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THE USE OF MOLECULAR MARKERS TO STUDY GENETIC DIVERSITY IN *MUCUNA*

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SUMMARY

Genetic diversity was estimated in a collection of 64 velvetbean accessions from different eco-geographical regions using amplified fragment length polymorphism (AFLP). Genetic relationships within the accessions were evaluated by generating a similarity matrix based on Nei and Li's (1979) similarity coefficient which ranges from 0 (no similarity) to 1 (perfect similarity). In the first study, the phenetic dendrogram (a system of classification of organisms based on overall or observable similarities) generated by UPGMA separated 40 velvetbean accessions into two main clusters. This grouping confirmed the existing phenological difference with regard to maturity. Similarity coefficients ranged from 0.87 to 0.97. In the second study, the phenetic dendrogram separated the 24 accessions into four main clusters. The cluster analysis indicated that velvetbean germplasm within the collection constitutes a broad genetic base with the values of genetic similarity ranging from 0.68 to 1.00. The grouping of a subcluster indicated differences with regard to growth habit. The level of genetic variability within the velvetbean accessions with AFLP analysis suggests that it is a reliable, efficient, and effective marker technology for delineating genetic relationships among genotypes and estimating genetic diversity, thereby enabling the formulation of appropriate strategies for velvetbean improvement programs.

Key words: velvetbean, *Mucuna,* AFLP, genetic diversity.

INTRODUCTION

World agriculture has been spectacularly successful in the past century in meeting the demand for more food. To maintain and increase future agricultural productivity, it will be necessary to use a broader range of the world's plant genetic diversity, particularly minor crop genetic resources such as velvetbean (*Mucuna* sp.). Velvetbean, described as a self-pollinated species (Duke, 1981), is a tropical legume. Originally, velvetbean came from China and eastern India, where it was widely cultivated as a green manure (Burkill, 1966; Duke, 1981; Wilmot-Dear, 1984). It has been used as source of food and as a soil-improving and pasture crop (Duggar, 1899). The taxonomy of velvetbean is confused with several synonyms at the generic and species levels (Duke, 1981). Burkill (1966) recorded *Mucuna nivea* as being synonymous with *Mucuna cochinchinensis* and *Mucuna lyonii* (Awang *et al*., 1997). In many instances, accessions are described only in terms of where they were grown (e.g., *Mucuna* sp. var Ghana) or by the many popular names under which they came to be known in various places, such as *M. cochinchinensis* in SE Asia or *M. deeringiana* in Florida. It is difficult to confirm that the name given to a species is representative of its genotype. Extensive exchange of seeds over the years probably led to the same species being given different names, according to the locality grown. On the opposite side, it is also highly plausible that species given the same name in two or more areas might in fact be different original stock or germplasm. This lack of information on taxonomy of *Mucuna* has impeded the effective utilization of *Mucuna*'s genetic resources. At the same time, the wide geographical and climatic distribution of the crop is likely to reflect a tremendous genetic diversity in it, which needs to be estimated before any cultivar development program. Because of the confusion surrounding the taxonomy of velvetbean, it is necessary to conduct research at the species level as well as to assess the genetic diversity and phenetic relationships among accessions before any breeding can be initiated.

The study of morphological variability is the classical way of assessing genetic diversity. For many species, especially minor crops, it is still the only approach used. An assessment of genetic diversity based only on morphological and agronomic traits might be biased because distinct morphotypes can result from a few mutations. However, with molecular marker techniques, powerful tools have been developed so that genetic resources can accurately be assessed and

characterized. Most of these techniques are based on the analysis of information-rich nucleic molecules and provide a reliable estimation of relatedness, phylogeny, and inheritance of genetic characteristics (Caetamo-Anollés and Gresshoff, 1998). With molecular maps and markers, we have a kind of X-ray vision that can detect genes controlling agronomic, morphological, and biochemical traits in the plant. Moreover, they are essential for explaining whether existing genetic variability, which is assessed by measuring biochemical factors and morphological traits, is related to genetic diversity, which is assessed by measuring allelic frequencies using molecular markers. This information can be used to construct a core collection, which serves as a base for future breeding programs.

Recently, Vos *et al*. (1995) developed the Amplified Fragments Length Polymorphisms (AFLP) method, which is a molecular marker technique equally applicable universally, is highly reproducible, and reveals very high level of polymophisms. AFLPs have proven to be extremely proficient in revealing diversity at below the species levels (Karp and Edwards, 1995). Molecular markers will, therefore, provide a classification of *Mucuna* accessions, which will also allow fuller use to be made of the genetic resources of this species. This study was undertaken to investigate the genetic diversity in velvetbean and determine the relatedness among its accessions.

MATERIALS AND METHODS

Samples used consisted of 64 accessions of *Mucuna* sp. obtained from various sources, including the USDA germplasm collection in Griffin, GA, collection from Alabama (U.S) farmers, the International Institute of Tropical Agriculture (IITA), Benin, and the International Center of Tropical Agriculture (CIAT), Colombia (Table 1). These accessions originated from various geographical regions in the world.

Genomic DNA was extracted from leaves according to the modified CTAB method of Doyle and Doyle (1990). AFLP analysis was performed according to Vos *et al*. (1995) with slight modifications. AFLP core reagent and starter primer kits were purchased from Life Technology (Gibco BRL, Gatherburg, MD). The technique involves three steps: restriction of the DNA and ligation of oligonucleotide adapters, selective amplification of sub-sets of restriction fragments, and gel analysis.

Polymorphisms are detected in AFLP analysis as the absence or presence of bands due to a difference in restriction sites (a place on a DNA molecule where a restriction enzyme acts) (Karp *et al*., 1998). This difference in restriction sites can be due to mutations around the restriction sites which match, or are different from the selective nucleotides added to the PCR primers, and insertions or deletions within the amplified restriction fragments. Each AFLP marker was treated as a unit character and scored as binary codes (1/0). Thus, the matrix values estimating the number of AFLP fragments shared (or not shared) between two accessions has been suggested as an appropriate estimator of relatedness under the assumption that the presence or absence of a discrete character in two or more accessions results from the same genetic changes (Skroch *et al*., 1992). The 1/0 matrix was used to estimate genetic similarity coefficients between accessions i and j according to Nei and Li (1979) [$GS_{NL} = 2a / (2a + b + c)$] where a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i. The resulting distance matrice was subjected to a clustering method by UPGMA (unweighted pair-group method analysis; Sokal and Michener, 1958). The goodness of fit of the clustering to the data matrix was calculated by the COPH and MXCOMP programs. Computations were done using the procedures in NTSYS-pc statistical package (version 2.0; Rohlf, 1998). The reliability and robustness of the phenograms were tested by bootstrap/jackknife analyses with 10,000 replications to assess branch support using the software PAUP (version 3.1; Swofford, 1993). Some workers consider that the confidence limits obtained in both bootstrap and jackknife must be over 95% in order to consider the grouping of taxa (a group of genetically similar organisms that are classified together as e.g. species, genus, or family) at a branch to be statistically significant (Felsenstein, 1985). Others use a lower limit (above 50% or at least 50%) as indicating statistical support for the topology at a node (Highton, 1993). In our study we used the lower limits to assess grouping of taxa to be statistically significant.

RESULTS AND DISCUSSION

Analysis of *Mucuna* accessions with 11 AFLP primer pairs identified a total of 508 fragments of which 251 were polymorphic (i.e., two or more discontinuous fragments or variants regularly and simultaneously in the same population between two or more accessions). Polymorphic fragments were generated by each of the primer pairs. The average number of fragments detected by an individual primer pair (Table 2) ranged from 28 to 70, thus confirming the high multiplex ratio produced by AFLP markers. The number of polymorphic fragments for each primer pair varied from 10 to 34 with an average of 23 per primer pair (Table 2). Gene diversity (which is a measure of gene variance of a population equal to the probability of non-identity of randomly chosen genes) ranged from 0.10 to 0.21 with average of 0.15 per primer combination (Table 2). Nei's genetic similarity between the U.S. landraces

and the exotic lines ranged from 0.98 to 0.87 with average of 0.92 per primer combination (Table 2) (Capo-chichi *et al*., 2001). In the second study comprising a set of 26 velvetbean accessions containing soybean (*Glycine max* L. Merr.) and cowpea (*Vigna unguiculata* (L.) Walp) as outgroups, AFLP detected a total of 1112 polymorphic fragments using 12 primer combinations. Polymorphic fragments were generated by each of the primer pairs. The number of polymorphic fragments detected by primer combination ranged from 35 to 133 with an average of 93 per primer pair.

AFLP fragment sizes

The size of the AFLP fragments was determined by comparing an AFLP standard marker to AFLP patterns. AFLP fragment sizes ranged from approximately 50 to 400 base pairs (bp). Polymorphic fragments were distributed across the entire size range with the major proportion between 75 and 300 bp. In the second set of accessions including soybean and cowpea, polymorphic AFLP fragment sizes ranged from 50 bp to 500 bp (Capo-chichi *et al*., 2001).

Phenetic analysis

To assess the usefulness of AFLPs as phenetic markers, a similarity matrix based on Nei and Li's coefficient (1979) was constructed to estimate the level of relatedness among accessions for each study. The calculation of Nei and Li's (1979) coefficient was based on the presence or absence of discrete characters (AFLP markers). This test was done to evaluate the goodness of fit of the resulting phylogenetic tree and reveal the reliability and stability of the inferred relationships. High co-phenetic correlation value was obtained (0.91) where $r > 0.9$ indicates a very good fit; $0.8 < r < 0.9$ indicates a good fit; $r < 0.8$ indicates a poor fit. The similarity matrix was then used to cluster the data using the UPGMA algorithm (Capo-chichi *et al*., 2001).

In the first study, the resulting dendrogram constructed by Nei and Li's coefficient and by UPGMA clustering method formed two main clusters (Figure 1). These two clusters were identified at the 87% similarity level. Cluster 1 is supported at 70% and 71% confidence interval limits in the bootstrap (BS) and jackknife (JK) analyses respectively. In cluster 2, the branch is supported at 69% (BS) and 71% (JK) levels. Within cluster 1, two subclusters were identified. Subcluster 1.1 is supported at 77% (BS) and 76% (JK) levels. The branch formed by the accessions "Rajada" and PI383272 in cluster 1 is strongly supported by bootstrap and jackknife values (93% confidence interval limits). In cluster 2, two subclusters were identified. Subcluster 2.2 supported at 92% confidence interval limits in both (BS) and (JK) and subcluster 2.1 consists of the single accession PI364362. A significant association was found within subcluster 2.2 between the accessions 19.W and 21.W in which the branch is supported at 98% and 97% confidence interval limits in the (BS) and (JK) analyses, respectively (Figure 1) (Capo-chichi *et al*., 2001).

In the second study, the resulting dendrogram constructed divides the 24 accessions obtained from CIAT into four distinct clusters (Figure 2). The cluster c1 is supported at <50% confidence interval limits, respectively, in the (BS) and (JK) analyses. The cluster c2 is supported at 59% and 59% confidence interval limits in the (BS) and (JK) analyses, respectively. Within the cluster c2, two subclusters were identified. Subcluster c2.1 comprised the accessions BV1, BV3, BV4, BV5 and 91080-991E, all of which have a 'bushy' growth type. Subcluster c2.2 grouped the F_1 hybrids derived from a controlled cross between PI364362 and an U.S. landrace accession from Alabama. The F_1 hybrids formed a strong association with the accession PI364362. Cluster c3 grouped two accessions; the branch is strongly supported at 100% (BS) and (JK). Cluster c4 consists of only the accession 00005-001E. This *Mucuna* cluster is supported at 88% (BS) and 89% (JK). As expected, soybean and cowpea separated from the velvetbean accessions.

Molecular markers have not been used to evaluate and characterize velvetbean germplasm, thus, this study was designed to measure the level of genetic variability in this species. The different accessions used were from cultivated species. Genetic diversity was evaluated with 11 primer combinations and 251 AFLP fragments were polymorphic. The genetic diversity was greater in the tropical lines compared to the U.S. landraces (Table 2), indicating that the tropical lines were more heterogeneous than the U.S. landraces. This may due to the wide range of geographical origins of the tropical lines. When genetic similarity between the U.S. landraces and tropical lines was compared, the highest value was obtained with the primer combination E-AAG/M-CTG and the lowest with E-AAG/M-CAT (Table 2).

Table 1. Characterization of accessions of velvetbean (*Mucuna* sp.) used for AFLP analysis, including the codes referred to in the text.

Taxon	Plant name	Accession number	Code	Donor [§]	Origin
Mucuna sp.	None	PI 227479	PI227479	USDA, ARS	Costa Rica
Mucuna sp.	Somerset [†]	PI 344047		USDA, ARS	Zimbabwe
Mucuna sp.	None	PI 337098	PI337098	USDA, ARS	Brazil
Mucuna sp.	None	PI 364362	PI364362	USDA, ARS	Mozambique
Mucuna sp.	Branco	PI 365411	PI365411	USDA, ARS	Mozambique
Mucuna sp.	Osccola	PI 365414	PI365414	USDA, ARS	Mozambique
Mucuna sp.	Verde Radio	PI 365415	PI365415	USDA, ARS	Mozambique
Mucuna sp.	None	PI 365573	PI365573	USDA, ARS	Brazil
Mucuna sp.	None	PI 366024	PI366024	USDA, ARS	Brazil
Mucuna sp.	African yellow	PI 383272	PI383272	USDA, ARS	USA
Mucuna pruriens	var. *deeringiena*		Deeringiena	CIEPCA	Brazil
Mucuna pruriens	var. *cochinchinensis*		Cochinchinensis	CIEPCA	Singapore
Mucuna pruriens	var. *utilis*		Utilis	CIEPCA	Nigeria
Mucuna sp.	var. rajada		Rajada	CIEPCA	Brazil
Mucuna sp.	var. Ghana		Ghana	CIEPCA	Ghana
Mucuna sp.	var. jaspada		Jaspada	CIEPCA	Brazil
Mucuna sp.	var. Georgia		Georgia	CIEPCA	Cimmyt (Mx.)
Mucuna sp.	var. IRZ		IRZ	CIEPCA	IITA
Mucuna sp.	var. veracruz-speckled			CIEPCA	Cimmyt (Mx.)
Mucuna sp.	var. veracruz-white		19.W	CIEPCA	Cimmyt (Mx.)
Mucuna sp.	var. preta		Preta	CIEPCA	Brazil
Mucuna sp.	Mexican (Chiapas)S.		21.S	AU	Chiapas (Mex.)
Mucuna sp.	Mexican (Chiapas)B.		21.B	AU	Chiapas (Mex.)
Mucuna sp.	Mexican (Chiapas)W.		21.W	AU	Chiapas (Mex.)
Mucuna sp.	USA (AL)-S.		22.S	AU	USA
Mucuna sp.	USA (AL)-B.		22.B	AU	USA
Mucuna sp.	USA (AL)-W.		22.W	AU	USA
Mucuna sp.	Edgar Farm (AL)S.		23.S	AU	USA
Mucuna sp.	Edgar Farm (AL)B.		23.B	AU	USA
Mucuna sp.	Edgar Farm (AL)W.		23.W	AU	USA
Mucuna sp.	90 day runner-S.		24.S	AU	USA
Mucuna sp.	90 day runner-B.		24.B	AU	USA
Mucuna sp.	90 day runner-W.		24.W	AU	USA

Table 1. (Cont.) Characterization of accessions of velvetbean (*Mucuna* sp.) used for AFLP analysis, including the codes referred to in the text.

Taxon	Plant name	Accession number	Code	Donor	Origin
Mucuna sp.	Belle Mina (AL)L.S.		25.LS	AU	USA
Mucuna sp.	Belle Mina (AL)L.B.		25.LB	AU	USA
Mucuna sp.	Belle Mina (AL)S-1		25.S1	AU	USA
Mucuna sp.	Belle Mina (AL)S-2		25.S2	AU	USA
Mucuna sp.	Belle Mina (AL)S-3		25.S3	AU	USA
Mucuna sp.	Belle Mina (AL)S-4		25.S4	AU	USA
Mucuna sp.	Belle Mina (AL)S-5		25.S5	AU	USA
Mucuna sp.	Belle Mina (AL)S-6		25.S6	AU	USA
Mucuna sp.	Belle Mina (AL)S-7		25.S7	AU	USA
Mucuna sp.	-	7160	CIAT7160	CIAT	Colombia
Mucuna sp.	-	8833	CIAT8833	CIAT	Colombia
Mucuna pruriens	-	9249	CIAT9249	CIAT	Colombia
Mucuna sp.	-	9327	CIAT9327	CIAT	Brazil
Mucuna pruriens	-	9349	CIAT9349	CIAT	Brazil
Mucuna sp.	-	18069	CIAT18069	CIAT	China
Mucuna pruriens	-	18243	CIAT18243	CIAT	Venezuela
Mucuna sloanei	-	18245	CIAT18245	CIAT	Venezuela
Mucuna pruriens	-	18680	CIAT18680	CIAT	Brazil
Mucuna pruriens	-	19088	CIAT19088	CIAT	Venezuela
Mucuna mutisiana	-	19370	CIAT19370	CIAT	Panama
Mucuna sp.	-	19372	CIAT19372	CIAT	Panama
Mucuna sp.	-	19837	CIAT19837	CIAT	Colombia
Mucuna sp.	-	20171	CIAT20171	CIAT	Colombia
Mucuna pruriens	-	21262	CIAT21262	CIAT	Colombia
Mucuna sp.	-	21338	CIAT21338	CIAT	Colombia
Mucuna pruriens	-	21686	CIAT21686	CIAT	Honduras
Mucuna pruriens	-	21883	CIAT21883	CIAT	Colombia
Mucuna sp.	-	33033	CIAT33033	CIAT	Thailand
Mucuna sp.	-	-	BV1	AU	-
Mucuna sp.	-	-	BV2	AU	-
Mucuna sp.	-	-	BV3	AU	-
Mucuna sp.	-	-	BV4	AU	-
Mucuna sp.	-	91080-991E	91080-991E	ECHO	-
Mucuna sp.	-	60008-A3	60008-A3	ECHO	-
Mucuna sp.	-	00005-001E	00005-001E	ECHO	-
Mucuna sp.	F_1 progeny [‡]	-	-	AU	USA
Mucuna sp.	23.W		23W.	AU	USA
Mucuna sp.	None	PI364362	PI364362	AU	USA

[†] These accessions did not germinate.
[‡] F_1: Progeny derived from a single cross between Edgar Farm (AL)W and PI364362.
[§] USDA, ARS: United States Department of Agriculture, Agricultural Research Service, CIEPCA: Center for Cover Crops Information and Seed Exchange in Africa, AU: Auburn University, CIAT: The International Center for Tropical Agriculture.
Source: Capo-chichi *et al.*, 2001

Table 2. Number of total and polymorphic fragments and gene diversity in overall accessions, U.S. landraces and tropical lines in the first study.

Primer pair	Total fragments	Overal accessions			U.S. landraces			Exotic lines			GS between U.S and exotic [¶]
		Poly [†]	% poly [‡]	H [§]	Poly	% poly	h	Poly	% poly	h	
E-AAG / M-CAA	55	32	58	0.21	25	45	0.16	32	58	0.23	0.96
E-AAG / M-CAG	35	16	46	0.15	23	23	0.08	16	46	0.15	0.94
E-AAG / M-CAT	70	28	40	0.15	19	19	0.05	28	40	0.14	0.87
E-AAG / M-CTC	56	34	61	0.20	30	30	0.11	33	59	0.18	0.88
E-AAG / M-CTG	46	27	59	0.14	33	33	0.10	26	57	0.15	0.98
E-AAG / M-CTT	53	19	36	0.10	26	26	0.08	19	36	0.09	0.96
E-ACT / M-CAG	44	25	57	0.18	32	32	0.10	24	55	0.18	0.92
E-ACT / M-CAT	42	20	48	0.16	36	36	0.13	16	38	0.13	0.96
E-ACT / M-CTC	37	17	46	0.15	35	35	0.12	13	35	0.11	0.91
E-ACG / M-CAG	42	23	55	0.18	33	33	0.11	22	52	0.17	0.90
E-AGT / M-CAG	28	10	36	0.11	21	21	0.06	9	32	0.11	0.94
Total	508	251	-	-	154	-	-	238	-	-	-
Mean	46	23	49	0.15	14	30	0.10	22	46	0.14	0.92
Number of observation	11	11	11	11	11	11	11	11	11	11	11
Maximum	70	34	61	0.21	45	45	0.16	33	59	0.23	0.98
Minimum	28	10	36	0.10	6	19	0.05	9	32	0.09	0.87
Standard deviation	11.69	7.2	9.3	0.03	4.8	7.57	0.03	7.8	0.33	0.03	0.03

[†] = total number of polymorphic fragments; [‡] = percentage of polymorphic fragments; [§] = gene diversity;
[¶] = Genetic similarity between U.S. landraces and exotic lines.
Source: Capo-chichi *et al.*, 2001

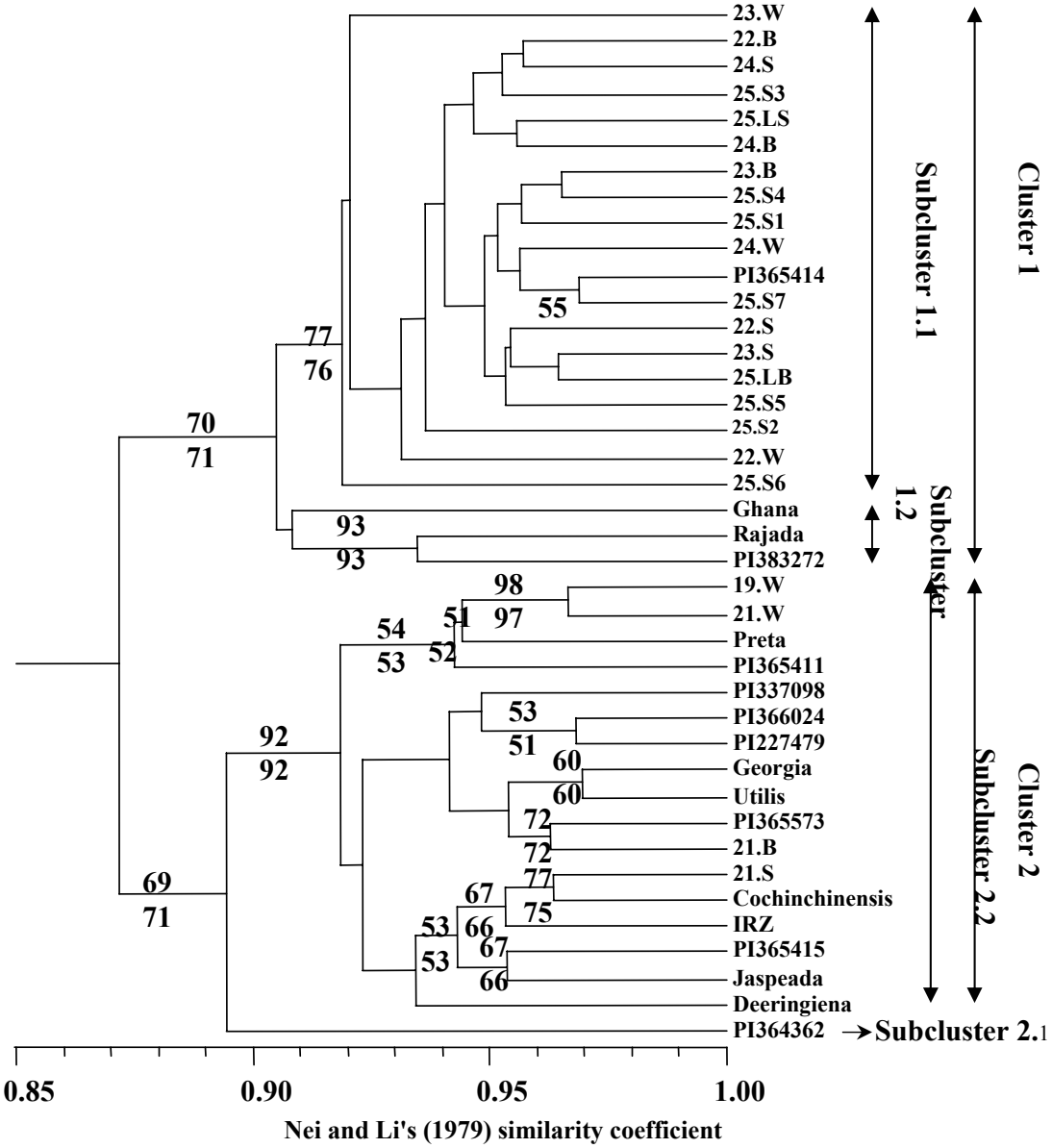

Figure 1. Phenogram of 40 velvetbean accessions revealed by UPGMA cluster analysis based on AFLP markers obtained with 11 primer combinations. Numbers shown above different branches represent percentage confidence limits obtained in the bootstrap analysis, those below branches are percentage confidence limits in the jackknife analysis. Branches lacking bootstrap and jackknife values received < 50% bootstrap and jackknife supports.
(Source: Capo-chichi *et al.*, 2001)

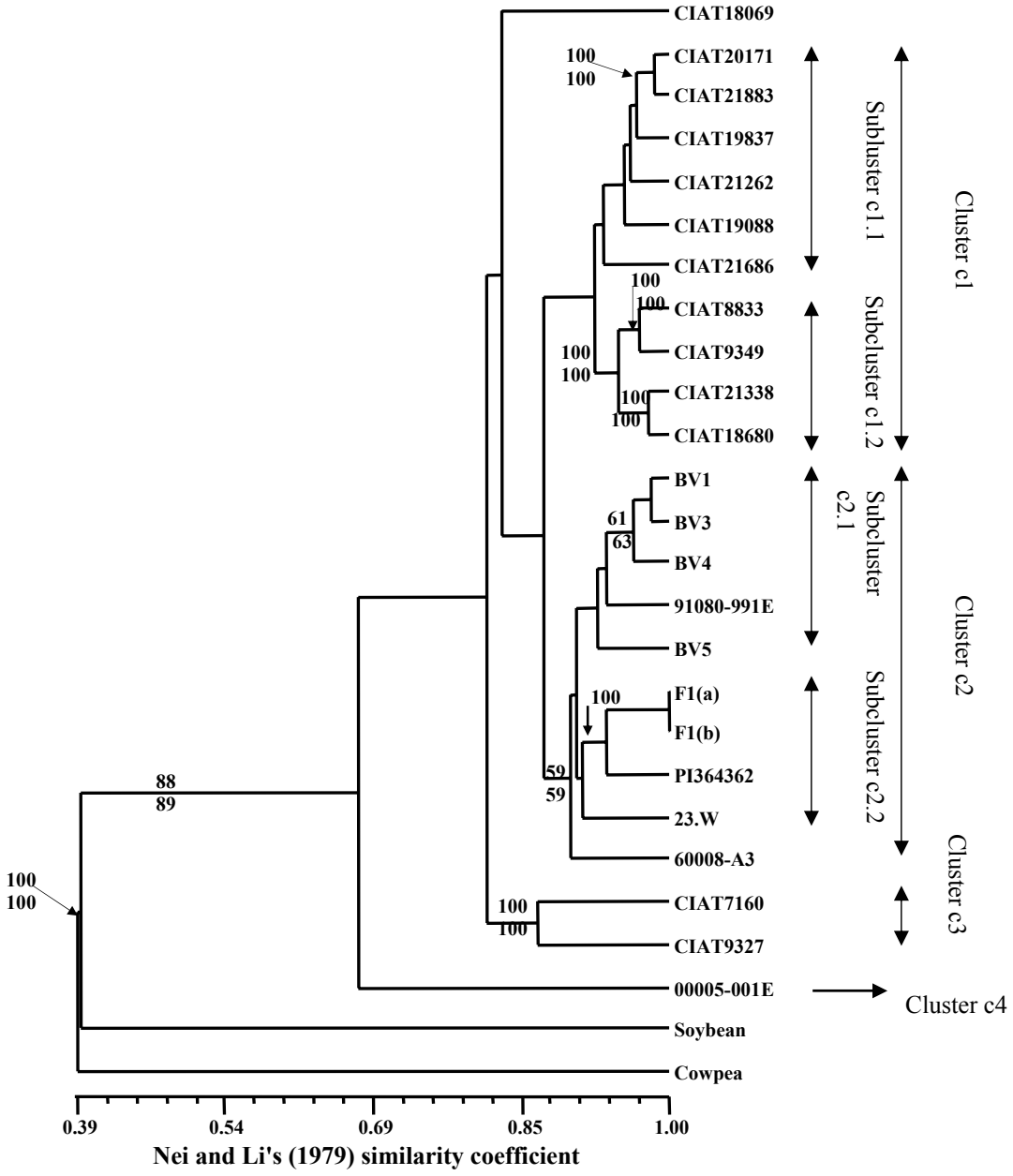

Figure 2. Phenogram of 24 velvetbean accessions revealed by UPGMA cluster analysis based on AFLP markers with 12 primer combinations. Numbers shown above different branches represent percentage confidence limits obtained in the bootstrap analysis, those below branches are percentage confidence limits in the jackknife analysis. Branches lacking bootstrap and jackknife values received <50% bootstrap and jackknife supports.

Cluster analyses of velvetbean accessions using UPGMA and Nei and Li's coefficients, as well as principal component analysis led to the separation of the accessions into two distinct groups. Clearly, all U.S landraces were clustered together (Subcluster 1.1, Figure 1). As can be seen, the three tropical accessions in cluster 1 were clearly discernible from the rest. The U.S. landrace accessions may be fewer generations removed from unknown ancestral introductions than the tropical lines. Early reports speculated that mutation is at the origin of the genetic variation observed in the landrace accessions (Coe, 1918). Within cluster 2, two separate subclusters are formed (Figure 1). The accession PI364362 forms a separate group at the 0.89 similarity level. This accession is different from the rest of the group by the color and shape of its pods. The accession PI364362 has greenish pod color, grey pod pubescence, and large pods while the rest of the accessions in this group have black pod, black pod pubescence, except IRZ which has green pod. The similarity coefficients were high, 0.89-0.97 and 0.87, respectively, within and among the main clusters, thereby indicating that all accessions used in this study should not be considered as different species. This is in agreement with the recommendations of Wilmot-Dear (1984). A number of taxa that were formerly considered separate species are now considered merely varieties of *Mucuna pruriens*, namely, *M. aterrima*, *M. cochinchinensis*, *M. hassjoo*, *M. nivea*, and *M. utilis*. Previous reports showed that the range of germplasm being exploited to date is quite restricted and derives from native genotypes in Central America, specially Honduras, with most accessions being nominally of the *Mucuna pruriens* var. *utilis* type (Kay, 1979; Buckles, 1995).

The two main clusters based upon AFLP analysis correspond to differences in maturity class. Thus, maturity is an important trait in differentiating velvetbean accessions. As can be seen, all the tropical accessions are grouped together except the accessions Ghana, Rajada, and PI 383272 (Figure 1). The earliness of the accessions Ghana, Rajada, and PI 383272 in the tropical lines may explain their separation (Capo-chichi *et al*., 2001).

Cluster analysis in study two (the accessions obtained from CIAT) separated accessions into four major groups. Within cluster c2, two separate subclusters are formed (Figure 2) corresponding to differences in growth habits. The subcluster c2.1 comprised the bush growth habit while the cluster c2.2 grouped the vining growth habit. Interestingly, in subcluster c2.2, the F_1 hybrids formed a strong association with the accession PI364362 (100% confidence interval limits in the bootstrap analysis). The F_1 hybrids and PI364362 have both black seed coat while the U.S. landrace has white seed coat. This may mean that seed coat color has a major contribution to that association.

The AFLP technique is an efficient and useful tool for detecting genetic diversity. AFLP analysis in the present study provided an estimate of genetic relationships in velvetbean accessions that was reliable and consistent. This supports the conclusions of previous studies which recommended AFLPs as an efficient, reliable, and useful tool compared to other molecular techniques, such as random amplified polymorphic DNAs (RAPD) and simple sequence repeats (SSR) (Jones *et al*., 1997). The results demonstrated that genetic resolution provided by AFLP is amenable to cluster analysis of closely related species.

CONCLUSIONS

Our results showed a clear classification between different taxa. Accessions from a broader geographical range, especially those from the tropics, have increased the variation, thereby increasing the scope for developing a breeding program. We have shown the genetic diversity and established relationships among the velvetbean collection using the AFLP techniques. Maturity seems to have an effect on the magnitude of this variation. Since the present project on the genetic diversity of *Mucuna* sp. was started recently, we can only show some results using AFLP markers. However, these results can be used to further study the genetic diversity of *Mucuna* and other related species, which are difficult to identify using morphological traits. This research represents one of the most comprehensive investigations of DNA diversity for velvetbean and is among the first to report on the effectiveness of the AFLP technique for determining genetic relationships in velvetbean. These results have direct implications in velvetbean improvement programs for anti-nutritional factors (e.g., lower L-Dopa content in seed, a step that would enhance the general utility of the crop) and specific cropping systems.

Additional aims are to organize germplasm collections to include the whole genus of *Mucuna* sp. including the wild related species, so that the potential of velvetbean accessions is fully characterized and useful for breeders.

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REFERENCES

Awang, D, Buckles, D, Arnason, JT. 1997. The phytochemistry, toxicology and processing potential of the cover crops velvetbean (cow(h)age, cowitch) (*Mucuna* Adans. Sp., Fabaceae). Paper presented at the International Workshop on Green Manure-Cover Crop Systems for small holders in tropical and subtropical regions, 6-12 Apr. Chapeco, Santa Catarina, Brazil. Rural Extension and Agricultural Research Institute of Santa Catarina, Santa Catarina, Brazil.

Buckles, D. 1995. Velvetbean: A "new" plant with a history. Economy Botany 49:13-25.

Burkill, IH. 1966. A dictionary of the economic products of the Malay Peninsula. Kuala Lumpur, Malaysia: Government of Malaysia and Singapore.

Caetamo-Anollés, G, Gressholff, PM. 1998. DNA analysis of turfgrass genetic diversity. Crop Science. 38:1415-1424

Capo-chichi LJA, Weaver, DB, Morton, CM. 2001. AFLP assessment of genetic variability among velvetbean (*Mucuna* sp.) accessions. Theoretical and Applied Genetics 103: 1180-1188.

Coe, HS. 1918. Origin of the Georgia and Alabama varieties of velvetbean. Journal of the American Society of Agronomy 12:175-179.

Doyle, JJ, Doyle, JL. 1990. Isolation of plant DNA from fresh tissue. Focus 12: 13-15.

Duggar, JF. 1899. Summer crops for green manure and soil improvement. U.S. Agricultural Experiment Station Bulletin No.104: 109-125.

Duke, JA. 1981. Handbook of legumes of world economic importance. New York, New York. Plenum Press.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783-791.

Highton, R. 1993. The relationship between the number of loci and the statistical support for the topology of UPGMA trees obtained from genetic distance data. Molecular Phylogenetics and Evolution 2:337-343.

Jones, CJ, Edwards, KJ, Castaglione, S, Winfield, MO, Sal, F, van de Wiel, C, Bredemeijer, G, Vosman, B, Matthes, M, Dally, A, Brettscneider, R, Bettini, P, Buiatti, M, Maestri, E, Malcevschi, A, Marmiroli, N, Aert, R, Volckaert, R, Rueda, J, Linacero, R, Vazquez, A, Karp, A. 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by network of European laboratories. Molecular Breeding 3:381-390.

Karp, A, Edwards, KJ. 1995. Molecular techniques in the analysis of the extent and distribution of genetic diversity. In Molecular genetic techniques for plant genetic resources. Report of an IPGRI workshop, 9-11 October 1995, Rome, Italy.

Karp, A, Isaac, PG, Ingram, DS. 1998. Molecular tools for screening biodiversity-plants and animals. 1st ed. Chapman & Hall GmbH, Weinheim, Germany.

Kay, D. 1979. Crop and product digest. No. 3-Food Legumes. Tropical Products Institute, London.

Nei, M, Li, W. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings National of Academic of Science 79:5269-5273.

Rohlf, FJ. 1998. NTSYS-pc Numerical taxonomy and multivariate analysis system. Version 2.0. Exerter Software, Setauket, N. Y.

Skroch, P, Tivang, J, Nienhui, S. 1992. Analysis of genetic relationships using RAPD marker data. In Proceedings. Symp. Applic. RAPD Techno. Plant Breed. Minneapolis, Minn. Crop Science Society of America, Madison, Wisconsin, pp 26-30.

Sokal, RR, Michener, CD. 1958. A statistical method for evaluating systematic relationships. University of Kansas Science Bulletin 38: 1409-1438.

Swofford, DL. 1993. PAUP: phylogenetic analysis using parsimony. Version 3.1 Illinois National History Survey, Champaign Illinois.

Vos, P, Hogers, R, Bleeker, M, Reijanas, M, van de Lee, T, Hornes, M, Freijters, A, Pot, J, Peleman, J, Kuiper, M, Zabeau, M. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research 23:4407-4414.

Wilmot-Dear, CM. 1984. A revision of *Mucuna* (Leguminosae-Phaseoleae) in China and Japan. Kew Bulletin. 39:23-65.

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