although Accessions 44 and 26 are more similar to Cluster 1 than Cluster 2, they somewhat resemble Cluster 2. Accession 44 resembles Cluster 2, probably because it was obtained from the geographical margin between the two clusters (Fig. 1). Accession 26 is isolated both genetically and geographically from the other accessions (Fig. 1 and Fig. 4). In a previous phenotypic study by Kulakow (1999), Accession 26 clustered with Accessions 24, 35, 36, and 39, which are found in Cluster 2 in this study. Therefore, Accession 26 is unique in its phenotypic resemblance to accessions in Cluster 2 and its genetic similarity to accessions in Cluster 1.

Table 3. Total gene diversity among and within (H_T) and gene diversity within (H_S) 50 accessions of Illinois bundleflower.

	H_T	H_S
All Accessions	0.0857 ± 0.0145†	0.0126 ± 0.0003
Cluster 1 Accessions	0.0363 ± 0.0055	0.0115 ± 0.0004
Cluster 2 Accessions	0.0592 ± 0.0164	0.0184 ± 0.0024

† Average ± standard deviation across all markers.

Gene Diversity and AMOVA

The total gene diversity across all accessions (H_T) was 0.086 and the within-accession gene diversity (H_S) was 0.013 (Table 3). Low H_S relative to H_T is consistent with the species being approximately 80% self-pollinating (DeHaan, unpublished data, 2000). The values of H_T and H_S for Cluster 2 exceed those of Cluster 1 by 61.3 and 62.5%, respectively, which indicates that Cluster 2 contains greater genetic diversity both among and within accessions. Although gene diversity estimates are commonly used to make comparisons between species, calculations derived from AFLP data should not be compared across studies because AFLP overestimates the number of loci and underestimates the number of alleles (Caicedo et al., 1999). Because of these factors and because fluorescence AFLP can detect many fragments that occur at low frequency, the low diversity estimates obtained may be due to the marker technology used rather than to low genetic diversity in the species. Thus relative, but not absolute, diversity estimates from this study should be considered reliable.

The AMOVA analysis showed that 83% of the molecular variance was explained by the two major clusters (Table 4). This result indicates that the majority of the genetic variation in the Illinois bundleflower accessions is found between two distinct types represented as Cluster 1 and Cluster 2. Kulakow (1999) described four clusters based on phenotypic data, but principal component

Table 4. Analysis of molecular variance (AMOVA) of 159 polymorphic AFLP markers from 50 Illinois bundleflower accessions.

Accessions included	Source of variance	Variance component†	Percent of total
All accessions	Among clusters	18.96	83.0
	Among accessions, within clusters	1.72	7.5
	Within accessions	2.18	9.5
Cluster 1 accessions	Among accessions	1.55	43.5
	Within accessions	2.01	56.5
Cluster 2 accessions	Among accessions	2.74	47.2
	Within accessions	3.06	52.8

† The probability of obtaining a more extreme variance component estimate by chance alone was <0.001 in all cases.

analysis showed that Clusters 1, 2, and 3 formed one large group. Cluster 4 was distinctly different from the other three clusters, which led to the conclusion that there are two distinct races of Illinois bundleflower. The AFLP analysis lends support to this conclusion. Four of the five accessions that Kulakow's (1999) analysis placed Cluster 4 were included in Cluster 2 in the current study (Table 1). Accession 26 was the exception, although ordination analysis revealed that this accession was distinctly different from most other Cluster 1 accessions (Fig. 4).

The AMOVA-derived estimate of population differentiation (Φ_{ST}) is 0.905, which is high when compared with studies of self-pollinated species. Nybom and Bartish (2000) reported that, across eight studies using random amplified polymorphic DNAs (RAPDs), average Φ_{ST} reported for selfing species was 0.70. The exceptionally large Φ_{ST} estimated for Illinois bundleflower can be attributed mainly to the existence of two very distinct clusters. Allocation of molecular variance within and between accessions is similar for both clusters. About 45% of the molecular variance is among accessions and about 55% is within accessions (Table 4). This means that when the two major clusters are disregarded, about 45% of the genetic variation is between accessions.

Geographic Distribution of Genetic Variation

The mean geographic distance between accessions is 763 km in Cluster 1 and 411 km in Cluster 2. Although accessions in Cluster 2 originate from a more localized geography, they are more diverse. Because accessions in Cluster 2 originated from the southeastern range of the area represented in this study, additional genetic diversity might be obtained by collecting seed of plants from the southeastern USA. According to the PLANTS Database (USDA, NRCS, 2001), the range of Illinois bundleflower extends south and east to Alabama, Georgia, Florida, South Carolina, North Carolina, Virginia, Maryland, and Pennsylvania. Accessions from these states are not available through the National Plant Germplasm System (USDA, ARS, 2001), but obtaining accessions from this region could increase the genetic diversity available to plant breeders working with the species.

Implications for Plant Breeding Programs

Accessions in Cluster 1 are the more common type among the accessions used in this experiment (Table 1) and in a previous study of 141 accessions (Kulakow, 1999). Kulakow (1999) described many of the accessions in this cluster as being erect, high seed yielding, and having good survivability, while accessions belonging to Cluster 2 were described as important for their large seed size and vigorous seedling growth. Because polymorphisms between the two races are abundant, the phenotypic differences between the clusters are likely to be a result of diversity across the genome rather than because of a major gene effect. Therefore, accessions of Cluster 2 are likely to be important sources of variation for many quantitative traits, including seed and forage yield.

The germplasm collections presently maintained by the University of Minnesota, The Land Institute, and the National Plant Germplasm System (USDA, ARS, 2001) are weighted heavily toward the more genetically uniform Cluster 1 type and contain few accessions of the more genetically diverse Cluster 2 type of Illinois bundleflower. To maximize the genetic diversity available for use by plant breeders, additional collections need to be made at the periphery of Illinois bundleflower's range in the southern, eastern, and western United States. The use of genetically uniform seed sources such as Sabine Illinois bundleflower for widespread roadside revegetation could threaten the diversity within the species by contaminating or overwhelming it. Local ecotypes should be used for this purpose whenever possible.

We obtained valuable information about several specific accessions. Although Accession 44 was placed in Cluster 1, it was nearly intermediate between the two clusters of Illinois bundleflower. It may be useful to examine the phenotype of this accession because of its potential to contain a combination of useful traits from the two clusters. Kulakow (1999) found Accession 26 to be most similar phenotypically to accessions that were placed in Cluster 2 in the present study. He also described it as being an important source of shatter resistance and large seed size. Because it is genetically similar to the high seed yielding accessions of Cluster 1, shatter resistance might be readily introduced from this accession into Cluster 1 types without substantially reducing seed yield or survival. Accessions 3 and 10 are genetically distant from other accessions collected in the northern United States. Therefore, they may be important sources of diversity within winter-hardy germplasm. The existence of these unique accessions underscores the importance of preserving populations of Illinois bundleflower native to the northern region. Illinois bundleflower has become a rare species in Minnesota because of draining wetlands and widespread herbicide usage (Coffin and Pfannmuller, 1988).

We have documented low within-accession genetic diversity relative to total genetic diversity present in Illinois bundleflower. This pattern underscores the importance of evaluating large numbers of accessions rather than many individuals per accession when searching for valuable traits for plant breeding programs.

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