

Evaluation of Diversity among and within Accessions of Illinois Bundleflower

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

ABSTRACT

Illinois bundleflower [*Desmanthus illinoensis* (Michx.) MacMillan] is a native herbaceous warm-season perennial legume that has potential as a forage and grain crop. Many research objectives with the species depend on knowledge of available genetic resources, but the diversity in northern accessions of Illinois bundleflower is unknown. Our objective was to test the hypothesis that genetic variation for selected characters exists within and among a set of northern accessions of Illinois bundleflower. From this, we could determine the distribution of variation among the accessions and examine the phenotypic correlations among characters. We evaluated 20 accessions at Becker and St. Paul, MN, and 18 accessions at Sioux Center, IA. Within-accession diversity was determined by a progeny test at Rosemount and St. Paul, MN. Every measured character was influenced by accession ($P < 0.05$) in at least one location. Therefore, the accessions evaluated can provide much of the genetic diversity needed to develop cultivars adapted to the northern USA. Much of the variation can be explained by latitude of origin. Southern accessions had the greatest forage and seed yield potential, were later maturing, but lacked persistence in Minnesota. Variation ($P < 0.05$) for characters including seed yield and survival was also found within accessions. Within year and location, average seed crude protein (CP) concentration and seed weight were correlated (average $r = 0.71$, $P < 0.05$). Within location in August, forage neutral detergent fiber (NDF) was negatively correlated with leaf fraction (average $r = -0.89$, $P < 0.001$), and leaf and pod fraction were negatively correlated (average $r = -0.80$, $P < 0.01$).

ILLINOIS BUNDLEFLOWER is an herbaceous perennial legume native to North America. It is found from the northern Great Plains south to Florida and New Mexico. It occurs most often in open wooded slopes, prairies, ravines, stream banks, roadsides, and waste places (Great Plains Flora Association, 1986). It is a warm-season plant that is adapted to a wide range of soil and climatic conditions (Stubbendieck and Conard, 1989). A mature plant has several erect stems growing to a height of 0.3 to 2.0 m (Great Plains Flora Association, 1986). The species is readily grazed by all classes of livestock and decreases in abundance when overgrazed (Stubbendieck and Conard, 1989).

Illinois bundleflower could become a valuable forage species because of its summer productivity and compatibility with warm-season grasses. It has been readily established in existing kleingrass (*Panicum coloratum* L.) swards in Texas, and stands have persisted for at least 4 yr (Dovel et al., 1990). Illinois bundleflower in biculture with three warm-season grasses in Kansas increased for-

age yield and CP concentration compared with grass monocultures (Posler et al., 1993).

Illinois bundleflower is also a promising perennial grain crop. Seed yields of 1700 kg ha⁻¹ have been obtained from unimproved accessions in Kansas (Kulakow et al., 1990). In Florida, annual seed yield with two harvests has exceeded 3000 kg ha⁻¹ (Adjei and Pitman, 1993). The seed contains no toxic levels of oxalates, cyanides, nitrates, or alkaloids (Kulakow et al., 1990) and has a crude protein content of about 380 g kg⁻¹ (Piper et al., 1988). As a potential multiple-use perennial grain and forage crop, Illinois bundleflower would provide flexibility and income for farmers while conserving natural resources (Soule and Piper, 1992).

Evaluation and selection have been performed with Illinois bundleflower accessions adapted to the southern portion of its range. The cultivar Sabine has been released for use in Texas, Oklahoma, and eastward (Muncrief and Heizer, 1985). It is useful for pasture mixes, wildlife plantings, and in revegetation of mined land. Kulakow (1999) evaluated diversity for characters related to grain production among 141 accessions obtained primarily from the Great Plains. Substantial variation was found in characters such as seed yield, seed size, shattering, and growth form, which led to the conclusion that the possibility of breeding Illinois bundleflower populations for use as a grain crop is promising. Accessions obtained from Texas and Oklahoma had poor survival at the study location in central Kansas. Lack of winter hardiness in accessions from slightly more southern latitudes than the study location suggests that a separate plant breeding program utilizing adapted accessions will be required to develop cultivars appropriate for the northern USA.

Diversity for agronomic characters in northern accessions of Illinois bundleflower is unknown, but would aid in determining plant breeding priorities. Information about the characteristics of specific northern accessions would enable plant breeders to make informed decisions about which crosses would aid in achieving plant improvement goals. Our objective was to test the hypothesis that genetic variation for selected characters within and among a representative set of northern accessions of Illinois bundleflower exists. From this, we could determine the geographic distribution of variation among the accessions and the phenotypic correlations among characters.

MATERIALS AND METHODS

Experiment 1

Seeds of 20 Illinois bundleflower accessions were obtained from the northern range of the species (Fig. 1). All accessions

Abbreviations: ADF, acid detergent fiber; CP, crude protein; NDF, neutral detergent fiber; NIRS, near infrared reflectance spectroscopy; PC, principal component; UPGMA, unweighted pair-group method, arithmetic average.

L.R. DeHaan, The Land Institute, 2440 E. Water Well Rd., Salina, KS 67401; N.J. Ehlke, C.C. Sheaffer, and D.L. Wyse, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108; R.L. DeHaan, Dordt College, 498 4th Ave NE, Sioux Center, IA 51250. Contribution of the Minnesota Agric. Exp. Stn. 1 Aug. 2002. *Corresponding author (dehaan@landinstitute.org).

were collected from wild populations, including five accessions obtained from the Land Institute, Salina, KS (Table 1). Seeds were scarified with sandpaper and planted in 40- by 205-mm cones in the greenhouse in early April 1998. The containers were surface-inoculated with appropriate rhizobium (Lipha-Tech, Milwaukee, WI) after planting. The accessions were transplanted in randomized complete block designs at three locations: St. Paul, MN; Becker, MN; and Sioux Center, IA. At St. Paul, there were five replications and six plants plot⁻¹, at Becker there were 10 replications and six plants per plot, and at Sioux Center there were seven replications and seven plants per plot. The soil at St. Paul was a Waukegan silt loam (fine-silty over sandy, mixed, mesic Typic Hapludoll) with pH, P, K, and organic matter levels of 7.6, 256 kg ha⁻¹, 352 kg ha⁻¹, and 28 g kg⁻¹, respectively. The soil at Becker was a Hubbard loamy sand (sandy, mixed, frigid Entic Hapludoll) with pH, P, K, and organic matter levels of 6.6, 88 kg ha⁻¹, 165 kg ha⁻¹, and 22 g kg⁻¹, respectively. The soil at Sioux Center was a Galva silty clay loam (fine-silty, mixed, superactive, mesic Typic Hapludoll) with pH, P, K, and organic matter levels of 7.0, 224 kg ha⁻¹, 672 kg ha⁻¹, and 60 g kg⁻¹, respectively. Because of limited seed supply, only entries 1 to 18 (Table 1) were established at Sioux Center and seed of entries 18 to 20 was only adequate for one to four plants per plot at St. Paul and Becker.

Illinois bundleflower plants were spaced 0.76 m apart in rows 1.52 m apart. This spacing minimized competition between adjacent plants and aided in measuring characters. Weeds were controlled with cultivation, hand weeding, mowing, and herbicide applications. Trifluralin [2,6-dinitro-*N,N*-dipropyl-4-(trifluoromethyl) benzenamine] was applied before planting at Becker and Sioux Center. Plants at St. Paul in 1998 and 1999 and at Becker in 1999 were covered and the plots were treated with imazethapyr [2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid], glyphosate [*N*-(phosphonomethyl) glycine], and pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine]. Plots at Sioux Center were treated with imazapic {(±)-2-[4,5-dihydro-4-methyl-4(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid} in 1999 and plots at Saint Paul and Becker were treated with imazapic and pendimethalin in 2000. At Becker, irrigation was provided according to the checkbook method (Wright and Bergsrud, 1991) in 1998 and 1999, but not in 2000.

We measured 58 characters, where the same measurements at different locations were considered unique characters (Table 2). Most characters were measured on every plant in

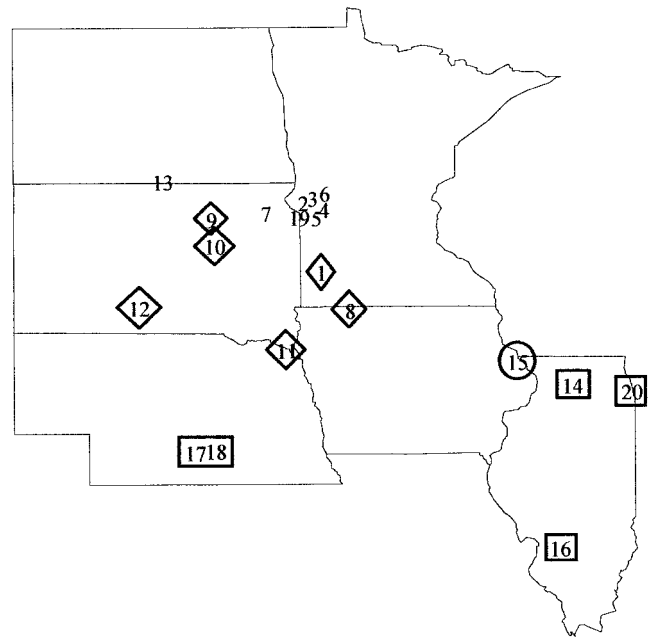


Fig. 1. Approximate locations of Illinois bundleflower entry origins. Clusters from UPGMA analysis are represented by shapes: Cluster 1, diamond; Cluster 2, square; Cluster 3, circle; Cluster 4, none.

each plot. Biomass yield, forage quality, and forage composition were determined by cutting one plant per plot to a height of 100 mm in mid-July 1999 and cutting a second plant in each plot in mid-August. Plants were not harvested in both months from plots containing fewer than four plants. Harvested plants were oven-dried at 60°C for 48 h before weighing to determine biomass yield. Growth staging was performed following the approach of Lancashire et al. (1991). Because the staging codes are not linear, we converted the codes to ranks before performing statistical calculations, resulting in a nonparametric analysis. To aid interpretation, the values presented (Table 2) were obtained by converting from ranks back to the growth stage code. Seed was harvested by manually cutting all plants not previously harvested for forage within a plot and mechanically threshing and cleaning the seed. Late flowering was rated in August 1998: plants with floral structures still in the bud stage were given a rating of one, and plants with all floral structures developed to the early pod stage were given a rating

Table 1. Entry numbers, accession numbers, and collection site information for Illinois bundleflower accessions evaluated for diversity.

Entry number	Accession identifier†	North latitude	West longitude	County	State
1	PNL532	44°15'00"	95°52'48"	Lyon	MN
2	PNL533	45°35'24"	96°29'24"	Traverse	MN
3	PNL534	45°32'24"	96°05'24"	Stevens	MN
4	PNL535	45°30'00"	96°00'00"	Stevens	MN
5	PNL536	45°23'24"	96°08'24"	Big Stone	MN
6	PNL537	45°49'48"	96°07'12"	Grant	MN
7	PNL538	45°24'36"	97°19'48"	Day	SD
8	PNL539	43°29'24"	95°06'00"	Dickenson	IA
9	PNL540	45°16'12"	98°45'00"	Edmonds	SD
10	PNL541	44°46'12"	98°42'00"	Spink	SD
11	PNL542	42°42'36"	96°48'00"	Union	SD
12	PNL543	43°33'36"	100°43'48"	Mellette	SD
13	PNL550	46°01'48"	100°04'48"	Emmons	ND
14	PNL544	42°00'00"	89°12'00"	Ogle	IL
15	PNL545	42°28'12"	90°39'36"	Dubuque	IA
16	LH1046	38°48'36"	89°33'00"	Bond	IL
17	LH1098	40°42'36"	99°07'48"	Buffalo	NE
18	LH1132	40°43'48"	98°49'48"	Buffalo	NE
19	LH1134	45°19'12"	96°27'00"	Big Stone	MN
20	LH1062	41°52'48"	87°36'36"	Cook	IL

† Accession origin: PNL = University of Minnesota Native Perennial Legume Collection, LI = The Land Institute, Salina, KS.

Table 2. Characters measured on Illinois bundleflower accessions at three locations, date measured, significance of accession effects, and mean and range of accession means.

Character description	Date	Becker, MN			St. Paul, MN			Sioux Center, IA		
		Significance	Mean	Range	Significance	Mean	Range	Significance	Mean	Range
Biomass yield (g plant ⁻¹)	July 1999	***	138	82.8–181	NS	78.8	27.9–139	***	52.6	16.9–101
Biomass yield (g plant ⁻¹)	Aug. 1999	*	395	295–495	NS	283	108–456	***	267	39.0–562
Forage acid detergent fiber (g kg ⁻¹)	July 1999	**	319	285–351	NS	258	214–302	***	262	184–319
Forage acid detergent fiber (g kg ⁻¹)	Aug. 1999	NS	444	415–480	NS	393	335–437	***	303	221–378
Forage neutral detergent fiber (g kg ⁻¹)	July 1999	*	364	333–409	NS	311	263–359	***	265	165–317
Forage neutral detergent fiber (g kg ⁻¹)	Aug. 1999	***	493	444–535	NS	436	333–501	***	333	244–394
Forage crude protein (g kg ⁻¹)	July 1999	*	168	151–190	*	192	166–236	***	241	209–298
Forage crude protein (g kg ⁻¹)	Aug. 1999	*	119	106–133	NS	150	125–175	***	218	183–265
Forage leaf fraction (g kg ⁻¹)	July 1999	***	587	543–658	NS	699	628–750	***	647	562–715
Forage leaf fraction (g kg ⁻¹)	Aug. 1999	***	200	128–310	**	311	159–454	***	473	325–646
Forage stem fraction (g kg ⁻¹)	Aug. 1999	***	370	287–484	*	405	305–561	***	351	252–421
Forage pod fraction (g kg ⁻¹)	Aug. 1999	***	430	205–540	***	302	63.4–513	***	179	67.4–286
Seed yield (g plant ⁻¹)	Oct. 1998	***	34.4	8.12–61.0	***	52.8	9.78–99.5	***	104	53.9–194
Seed yield (g plant ⁻¹)	Oct. 1999	***	70.6	42.4–97.1	***	59.2	32.7–108	***	—	—
Seed yield (g plant ⁻¹)	Oct. 2000	***	72.8	29.7–130	***	54.9	24.3–108	***	—	—
Seed weight (g 1000 seeds ⁻¹)	1998	***	6.66	5.79–7.47	***	5.77	4.59–6.52	—	—	—
Seed weight (g 1000 seeds ⁻¹)	1999	***	5.52	4.96–6.17	***	5.62	4.76–6.28	***	—	—
Seed weight (g 1000 seeds ⁻¹)	2000	***	5.69	4.93–5.98	***	5.94	5.21–6.28	***	6.06	4.89–6.85
Seed crude protein (g kg ⁻¹)	1998	***	337	299–363	***	350	314–376	***	—	—
Seed crude protein (g kg ⁻¹)	1999	***	302	282–332	NS	321	305–338	***	360	343–372
Seed crude protein (g kg ⁻¹)	2000	***	323	301–345	***	351	333–371	***	—	—
Maturity (1 = veg., 3 = flower, 5 = pod)	July 1998	***	2.73	1.10–4.03	***	1.99	1.07–3.24	—	—	—
Maturity (1 = veg., 3 = flower, 5 = pod)	Aug. 1998	***	3.24	1.00–5.00	***	3.36	1.00–4.79	—	—	—
Late flowering (1 = buds, 5 = no flowers)	Aug. 1998	***	1.99	1.15–3.23	***	4.03	2.26–4.97	—	—	—
Pod ripening (1 = none, 5 = 100%)	Sept. 1998	***	2.84	1.06–3.71	***	3.61	1.96–4.67	—	—	—
Senescence (1 = no leaf drop, 5 = 100%)	Sept. 1999	***	5.5	5.1–5.7	***	5.5	3.9–5.7	***	5.5	3.9–5.7
Growth stage	July 1999	***	8.0	7.5–8.5	***	7.5	6.9–8.1	***	—	—
Early growth	Aug. 1999	***	89.1	52.5–112	***	119	87.5–160	***	106	45.4–147
Lower leaf drop (mm from crown)	May 1999	***	670	460–835	***	512	270–683	—	—	—
Height of plants cut in July 1999 (m)	Aug. 1999	***	0.59	0.38–0.80	***	0.629	0.26–1.00	—	—	—
Height of never-cut plants (m)	Aug. 1999	***	1.16	0.57–1.67	NS	0.94	0.69–1.07	—	—	—
Height of plants cut in July 1999 (m)	Aug. 2000	***	0.98	0.51–1.38	***	1.10	0.58–1.60	—	—	—
Height of never-cut plants (m)	Aug. 2000	***	0.91	0.47–1.17	***	1.07	0.68–1.25	—	—	—
Width of plants cut in July 1999 (m)	Aug. 1999	*	0.78	0.64–0.93	*	0.83	0.26–1.52	—	—	—
Width of never-cut plants (m)	Aug. 1999	***	1.45	1.16–1.83	NS	1.38	1.05–1.64	—	—	—
Width of plants cut in July 1999 (m)	Aug. 2000	NS	1.53	1.11–1.81	NS	1.49	1.02–1.95	—	—	—
Width of never-cut plants (m)	Aug. 2000	***	1.48	0.96–1.81	*	1.39	0.94–1.85	***	0.27	0.11–0.46
Stem length (m)	July 1998	***	0.61	0.20–0.91	—	—	—	—	—	—
Stem length, plants cut July 1999 (m)	Sept. 1999	***	0.64	0.47–0.94	***	0.68	0.28–1.08	—	—	—
Stem length, never-cut plants (m)	Sept. 1999	***	1.22	0.85–1.75	*	1.09	0.94–1.25	—	—	—
Number of stems from crown	Aug. 1999	***	13.5	6.35–17.5	***	9.66	5.64–12.8	***	3.23	1.61–4.75
Weight of one bundle (g)	Sept. 1998	***	1.00	0.68–1.37	—	—	—	—	—	—
Number of pods per bundle	Sept. 1998	***	30.9	16.8–39.9	—	—	—	—	—	—
Length of five pods (mm)	Sept. 1998	***	170	152–207	—	—	—	—	—	—
Width of five pods (mm)	Sept. 1998	***	61.5	53.6–68.0	—	—	—	—	—	—
Length of one peduncle (mm)	Sept. 1998	***	50.4	41.3–58.0	—	—	—	—	—	—
Dehiscence rating (1 = none, 5 = 100%)	Sept. 1998	***	4.61	3.13–5.00	—	—	—	—	—	—
Length of three leaves (mm)	July 1999	***	240	182–286	***	260	224–287	—	—	—
Width of three leaves (mm)	July 1999	***	215	176–252	***	239	205–265	—	—	—
Number of pinnae on three leaves	July 1999	***	77.8	65.3–92.7	***	71.6	53.9–79.2	—	—	—
Vigor (1 = dead, 5 = vigorous)	Sept. 1998	***	4.62	3.00–5.00	***	4.65	3.00–5.00	***	3.65	1.86–4.9
Survival, proportion of plants still alive	July 1999	—	—	—	—	—	—	—	—	—
Survival, plants cut July 1999	July 2000	NS	0.43	0.00–0.88	NS	0.91	0.60–1.00	—	—	—
Survival, never-cut plants	July 2000	NS	0.02	0.00–0.11	***	0.22	0.00–1.00	—	—	—
Survival, plants cut July 1999	July 2001	—	0.63	0.16–0.88	—	—	—	—	—	—
Survival, plants cut August 1999	July 2001	—	—	—	—	—	—	—	—	—
Survival, never-cut plants	July 2001	—	—	—	—	—	—	—	—	—

*, Significant at the 0.05 probability level.
 **, Significant at the 0.01 probability level.
 ***, Significant at the 0.001 probability level.
 NS, not significant.
 † Character not measured at this location.

of five. Early season growth was estimated by measuring the length of the longest stem developing from each crown in May 1999. The lower leaves on Illinois bundleflower plants often yellow and drop as the plant matures. Distance measured from the crown to the lowest remaining leaf on each plant was used as an indication of the extent of leaf drop in August 1999. Plant height was measured as the distance from the soil surface to the top of the longest stem. Plant width was measured as the distance across each plant when measured perpendicular to the plot row. Stem length was measured as the length of the longest stem on a plant, from the crown to its apex. Measurements on bundles refer to the peduncle and all the pods attached to it in a single inflorescence. Two representative bundles were harvested from each plant at maturity to determine bundle weight and number of pods per bundle. The length and width of five pods were determined by measuring five representative pods from each plant. Peduncle length was determined by measuring two representative peduncles from each plant. Leaf characters were measured from three healthy, fully expanded leaves from each plant. Survival was recorded as the proportion of plants remaining alive within a plot at various times.

Experiment 2

In 1998, seed was collected from four randomly selected plants of each accession at the Becker location of Exp. 1. Progeny from a single maternal plant comprised one family, which was nested within accession. Using a protocol similar to Exp. 1, we established experiments at St. Paul, MN, and Rosemount, MN, in 1999. There were five replications and one plant per plot in a randomized complete block design. At St. Paul, weeds were controlled with imazapic and pendimethalin. At Rosemount, weeds were controlled with trifluralin in 1999 and imazapic in 2000. In August 1999, plant height was measured. In September 2000, plant height, plant width, seed yield, weight of 1000 seeds, and seed CP were measured. In June 2001, early growth (length of longest stem) and survival were measured.

Analysis

Forage CP (AOAC, 1985), NDF, and ADF (Goering and Van Soest, 1970), forage composition (leaf, stem, and pod fraction), seed CP, and weight of 1000 seeds in 2000 (both experiments) were determined by means of near infrared reflectance spectroscopy (NIRS). A 500-g subsample of the forage harvested from each plant in July and August 1999 was ground in a Wiley mill with a 1 mm screen. The samples were tumbled in a drum turning at 15 rpm for 20 min to obtain homogeneity. Seeds were scanned whole but ground before CP analysis of reference samples. Spectra were collected on all samples with a NIRS scanning monochromator, model 6500 (Foss North America, Inc., Eden Prairie, MN) and NIRS version 4.0 software (Infrasoft International, Port Matilda, PA 16870). Reflectance data were recorded between 400- and 2500-nm wavelengths at 2-nm intervals. Equations for leaf, stem, and pod fractions were developed using 50 samples that were hand separated, weighed, ground, and reconstituted. Reference procedures for all other characters were performed on 50 samples selected by the software and prediction equations were developed. The 1 - VR values for the prediction equations were 0.97, 0.99, 0.98, 0.94, 0.74, 0.97, 0.90, and 0.84 for forage CP, NDF, ADF, leaf fraction, stem fraction, pod fraction, seed CP, and weight of 1000 seeds, respectively.

For Exp. 1, the mean, maximum, and minimum values of each entry were calculated for each character at each location to assess the range and distribution of values for each charac-

ter. An analysis of variance (ANOVA) was performed on plot means of every character for each location in which it was measured with PROC MIXED (Littell et al., 1996). Entry was treated as a fixed effect and block as random. Adjusted means were calculated for the entries at each location.

Principal component (PC) analysis across all locations was performed on standardized [(accession mean - character mean)/standard deviation] adjusted means using only the character-location combinations that differed among accessions (Table 2) by NTSYSpc (Rohlf, 2000). Correlations between the first three PCs and the initial characters were calculated to aid in interpretation of the analysis. To examine relationships among selected characters further, a correlation analysis was performed with Spearman-rank correlations (SAS Institute, 1990). Spearman-rank correlations were used because the data were not normally distributed.

Cluster analysis across all locations was performed with all entries from the character-location combinations that were influenced by entry in NTSYSpc. The average taxonomic distances (Rohlf, 2000) between all accessions were calculated from adjusted means. The resulting distance matrix was used to generate a tree by means of the UPGMA (unweighted pair-group method, arithmetic averages) clustering procedure. An average taxonomic distance of 1.3 was selected arbitrarily to divide the entries into seven clusters that were consistent with the principal component analysis.

For Exp. 2, PROC MIXED was used to calculate variance components for each character. The model consisted of the following effects, all considered random: location, block within location, accession, accession by location, family within accession, family within accession by location, and pooled error. Significance of variance component estimates was tested with likelihood ratio tests (Littell et al., 1996).

RESULTS AND DISCUSSION

Diversity among Accessions

In Exp. 1, every measured character was influenced by entry in at least one location (Table 2). Fifty-one of 54 characters were influenced by entry at Becker, 38 of 51 at St. Paul, and 20 of 20 at Sioux Center ($P < 0.05$). Thus, substantial genetic diversity exists among northern accessions of Illinois bundleflower for a wide array of morphological, agronomic, and phenological characters.

Differences among entries in maturity, height, and width were observed in the field, with some examples shown (Fig. 2). Two unique phenotypes that appeared to be qualitative traits were also observed. Two plants were identified within Entry 14 that were highly branched and had small leaves and pods (Fig. 2). Three completely prostrate plants were identified within Entry 7 (Fig. 2). The qualitative inheritance of these traits was confirmed (DeHaan et al., 2002, unpublished data), and they may be useful as markers in genetic studies.

Maximum biomass yield of 562 g plant⁻¹ was obtained from Entry 16 in August at Sioux Center. At the planting density used (0.87 plants m⁻²), this yield translates to 4.9 Mg ha⁻¹. The maximum yield obtained from a single August cutting of Illinois bundleflower is about 50% of yields of alfalfa (*Medicago sativa* L.) cut three times per year in a similar location (Sheaffer et al., 2000). Optimal planting density of Illinois bundleflower may be much higher than the density used in this study. Therefore, the highest yields observed in this study on an area basis

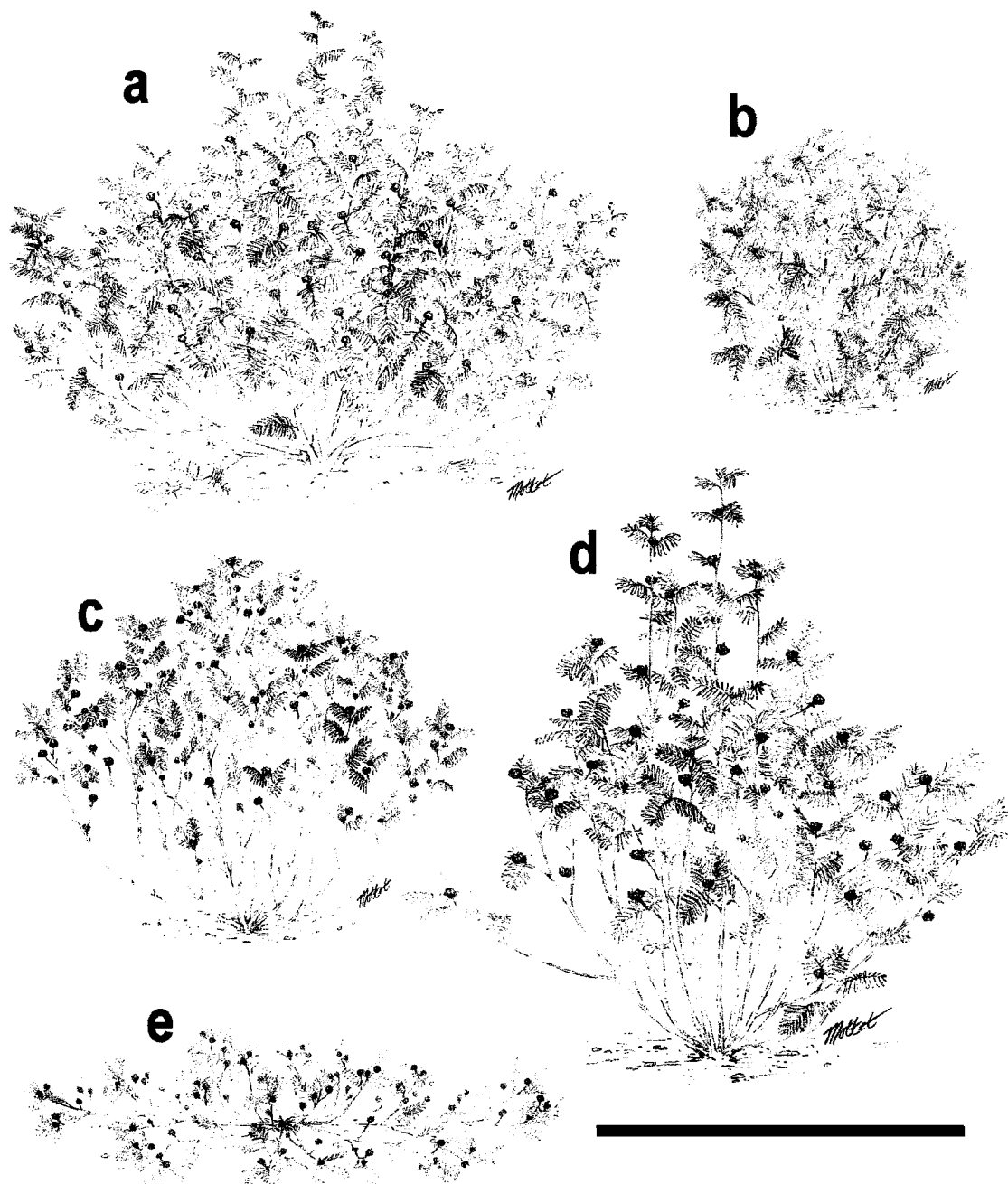


Fig. 2. Illustrations of diverse third-year Illinois bundleflower plants in late August. (a) Entry 16 typical plant. (b) Highly branched plant from Entry 14. (c) Entry 6 typical plant. (d) Entry 20 typical plant. (e) Prostrate plant from Entry 7 (bar = 1 m).

may be below potential yields under optimal management. The importance of selection for high biomass yield is clear because the lowest yielding entry at Sioux Center yielded only 7% of Entry 16 in August. Across all three locations, average August biomass was more than 300% greater than average July biomass, demonstrating the potential of Illinois bundleflower to supply forage in the hot summer months.

In July, location average CP, ADF, and NDF values (Table 2) were similar or superior to those of alfalfa harvested at the early flower stage (Sheaffer et al., 2000). Average leaf fraction across entries and locations in July was 644 g kg^{-1} , which is high relative to the 500 g kg^{-1} that is typical of alfalfa at the early flower stage

(Sheaffer et al., 2000). Low plant density may have contributed to the high leaf fractions relative to alfalfa in dense plots.

August forage quality varied widely by location (Table 2). Plants at Becker had the lowest forage quality, which was visually associated with earlier maturity and leaf drop at this location. At St. Paul, average August ADF and NDF concentrations were 393 and 436 g kg^{-1} , superior to alfalfa harvested at the late flower stage (Sheaffer et al., 2000). However, CP concentration was only 150 g kg^{-1} , lower than alfalfa harvested at the late flower stage [about 170 g kg^{-1} (Sheaffer et al., 2000)]. August forage quality was the highest at Sioux Center. Entry 16, which had the highest August biomass yield,

had CP, ADF, and NDF concentrations of 190, 353, and 394 g kg⁻¹, which is similar to alfalfa harvested at the early flower stage (Sheaffer et al., 2000). High August forage quality at Sioux Center was probably due to slow late-season maturation at this location. In August, average pod fraction at Sioux Center was only 179 g kg⁻¹, compared with 430 g kg⁻¹ at Becker. Although more data are needed, these results indicate that harvesting Illinois bundleflower when the pod fraction is about 180 g kg⁻¹ may optimize forage yield and quality.

Seed yield in 1998 at St. Paul and Becker averaged 43.6 g plant⁻¹, and increased in the following 2 yr at these locations (Table 2). Highest seed yields were in 1999 at Sioux Center. Entry 16 had the highest seed yield at this location, 194 g plant⁻¹. This yield corresponds to 1.7 Mg ha⁻¹. The maximum seed yield recorded by Kulakow (1999) in Kansas was also 1.7 Mg ha⁻¹, but in the first year rather than the second. Average second year seed yield across accessions and locations was 0.7 Mg ha⁻¹, similar to the average seed yield of 0.6 Mg ha⁻¹ obtained with primarily southern accessions in Kansas. These results indicate that seed yield of Illinois bundleflower is not necessarily lower in the northern range of its distribution, although an establishment year may be required to obtain maximum seed yield in this region. Maximum seed yields on an area basis may be substantially higher at higher planting densities.

Average seed yield at Becker in 2000 in the absence of irrigation was 72.8 g plant⁻¹. This yield was nearly equal to seed yield the previous year when irrigation was supplied. The highest yielding accession produced the equivalent of 1.1 Mg ha⁻¹ at Becker with no irrigation. For comparison, dryland soybean [*Glycine max* (L.) Merr.] at the same location yielded about 1.4 Mg ha⁻¹ and irrigated soybean yielded about 3.4 Mg ha⁻¹ (J.H. Orf, personal communication, 2001). These results indicate that seed yield of Illinois bundleflower may be more stable than annual crops in dry environments.

Average seed weight in 1999 was 5.7 g 1000 seeds⁻¹, similar to the average seed weight of 6.1 g 1000 seeds⁻¹ with southern accessions (Kulakow, 1999). The range in seed size across accessions and locations in 1999 was 4.8 to 6.9 g 1000 seeds⁻¹, whereas previously evaluated, primarily southern, accessions in a very different environment had a range from 4.3 to 9.7 g 1000 seeds⁻¹ (Kulakow, 1999). Thus, previously evaluated accessions may be an important source of diversity for improving this character.

Seed CP concentration within northern Illinois bundleflower accessions was less than reported previously for southern accessions. Average CP concentration across entries and locations in 1999 was 328 g kg⁻¹, whereas two southern accessions had seed CP concentrations of 367 and 389 g kg⁻¹ (Piper et al., 1988). Southern accessions may be an important source of diversity for improving the CP content of seed, or northern environments may limit seed CP concentration. For comparison, the average CP concentration of soybeans grown in central Minnesota is about 360 g kg⁻¹ (Pazdernik et al., 1997).

Survival varied widely by accession and location. No plants were lost in 1999 at Becker. At St. Paul in 1999,

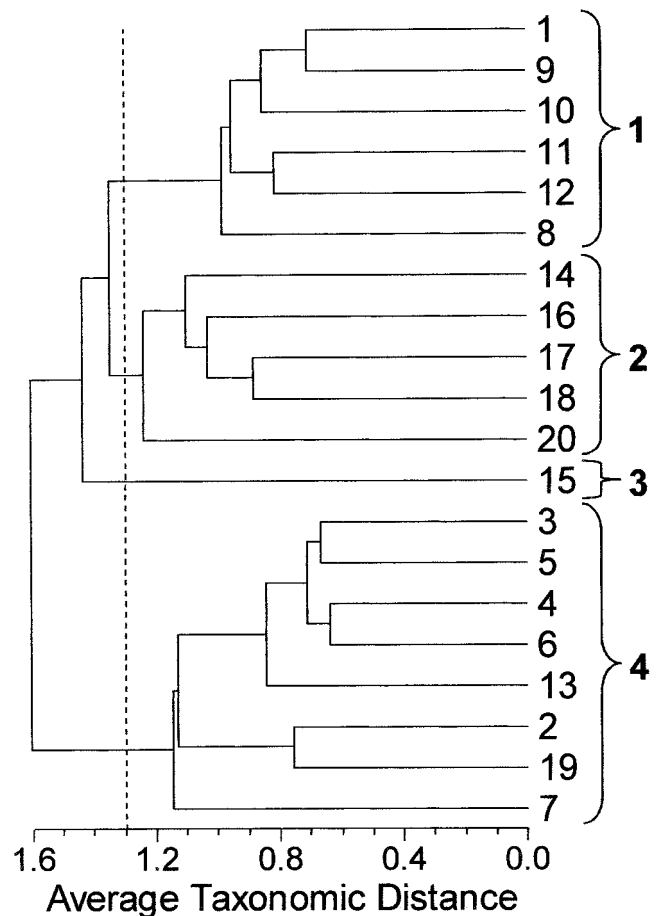


Fig. 3. Illinois bundleflower entries clustered by UPGMA. The four clusters were created by selecting a cutoff of 1.3 (indicated by dashed line).

survival ranged from 13 to 97%. In 2000, survival of plants cut in July was consistently higher than survival of plants cut in August (Table 2), indicating that later forage harvests may reduce stand persistence. At St. Paul, most entries had less than 20% survival when cut in August. However, August-cut plants of entries 3, 7, and 19 had survival of 80% or higher. Therefore, selection for persistence under late-season cutting or grazing appears possible.

Cluster and Principal Component Analysis

Cluster analysis by UPGMA (Fig. 3) provided an adequate grouping of the accessions (cophenetic correlation = 0.74). Accessions within the major clusters were derived from similar geographic origins (Fig. 1). Accessions in Cluster 1 originated from southern and eastern South Dakota and nearby regions of Iowa and Minnesota. Cluster 2 accessions were obtained from $\leq 42^\circ$ N lat. The single accession in Cluster 3 came from eastern Iowa. Accessions in Cluster 4 were derived from a localized region of west central Minnesota and nearby South Dakota, with one accession from south central North Dakota. The clear agreement of the clusters with geographical origin indicates that additional collections from other regions would be likely to provide further diversity for plant breeding. Regions that were under-

Table 3. Correlations of the first three principal components (PCs) with the original Illinois bundleflower characters from which the PCs were derived, and date the characters were measured.

Trait	Date	Becker, MN			St. Paul, MN			Sioux Center, IA		
		PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
Biomass yield (g plant ⁻¹)	July 1999	0.32	0.74†	0.13				0.87	0.05	-0.38
Biomass yield (g plant ⁻¹)	Aug. 1999	0.65	0.34	0.11				0.85	-0.04	-0.14
Forage acid detergent fiber (g kg ⁻¹)	July 1999	0.59	0.58	0.29				0.88	0.11	-0.27
Forage acid detergent fiber (g kg ⁻¹)	Aug. 1999							0.94	-0.04	-0.05
Forage neutral detergent fiber (g kg ⁻¹)	July 1999	0.46	0.62	0.29	-0.20	0.57	0.30	0.87	0.17	-0.21
Forage neutral detergent fiber (g kg ⁻¹)	Aug. 1999	-0.46	0.45	0.56				0.93	-0.03	-0.09
Forage crude protein (g kg ⁻¹)	July 1999	-0.47	-0.09	-0.27	0.55	-0.31	-0.05	-0.86	-0.09	0.30
Forage crude protein (g kg ⁻¹)	Aug. 1999	0.13	-0.29	-0.42				-0.92	0.06	0.10
Forage leaf fraction (g kg ⁻¹)	July 1999	-0.54	-0.44	-0.18				-0.90	0.08	0.28
Forage leaf fraction (g kg ⁻¹)	Aug. 1999	0.54	-0.52	-0.29	0.66	-0.16	-0.02	-0.84	0.03	0.28
Forage stem fraction (g kg ⁻¹)	Aug. 1999	0.83	-0.18	0.26	0.67	-0.23	-0.02	0.88	0.01	0.23
Forage pod fraction (g kg ⁻¹)	Aug. 1999	-0.80	0.38	-0.01	-0.75	0.22	0.08	0.39	-0.06	-0.49
Seed yield (g plant ⁻¹)	Oct. 1998	0.87	0.29	-0.02	0.88	0.24	-0.11			
Seed yield (g plant ⁻¹)	Oct. 1999	0.71	0.19	0.12	-0.20	0.71	-0.14	0.59	-0.14	-0.25
Seed yield (g plant ⁻¹)	Oct. 2000	0.46	0.56	-0.63	0.27	0.27	-0.15			
Seed weight (g 1000 seeds ⁻¹)	1998	-0.78	0.15	-0.41	-0.77	0.35	-0.25			
Seed weight (g 1000 seeds ⁻¹)	1999	0.13	-0.05	-0.66	-0.52	0.28	-0.51	-0.55	0.21	-0.32
Seed weight (g 1000 seeds ⁻¹)	2000	-0.40	0.46	-0.33	-0.62	0.51	-0.33			
Seed crude protein (g kg ⁻¹)	1998	-0.90	0.17	-0.26	-0.93	0.23	-0.13			
Seed crude protein (g kg ⁻¹)	1999	0.50	-0.39	-0.19				-0.44	0.49	-0.40
Seed crude protein (g kg ⁻¹)	2000	-0.28	0.18	-0.74	-0.40	0.21	-0.56			
Maturity (1 = veg., 3 = flower, 5 = pod)	July 1998	0.74	0.50	-0.28	0.30	0.57	-0.53			
Late flowering (1 = buds, 5 = no flowers)	Aug. 1998	-0.76	0.51	0.05	-0.73	0.54	0.05			
Pod ripening (1 = none, 5 = 100%)	Sept. 1998	0.19	0.71	-0.39	-0.70	0.50	-0.15			
Senescence (1 = no leaf drop, 5 = 100%)	Sept. 1998	0.04	0.88	0.17	-0.28	0.87	-0.10			
Growth stage	July 1999	-0.77	0.38	-0.21	-0.93	0.12	-0.13	0.62	0.28	-0.31
Growth stage	Aug. 1999	-0.89	0.32	-0.19	-0.94	-0.02	-0.12			
Early growth, longest stem length (mm)	May 1999	0.24	0.73	0.28	-0.72	0.27	0.08	0.81	0.22	0.03
Lower leaf drop (mm from crown)	Aug. 1999	-0.13	0.78	0.08	-0.64	0.29	0.24			
Height of plants cut in July 1999 (m)	Aug. 1999	0.88	0.28	-0.23	0.81	0.09	-0.20			
Height of never-cut plants (m)	Aug. 1999	0.87	0.07	0.25						
Height of plants cut in July 1999 (m)	Aug. 2000	0.82	0.39	0.16	0.82	0.24	0.24			
Height of never-cut plants (m)	Aug. 2000	0.66	0.35	0.47	0.69	0.07	-0.08			
Width of plants cut in July 1999 (m)	Aug. 1999	0.09	0.28	-0.70	0.49	0.11	-0.09			
Width of never-cut plants (m)	Aug. 1999	0.87	-0.01	0.00						
Width of never-cut plants (m)	Aug. 2000	0.37	0.63	-0.07	0.03	0.57	0.20			
Stem length (m)	July 1998	0.90	0.32	0.06				0.91	-0.04	-0.27
Stem length, plants cut July 1999 (m)	Sept. 1999	0.90	0.00	-0.40	0.80	0.01	-0.20			
Stem length, never-cut plants (m)	Sept. 1999	0.91	-0.03	0.28	0.37	0.14	0.58			
Number of stems from crown	Aug. 1999	0.44	0.72	0.02	-0.28	0.59	0.16	0.79	-0.05	-0.20
Weight of one bundle (g)	Sept. 1998	0.82	0.31	0.13						
Number of pods per bundle	Sept. 1998	0.84	0.29	0.32						
Length of five pods (mm)	Sept. 1998	0.28	0.32	-0.68						
Width of five pods (mm)	Sept. 1998	-0.51	-0.02	-0.40						
Length of one peduncle (mm)	Sept. 1998	0.64	0.49	0.15						
Dehiscence rating (1 = none, 5 = 100%)	Sept. 1998	-0.14	-0.12	-0.54						
Length of three leaves (mm)	July 1999	0.13	0.44	0.27	-0.39	0.43	0.10			
Width of three leaves (mm)	July 1999	0.49	-0.09	-0.03	0.29	0.20	0.46			
Number of pinnae on three leaves	July 1999	-0.04	0.47	0.34	-0.55	0.09	-0.21			
Vigor (1 = dead, 5 = vigorous)	Sept. 1998	0.73	0.46	-0.09	0.63	0.55	-0.16	0.91	0.16	-0.08
Survival, proportion of plants still alive	July 1999				-0.82	0.31	0.20			
Survival, plants cut July 1999	July 2000	-0.83	-0.15	0.21						
Survival, plants cut August 1999	July 2000				-0.72	-0.31	-0.14			
Survival, never-cut plants	July 2000	-0.69	0.56	0.26	-0.86	0.29	0.02			
Survival, plants cut August 1999	July 2001				-0.67	-0.33	-0.12			
Survival, never-cut plants	July 2001				-0.89	0.32	-0.08			

† Correlations ≤ -0.55 and ≥ 0.55 are underscored.

represented in the current study, such as central Iowa and eastern Minnesota, should be targeted for additional collecting. Further support for collecting from new regions to enhance available diversity is that the geographic distances between accessions were correlated with the taxonomic distances ($r = 0.55$, $P < 0.001$ by Mantel test). The correlation indicates that geographically adjacent accessions will be more similar than geographically distant accessions.

The first three PCs explained 44.9, 14.0, and 8.5% of the variance, respectively, and explained 66.9% of the total variance. Most characters were correlated ($r > 0.55$ or $r < -0.55$, $P < 0.05$) with one of the first three PCs (Table 3). All but Clusters 2 and 3 were clearly

separated by the first two PCs (Fig. 4), and Cluster 3 was clearly separated from all others by the first and third PCs (Fig. 5). Clusters that are separated by a given PC will differ for characters highly correlated with that PC. Most characters were well correlated with at least one of the first three PCs. Therefore, positions of the clusters on the plots of the first three PCs can be used to infer relative values for many characters. Accessions in Cluster 4 had very low values for PC 1, so these accessions are generally early maturing, have short stems, have low first-year vigor, and have a high survival rate. Accessions in Clusters 2 and 3 had the highest values for PC 1, indicating that they were late maturing, had low survival, and had the highest biomass and seed

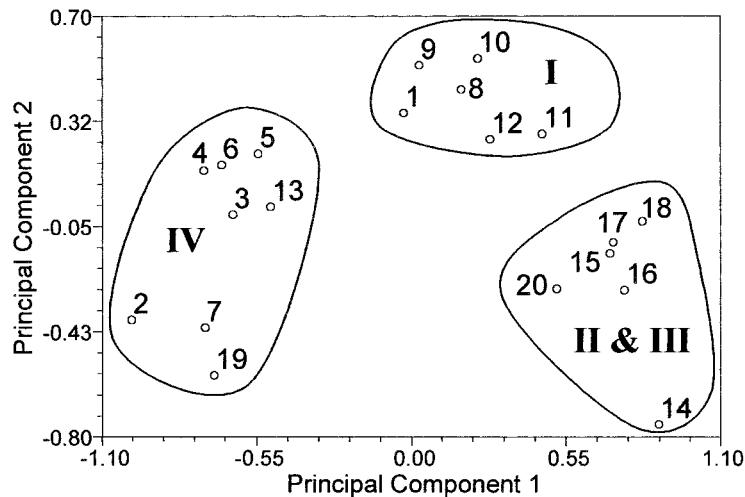


Fig. 4. Plot of the first two principal components from analysis of Illinois bundleflower characters listed in Table 3. Clusters from UPGMA analysis are labeled with Roman numerals.

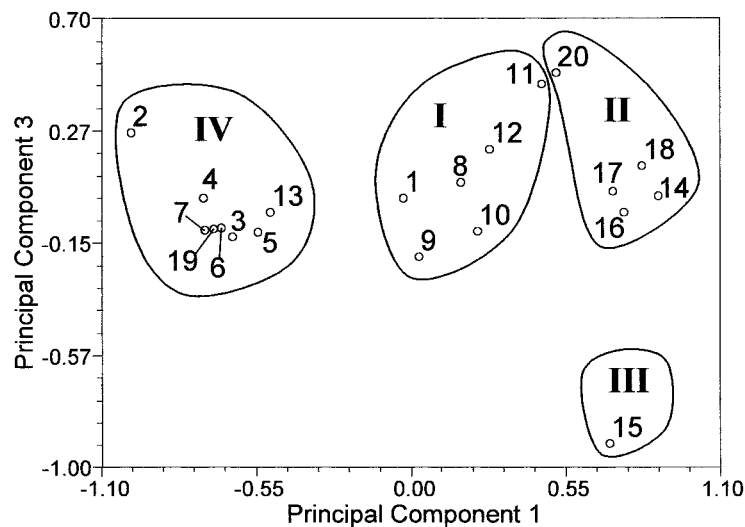


Fig. 5. Plot of the first and third principal components from analysis of Illinois bundleflower characters listed in Table 3. Clusters from UPGMA analysis are labeled with Roman numerals.

yields and lowest forage quality at Sioux Center. The first PC was well correlated with latitude of accession origins ($r = -0.88$, $P < 0.001$), which indicates that the late maturity, low survival, and occasionally high yields of accessions in Clusters 2 and 3 were probably due to their southern origin. Cluster 1 accessions were intermediate for many characters, having moderate winter survival, intermediate maturity, and average seed and forage yields. Cluster 3, consisting only of Accession 15, was clearly separated from all other accessions by PC 3, indicating that this cluster is extreme for characters well correlated with PC 3, such as seed CP in 2000.

Many characters had similar PC correlations across all three locations, indicating that for these characters location by accession interactions were small. In instances where PC correlations differed by location, we can use the PC analysis to examine the interaction among accessions and locations. At Sioux Center, all but two characters were well correlated with the first principal component. At this location, first-year vigor appeared to have an overwhelming influence on almost

all other characters. Accessions with large values for PC1 had good first-year vigor and continued to be the largest and most rapidly developing in the following year. At Becker and St. Paul, seed yield was correlated with PC1 in 1998. However, in 1999, seed yield at St. Paul was correlated with PC2 and in 2000 seed yield at Becker was correlated with PC2. These changes most likely occurred because of winter injury to accessions with the highest values for PC1. We conclude that in the absence of winter injury, the southern accessions with high values for PC1 have the highest potential for seed yield, but when winter injury occurs accessions with high values for PC2 will generally produce the most seed.

Correlation Analysis

Forage yield, forage quality, seed yield, and seed quality characters were often not well correlated with one of the first three PCs. Therefore, relationships among these characters cannot be easily determined from the

PC analysis. To examine relationships among these characters, Spearman-rank correlations were calculated.

Correlations of seed yields across locations and years were not consistent. Seed yields were correlated across years at Becker (average $r = 0.65$, $P < 0.01$), but not at St. Paul, which was probably due to winter injury. Across locations within years, St. Paul yields were correlated with Becker yields in 1998 ($r = 0.73$, $P < 0.001$) and 2000 ($r = 0.48$, $P < 0.05$). Instances where correlations in seed yield are low can often be explained by differences in winter injury. Therefore, selecting for survival will be an important means of insuring consistent seed yield in Illinois bundleflower in northern environments.

Seed CP concentration of accessions was generally well correlated across both years and locations with the exception of seed produced at Becker in 1999. The seed CP from this year and location was not positively correlated with any other year or location. If Becker 1999 is excluded, seed CP of all other years and locations was correlated (average $r = 0.66$, $P < 0.01$). We conclude that breeding for consistently high seed CP across locations would be a reasonable objective, although seed CP may be unpredictable in some environments.

To examine the influence of seed yield and seed weight on seed CP, we calculated correlations for these characters within each year and location. Within year and location, seed CP was always correlated with seed weight (average $r = 0.71$, $P < 0.05$). The strong positive correlation indicates that selection for seed weight would likely have a positive influence on seed CP. Seed yield was negatively correlated with seed CP at St. Paul and Becker in 1998 (average $r = -0.78$, $P < 0.001$) but not correlated in the second and third years. Therefore, selection for seed yield in mature plants would not necessarily produce a correlated reduction in seed CP.

Correlations were calculated between biomass yield, forage CP, NDF, and forage composition (leaf, stem, and pod) within and among locations within the July and August harvests. For both harvests, none of these characters was consistently correlated across locations. The lack of correlation across locations could have been due to different maturities at the three locations. Lack of correlation across locations indicates that selection will necessarily have to be conducted in multiple locations. Within location in July, biomass yield (average $r = 0.73$, $P < 0.05$) and leaf fraction of the forage (average $r = -0.75$, $P < 0.05$) were correlated with NDF. Within location in August, leaf fraction of the forage was correlated with NDF (average $r = -0.89$,

$P < 0.001$) and forage CP (average $r = 0.67$, $P < 0.05$), and forage pod fraction was correlated with forage leaf fraction (average $r = -0.80$, $P < 0.01$). These correlations indicate that forage nutritive value declines with advancing plant maturity, as occurs in many species.

Diversity within Accessions

Experiment 2 evaluated progeny of four maternal parents from each accession used in Exp. 1. If we assume 100% self-pollination, progeny of maternal plants (families) would be genetically identical, and differences among families from different maternal plants within an accession would estimate within-accession diversity. Because Illinois bundleflower is about 80% self-pollinating, we used among-family diversity as a conservative estimate of within-accession diversity.

We found within-accession diversity for seven of eight measured characters (Table 4). Within-accession diversity for survival indicates that selection within southern populations could improve survival when planted farther north. Seed yield showed within-accession diversity but not between-accession diversity, in contrast to Exp. 1. Therefore, selection for seed yield could be successfully performed within accessions that have other desirable characters. For height in 1999 and 2000, only 16 and 12%, respectively, of the genetically controlled variance was within accession. Selection for plant height within accession would produce limited gains compared with selection for plant height between accessions. Of the genetically controlled variance, 29% for seed weight and 36% for seed CP was within accession. Relatively high within-accession diversity for these characters indicates that they could be improved by selection both between and within accessions.

DeHaan et al. (2003) have studied within- and between-accession genetic diversity in Illinois bundleflower using AFLP (amplified fragment length polymorphism) molecular markers. An analysis of molecular variance (Excoffier et al., 1992) indicated that 57% of the diversity in northern accessions of Illinois bundleflower was within accessions. A second statistic, the within-accession diversity estimate, (Nei, 1973) calculated for northern populations indicated that 31% of the diversity was within accessions. Across all characters measured in Exp. 2, 33% of the genetically controlled variance was found within accession.

CONCLUSIONS

Variation is present among northern Illinois bundleflower accessions for characters including forage

Table 4. Variance component estimates for Illinois bundleflower families grown at two locations. Characters measured were seed yield, seed weight, seed crude protein, height, and width in 2000; height in 1999; and early growth and survival in 2001.

Variance Component	Seed yield	Seed weight	Seed CP	Height 1999	Height 2000	Width	Early growth	Survival
Location	4.469***	0.001	0.036	0.1	95.5***	1030.0***	7.10***	0.000
Block(Loc)	0.023	0.002*	0.029	0.4	2.2	0.0	0.13	0.000
Accession	0.149	0.082***	0.500*	289.0***	256.1***	34.3	3.85	0.031***
Loc×Acc	0.130	0.007	0.050	3.6	39.8***	61.9***	4.23**	0.000
Family(Acc)	0.327**	0.034***	0.278***	56.1***	36.4***	48.2*	1.02	0.009***
Loc×Family(Acc)	0.149	0.005	0.000	0.0	13.0	33.5	2.11	0.000
Residual	2.486	0.145	1.633	283.3	241.2	697.7	33.78	0.106

*, Significant at the 0.05 probability level.

**, Significant at the 0.01 probability level.

***, Significant at the 0.001 probability level.

yield, growth form, maturity, survival, seed yield, seed size, and seed CP concentration. We conclude that the accessions we evaluated could provide much of the genetic diversity required for improving the performance of Illinois bundleflower as a perennial grain or forage crop in the north central USA. The seed and forage yield and forage quality of unimproved accessions is remarkably high, indicating that Illinois bundleflower could become an economically important species after a modest plant breeding effort.

Much of the variation among northern Illinois bundleflower accessions can be explained by latitude of origin. The first PC was highly correlated with latitude of origin and explained 44.9% of the total variance. Accessions from lower latitudes have greater forage and seed yield potential, are later maturing, but often lack persistence. Accessions from higher latitudes are typically less productive and more persistent. Because geographic diversity was associated with phenotypic diversity of accessions, collections should be made from under-represented geographic regions to increase the available genetic diversity. Although these generalities are useful, they must not be overstated. Even among accessions from South Dakota, there is a wide range of seed yield. Tables of the adjusted accession means and their standard errors for all measured characters have been published (DeHaan, 2001), and are available online (http://www.landinstitute.org/pages/IBF_Tables.PDF; verified 6 February 2003). Substantial diversity for characters including seed yield and survival also exists within some accessions, indicating that these characters could be improved by selecting from within accessions that are otherwise desirable.

Knowledge of the associations among characters in Illinois bundleflower will be valuable in planning future work with the species. Seed yield was associated with lower winter injury in many locations and years. Therefore, selection for survival will be an important means to ensure consistent seed yield. Seed CP concentration was correlated across most locations, indicating that this character could be readily improved in cultivars planted across a wide geographic range. The strong correlation between seed size and seed CP concentration indicates that selection should be effective in increasing both characters simultaneously. Seed yield and seed CP were not negatively correlated in the second or third years, an indication that simultaneously increasing these characters via selection is promising. Leaf and pod fractions were correlated with forage quality characters. Changes in forage composition with advancing maturity will impact forage quality and must be considered in future agronomic and plant breeding research.

ACKNOWLEDGMENTS

We gratefully acknowledge funding provided by the Minnesota Environment and Natural Resources Trust Fund, as recommended by the Legislative Commission on Minnesota Re-

sources; North Central Region Sustainable Agriculture Research and Education (NCR SARE); The Land Institute, Salina, KS; and the University of Minnesota Graduate School. David Mottet drew the illustrations.

REFERENCES

- Adjei, M.B., and W.D. Pitman. 1993. Response of *Desmanthus* to clipping on a phosphatic clay mine-spoil. *Trop. Grassl.* 27:94–99.
- AOAC. 1985. Official methods of analysis. 14th ed. Association of Official Analytical Chemists. Washington, DC.
- DeHaan, L.R. 2001. Analysis of diversity among accessions of *Amorpha fruticosa* L. and *Desmanthus illinoensis* (Michx.) Macmillan. Ph.D. diss. (Diss. Abstr. AAT 3020586). Univ. of Minnesota, St. Paul.
- DeHaan, L.R., N.J. Ehlke, C.C. Sheaffer, G.J. Muehlbauer, and D.L. Wyse. 2003. Illinois bundleflower genetic diversity determined by AFLP analysis. *Crop Sci.* 43:402–408.
- Dovel, R.I., M.A. Hussey, and E.C. Holt. 1990. Establishment and survival of Illinois bundleflower interseeded into an established kleingrass pasture. *J. Range Manage.* 43:153–156.
- Excoffier, L., P.E. Smouse, and J.M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131:479–491.
- Goering, H.K., and P.J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures and some applications). USDA Agric. Handb. 379. U.S. Gov. Print. Office, Washington, DC.
- Great Plains Flora Association. 1986. Flora of the Great Plains. University Press of Kansas, Lawrence, KS.
- Kulakow, P.A. 1999. Variation in Illinois bundleflower (*Desmanthus illinoensis* (Michaux) MacMillan): A potential perennial grain legume. *Euphytica* 110:7–20.
- Kulakow, P.A., L.L. Benson, and J.G. Vail. 1990. Prospects for domesticating Illinois bundleflower. p. 168–171. *In* J. Janick and J.E. Simon (ed.) *Advances in new crops*. Timber Press, Portland, OR.
- Lancashire, P.D., H. Bleiholder, T. van den Boom, P. Langelüddeke, R. Stauss, E. Weber, and A. Witzemberger. 1991. A uniform decimal code for growth stages of crops and weeds. *Ann. Appl. Biol.* 119: 561–601.
- Littell, R.C., G.A. Milliken, W.W. Stroup, and R.D. Wolfinger. 1996. SAS system for mixed models. SAS Inst., Cary, NC.
- Muncrief, J.B., and R.B. Heizer. 1985. Registration of 'Sabine' Illinois bundleflower. *Crop Sci.* 25:1124.
- Nei, M. 1973. Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. (USA)* 70:3321–3323.
- Pazdernik, D.L., L.L. Hardman, and J.H. Orf. 1997. Agronomic performance and stability of soybean varieties grown in three maturity zones of Minnesota. *J. Prod. Agric.* 10:425–430.
- Piper, J., J. Henson, M. Bruns, and M. Bender. 1988. Seed yield and quality comparison of herbaceous perennials and annual crops. p. 715–719. *In* P. Allen and D. Van Dusen (ed.) *Global perspectives on agroecology and sustainable agricultural systems*. Univ. of California, Santa Cruz.
- Posler, G.L., A.W. Lenssen, and G.L. Fine. 1993. Forage yield, quality, compatibility, and persistence of warm-season grass-legume mixtures. *Agron. J.* 85:554–560.
- Rohlf, F.J. 2000. NTSYSpc numerical taxonomy and multivariate analysis system version 2.1. Exeter Software, Setauket, NY.
- SAS Institute. 1990. SAS user's guide: Statistics. Fourth ed. SAS Inst., Cary, NC.
- Sheaffer, C.C., N.P. Martin, J.F.S. Lamb, G.R. Cuomo, J.G. Jewett, and S.R. Quring. 2000. Leaf and stem properties of alfalfa entries. *Agron. J.* 92:733–739.
- Soule, J., and J.K. Piper. 1992. Farming in nature's image: An ecological approach to agriculture. Island Press, Washington, DC.
- Stubbenieck, J., and E.C. Conard. 1989. Common legumes of the Great Plains: An illustrated guide. Univ. of Nebraska Press, Lincoln.
- Wright, J., and F. Bergsrud. 1991. Irrigation scheduling: Checkbook method. AG-FO-1322-G. Minn. Ext. Serv., St. Paul.