Indian J. Genet., 71(3): 279-282 (2011)

Short Communication

The genotype x environment interaction and stability analysis for L-Dopa trait in Velvetbean (*Mucuna pruriens*) seeds

S. Mahesh and N. Sathyanarayana*

Department of Bio-Technology, Sir M Visvesvaraya Institute of Technology, Bangalore 562 157

(Received: September 2010; Revised: May 2011; Accepted: June 2011)

Legumes, unlike other crops, fix nitrogen, need little fertilizer and help maintain the soil fertility. They are also an important source of protein and iron. Velvetbean (*Mucuna pruriens*) is a promising plant with multiple uses. Its potential as rich source of protein supplement in food and feed has been well documented [1, 2]. The plant also constitutes excellent raw material for some of the indigenous ayurvedic drugs due to presence of L-Dopa as L-3, 4-dihydrophenylalanine which provides symptomatic relief in Parkinson's disease [3, 4]. L-Dopa is present in variable concentrations within different plant parts, minimal in dried leaves and pods to maximal in raw seeds ranging between 1.9 and 9% dry matter [5-8]. Although pharmacologically an active ingredient [9], it is potentially anti-nutritional and toxic if ingested in large amounts, and concentration greater than 1% is reported unsafe for human consumption [10, 11]. Past researches have shown this as major bottleneck for popularization of Velvetbean cultivation among the farmers and thus efforts are needed to breed improved varieties with safe levels of L-Dopa (<0.5%) to make its cultivation broad based and acceptable.

Very little is known about the genetics and synthesis of L-Dopa in *Mucuna* species. Specifically, information on genotype x environment interaction on its expression lack consensus. Lorenzetti *et al*. [12] found both environment and genotype influence on L-Dopa production. In their study, latitude difference - an environmental factor was shown to have impact. This study was supported by St. Laurent *et al.* [7] who found slight impact of latitude on L-Dopa expression but concluded that other factors too influences its synthesis. Capo-chichi *et al.* [13], however, noted that genotype had a greater influence on L-Dopa synthesis while, genotype x environment interaction effect was minimal when compared to genotype/accession main effect. These conflicting views have rendered significant problem in defining strategies for L-Dopa breeding in Velvetbean. A pilot study thus was envisaged to examine the role of G x E interaction on L-Dopa production in *Mucuna* seeds in addition to assessing the stability of genotypes across different environmental conditions.

Twenty six accessions (Table 1) were initially screened for L-Dopa content and five accessions which showed significant difference *viz*., 500153AP, 500149AP, 500150AP, 500101KA and IC385841 were selected for plantation during *Kharif* season (period from 1st August–15th September, 2009-10). Plantation was carried out at five locations (Bangalore, Gulbarga, Moornad, Mangalore and Basarikatte) in Karnataka differing in agro-climatic and edaphic conditions to support evaluation of differential expression of genotypes, if any, across the environments.

Seeds of individual accessions were planted in 8" plastic pots [Diameter - top: bottom: height- 7 ¾":7 ¼":9"] (except in Bangalore) filled with native soil and were maintained as per the report of Capo-chichi *et al*. [13]. In Bangalore, 25 plants were directly planted on to soil

*Corresponding author's e-mail: sathya_dixit@yahoo.com
Published by Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com

Table 1. Percentage of l-DOPA content in seeds collected from different trial locations

S.No.	Accessions	L-DOPA percentage (Mean Percentage ± standard deviation)^{Y}				
		Bangalore	Basarikatte	Gulbarga	Mangalore	Moornad
1	500153AP	3.018 ± 0.011^{C}	3.136 ± 0.018^{B}	3.641 ± 0.013^{A}	3.102 ± 0.024^{B}	3.134 ± 0.017^{B}
2	500149AP	2.401 ± 0.016^{J}	2.688 ± 0.011^{F}	3.006 ± 0.011^{c}	2.932 ± 0.011^{D}	2.855 ± 0.015^{E}
3	500150AP	2.151 ± 0.017^{K}	2.470 ± 0.017^{HI}	2.527 ± 0.018^{G}	2.502 ± 0.016^{GH}	2.435 ± 0.120^{IJ}
4	500101KA	1.668 ± 0.014^{P}	2.085 ± 0.014^{L}	2.097 ± 0.009^{L}	2.042 ± 0.001^{M}	1.993 ± 0.014^{N}
5	IC385841	1.564 ± 0.010^{Q}	1.841 ± 0.016^{O}	1.841 ± 0.016^{O}	1.844 ± 0.013^{O}	1.830 ± 0.010^{O}

^{Y}Values are mean ± standard deviation of 4 independent experiments. Means followed by same letter are not significantly different at 1% significance level as determined by Tukey's HSD test.

with 5 plants in each row with plant to plant and row to row distance of 1.83m in a plot size of 6m^2. All the plants were maintained under natural climatic conditions prevailing at the respective trial location and irrigated twice a week (except rainy season). After 6 months, fully grown mature pods in each plant were harvested separately and the seeds were collected.

For L-Dopa analysis, seeds from each plant were bulked separately, and, the powdered sample from each bulk was used for the analysis. The L-Dopa content was determined using standardized Daxenbichler *et al.* [14] method. The data were subjected to analysis of variance separately for each environment and combined over environments (Table 2). The statistical model used for ANOVA is $Y_{ijk} = \mu + G_i + E_j + GE_{ij} + Bk_{(j)} + \varepsilon_{ijk}$ Where, Y_{ijk} = observed value of genotype i in block k of environment (location) j; μ = grand mean; G_i = effect of genotype i; E_j = environment or location effect; GE_{ij} = the interaction effect of genotype i with environment j; $Bk_{(j)}$ = the effect of block k in location (environment) j and e_{ijk} = error (residual) effect of genotype i in block k of environment j.

First descriptive statistics (mean, standard deviation) were generated using software JMP version 8 [15]. Stability analysis was later performed using SPAR 2.0 [16] software that provides a general summary of the response patterns of genotypes to environmental changes. Eberhart and Russel [17] have proposed pooling of the sums of squares for environments and genotype environment interaction (GEI) and subdividing it into a linear effect between environments (with 1 df) and linear effect for genotype x environment (with *E-2* df). In effect the residual mean squares from the regression model across environments is used as an index of stability, and a stable genotype is one in which the deviation from regression mean squares (S^2di) is small. This approach has been adopted in this study.

Genotype environment interaction is an important aspect in any selection program seeking to exploit genotype performance across several environments. Significant GEI results from the changes in the magnitude of differences between genotypes in different environments or changes in the relative ranking of the genotypes [18]. GEI makes it difficult to select the best performing and most stable genotypes and is an important consideration in plant breeding programs because it reduces the progress from selection in any one environment [19]. Therefore, it is necessary to assess the environmental sensitivity of genotypes in terms of stability. Most plant breeders use regression method as proposed by Eberhart and Russel [17] for interpreting the genotype and environment interaction as it is shown to be the most reliable one [20, 21].

In this study, we used 5 *Mucuna* genotypes having L-Dopa content in a range of 1.564% to 3.018% and tested them for their stability across different locations with varying environmental conditions (Table 3). Significant differences in mean L-Dopa content indicated the variability among the genotypes. The mean percentage of L-Dopa content in seeds obtained from different trial locations ranged from 1.564% to 3.641%. Genotype IC385841 produced the lowest L-Dopa content of 1.564% while genotype 500153AP produced the highest content of 3.641% averaged across different environments. Despite variation in the L-Dopa content among the seeds from different trial locations, the ranking of the genotypes remained consistent without any crossovers (Table 1). The combined analysis of variance indicated that the effect of genotypes and environments were significant at 0.05 probability levels whereas it was non-significant in case of GEI (Table 2) suggesting preponderance of genotype/accession main

Table 2. Summary table on analysis of variance

Source	df	SS	MS	F-value	L-DOPA
Genotype	4	27.68	6.92	133.08	*
Environment	4	2.04	0.51	9.81	*
Interaction (G X E)	16	0.95	0.059	1.135	NS
Residual	15	0.772	0.052		

*Significant at 0.05 probability levels; NS: non-significant

effect over GEI effect which is negligible. The result is in consensus with the earlier work of Capo-chichi *et al.* [13].

The stability analysis performed according to Eberhart and Russel's model revealed that all the accessions under study were stable across varying environments as the values for regression co-efficient was close to unity and mean square deviation from regression value was also nearing to zero (Table 3). The high L-Dopa content with high value of regression co-efficient in case of 500149AP and 500150AP indicated that these accessions are adaptable to high performing environments. The medium L-Dopa content and lower regression coefficient difference in the genotype 500101KA shows their adaptability to low performing environments. The accessions 500153AP and IC385841 has regression co-efficient value almost closer to unity and also the mean square deviation from regression value approaching very close to zero, clearly indicating that they have above average stability and is especially adaptable to low performing environments. Similar analysis has been performed to determine the stability of seed yield in case of mungbean [22], chickpea [23], garden pea [24] and cowpea [25].

From the above results it can be suggested that these Velvetbean accessions are relatively stable across the environments. The strong influence of genotype on L-Dopa production offers significant scope for breeding varieties with low L-Dopa content (< 0.5%) that would permit their utilization as food and feed. Further analysis, however, with respect to different seasons should be carried out to confirm the same for seasonal variations. The stable genotypes revealed in the present study can be promising parental lines for breeding *Mucuna pruriens* for decreased L-Dopa content.

Table 3. Eberhart and Russel's model for stability parameters

S.No.	Genotypes	Mean L-Dopa content (%)	Regression coefficient (b)	Deviation from regression (s^2di)
1	500153AP	3.206	1.052	0.0033
2	500149AP	2.776	1.429	0.0068
3	500150AP	2.417	1.116	0.0392
4	500101KA	1.977	0.5141	0.0113
5	IC385841	1.784	0.887	0.0019

References

1. **Siddhuraju P. and Becker K.** 2001. Preliminary evaluation of *Mucuna* seed meal (*Mucuna pruriens* var. *utilis*) in common carp (*Cyprinus carpio* L.): An assessment by growth performance and feed utilization. Aquaculture, **196:** 105-123.
2. **Bressani R.** 2002. Factors influencing nutritive value in food grain legumes: *Mucuna* compared to other grain legumes. *In:* Food and Feed from *Mucuna*: Current Uses and the Way Forward, Flores B. M., Eilittä M., Myhrman R., Carew L. B. and Carsky R. J. (eds.). Workshop, CIDICCO, CIEPCA and World Hunger Research Center, Tegucigalpa, Honduras, 164-188.
3. **Shaw B. P. and Bera C. H.** 1993. A preliminary clinical study to cultivate the effect of vogorex-SF in sexual disability patients. Ind. J. Internal Medicine, **3:** 165-169.
4. **Prakash D. and Tewari S. K.** 1999. Variation on L-DOPA content in *Mucuna* species. J. Med. Aromatic. Plant Sci., **21:** 343-346.
5. **Bell E. A. and Janzen D. H.** 1971. Medical and ecological considerations of L-Dopa and 5-HTP in seeds. Nature, **229:** 136-137.
6. **Duke.** 1981. Handbook of Legumes of World Economic Importance. Plenum Press, New York, 170-173.
7. **St Laurent L., Livesey J., Arnason J. T. and Bruneau A.** 2002. Variation in L-Dopa concentration in accessions of *Mucuna pruriens* (L) DC. and in *Mucuna brachycarpa* Resh., *In:* Food and Feed from *Mucuna*: Current Uses and the Way Forward, Flores B. M., Eilittä M., Myhrman R., Carew L. B. and Carsky R. J. (eds.), Workshop, CIDICCO, CIEPCA and World Hunger Research Center, Tegucigalpa, Honduras, 252-375.
8. **Szabo N. J. and Tebbett I. R.** 2002. The chemistry and toxicity of *Mucuna* species. *In:* Food and Feed from *Mucuna*: Current Uses and the Way Forward, Flores B. M., Eilittä M., Myhrman R., Carew L. B. and Carsky R. J. (eds.), Workshop, CIDICCO, CIEPCA and World Hunger Research Center, Tegucigalpa, Honduras, 120-141.

9. **Pieris N., Jansz E. R. and Dharmadasa H. M.** 1980. Studies on *Mucuna* species of Sri Lanka 1 - The L-Dopa content of seeds. J. Nat. Sci., **8:** 35-40.

10. **Versteeg M. N., Amadji F., Eteka A., Houndehon V. and Manyong V. M.** 1998. Collaboration to increase the use of *Mucuna* in production systems in Benin. *In:* Cover crops in West Africa: contributing to sustainable agriculture, Buckles D., Eteka A., Osiname O., Galiba M. and Galimo W. (eds.), Ottawa, Canada. IDRC: 1-32.

11. **Mary Josephine R. and Janardhanan K.** 1992. Studies on chemical composition and antinutritional factors in three germplasm seed materials of the tribal pulse, *Mucuna pruriens* (L.) DC. Food Chem., **43:** 13-18.

12. **Lorenzetti F., MacIsaac S., Arnason J. T., Awang D. V. C. and Buckles D.** 1998. The phytochemistry, toxicology and processing potential of the cover crop velvet bean (Cowhage, Cowitch) (*Mucuna adans*, Fabaceae). *In:* Cover Crops in West Africa - Contributing to Sustainable Agriculture, Buckles D., Et'eka A., Osiname O., Galiba M. and Galiano N. (eds.), IDRC, Ottawa, Canada: 67-84.

13. **Capo-chichi L. J. A., Eilittä M., Carsky R. J., Gilbert R. A. and Maasdorp B.** 2003. Effect of genotype and environment on L-Dopa concentration in *Mucuna*'s (*Mucuna* sp.) seeds. Trop. Subtrop. Agroecosyst., **1:** 319-328.

14. **Daxenbichler M. E., Vanetten C. H., Hallinan, Earle F. R., Barclay A. S.** 1971. L-Dopa recovery from *Mucuna* seed. J Med. Chem., 14: 463.

15. **SAS Institute Inc.** 2008. JMP statistics and graphics guide version 8.0, SAS Institute Inc. Cary, NC, USA.

16. **Ahuja, Sangeeta, Malhotra P. K., Bhatia V. K., Parsad, Rajender and Gupta V. H.** 2005. Development of Statistical Package for Agricultural Research (Windows Version) - SPAR 2.0. Final Project Report, IASRI: 1-40.

17. **Eberhart S. and Russell W. A.** 1966**.** Stability parameters for comparing varieties. Crop Sci. **6:** 36-40.

18. **Falconer D. S. and Mackay T. F. C.** 1996. Introduction to Quantitative Genetics.4th edition, Longman, New York: 132-133.

19. **YAU S. K.** 1995. Regression and AMMI analyses of genotype x environment interactions: An empirical comparison. Agron. J., **87:** 121-126.

20. **Freeman G. H. and Perkins J. M.** 1971. Environmental and genotype-environmental components of variability. VIII. Relations between genotypes grown in different environments and measure of these environments. Heredity, **26:**15-23.

21. **Westcott B.** 1986. Some methods of analyzing genotype × environment interactions. Heredity, **56:** 243–253.

22. **Saleem M., Sadiq M. S., Sarwar G. and Abbas G.** 2002. Performance of elite mungbean (*Vigna radiata* L. Wilczek) germplasm in multilocational experiments. Biologia, **48:** 9-13.

23. **Prakash V.** 2006. Stability analysis for grain yield and contributing traits in chickpea (*Cicer arietinum* L.). Indian J. Genet., **66:** 239-240.

24. **Rana A., Jamwal R. S. and Sharma A.** 2006. Genotype x environment interactions for pod yield and quality traits in garden pea (*Pisum sativum* L.). Indian J. Genet., **6:** 247-248.

25. **Ali Y. and Sarwar G.** 2008. Genotype x environment interaction of cowpea genotypes. Int. J. Environ. Res., **2:** 125-132.