Tropical and Subtropical Agroecosystems, 1 (2003): 319 - 328

Tropical and Subtropical Agroecosystems

EFFECT OF GENOTYPE AND ENVIRONMENT ON L-DOPA CONCENTRATION IN *MUCUNA*'S (*Mucuna* sp.) SEEDS

L.J.A. Capo-chichi[1]*, M. Eilittä[2], R.J. Carsky[3], R.A. Gilbert[4] and B. Maasdorp[5]

[1]*Department of Agronomy and Soils, Auburn University, AL 36849, USA*
E-mail: cludovic@acesag.auburn.edu
[2]*Post-doctoral fellow funded by the Rockefeller Foundation, Accra, Ghana*
[3]*International Institute of Tropical Agriculture, Cotonou, Bénin*
[4]*Everglades Research and Education Center, Department of Agronomy, University of Florida, Belle Glade, FL, USA*
[5]*Crop Science Department, University of Zimbabwe, Harare, Zimbabwe*
**Corresponding author*

SUMMARY

High L-Dopa content in *Mucuna* seeds is most likely the greatest impediment to increasing their utilization as a food and feed. Breeding *Mucuna* to lower the L-Dopa content of the seeds would be one way to increase the utility of this cover crop as a food and feed. Breeding efforts would be aided by the evaluation of the natural range of genetic variability in L-Dopa content and its relationship to the growing environment. Our objective was to determine whether genotype x environment (G x E) interactions are present for L-Dopa in very different sites and with different management across a range of latitudes and, if so, to interpret the nature of the interactions as well as to determine their implications for a *Mucuna* breeding program. Several accessions (four in 2000-2001 and eight in 2001-2002) were grown in numerous sites (four and six, respectively) in latitudes ranging from 18°S to 30°N. The relative magnitude of the G x E interaction components, namely genotype x site (G x S), genotype x years (G x У), and genotype x site x years (G x S x У), was studied using variance component analysis. A standard multi-factor analysis of variance (ANOVA) test revealed that all first order interactions (G x S, G x У, S x У) as well as second order interactions (G x S x У) were either absent or not highly significant and accounted for a minimal amount of the variance, indicating that genotype performance was not dependent upon sites or years. Genotypes performance for L-Dopa synthesis fluctuated across sites inconsistently. As latitude increased, *Mucuna* L-Dopa fluctuations in seeds were more pronounced in some accessions than others. However, this response was not consistent for all accessions, indicating the need to test genotypes or clones at multi-environments over longer periods of time. At all sites, the early-maturing accession Rajada had the lowest L-Dopa content in seeds and its values ranged from 2.4% to 4.4% of seed dry weight (DW), averaging 3.5%. This was followed by Ghana, another early-maturing accession, for which L-Dopa content ranged from 3.1% to 5.6% (average 4.6%, all DW). The highest L-Dopa content was observed for the late-maturing accessions Cochinchinensis, Deeringiana, Preta, and Utilis, respectively averaging 5.4%, 5.4% 5.5%, and 5.2% DW. Thus, maturity time may be tested as a physiological trait for predicting the level of L-Dopa in seeds. For all accessions, the regression of seed L-Dopa content on latitude was not significant. Thus, the relationship between L-Dopa concentration and the environment may be more complex than a simple linear correlation to latitude.

Key words: Genotype, environment, L-Dopa, *Mucuna,* latitude.

INTRODUCTION

Mucuna species have been shown to produce L-Dopa (L-3,4-dihydrophenylalanine), a non-protein amino acid which is a precursor of the neurotransmitter dopamine and is used in the treatment of Parkinson's disease (Barbeau and McDowell, 1970). In a screening survey of more than 1000 species in 135 plant families, *Mucuna* was the only species with sufficient L-Dopa to suggest a possible use for commercial production (Daxenbichler *et al*., 1971). Those using L-Dopa to alleviate symptoms of Parkinson's disease, as well as people and monogastric animals consuming improperly processed *Mucuna* seeds, are known to suffer from gastrointestinal disturbances, notably, nausea, vomiting, and anorexia, as well as more serious effects, such as paranoid delusions, hallucinations, delirium, and unmasking of dementia (Reynold, 1989). Therefore, *Mucuna*'s high content of L-Dopa is seen as the greatest impediment to its increased utilization as a food and feed (Carsky *et al*., 1998; Flores *et al*., 2002). Interestingly, it has proven potential as food and feed, as it was a common feed crop in the southern United States and in several tropical countries in the early part of the 20th century and has been consumed as a minor food crop in a number of African and Asian countries (Buckles,

1995; Eilittä and Sollenberger, 2002; Eilittä *et al*., 2002).

Several studies have focused on L-Dopa quantification in plant parts of various *Mucuna* species. Bell and Janzen (1971), in surveying six accessions, found a range from 5.9 to 9.0% in the seed, while Daxenbichler *et al*. (1971; 1972), in their above-mentioned survey, observed variability between 3.1 and 6.7%. The ranges of from 2.2 to 7.2% were found in a survey of 36 accessions (Lorenzetti *et al*., 1998) and from 1.9 to 7.6% in a survey of 38 accessions (St-Laurent *et al*., 2002).

However, little is known of the production of L-Dopa in *Mucuna* species. Specifically, there is little information on the impact of genotype and environment on the production of L-Dopa, as only two studies to date have addressed the topic (Lorenzetti *et al*., 1998; St. Laurent *et al*., 2002). Both studies found both environmental and genetic control of L-Dopa production. Of the environmental factors, both studies have been particularly interested in the impact of latitude where the genotype was grown. Lorenzetti *et al*. (1998) found both environmental and genotypic impact on L-Dopa production; of the environmental factors, only latitude was considered and was found to have impact. Similarly, St. Laurent *et al*. (2002) found a slight impact of latitude and concluded that other factors were influential also. Lorenzetti *et al*. (1998) hypothesized, based on the laboratory work by Pras *et al*. (1993) and Liu and McClure (1995), that variation in the intensity of light and in backscattered ultraviolet radiation, both generally higher near the equator, may be among factors explaining why L-Dopa content was found to be higher at lower latitudes. Also Wichers *et al*. (1985) showed that environmental parameters (e.g., nature of nitrogen source and presence/absence of illumination) affected the production of L-Dopa by cell suspension cultures of *M. pruriens*. Many other environmental and management factors could impact the production of L-Dopa in *Mucuna* seed but their role has been poorly studied.

These two past studies that explored the role of environment and genotype on L-Dopa production utilized seed that was available at different locations worldwide. Thus, location was confounded with genotype. In addition, both studies took seed color as an indicator of genetic variability. To determine with certainty the comparative influence of genotype, environment and their interactions, the same genotypes need to be grown in all environments.

Relatively little recent taxonomic work has been conducted on *Mucuna* and there have been very few recent breeding efforts on the crop. Taxonomy classification of the various accessions of *Mucuna* is uncertain and it is unknown if the variability of the accessions whose L-Dopa content has been quantified has been at species, sub-species, or varietal level, or if some accessions in the same study have been the same varieties (albeit with a different seed color or name). Fortunately, a recent study (Capo-chichi *et al*., 2001) and ongoing work at Auburn University are clarifying the confusion surrounding the taxonomy of *Mucuna*, suggesting that currently available accessions are mere varieties of the species *Mucuna pruriens*. Improved understanding of the *Mucuna* taxonomy is necessary before a breeding program on the crop can be initiated.

Genotype x environment interactions (G x E) are a matter of concern in any selection program seeking to exploit genotype performance across several environments. Genotype x environment interactions affect selection decisions when the rank of a genotype changes across environments. In such a situation, it becomes necessary to evaluate genotypes across a range of environments to determine their true value. For example, the measured L-Dopa concentration of each accession in each tested environment is due to a mixture of environment main effect (E), genotype main effect (G), and genotype x environment interaction (GE). Typically, the explanation of E on a given phenotype is still unknown. In general, it is G and G x E that are relevant to cultivar evaluation. Moreover, G and G x E must be considered simultaneously when making cultivar selection decisions.

To determine what the impact of genotype and environment are on L-Dopa production, the same genotypes need to be grown in a multitude of environments. Previous studies have found that of environmental factors, latitude may be particularly important in determining the content of L-Dopa. Therefore, the objectives of this study were 1) to estimate L-Dopa content of numerous accessions of *Mucuna* grown at different sites from a range of latitudes 2) to determine the relative impact of genotype and environment and their interactions on the production of L-Dopa.

MATERIALS AND METHODS

The experiment included four accessions of *Mucuna* (Rajada, Ghana, Jaspeada, and Utilis) in the year 2000 experiment and eight accessions of *Mucuna* (Rajada, Ghana, Jaspeada, Utilis, IRZ, Cochinchinensis, Preta, and Deeringiana) in the year 2001 experiment. These accessions, which were obtained from the Center for Cover Crop Information and Seed Exchange (CIEPCA) at the International Institute of Tropical Agriculture station in Benin, vary with regard to time to maturity (e.g. Rajada and Ghana are earlier maturing accessions, while Utilis and Cochinchinensis are very late-maturing), in seed and flower color, and in other characteristics. Seeds of the same accession came from the same seedlot to ensure uniformity

across sites in any given year. The trial was conducted in seven locations that vary in latitude between 18°S and 30°N and other environmental parameters (e.g., rainfall and soil type). In 2000, locations included Florida-Gainesville, Florida-Belle Glade, Honduras, Bénin, and Zimbabwe. In 2001, locations were Florida-Bell Glade, Honduras, Bénin, Zimbabwe, Chiapas (Mexico), Yucatan (Mexico), and Cali (Colombia) (Table 1).

Table 1. Country, latitude and institution of sites and collaborators in the genotype by environment trial.

Country	Latitude	Institution
Colombia	3°N	CIAT
Benin	6°N	CIEPCA/IITA
Honduras	14°N	CIDICCO
Mexico (Chiapas)	16°N	Universidad Autonoma de Chiapas
Zimbabwe	18°S	University of Zimbabwe
Mexico (Yucatan)	20°N	Universidad Autonoma de Yucatan
USA (Belle Glade, FL)	26.4°N	University of Florida
USA (Gainesville, FL)	29.6°N	University of Florida

Note: CIAT = Centro Internacional de Agricultura Tropical,
CIEPCA = Center for Cover Crops Information and Seed Exchange in Africa,
IITA =International Institute of Tropical Agriculture,
CIDICCO = International Cover Crops Clearinghouse for Information.

In 2000, *Mucuna* seeds were planted in plots of variable size except in the two locations in Florida, where the trial was planted in pots (one in a greenhouse for avoidance of late-season frosts and another one outside to avoid water logging). The experimental design was a randomized complete block design with four blocks. In 2001, *Mucuna* was either direct-seeded in the field or planted in pots in a greenhouse and then transplanted in the field. In one location (Belle Glade-Florida), the trial was planted in pots outside. In the case of direct seeding, 5 to 6 seeds were planted per hill initially, and then thinned to two per hill, then to one plant per hill. In the case of transplanting, 2 to 3 seeds were planted per pot with 4 replicates (4 pots) in a greenhouse. As soon as field conditions were appropriate, 1 to 2 greenhouse seedlings were transplanted into each hill plot. These methods were done simply to improve seedling establishment. In 2001, plots consisted of a single plant, typically supported by a 2.5 m high metal frame, 1 m^2 at the base. A corridor of 1.5 m between hills/trellises was maintained as *Mucuna* tends to spread very aggressively. Such corridors were slashed to make sure that *Mucuna* accessions did not cross to the adjoining plots (trellises). The experimental design in 2001 was a randomized complete block design with two blocks. In both years, management varied by location. In trials planted in pots, fertilizer was added and *Mucuna* was cut back when necessary. *Mucuna* seeds were harvested at maturity. In 2000, Utilis did not reach maturity in the Florida sites.

Sample preparation and L-Dopa extraction

Sample preparation, extraction and determination of L-Dopa were conducted at Judson College, Illinois, USA. *Mucuna* seeds were finely ground in a Wiley laboratory mill (Model 3383-10) after the seed coat had been removed. Material that passed through a 40-mesh screen was collected for analysis. L-Dopa extraction was performed in quadruplicate for each accession. Approximately 0.1 g of flour was weighed in a 27 x 95 mm glass screw-top vial. Twenty mL of distilled-deionized water was added. The cap vial was subjected to 10 minutes of sonification in a Branson Model 2510R-DTH Ultrasonic Cleaner. The contents of the vial were diluted with water to 100 mL in a volumetric flask, filtered using a 0.45 μm syringe filter unit with nylon membrane (Xpertek 9440721, P.J Cobert Associates, St. Louis, MO 63146), and transferred to an autosampler vial for HPLC analysis.

Determination of L-Dopa by HPLC

The concentration of L-Dopa in *Mucuna* flour extract was determined by HPLC on a Zorbak stableBond SB-C18 column (4.6 x 150 mm, 3 μm particles No 86953-902, with guard column No 820950-920, Mac-Mod Analytical, Chadds Ford, PA 19317) at 1 $mL.min^{-1}$ and 30°C with a mobile phase consisting of 9 parts buffer [0.1 M phosphoric acid, 1 mM 1-octanesulfonic acid (No 08380, Sigma-Aldrich, St., MO 63178), 2 mM disodium EDTA, adjusted to pH 3.0]. The system included Rainin HXps (Varian Instruments, Palo Alto,

CA 94304), a Waters 717 + autosampler with refrigeration set at 15˚C (Waters Corporation, Milford, MA 01757) and a Perkin-Elmer Model 200 tunable absorbance detector set at 279 nm. Injection volume was 40 µl, and a 0.5 µm inline filter (NoA103, Upchurch Scientific, Oak Harbor, WA 98277) was positioned between the autosampler and the column. L-Dopa concentrations were determined with reference to standard (NoD9378, Sigma-Aldrich) using Star Chromatography software (Varian).

Data analysis

The data were subjected to analyses of variance (ANOVA) using models for a randomized complete block design. The model to analyze data from both years was as follows:

$$Y_{jskil} = \mu + Y_i + R(Y)_{ki} + S_l + R(YS)_{kil} + G_j + GS_{jl} + GC_{ji} + GSY_{jli} + GR(SY)_{jkli} + E_{jskil} \quad \text{(Model I)}$$

Where:
Y_{jskil} is the observation for genotype *j*, in sampling *s* nested within rep *k*, year *i*, and site *s*; µ is the overall mean; Y*i* is the effect of the *i*th crop-year; $R(Y)_{ki}$ is the effect of the *k*th rep nested within the *i*th year; S_l is the effect of the *s*th site; YS_{il} is the effect of the interaction between the $_i$th crop-year and the $_l$th site; $R(YS)_{kil}$ is the effect of the *k*th rep nested within the interaction between the *i*th year and the *l*th site; G*j* is the effect of the *j*th genotype; GS_{jl} is the effect of the interaction between the *j*th genotype and the *l*th site; GY_{ji} is the effect of the interaction between the *j*th genotype and the *i*th year; GSY_{jli} is the effect of the interaction between the *j*th genotype, the *l*th site, and the *i*th year; $GR(SY)_{jkli}$ is the interaction effect between the *j*th genotype, and the *k*th rep nested within the interaction between the *l*th site and the *i*th year; E*jskil* is the residual term.

The model to analyze the data by year (either 2000 or 2001) was as follows:

$$Y_{ikl} = \mu + S_l + R(S)_{kl} + G_i + GS_{il} + E_{ikl} \quad \text{(Model II for year 2000 and III for 2001)}$$

Where:
Y_{ikl} is the observation for genotype *j*, in rep *k* nested within site *l*; µ is the overall mean; S*j* is the effect of the *l*th site; $R(S)_{kl}$ is the effect of the *k*th rep nested within the *l*th site; G*j* is the effect of the *j*th genotype; GS_{il} is the effect of the interaction of the *j*th genotype and the *l*th site; E_{ikl} is the residual term. The analyses were carried out using the General Linear Model Procedure (SAS Institute Inc., 1997). Since ANOVA is incapable of breaking down an interaction into several parts, regression was used to explore the effect of environment (site and year) for the genotypes and to further study the possible effect of latitude as an environmental factor. In this approach, L-Dopa content of each accession was regressed on the absolute value of the latitudes of each site using non-linear regression models (Neter *et al*., 1996; SAS Institute, 1997). Note however, as mentioned earlier, the sites varied not only in latitude, but also in other environmental factors and in management.

RESULTS AND DISCUSSION

In the combined analysis of variance including both years (model I), genotypes differed significantly ($P < 0.001$) in mean L-Dopa concentration in *Mucuna* seeds (Table 2). Sites and years were not significantly different (Table 2). One first order interaction (G x S) and the second order interaction (G x S x Y) were not significant for L-Dopa concentration using model I (data from 2000 and 2001) and II (2000 only), while the other first order interactions (G x Y) and (S x Y) were significant at 5% probability level using model I (Table 2). In the individual year ANOVAs (models II and III), genotype was again highly significant. The G x S was significant for model III only ($P < 0.05$). The slight or non-significance of these interactions relative to the strong genotype main effect ($P<0.001$) indicates that not too much emphasis should be placed on these interactions and that accessions/genotypes concentration for L-Dopa in seeds was not dependent upon environment. In model I (which included both years), the analysis of variance showed that out of the total sum of squares, 42.6%, 13.2%, 6.8%, 3.4%, 3.0%, 2.0%, and 1.5% was attributable to G, S, G x S, G x Y, S x Y, and G x S x Y, respectively (Table 2). In model II (year 2000), 42.7%, 5.3% and 5.0% was attributable to G, S, G x S, respectively (Table 2). In model III (year 2001), 49.1%, 19.6% and 16.5% was attributable to G, G x S, and S, respectively (Table 2). Thus, genotypes/accessions explained 42.6% to 49.1% of the total variation of L-Dopa concentration in *Mucuna*'s seeds (i.e. 2 to 14 times the variation explained by the first order interactions and 21 times the variation explained by the second order interaction). The size of genotype sum of squares in relation to the first and second order interactions indicated substantial differences in genotype response for L-Dopa

concentration in seed in different environments. The slight variation due to the interactions could also be attributable to site-related factors. The magnitude of variances among genotypes that do not bring about changes in genotype ranking would not affect genotype selection decisions for breeding to reduce L-Dopa content. All accessions may be widely adapted for L-Dopa production because of their stability in L-Dopa production across all environments (Figure 1). These accessions slightly vary without changing their rank.

Table 2. Analysis of variance for L-Dopa content in dry seed of *Mucuna* grown at seven sites: I) 2000, 2001, and four accessions, II) 2000 and four accessions, III) 2001 and eight accessions.

Model	Source	df	SS	L-Dopa	% of total SS
	Year (У)	1	6.1	NS	1.5
	Block (Year)	4	4.2	NS	
	Site (S)	6	50.8	NS	13.2
	S x У	3	12.0	*	3.0
	Block (S x У)	15	16.0	NS	
I	Genotype (G)	3	166.7	***	42.6
	G x S	18	26.8	NS	6.8
	G x У	3	13.4	*	3.4
	G x S x У	8	7.9	NS	2.0
	G x Block (S x У)	54	58.5	NS	
	Sampling (G x Block x У x S)	284	12.8	NS	
	Residual	21	15.0		
	Total	420	391.2		
	Site (S)	3	9.0	*	5.3
	Block (Site)	12	9.7	***	
	Genotype (G)	3	71.4	***	42.7
II	G x S	9	8.5	NS	5.0
	G x Block (Site)	36	40.2	***	
	Residual	149	20.2	NS	
	Total	212	167.2		
	Site (S)	6	64.2	NS	16.5
	Block (Site)	7	11.5	*	
	Genotype (G)	7	190.2	***	49.1
III	G x S	39	76.0	*	19.6
	G x Block (Site)	37	34.4	***	
	Residual	291	19.1		
	Total	387	387.6		

*, **, *** significant at 0.05, 0.01, and 0.001 probability levels, respectively
NS: non significant

In 2000, L-Dopa concentration in *Mucuna*'s seeds varied from 5.9% of dry weight for the accession Jaspeada to 3.3% for Rajada (Table 3). In 2001, L-Dopa concentration varied from 6.4% for Cochinchinensis to 2.4% for Rajada (Table 3). Across sites, L-Dopa concentration averaged 5.4% for Cochinchinensis, 5.4% for Deeringiana, 5.5% for Preta, 5.2% for Utilis and 5.0% for Jaspeada, 4.7% for IRZ, 4.5% for Ghana, and 3.5% for Rajada (Table 3). The accessions Ghana and Rajada had the lowest L-Dopa concentration. Therefore, selection for low L-Dopa should favor the accessions Ghana and Rajada. In sites where two-year experiments were conducted, L-Dopa content in seeds was presented per year. There was no consistent pattern across accessions between years. In Zimbabwe, L-Dopa concentrations were similar in both years for Utilis, Jaspeada, Ghana and Rajada, whereas in Bénin, Florida, and Honduras, L-Dopa concentration in seeds was slightly higher for Jaspeada, Ghana, and Rajada in 2000 than in 2001. The exception was in Belle Glade-Florida where Rajada tended to synthesize more L-Dopa and in Bénin where Jaspeada levels were similar in both years (Table 3; Figure 1). This difference may be explained by the fact that seeds for the two-year experiments were obtained from different seed lots. Thus, there may be seed-to-seed variability for L-Dopa within the same accession.

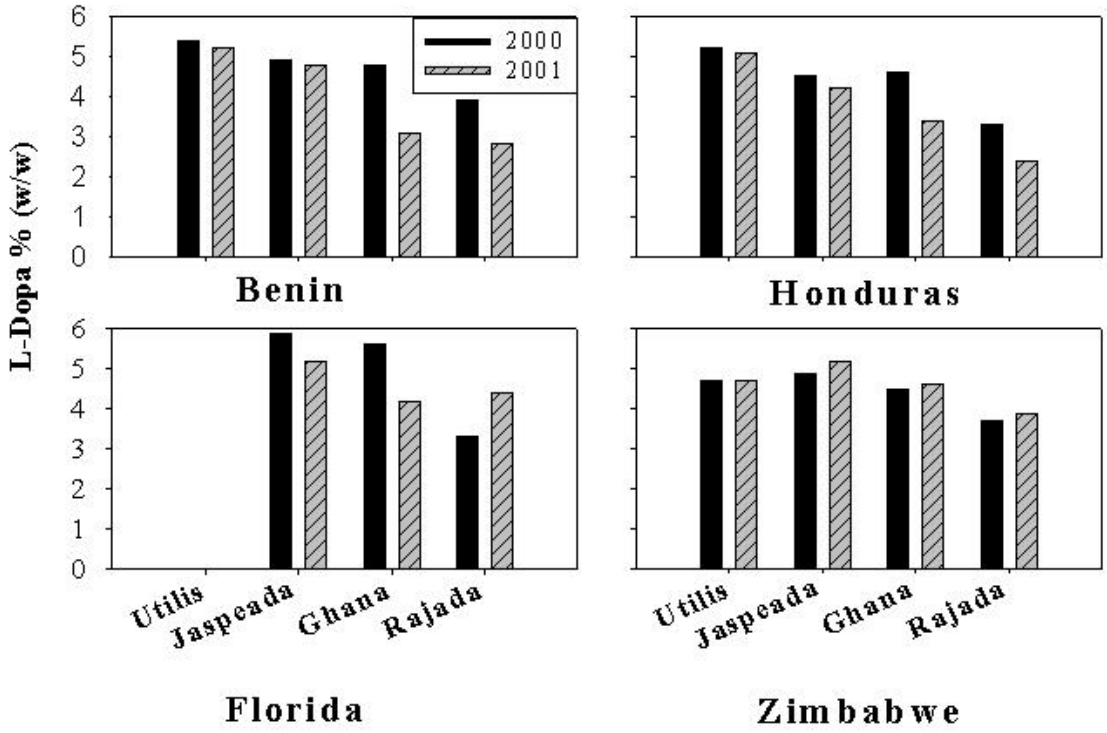

Figure 1. L-Dopa content of seed dry weight of four *Mucuna* accessions grown at four locations in two years.

Table 3. Means of L-Dopa in seeds of eight *Mucuna* accessions within sites grown in two years.

Year	Location	Accession								LSD (0.05)
		Cochin-chinensis	Deeringiana	Preta	Utilis	Jaspeada	IRZ	Ghana	Rajada	
		% (w/w)								
	Cotonou (Bénin)	-	-	-	5.4	4.9	-	4.8	3.9	0.2
	Honduras	-	-	-	5.2	4.5	-	4.6	3.3	0.6
2000	Zimbabwe	-	-	-	4.7	4.9	-	4.5	3.7	0.3
	Belle Glade (Florida)	-	-	-	5.8	4.5	-	5.1	3.8	0.5
	Gainesville (Florida)	-	-	-	-	5.9	-	5.6	3.3	0.3
	Colombia	6.4	5.7	5.8	5.4	5.7	4.5	5.3	3.3	0.5
	Cotonou (Bénin)	6.1	5.9	5.5	5.2	4.8	4.1	3.1	2.8	0.4
	Honduras	4.5	4.7	5.7	5.1	4.2	3.4	3.4	2.4	0.4
2001	Chiapas (Mexico)	4.8	5.5	5.1	4.9	4.9	-	4.0	3.0	0.2
	Zimbabwe	5.7	-	4.9	4.7	5.2	5.1	4.6	3.9	0.3
	Yucatan (Mexico)	5.4	5.9	5.6	5.8	5.2	5.7	5.4	4.2	0.4
	Gainesville (Florida)	5.0	4.9	6.0	-	5.2	5.5	4.2	4.4	0.7
Mean		5.4	5.4	5.5	5.2	5.0	4.7	4.5	3.5	

The regression analysis of L-Dopa in seeds against latitude was not statistically significant for any accession (Figure 2). Increasing latitude did not result in statistically higher or lower L-Dopa concentration for any of the accessions. This may suggest that variation in latitude is not an important factor influencing L-Dopa concentration in *Mucuna* seeds, although the results should be interpreted with caution since the sites also varied in many other environmental factors and in management. Lorenzetti *et al.* (1998) and St-Laurent *et al*. (2000) observed a slight variation in L-Dopa concentration due to the variation in latitude, but, as mentioned earlier, they utilized genotypes that were available at different latitudes. As discussed previously, latitude-related factors, such as light intensity, might have an effect on the L-Dopa synthesis in the seeds of *Mucuna* (Lorenzetti *et al*., 1998). Temperature could be another influential factor. Flowering in *Mucuna* may be hastened by cool nights (< 21°C), but the reproductive responses of *Mucuna* to photoperiod are unknown (Keatinge *et al*., 1997). At 3 °N (Colombia), Cochinchinensis synthesized the highest L-Dopa in the seeds (6.4% of the dry weight). At all sites, the accession Rajada produced the lowest L-Dopa, followed by Ghana. This may be due to the fact that they mature earlier compared to other accessions used in the present study (data not shown).
If the role of L-Dopa in the plant is to protect it from insects (Temple and Huyck, 2002), then an early maturing variety would not need as much L-Dopa because it would be more likely to escape insect pressure. Alternatively, lower L-Dopa in Rajada may occur for some other reason and low L-Dopa may not even be linked with maturity. A study on genetic diversity using molecular markers showed that the accessions Rajada and Ghana formed a separate cluster within the main cluster composed of early-maturating accessions (Capo-chichi *et al*., 2001). It should be further tested to reveal if maturity could be used as a physiological indicator in predicting the level of L-Dopa in *Mucuna*'s seeds. The stability across all environments (Figure 2) indicates that all accessions may be widely adapted for L-Dopa production.

CONCLUSIONS

Trials were conducted in several sites in Africa, Latin America, and USA to estimate L-Dopa content of numerous accessions of *Mucuna* grown at different sites and to determine the relative impact of genotype and environment on the production of L-Dopa. Genotype had the greater influence on L-Dopa content, accountings for 44.6-49.1% of the total variance of L-Dopa at different sites. Overall, L-Dopa was lower in seeds of the accessions Rajada and Ghana and higher in seeds of Cochinchinensis, Deeringiana, Utilis, IRZ, Jaspeada, and Preta. The genotype x environment interaction effect appeared minimal when compared with the genotype/accession main effect. This finding is particularly germane to breeding decisions as it implies that *Mucuna* accessions are relatively stable across environments. The low L-Dopa concentration in seeds of Rajada and Ghana is perhaps due to the fact that they are early-maturing accessions. This should be confirmed. Regression analyses of L-Dopa content indicated that variation in latitude did not result in significant variation in L-Dopa concentration in *Mucuna* seeds; as discussed earlier in this analysis, latitudes were represented by sites which differed in a number of other environmental factors and in management. Increasing latitude from the equator did not result in significantly higher or lower L-Dopa concentration in *Mucuna* seeds. The strong influence of genotype on L-Dopa production in *Mucuna* offers hope that breeding can contribute toward decreasing L-Dopa in *Mucuna* seeds to a level that would permit their increased utilization as a food and feed.

ACKNOWLEDGMENTS

The authors wish to acknowledge funding from the Rockefeller Foundation through the project "Increasing *Mucuna*'s Potential as a Food and Feed Crop, coordinated by the Center for Cover Crops Information and Seed Exchange in Africa (CIEPCA) at the International Institute for Tropical Agriculture (IITA). We also acknowledge Dr. Peters Michael from the Tropical Grasses and Legume Program of the Centro de Investigacion Tropical (CIAT), Milton Flores from the Centro Internacional de Informacion Sobre Cultivos de Coberture (CIDICCO), Dr. Ricardo Quiroga-Madrigal from Facultad de Ciencias Agronomicas, Universidad Autonoma de Chiapas, Jose Castillo-Caamal from PROTOPICO-Facultad de Medicina Veterinaria y Zootechnia, Universidad Autonoma de Yucatan, Dr. Steven R. Temple, Agronomy and Range Science Department, University of California, Davis, and Mr. Albert C. Eteka, CIEPCA-IITA, for managing the trial at their respective experimental stations. We thank Dr. Rolf Myhrman, Judson College, Illinois, for the L-Dopa analysis.

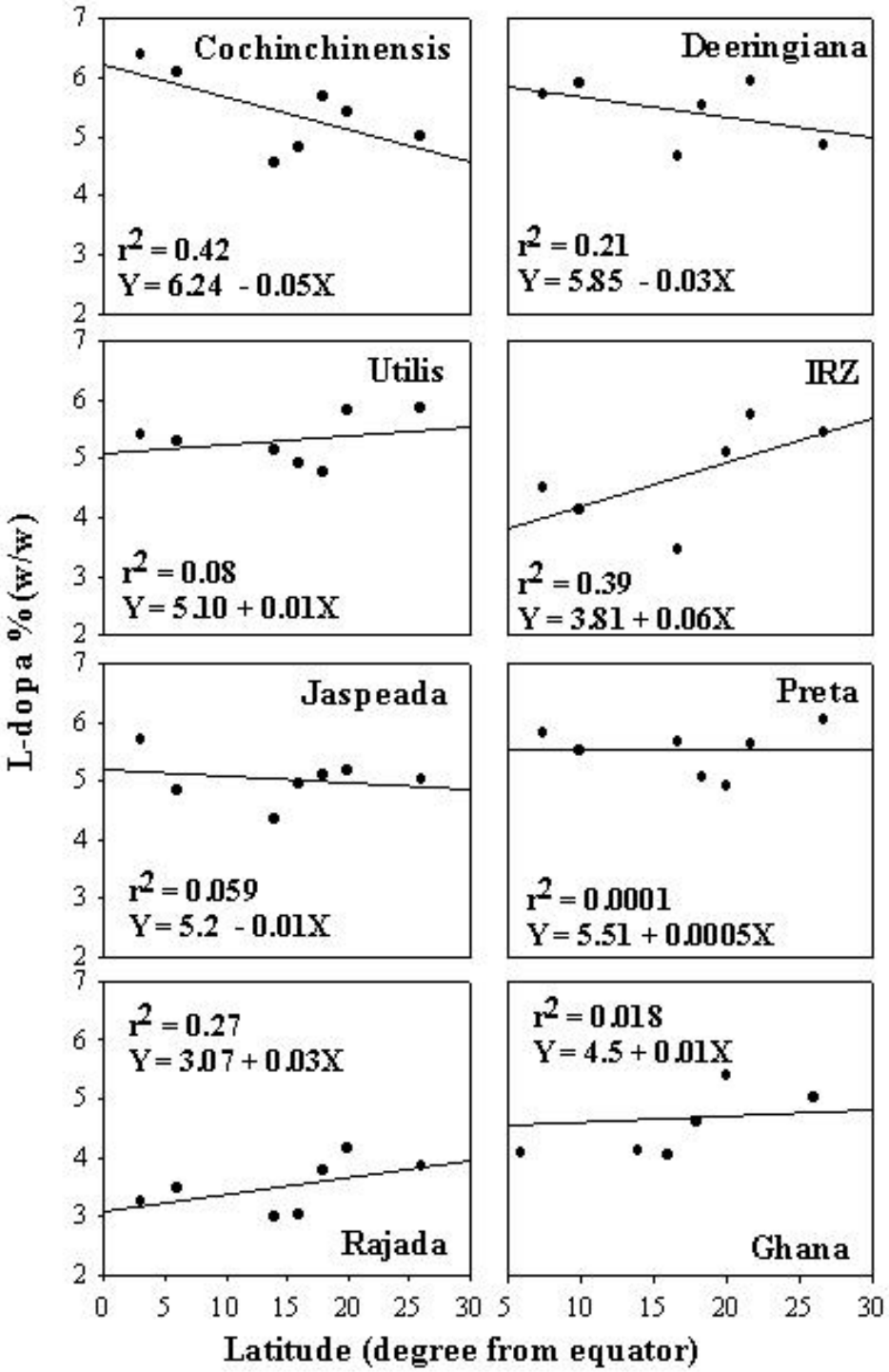

Figure 2. Regression analysis of L-Dopa content in seeds (as per cent of dry weight) of eight *Mucuna* accessions against variation in latitude.

REFERENCES

Barbeau, A, McDowell, FH. 1970. L-Dopa and Parkinsonism. FA Davis Company, Philadelphia, PA, USA. 403p.

Bell, EA, Janzen, DH. 1971. Medical and ecological considerations of L-Dopa and 5-HTP in seeds. Nature 229:136-137.

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

Capo-chichi, LJA, Weaver, DB, Morton, CM. 2001. AFLP Assessment of genetic variability among Velvetbean (*Mucuna* sp.) accessions. Theoretical and Applied Genetics. 103(8): 1180-1188.

Carsky, RJ, Tarawali, SA, Becker, M, Chikoye, D, Tian, G, Sanginga, N. 1998. *Mucuna* – herbaceous cover legume with potential for multiple uses. Resource and Crop Management Research Monograph 25. International Institute of Tropical Agriculture, Ibadan, Nigeria.

Daxenbichler, ME., Van Etten, CH, Earle, FR, Tallent, WH. 1971. L-Dopa recovery from *Mucuna* seed. Journal of Agricultural and Food Chemistry. 20(5):1046-1048.

Daxenbichler, ME, Van Etten, CH, Earle, FR, Tallent, WH. 1972. L-Dopa recovery from *Mucuna* Seed. Journal of Agriculture and Food Chemistry. 20(5):1046-1048.

Eilittä, M, Sollenberger, LE. 2002. The many uses of *Mucuna*: velvetbean in the Southern United States in the early 20th Century. In: Food and Feed from *Mucuna*: Current Uses and the Way Forward. Flores B, M, Eilittä, M, Myhrman, M, Carew, LB, Carsky, RJ (Eds). Proceedings of an International Workshop. CIDICCO, CIEPCA, and World Hunger Research Center. Tegucigalpa, Honduras, April 26-29,2000. Pp 73-110.

Eilittä, M, Bressani, R, Carew, LB, Carsky, RJ, Flores, M, Gilbert, R, Huyck, L, St-Laurent, L, Szabo, NJ. 2002. *Mucuna* as a food and feed crop: an overview. In: Food and Feed from *Mucuna*: Current Uses and the Way Forward. Flores B, M, Eilittä, M, Myhrman, M, Carew, LB, Carsky, RJ (Eds). Proceedings of an International Workshop. CIDICCO, CIEPCA, and World Hunger Research Center. Tegucigalpa, Honduras, April 26-29,2000. Pp 18-47.

Flores, L, Esnaola, MA, Myhrman, R. 2002. Growth of pigs fed diets with *Mucuna* bean flour (*Mucuna pruriens*) compared to soybean meal. In: Food and Feed from *Mucuna*: Current Uses and the Way Forward. Flores B, M, Eilittä, M, Myhrman, M, Carew, LB, Carsky, RJ (Eds). Proceedings of an International Workshop. CIDICCO, CIEPCA, and World Hunger Research Center. Tegucigalpa, Honduras, April 26-29,2000. Pp 288-303.

Keatinge, JDH, Aiming, Q, Wheeler, TR, Ellis, H, Craufurd PQ, Summerfield, RJ. 1997. Photothermal effects on the phenology of annual legume crops with potential for use as cover crops and green manures in tropical and sub-tropical hillside environments. Hillside Production System, ODA Natural Resources Systems Programme, Renewable Natural Resources Research Strategy. University of Reading, Department of Agriculture. Pp 6-7.

Liu, L., McClure, J.W. 1995. Effects of UV-B on activities of enzymes of secondary phenolic metabolism in barley primary leaves. Physiologia Plantarum 93: 734–739.

Lorenzetti, F, MacIsaac, S, Arnason, JT, Awang, DVC, Buckles, D. 1998. The phytochemistry, toxicology and food potential of velvetbean (*Mucuna* Adans. spp, Fabaceae), In: Cover Crops in West Africa Contributing to Sustainable Agriculture. Buckles, D, Eteka, A, Osiname, O, Galiba, M, and Galiano, N (Eds.). IDRC, Ottawa, Canada. Pp 67-84.

Neter, J, Kutner, ,MH, Nachtsheim, CJ, Wasserman, C. 1996. Applied Linear Statistical Models. Fourth Edition. IRWIN, USA. 1408 pp.

Pras, N, Woerdenbag, HJ, Batterman, S, Visser, JF, Vanuden, W. 1993. Mucuna pruriens: Improvement of the biotechnological production of the anti-parkinson drug L-dopa by plant cell selection. Pharm. World Science 15:263–268.

SAS Institute. 1990. SAS/STAT User's Guide. Version 6. Fourth Edition. SAS, Cary, NC, USA.

St-Laurent, L, Livesey, J, Arnason, JT, Bruneau, A. 2002. Variation in L-Dopa concentration in accessions of *Mucuna pruriens* (L) DC. and in *Mucuna brachycarpa* Rech, In: Food and Feed from *Mucuna*: Current Uses and the Way Forward, Flores B, M, Eilittä, M, Myhrman, R, Carew, LB, Carsky, RJ (Eds). Proceedings of an International Workshop. CIDICCO, CIEPCA, and World Hunger Research Center. Tegucigalpa, Honduras, April 26-29, 2000. Pp 252-375.

Temple, SR, Huyck, LM. 2002. Genetic improvement of Mucuna to reduce antinutritional factors and its ecological significance. In: Food and Feed from *Mucuna*: Current Uses and the Way Forward, Flores B, M, Eilittä, M, Myhrman, R, Carew, LB, Carsky, RJ (Eds). Proceedings of an International Workshop.

CIDICCO, CIEPCA, and World Hunger Research Center. Tegucigalpa, Honduras, April 26-29, 2000. Pp 306-338.

Wichers, HJ, Wijnsma, R, Visser, JF, Malingré, THM, Huizing, HJ. 1985. Production of L-DOPA by cell suspension cultures of *Mucuna pruriens*. II. Effect of environmental parameters on the production of L-DOPA. Plant Cell Tissue Organ Culture 4:75-82.

Submitted September 4, 2002 - Accepted October 4, 2002