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Short Communication

RAPD markers for genetic characterization in *Mucuna* species

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The genus *Mucuna* belongs to the Family Fabaceae (Leguminoceae) and includes about 150 species of annual and perennial legumes of pantropical distribution. Many species exhibits properties such as high nitrogen fixing ability, aggressive growth habit and high productivity of vegetative matter rendering them as an excellent source of green-manure cover crop (GMCC) [1]. Almost all the species are also known to produce L-Dopa (L 3,4 dihydroxy phenylalanine), a non protein amino acid that acts as a precursor to the neurotransmitter drug dopamine used in the treatment of Parkinson's disease [2]. Even though *Mucuna* has long history of usage in India, most of the research until now has been focused mainly on determining its nutritional potential [3, 4]. There is serious paucity of systematic efforts to collect and evaluate *Mucuna* germplasm on all India basis except for some regional efforts. The scenario call for an immediate need to embark on "*Mucuna* germplasm program" in India starting with collection, documentation and characterization of the germplasm grown at different geographic regions of India.

Molecular markers offer excellent tool for characterization of germplasm for various utilities. Randomly Amplified Polymorphic DNA (RAPD), a PCR based DNA marker technology offers advantages in speed, technical simplicity and low cost [5]. However, it is often reported to reveal low level of polymorphism and lacks reproducibility [6]. Thus, there is a need to confirm its suitability for desired application in relevant crop before a detailed study is undertaken. In this investigation, we have assessed the efficacy of RAPD marker system for characterization of *Mucuna* species in addition to morphometric analysis.

Eight *Mucuna* accessions; four belonging to *M.pruriens* var. *pruriens*, three of *M.pruriens* var. *utilis* and one of *M. deeringiana* collected from wild in Karnataka and Kerala states and NBPGR were used in the study. Total genomic DNA from individual plant was isolated from young leaves using modified CTAB extraction method [7]. DNA concentration was approximated by gel electrophoresis on 0.8% agarose, staining with ethidium bromide, and comparison with a set of concentration standards. RAPD primers (A, B kits) were procured from Qiagen-Operon Tech., CA, USA and were screened before deployment on the basis of the best profiles with each template DNAs tested. All amplification were performed on a PTC-200^{TM} (MJ Research Inc., USA) with a thermal profile of denaturation at 94°C, annealing at 36°C and extension at 72°C. Amplified products were resolved on 1.8% agarose gel in 1X TAE buffer at constant voltage of 5V/cm for 3 h. After electrophoresis, the gel was stained with ethidium bromide, visualized, photographed and archived using BIO-RAD^{TM} gel documentation system. Fragment sizes of the amplification products obtained using RAPD primers were estimated from gel by comparison with standard molecular weight marker (Fermentas Life Sciences Inc., USA). Each RAPD marker was treated as a unit character and scored as a binary code (1/0). Genetic similarity matrix generated based on Jaccard's coefficients [8] was subjected to clustering method by UPGMA using NTSYS-pc statistical package [9] and the resultant tree was computed after allowing a 1000 bootstrap test using WINBOOT software [10]. For variability in morphometric characters, eight accessions were scored for leaf shape, leaf size, plant hairiness, inflorescence length, flower buds per cluster, maturity levels, flower color, pod size, pod shape and pod itchiness using standard descriptor prepared for this purpose (unpublished). Average values of morphometric traits were analyzed alike RAPD data to evaluate the phenetic relationships among the accessions.

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Of the 20 random primers, 17 generated 164 reproducible markers with 85.32% polymorphism. The number of products ranged from 1 in OPA-04 to 11 in OPAK-17. On an average, single primer generated 9.4 products of which nearly 7.2 were polymorphic. Cluster analysis based on UPGMA dendrogram (Fig. 1) grouped 8 accessions into two distinct groups which by and large agreed with the morphometric analysis (Fig. 2). All the var. *utilis* accessions obtained from NBPGR, New Delhi (IC471876, IC369144 and IC385929) grouped in cluster-I along with lone accession of *M. deeringiana* (500102 KA). The latter has been suggested as probable taxonomical synonym of *M. pruriens* particularly of the var. *utilis* [11]. This may be due high similarities they share in floral and pod characteristics as established even by present morphometric analysis (Fig. 2). However, its distinct placing with similarity value of 60.5% in UPGMA dendrogram indicate certain level of divergence between these two at genetic level requiring consideration to place it among the botanical varieties

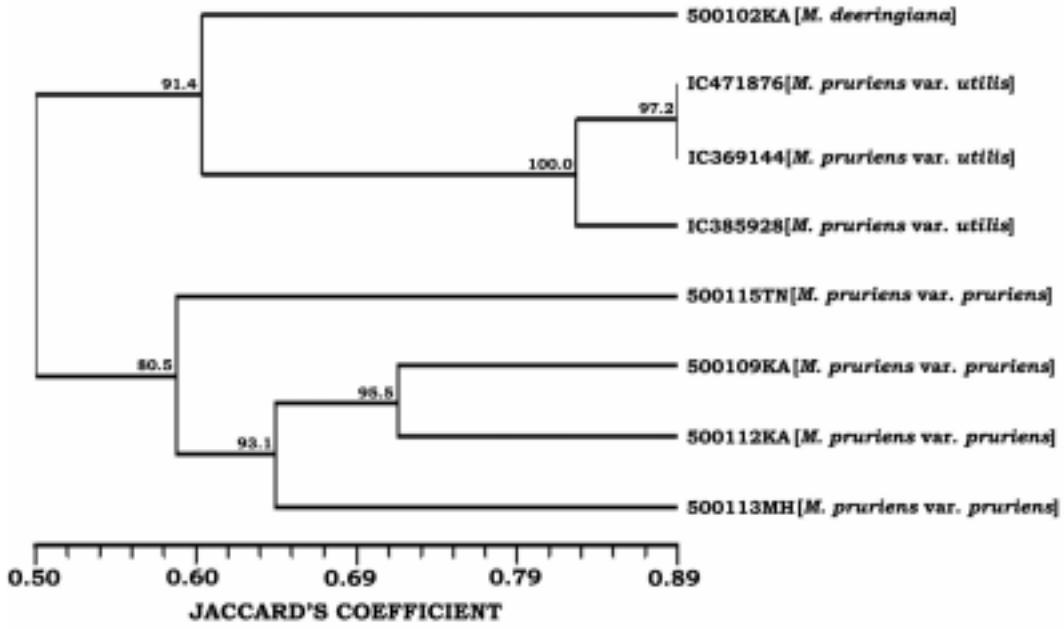

Fig. 1. UPGMA dendrogram of *Mucuna* accessions based on RAPD data

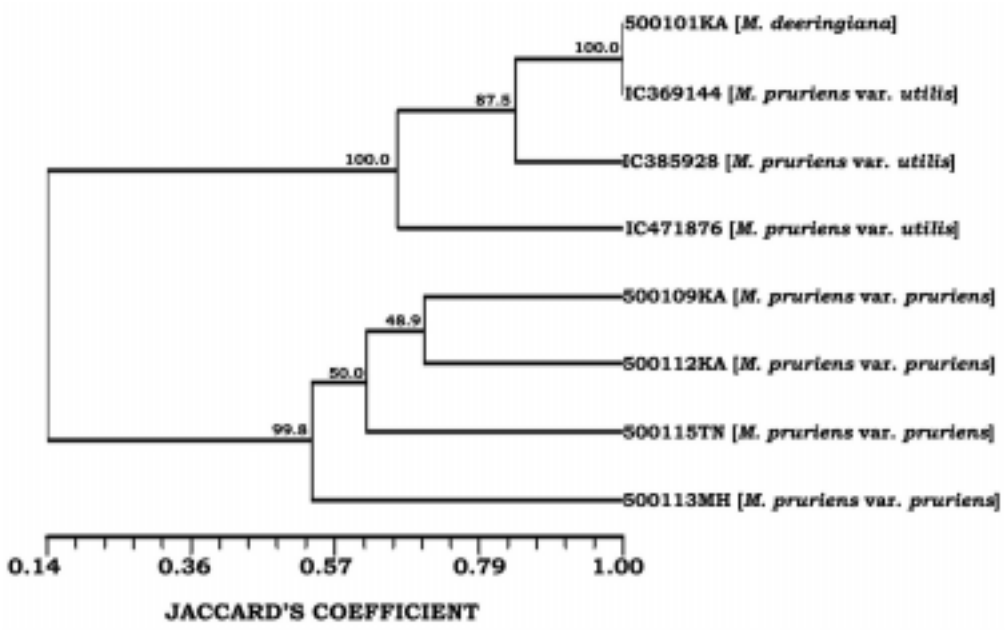

Fig. 2. UPGMA dendrogram based on morphometric data

of *M. pruriens*. On the other hand, all the *M.pruriens* var. *pruriens* accessions grouped in cluster-II at confidence interval limit of 80.5% without any notable deviation. On the whole, the RAPD analysis placed evidently all the *Mucuna* accessions into their respective morphometric and/or taxonomic assemblage without much discrepancy. Geographical affiliation was found to be having minimal impact on the clustering pattern. Some of the accessions, in both the analysis, showed high similarity with each other (IC471876: IC369144; 500109KA: 500112KA). It needs to be confirmed whether these represent duplicate collections in the germplasm. Redundancy is a common problem in velvetbean collections due to high degree of nomenclatural errors [12]. Molecular markers might provide valuable clues to deal with them as seen in other plant species [13, 14].

The high levels of polymorphism seen in otherwise self pollinated members of *Mucuna* suggests broad genetic base among its different members, possibly due to accumulation of novel gene combinations in response to dynamic pressures of natural selection. The observation is in consistence with earlier reports [15, 16]. Attempts to resolve taxonomic status of *M. pruriens* by amplified ITS regions of nuclear DNA showed no variation between the accessions [17]. Superiority of fingerprinting methods over ITS sequences for such a purpose has been well documented in number of earlier reports [16, 17]. Our study reaffirms the utility of relatively simple, low-cost fingerprinting technique like RAPD for such studies.

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