

A Core Collection for the United States Annual *Medicago* Germplasm Collection

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ABSTRACT

The United States National Plant Germplasm System contains 3159 accessions from 36 species of annual *Medicago*. Although there is increasing interest in the annual medics for use in sustainable agriculture, the U.S. collection is under utilized because of lack of agronomic information. Development of a core collection could facilitate easier access to the germplasm collection and enhance its use. The core collection should consist of a sample of accessions that represents the range of variability within the germplasm collection with minimum redundancies. To select a core collection of annual *Medicago* species, a subset of 1240 accessions was evaluated during the summer of 1990 for 16 agronomic and morphological traits. Accessions within species were grouped by cluster analysis utilizing an unweighted pair group method with arithmetic averages. Intraspecific phenotypic diversity determined the number of accessions for that species selected for the core collection. One accession per cluster was selected for each species for the core collection. Accessions were chosen within a species to represent the greatest diversity in geographical regions. The selected core collection of 211 accessions was reevaluated during the summer of 1991. The core collection was found to represent the variability of the germplasm collection and to remain stable between the two evaluation years. This study can be used as a model method for selecting a core collection for multispecies germplasm collections.

THE GENUS *MEDICAGO* (Leguminosae) consists of 83 herbaceous annual and perennial species including alfalfa (*M. sativa* L.), and two woody species (Small and Jomphe, 1988). The annual species of *Medicago* are endemic to the Mediterranean, and western and central Asia regions (Heyn, 1963). Many of the annual *Medicago* species are fast growers, produce large amounts of biomass with many pods, can supply N to other crops through their association with *Rhizobium* spp., and have hard seeds which can remain viable in the soil for long periods of time. Therefore, many annual *Medicago* species are excellent candidates for use in sustainable agriculture systems as forage and cover crops. There is very little information about the agronomic performance of the annual *Medicago* species in the USA. Medics evaluated for their potential use in Texas (Ocumpaugh et al., 1987) and in Utah (Rumbaugh and Johnson, 1986) produced forage earlier than most of the true clovers. In Australia, medics are planted extensively to improve soil structure, increase soil N, and control soil erosion in permanent pastures and in rotation with cereals (Crawford et al., 1989). The species most widely grown in Australia are *M. littoralis* Rohde ex Lois., *M. polymorpha* L., *M. rugosa* Desr., *M. scutellata* (L.) Miller, *M. italica* (Miller) Fiori, and *M. truncatula* Gaerth.

The U.S. annual *Medicago* germplasm collection, located in Pullman, WA, consists of 3159 accessions. Most accessions in the germplasm collection are part of the

Lesins collection, assembled by Karl and Irma Lesins (Lesins and Lesins, 1979). All annual species recognized by Lesins and Lesins, as well as *M. lesinsii* E. Small (Small and Brookes, 1985) are represented in the U.S. National Germplasm System collection. However, some rare annual *Medicago* species recognized by Small and Jomphe (1988) are not present in that collection. The number of accessions per species in the U.S. germplasm collection ranges from 1 to 651 (Table 1).

Despite increasing interest since this study began in the annual *Medicago* species, the U.S. annual *Medicago* germplasm collection was rarely used, primarily because of the lack of information about the accessions in the collection (R. Johnson, curator of the *Medicago* germplasm collection, personal communication). Utilization of this germplasm collection for plant breeding and other studies might increase once the collection is evaluated and a core collection of annual *Medicago* species assembled.

A core collection (Frankel, 1984) is a small collection that contains most of the alleles in the germplasm collection. The rest of the collection is then used as a reserve collection. Brown (1989a) suggested that the core collection should contain 5 to 10% of the germplasm collection or no more than 3000 accessions, and that this size of a core collection should retain over 75% of the variability in the whole collection. A good core collection should have maximum genetic diversity and no genotypically redundant entries, should represent the whole collection (species, subspecies, geographical regions, etc.), and should be small enough to manage easily (Brown, 1989b). Although Frankel (1984) and Brown (1989a,b) based their core collection theory on allelic frequencies, phenotype rather than genotype has been used to select some core collections. The perennial *Glycine* species core collection was selected based on a combination of morphological, cytological and isozyme variation, and passport data (Brown et al., 1987), and an okra (*Abelmoschus esculentus*) core collection was selected based on passport data and phenotypic descriptors (Hamon and van Sloten, 1989).

The objectives of this study were to select a core collection for the U.S. National Plant Germplasm System annual *Medicago* species collection based on the phenotypic evaluation of 40% of the accessions in this germplasm collection, assess the stability of the traits used to evaluate the accessions, ascertain which of these traits were useful for the core collection assemblage, and characterize the agronomic performance of the core collection with spring planting in Maryland.

MATERIAL AND METHODS

Plant Material

Thirty six of the annual *Medicago* species from sections *Spirocarpos* Ser., *Lupularia* Ser. in DC., and *Platycarpae* E. Small (Small and Jomphe, 1988) were represented in this study (Table 1). *Medicago rigidula* (L.) All., which was included in

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Table 1. The number of accessions of studied annual *Medicago* species in the U.S. National Plant Germplasm System collection, in the initial subset, and in the core collection.

<i>Medicago</i> Species	U.S.	Initial	Core
	collection	subset	collection
	Number		
<i>arabica</i> (L.) Huds.	71	35	2
<i>blancheana</i> Boiss.	18	18	8
<i>ciliaris</i> (L.) Krockner	73	31	6
<i>constricta</i> Durieu	48	30	3
<i>coronata</i> (L.) Bart.	23	3	2
<i>disciformis</i> DC.	50	30	4
<i>doliata</i> Carmign.	127	40	3
<i>granadensis</i> Willd.	14	13	4
<i>heyriana</i> Greuter	2	2	1
<i>intertexta</i> (L.) Miller	22	19	6
<i>italica</i> (Miller) Fiori	83	32	9
<i>laciniata</i> (L.) Miller	130	52	10
<i>lanigera</i> Winkl. & Fedtsch	1	1	1
<i>lesinsii</i> E. Small	5	2	2
<i>littoralis</i> Rohde ex Lois.	120	50	6
<i>lupulina</i> L.	170	63	14
<i>minima</i> (L.) Bart.	274	101	4
<i>murex</i> Willd.	73	34	6
<i>muricoleptis</i> Tin.	7	7	1
<i>noeana</i> Boiss.	19	14	3
<i>orbicularis</i> (L.) Bart.	251	86	8
<i>platycarpa</i> (L.) Trautv.	6	5	1
<i>polymorpha</i> L.	651	217	36
<i>praecox</i> DC.	21	20	2
<i>radiata</i> L.	12	11	4
<i>rigidula</i> (L.) All.	329	104	6
<i>rotata</i> Boiss.	21	20	8
<i>rugosa</i> Desr.	43	28	11
<i>sauvagei</i> Negre	5	5	2
<i>scutellata</i> (L.) Miller	60	37	18
<i>secundiflora</i> Durieu	2	2	1
<i>shepardii</i> Post	4	4	1
<i>soleirolii</i> Duby	10	10	3
<i>tenoreana</i> Ser.	6	5	1
<i>truncatula</i> Gaerth.	325	71	8
<i>turbinata</i> (L.) All.	83	38	6
Total	3159	1240	211

this study, has since been divided into two species *M. rigidula* and *M. rigiduloides* E. Small (Small, 1990; Small et al., 1990). This latest division was not included in this study.

Due to constraints in space, time, and seed, an initial subset of 40% (1240 accessions) of the germplasm collection was selected for field evaluation. The initial subset included accessions of all annual *Medicago* species in the germplasm collection. Accessions within species were chosen to represent proportionally the countries of origin for that species in the germplasm collection. Specific accessions within the country of origin were randomly selected. The number of accessions of each species chosen for the initial subset was based on the number of accessions in the germplasm collection (Table 2). The number of accessions evaluated per species was limited to 100, except for *M. polymorpha*. Because of the agronomic importance and large phenotypic variability of *M. polymorpha* (Lesins and Lesins, 1979), one third of the 651 accessions in the germplasm collection were selected for field evaluation.

Field Evaluation

The 1240 selected accessions were grown in Beltsville, MD, on Mattapex silt loam soil (fine silty, mixed, mesic Aquic Hapludult) with pH of 6.5. The field was sprayed with glyphosate [*N*-(phosphonomethyl) glycine], plowed, and fumigated with methyl bromide to control weeds. About 50 seeds of each accession were scarified, inoculated with a standard strain of *Rhizobium meliloti* Dang, generally used to inoculate alfalfa, and sown in June 1990, with no replication, in 1.5-m rows with 1.2-m row spacing.

Traits were evaluated on a row basis as follows.

Table 2. Number of accessions per species from the germplasm collection in the initial subset.

Germplasm collection	Initial subset
Number of accessions per species	
under 30	all
30-60	30
60-100	1/2(N)†
100-150	50
150-300	1/3(N)
more than 300	100‡

† N = Group size in germplasm collection.

‡ *M. polymorpha* had 218 accessions evaluated.

1. *Days to flower*: days from seeding day to first open flower.
2. *Days to full pod production*: days from seeding to the first plant in full pod stage.
3. *Growth habit*: scored as 1 = erect, 3 = less erect, 5 = semi-erect, 7 = decumbent, 9 = prostrate (5 classes).
4. *Biomass within species*: scored as 1 = highest to 9 = lowest (9 classes), compared to other accessions of that species.
5. *Variability within accession*: scored as 1 = yes 0 = no (2 classes), for any measured trait.
6. *Pod production*: scored as 1 = good to 9 = poor (9 classes).
7. *Pod spines*: scored as 1 = smooth, 5 = very short spines, 9 = spiny (3 classes).
8. *Plant height*: mean of mature plants (cm).
9. *Plant maximal spread*: width of the largest mature plant (cm).
10. *Length of middle leaflet*: mean of three mature leaflets from different plants.
11. *Width of middle leaflet*: mean of three mature leaflets from different plants.
12. *Number of flowers per raceme*: mean of five randomly chosen flowering racemes from different plants.
13. *Number of pods per raceme*: mean of five randomly chosen fruiting racemes from different plants.
14. *Internode length*: third internode from the plant base, mean of three randomly chosen plants.
15. *Seed size*: scored as 1 = smallest to 5 = largest (5 classes).
16. *Biomass among species*: scored as 10 = highest to 99 = lowest (89 classes).

Traits 2 to 9, 15, and 16 were measured at full pod stage, when flower production had slowed and the racemes bore mostly mature green pods. Traits 3 to 7, 15, and 16 were scored by the same person. The traits evaluated were chosen from the alfalfa descriptors list developed by the Alfalfa Crop Advisory Committee and adopted by the USDA Germplasm Resources Information Network (U.S. Department of Agriculture, 1989).

The core collection was assembled based on cluster analysis using traits 1 to 12, 14 and 15. This core collection was seeded in May 1991 at Beltsville, MD. Field location, planting preparation, and soil type were the same as in the summer of 1990. The core collection was sown in two blocks in a group balanced design where species were randomized within the blocks and accessions were randomized within the species in the field. Fifty scarified seeds of each accession per block were inoculated with the N strain of *R. meliloti* from Liphatech, Inc. (Milwaukee, WI) plus USDA Strains 1060 and 1167, and then sown in a 1.5-m row with 1.2-m row spacing. During the 1991 growing season all traits except seed size were reevaluated.

Statistical Analysis

Accessions were grouped into clusters based on traits 1 to 12, 14, and 15. Number of pods per raceme was not used in the cluster analysis because it is totally dependent upon number

Table 3. Pearson correlation coefficients between 15 phenotypic traits measured for the annual *Medicago* germplasm collection.†

	VAR‡	BIOMSP	PODPR	SPN	FLDT	PODDT	GRH	SPRD	HT	SDSZ	LLF	WL	INTN	FL
BIOMSP	-0.20													
PODPR	0.03	0.14**												
SPN	0.01**	0.04	0.00											
FLDT	-0.23**	-0.07	-0.22**	0.00										
PODDT	-0.26**	-0.10*	-0.24**	0.02	0.85**									
GRH	-0.16**	-0.09**	-0.18**	-0.07	0.41**	0.36**								
SPRD	-0.04	-0.64**	-0.15**	-0.09	0.11*	0.21**	-0.04							
HT	0.12**	-0.45**	-0.06	0.02	-0.28**	-0.10*	-0.50**	0.56**						
SDSZ	0.03	-0.16**	-0.04	0.05	-0.34	-0.04	-0.21**	0.31**	0.35					
LLF	0.01	-0.39**	-0.11*	-0.15**	0.11**	0.15**	-0.21**	0.61**	0.59**	0.47**				
WL	-0.04	-0.30**	-0.18**	-0.17**	0.31**	0.31**	-0.01	0.46**	0.26**	0.29**	0.83**			
INTN	0.16	-0.07*	-0.13**	0.08	-0.08	-0.04	-0.19**	0.21**	0.23**	0.15**	0.17**	0.12**		
FL	-0.35**	-0.07	-0.11*	-0.18**	0.13**	0.11*	0.16**	0.06	-0.08	-0.35**	0.10*	0.16**	0.69	
POD	-0.38**	-0.07	-0.10*	-0.19**	0.12*	0.09	0.19**	0.03	-0.13**	-0.37**	0.04	0.09	0.68	0.96**

*,** significantly different from zero at the 0.05 and 0.01 levels, respectively.

† Correlations derived from character means across all 36 species.

‡ VAR-variability within accession, BIOMSP-biomass within a species, PODPR-pod production, SPN-spines, FLDT-flowering date, PODDT-pod date, GRH-growth habit, SPRD-spread, HT-height, SDSZ-seed size, LLF-length of middle leaflet, WL-width of middle leaflet, INTN-internode length, FL-flowers per raceme, POD-pods per raceme.

of flowers per raceme (Table 3). Biomass among species was also excluded from the analysis because accessions were clustered within species. Although flowering date and full pod date, as well as length of middle leaflet and width of middle leaflet were highly correlated (Table 3), these traits were included in the analysis because they were partially independent (Sneath and Sokal, 1973). The data for the 14 evaluated traits were standardized with a mean of 0, and a variance of 1. A Statistical Analysis System (SAS) macro (Jacobs, 1990) calculated a distance matrix for each of the *Medicago* species based on Euclidean distances between all 14 traits. The distance matrices were entered into another SAS macro (Jacobs, 1990) to conduct cluster analysis using an unweighted pair group method with arithmetic averages.

The number of accessions per species selected for the core collection was based on the diversity for the 14 measured traits within that species. Diversity within each species was determined by the number of clusters in each species. A Euclidean distance of 3.0 was used to determine clusters because it generated the desired core collection size of 200 to 250 accessions. Species with more clusters were considered more diverse than those with fewer clusters. One accession per cluster was selected for the core collection. In order to achieve the greatest representation of geographical regions for the species in the germplasm collection, countries of origin as recorded in the passport data were grouped into geographical regions (Table

4). Within a species, accessions were chosen from different geographical regions.

Means, variances, and ranges of the 14 measured traits were compared between the core collection and the initial subset of 1240 accessions using a Wilcoxon rank-sum non-parametric test, in order to determine whether the core collection represents the germplasm collection. The Wilcoxon test was also used to compare the 1990 and 1991 core collection evaluation means to determine if the core collection remained stable over the two evaluation years. The Wilcoxon rank-sum test was performed using the SAS NPAR1WAY procedure, Wilcoxon option (SAS Institute, 1989). All tests of significance were conducted at an alpha of 0.05.

RESULTS AND DISCUSSION

A core collection of 211 accessions containing 36 annual *Medicago* species was assembled for the annual *Medicago* germplasm collection (Table 1). Phenotypic diversity within species, measured by cluster analysis, differed among species. Some species in the germplasm collection contained many redundant phenotypes, while others were quite diverse. Although the number of accessions of *M. scutellata* and *M. turbinata* (L.) All. in the initial subset was similar (37 and 38), analysis of *M. scutellata* traits produced three times as many clusters as *M. turbinata*. Therefore, 18 accessions of *M. scutellata* were chosen for the core collection, compared to six accessions of *M. turbinata*. *Medicago blanchiana* Boiss and *M. rotata* Boiss were two of the most variable species for the 14 traits measured. Therefore, a large proportion of the accessions of these species are represented in the core collection. Some of the variability in *M. blanchiana* and *M. rotata* may be due to natural hybridization between the two species (Small, 1984). Such hybrids may have been classified as *M. blanchiana* by Lesins and Lesins (1979). In contrast, *M. minima* (L.) Bart. accessions were relatively uniform in the field and are represented in the core collection by only few accessions (Table 1). The number of accessions per species chosen for the annual *Medicago* core collection ranged from 1 to 36 (Table 1).

Differences between means of the core collection and the initial subset of accessions were found significant for eight of the 36 species (Table 5). Three or fewer traits of the 14 measured traits were found significantly different for these eight species. Overall, only 3% of the

Table 4. Countries of origin from passport data grouped into geographical regions.

Geographical region	Country of Origin
North Africa	Morocco, Algeria, Tunisia, Libya
East Africa	Egypt, Kenya, Ethiopia
East Mediterranean	Israel, Lebanon, Syria, Jordan, Cyprus, Turkey
Middle Mediterranean	Greece, Former Yugoslavia, Italy, Sicily, Malta, Crete
West Mediterranean	Spain, Portugal
West Europe	France, Holland, Belgium, United Kingdom
Central Europe	Germany, Czechoslovakia, Austria, Switzerland, Bulgaria, Poland, Hungary, Romania
USSR	Former Soviet Union
Northern Europe	Sweden, Denmark
Western South America	Chile, Peru, Ecuador
Eastern South America	Brazil, Bolivia, Argentina, Uruguay
USA	USA
Central Asia	Iraq, Iran, Afghanistan, Pakistan
China	China
Australia	Australia
Canada	Canada
Nepal	Nepal
Unknown	Unknown

Table 5. Species with traits that were significantly different ($\alpha = 0.05$) between the core collection and the germplasm collection, based on Wilcoxon rank-sum non-parametric test.

<i>Medicago</i> species	Traits†
<i>disciformis</i>	Seed size
<i>laciniata</i>	Growth habit
<i>littoralis</i>	Pod date, growth habit
<i>lupulina</i>	Variability within accession
<i>minima</i>	Variability within accession, height, internode length
<i>orbicularis</i>	Variability within accession, height, length of middle leaflet
<i>polymorpha</i>	Variability within accession
<i>truncatula</i>	Seed size, pod production, growth habit
Total	15

† Trait means were compared for 14 traits and 36 species.

trait means differed significantly between the germplasm collection and the core collection, which is within the 5% type I error rate. Only two of the overall species variances and two of the overall species ranges for each measured traits were different between the core and the initial subset. The variances for spread and variability

Table 6. Species with traits that were significantly different ($\alpha = 0.05$) between 1990 and 1991 for the core collection. Based on Wilcoxon rank-sum non-parametric test.

<i>Medicago</i> Species	Traits†
<i>ciliaris</i>	Length of middle leaflet
<i>constricta</i>	Length of middle leaflet, internode length
<i>disciformis</i>	Internode length
<i>italica</i>	Length of middle leaflet, width of middle leaflet, internode length
<i>laciniata</i>	Pod production, internode length
<i>lupulina</i>	Length of middle leaflet, width of middle leaflet, internode length, number of flowers per raceme
<i>minima</i>	Internode length
<i>noeana</i>	Growth habit
<i>orbicularis</i>	Width of middle leaflet
<i>polymorpha</i>	Pod date, internode length
<i>radiata</i>	Internode length
<i>rigidula</i>	Internode length
<i>rotata</i>	Growth habit
<i>scutellata</i>	Pod production
<i>truncatula</i>	Pod date
<i>turbinata</i>	Number of flowers per raceme
Total	24

† Trait means were compared for 14 traits (traits 1 to 12, 14, and 16 as defined in Material and Methods), and 36 species.

Table 7. Means and standard deviations of nine vegetative traits of the annual *Medicago* species core collection grown in Maryland during the spring and summer of 1991.

Trait measured†	BIOMAM	GRH	SPRD	HT	INTN	LLF	WL	VAR	SDSZ‡
<i>Medicago</i> species	score‡		cm			score‡			
<i>arabica</i>	72.0 ± 1.5	9.0 ± 0.0	48 ± 10	6.5 ± 1.3	0.92 ± 0.77	1.29 ± 0.26	1.94 ± 0.49	—	2.0 ± 0.0
<i>blancheana</i>	25.3 ± 2.6	5.3 ± 1.8	51 ± 10	18.0 ± 6.6	1.73 ± 0.57	1.63 ± 0.26	0.87 ± 0.12	0.0 ± 0.00	4.0 ± 0.0
<i>ciliaris</i>	33.8 ± 2.3	7.8 ± 2.3	66 ± 29	11.2 ± 6.1	1.06 ± 0.51	1.05 ± 0.16	1.09 ± 0.16	0.0 ± 0.00	4.8 ± 0.0
<i>constricta</i>	74.3 ± 3.3	8.3 ± 1.6	55 ± 9	6.0 ± 3.3	0.61 ± 0.48	0.54 ± 0.28	0.91 ± 0.13	0.0 ± 0.00	3.1 ± 0.4
<i>coronata</i>	72.0 ± 1.5	5.0 ± 0.0	61 ± 18	12.1 ± 3.3	0.33 ± 0.46	0.64 ± 0.05	0.63 ± 0.07	0.0 ± 0.00	1.0 ± 0.0
<i>disciformis</i>	44.5 ± 3.0	9.0 ± 0.0	62 ± 24	6.5 ± 5.2	0.21 ± 0.28	0.65 ± 0.13	0.60 ± 0.09	0.0 ± 0.00	2.4 ± 0.5
<i>doliata</i>	53.6 ± 3.0	8.3 ± 1.6	59 ± 18	8.3 ± 4.5	0.92 ± 0.24	1.22 ± 0.26	0.90 ± 0.18	0.0 ± 0.00	4.7 ± 0.6
<i>granadensis</i>	55.3 ± 2.9	4.3 ± 3.0	35 ± 18	12.4 ± 4.1	0.80 ± 0.36	1.01 ± 0.31	0.60 ± 0.13	0.0 ± 0.00	3.4 ± 0.5
<i>heyriana</i> ¶	62.0 ± 1.4	9.0 ± 0.0	43 ± 4	5.4 ± 2.3	1.45 ± 0.02	0.77 ± 0.05	0.73 ± 0.00	0.0 ± 0.00	3.0 ± 0.0
<i>intertexta</i>	34.3 ± 3.0	8.5 ± 1.4	52 ± 21	10.0 ± 4.6	0.81 ± 0.23	0.92 ± 0.22	0.93 ± 0.27	0.0 ± 0.00	4.3 ± 0.6
<i>italica</i>	23.9 ± 2.5	6.3 ± 2.6	79 ± 27	22.6 ± 11.7	0.67 ± 0.60	1.01 ± 0.25	0.73 ± 0.19	0.0 ± 0.00	2.8 ± 0.6
<i>lesinsii</i> ¶	44.0 ± 1.4	7.0 ± 2.8	69 ± 17	12.8 ± 6.4	0.92 ± 0.12	1.33 ± 0.09	1.40 ± 0.00	0.0 ± 0.00	3.0 ± 0.0
<i>laciniata</i>	64.1 ± 2.1	5.6 ± 2.4	35 ± 16	10.6 ± 2.0	0.44 ± 0.44	0.79 ± 0.20	0.45 ± 0.07	0.0 ± 0.00	1.5 ± 0.5
<i>lanigera</i>	91.0 ± 0.0	9.0 ± 0.0	26 ± 4	6.2 ± 0.2	0.15 ± 0.02	0.65 ± 0.03	0.47 ± 0.05	0.0 ± 0.00	3.0 ± §
<i>littoralis</i>	53.0 ± 2.3	7.0 ± 2.3	50 ± 32	9.3 ± 1.5	1.06 ± 0.37	1.25 ± 0.32	0.94 ± 0.21	0.0 ± 0.00	2.6 ± 0.5
<i>lupulina</i>	64.8 ± 2.6	7.9 ± 2.1	57 ± 30	8.3 ± 4.9	1.14 ± 2.74	1.17 ± 0.25	0.95 ± 0.24	0.0 ± 0.00	1.1 ± 0.2
<i>minima</i>	85.6 ± 3.9	7.3 ± 2.7	22 ± 18	5.7 ± 2.3	0.50 ± 0.65	0.67 ± 0.22	0.95 ± 0.24	0.0 ± 0.00	1.4 ± 0.5
<i>murex</i>	43.0 ± 2.1	7.2 ± 2.0	68 ± 14	15.9 ± 8.3	0.98 ± 0.36	1.31 ± 0.33	1.18 ± 0.28	0.7 ± 0.99	3.4 ± 0.6
<i>muricoleptis</i> ¶	64.1 ± 2.5	9.0 ± 0.0	53 ± 12	5.3 ± 1.6	1.02 ± 0.33	1.46 ± 0.12	1.30 ± 0.13	0.0 ± 0.00	3.6 ± 0.5
<i>noeana</i>	84.5 ± 2.0	9.0 ± 0.0	47 ± 32	4.0 ± 0.0	0.63 ± 0.23	0.91 ± 0.19	0.90 ± 0.17	—	2.7 ± 0.9
<i>orbicularis</i>	64.4 ± 2.5	6.4 ± 2.4	47 ± 22	10.7 ± 5.5	0.50 ± 0.48	1.17 ± 0.18	0.88 ± 0.14	0.0 ± 0.00	2.3 ± 0.5
<i>platycarpa</i>	93.8 ± 3.0	7.0 ± 0.0	27 ± 12	3.6 ± 1.6	0.71 ± 0.37	1.63 ± 0.34	1.63 ± 0.98	0.0 ± 0.00	—
<i>polymorpha</i>	24.4 ± 2.2	5.3 ± 2.4	49 ± 21	14.3 ± 5.4	0.89 ± 0.67	1.26 ± 0.31	1.11 ± 0.26	0.0 ± 0.21	2.7 ± 0.5
<i>praecox</i>	96.0 ± 1.2	9.0 ± 0.0	24 ± 10	2.5 ± 0.9	0.53 ± 0.01	0.33 ± 0.07	0.41 ± 0.09	—	2.0 ± 0.0
<i>radiata</i>	83.0 ± 1.5	3.5 ± 0.9	44 ± 17	14.7 ± 4.2	0.69 ± 0.20	0.96 ± 0.10	0.65 ± 0.09	0.0 ± 0.00	3.0 ± 0.0
<i>rigidula</i>	73.4 ± 2.3	8.6 ± 1.3	58 ± 28	6.9 ± 4.5	0.76 ± 0.47	0.78 ± 0.21	0.77 ± 0.20	0.0 ± 0.00	3.1 ± 0.6
<i>rotata</i>	33.4 ± 2.6	4.6 ± 1.3	63 ± 17	19.6 ± 5.6	1.21 ± 0.39	1.60 ± 0.46	0.92 ± 0.35	0.1 ± 0.35	3.6 ± 0.5
<i>rugosa</i>	25.1 ± 2.6	5.6 ± 2.1	66 ± 28	15.6 ± 8.8	1.33 ± 0.60	1.37 ± 0.43	1.03 ± 0.24	0.0 ± 0.00	3.3 ± 0.7
<i>sauvagei</i>	93.0 ± 1.6	5.5 ± 4.1	48 ± 24	18.0 ± 3.7	0.58 ± 0.19	1.03 ± 0.10	0.59 ± 0.11	0.0 ± §	3.0 ± 0.0
<i>scutellata</i>	14.2 ± 2.8	5.5 ± 1.4	93 ± 44	24.8 ± 7.9	1.71 ± 0.89	2.07 ± 0.35	1.23 ± 0.28	0.0 ± 0.21	4.9 ± 0.3
<i>secundiflora</i> ¶	98.0 ± 1.4	9.0 ± 0.0	—	—	0.13 ±	0.37 ± §	0.30 ± §	—	1.0 ± 0.0
<i>shepardii</i> ¶	93.0 ± 2.8	9.0 ± 0.0	20 ± §	—	0.80 ± 0.24	0.78 ± 0.02	0.57 ± 0.05	—	2.0 ± 0.0
<i>soleirolii</i>	83.0 ± §	5.0 ± §	30 ± §	13.0 ± §	1.90 ± §	1.35 ± §	1.40 ± §	—	3.9 ± 0.4
<i>tenoreana</i> ¶	99.0 ± 0.0	9.0 ± 0.0	—	—	—	0.60 ± §	0.60 ± §	—	2.0 ± 0.0
<i>truncatula</i>	53.3 ± 2.3	7.3 ± 2.6	72 ± 26	13.3 ± 4.2	1.02 ± 0.45	1.36 ± 0.23	1.01 ± 0.20	0.0 ± 0.00	3.0 ± 0.4
<i>turbinata</i>	74.2 ± 3.0	7.0 ± 2.8	49 ± 30	21.9 ± 17.9	1.37 ± 1.65	1.19 ± 0.27	0.85 ± 0.11	0.0 ± 0.00	3.9 ± 0.8

† BIOMAM-biomass among species, BIOMSP-biomass within a species, GRH-growth habit, SPRD-spread, HT-height, INTN-internode length, LLF-length of middle leaflet, WL-width of middle leaflet, VAR-variability within an accession, SDSZ-seed size.

‡ BIOMAM scored 10-90;- 10 = highest, 99 = lowest. GRH scored 1-9;- 1 = erect, 3 = less erect, 5 = semi-erect, 7 = decumbent, 9 = prostrate.

§ based on one observation.

¶ based on 1990 evaluation data.

Table 8. Means and standard deviations of six characters associated with flowering of the annual *Medicago* species core collection grown in Maryland during the spring and summer of 1991.

Character measured†	FLDT	PODDT	PODPR	SPN	FL	POD
<i>Medicago</i> Species	days		score‡		number	
<i>arabica</i> §	85 ± ¶	—	—	—	2.0 ± ¶	1.0 ± ¶
<i>blancheana</i>	45 ± 13	73 ± 17	1.3 ± 0.7	2.4 ± 2.5	2.2 ± 1.2	1.2 ± 0.6
<i>ciliaris</i>	63 ± 31	101 ± 33	2.5 ± 1.9	9.0 ± 0.0	2.1 ± 1.5	0.9 ± 0.8
<i>constricta</i>	67 ± 17	111 ± 15	1.4 ± 1.1	6.9 ± 4.2	1.6 ± 1.0	1.0 ± 0.0
<i>coronata</i>	67 ± 40	113 ± 1	1.0 ± 0.0	9.0 ± 0.0	14.2 ± 0.7	11.4 ± 3.3
<i>disciformis</i>	67 ± 15	99 ± 18	1.8 ± 1.8	8.2 ± 1.8	1.7 ± 0.5	1.5 ± 0.3
<i>doliata</i>	73 ± 30	100 ± ¶	3.0 ± 2.8	3.7 ± 4.6	2.2 ± 0.3	1.0 ± 0.0
<i>granadensis</i>	89 ± 18	115 ± 11	2.6 ± 1.7	9.0 ± 0.0	2.8 ± 1.8	1.5 ± 0.8
<i>heyriana</i> §	81 ± 4	102 ± 12	7.0 ± 2.8	9.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.3
<i>intertexta</i>	128 ± ¶	—	1.4 ± 0.2	1.3 ± 0.3	—	—
<i>italica</i>	62 ± 23	110 ± 16	1.0 ± 0.0	6.5 ± 3.7	6.5 ± 3.1	3.4 ± 1.7
<i>lesinsii</i> §	99 ± ¶	—	—	1.0 ± ¶	1.6 ± ¶	1.0 ± ¶
<i>laciniata</i>	48 ± 11	81 ± 16	1.0 ± 0.0	9.0 ± 0.0	1.7 ± 0.5	1.6 ± 0.6
<i>lanigera</i>	69 ± 5	94 ± 1	1.0 ± 0.0	1.0 ± 0.0#	1.0 ± 0.0	1.0 ± 0.0
<i>littoralis</i>	56 ± 26	66 ± 8	1.0 ± 0.0	9.0 ± 0.0	1.7 ± 1.2	1.2 ± 0.4
<i>lupulina</i>	62 ± 23	89 ± 19	1.0 ± 0.0	1.0 ± 0.0	29.2 ± 6.4	23.2 ± 7.4
<i>minima</i>	51 ± 23	71 ± 29	1.0 ± 0.0	9.0 ± 0.0	3.2 ± 0.6	2.6 ± 0.3
<i>murex</i>	104 ± 17	22 ± 11	2.0 ± 1.4	5.7 ± 3.0	2.2 ± 1.4	1.3 ± 0.4
<i>orbicularis</i>	58 ± 13	84 ± 17	1.8 ± 1.3	1.0 ± 0.0	1.7 ± 0.6	1.3 ± 0.3
<i>platycarpa</i>	77 ± 3	117 ± 4	3.0 ± ¶	1.0 ± 0.0	1.6 ± 0.3	1.6 ± 1.0
<i>polymorpha</i>	58 ± 22	89 ± 20	1.0 ± 0.0	7.8 ± 2.7	3.4 ± 2.0	2.1 ± 1.2
<i>praecox</i> §	75 ± 8	93 ± 4	1.0 ± 0.0	9.0 ± 0.0	—	0.8 ± 0.0
<i>radiata</i>	63 ± 17	99 ± 20	1.5 ± 0.9	9.0 ± 0.0	2.2 ± 1.3	1.7 ± 0.6
<i>rigidula</i>	80 ± 32	99 ± 40	1.5 ± 1.0	8.0 ± 2.0	2.5 ± 0.9	1.2 ± 0.2
<i>rotata</i>	47 ± 5	89 ± 12	1.8 ± 1.0	6.5 ± 2.1	3.6 ± 1.2	1.9 ± 0.6
<i>rugosa</i>	57 ± 25	85 ± 37	1.9 ± 1.5	1.0 ± 0.0	1.9 ± 1.3	1.7 ± 0.8
<i>sauvagei</i>	91 ± ¶	114 ± ¶	5.0 ± ¶	9.0 ± ¶	1.4 ± ¶	2.0 ± ¶
<i>scutellata</i>	47 ± 22	73 ± 31	1.6 ± 1.2	1.0 ± 0.0	1.9 ± 1.2	1.4 ± 0.4
<i>truncatula</i>	55 ± 17	119 ± 12	1.0 ± 0.0	9.0 ± 0.0	2.6 ± 0.6	1.3 ± 0.2
<i>turbinata</i>	57 ± 24	91 ± 4	3.8 ± 3.3	5.8 ± 4.3	1.5 ± 0.4	0.8 ± 0.5

† FLDT-flowering date, PODDT-full pod date, PODPR-pod production, SPN-spines, FL-flowers per raceme, POD-pods per raceme.

‡ PODPR scored 1-9: 1 = good, 9 = poor. SPN scored 1-9: 1 = smooth, 5 = very short spines, 9 = spiny.

§ based on 1990 evaluation data.

¶ based on one observation.

M. lanigera pods are spineless and densely covered with hairs up to 5 mm long.

within accession, and the ranges for biomass within species and width of middle leaflet were significantly different. This accounted for 13% differences in variances and ranges between the core collection and the 1240 evaluated accessions. These differences between the core and the germplasm collections are acceptable, since the core collection should retain at least 75% of the variability in the whole collection (Brown 1989a).

To evaluate stability of the core collection, a Wilcoxon rank-sum test was used for detecting significant differences in trait means for each species between the years 1990 and 1991. Sixteen of the 36 species had one to five trait means which were significantly different between the 1990 and 1991 (Table 6). Internode length was found to be significantly different for nine out of the 36 species. These differences were probably due to the difficulty of identifying the third internode from the base of plants with different growth habits. Also, this trait was measured by different people across the field. Therefore, internode length as measured was an unreliable trait and probably should not be used to evaluate the annual *Medicago* species. Overall, only 5% of the means of the traits measured (including internode length) were significantly different between 1990 and 1991. These data show that the core collection generally remained stable between the two years, and that the 1-yr evaluation trial with one replicate was sufficient for the assemblage of the annual *Medicago* core collection.

Since field evaluations are labor intensive, it is im-

portant to determine if all traits used in the evaluation contributed information to the assemblage of the core collection or if it is possible to reduce the number of traits measured. Correlations between most traits were generally low (Table 3). Flowering date and full pod date, length and width of middle leaflet, and number of flowers and pods per raceme are the only traits that were highly correlated (Table 3). Because of the associations found between flowering and full pod dates, and between number of flowers and pods per raceme, future evaluations do not need to examine each of the correlated traits, but can be limited to one trait from each pair of correlated traits. Future evaluations of annual *Medicago* species would be more efficient if all measurements were taken at full pod stage and traits associated with flowering were eliminated from the evaluations. Most evaluated traits were measured at full pod, and spines and pod production, which are important traits, can be scored only when the plants bear mature pods.

The 36 species in the U.S. National Germplasm System annual *Medicago* core collection varied considerably in their agronomic performance. The core collection field evaluation data for 1991 is summarized in Tables 7 and 8 for traits associated with vegetative and reproductive growth stages, respectively. It should be noted that in nature the annual *Medicago* species are adapted to Mediterranean climate and many are winter annuals (Crawford et al., 1989). However, this study was a trial conducted in spring and summer since this is the appro-

appropriate growing season for these species in Maryland. *Medicago scutellata* produced more forage on average than any other species in the core collection, and was also one of the first species to flower and to produce pods. On the average, other good forage producers were *M. blanchiana*, *M. italica*, *M. polymorpha*, and *M. rugosa*. Other early flowering species were *M. blanchiana*, *M. laciniata* (L.) Miller, and *M. rotata*. In comparison, the annual *Medicago* species in the core collection having very low forage production potential were *M. lanigera* Winkl. & Fedtsch, *M. sauvagei* Nerge, *M. platycarpa* (L.) Trautv., *M. tenoreana* Ser., *M. secundiflora* Durieu, *M. praecox* DC., and *M. shepardii* Post. Black medic (*M. lupulina* L.) produced the most flowers and pods compared to all other species evaluated. The agronomic information presented in Tables 7 and 8 could be of use for plant breeders in the selection of species and accessions within species for germplasm development.

Molecular markers can be used more directly than morphological traits to evaluate genetic diversity, and understand the evolution of crop species (Bernatsky and Tanksley, 1989; Skroch et al., 1992). However, in the case of the annual *Medicago* species, a lack of information about agronomic and morphological characters hinders usage. Therefore, evaluation of the collection for agronomic traits was used rather than molecular and biochemical evaluations to develop the core collection. Morphological measurements in other crops have revealed much greater variability than isozyme data (Jain et al., 1980; Giles, 1984; Schwaegerle et al., 1986; Perry and McIntosh, 1991a,b). Furthermore, in cultivated common bean considerable morphological variation was found to exist between some cultivars in spite of their uniformity at the allozyme level (Singh et al., 1991b). It was suggested that most of the cultivars with the same allozyme genotype have undergone further diversification for morphological traits but not for molecular markers (Singh et al., 1991a). These differences in diversification levels between morphological and molecular data probably exist because allozyme genes do not have strong effects on phenotype and are most likely selectively neutral (Kimura, 1983). Conversely, bean genotypes that appear to be very diverse based on their phenotype in some cases are genetically closely related, and genotypes with similar morphological traits may be evolutionarily distant as revealed by allozyme data (Singh et al., 1991b). Perry and McIntosh (1991b) pointed out that unless the enzyme loci are genes involved in the expression of the morphological characters, or show high linkage with them, both morphological and enzyme allele frequency data should be used when describing variation in germplasm collections. Singh et al. (1991b) also concluded that gene banks should integrate morphological, agronomical, biochemical, and molecular evaluations of their genetic resources because the different types of traits provide complementary information.

We have developed a core collection for the U.S. National Plant Germplasm System annual *Medicago* species collection, using phenotypic diversity as the basis for the core collection selection. The core collection contains 211 accessions, from 36 annual *Medicago* species. The core collection was shown to represent the germplasm collection for the evaluated traits and to remain stable

over the 2 yr of evaluation. Some of the annual species (*M. scutellata*, *M. blanchiana*, *M. italica*, *M. rugosa*, and *M. polymorpha*) showed promising agronomic potential in Maryland. The assemblage of the annual *Medicago* core collection should facilitate further investigations concerning their many potential uses in the USA, especially for sustainable agriculture programs. This study may be used as a model for assemblage of core collections where diversity within a group can be estimated for germplasm collection composed of well defined groups such as species, subspecies, or geographical regions.

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