

# Role of genetic resources and molecular markers in *Mucuna pruriens* (L.) DC improvement

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## Abstract

*Mucuna pruriens* (L.) DC is a tropical legume cover crop with promising nutritional and agronomic potentials. It is also a key source of 3,4 dihydroxy-L-phenylalanine (L-Dopa) – a precursor of dopamine used in the treatment of Parkinson's disease. However, lack of well-characterized germplasm plus poor accessibility to genomic resources has hindered its breeding programs. Furthermore, the cause and effect of various biotic and abiotic stresses impacting yield is also little studied. Systematic collection and evaluation of Indian germplasm by our group revealed presence of a diverse gene pool in India that can support a variety of breeding needs. The stability of L-Dopa trait across environments examined through Genotype and environment (G × E) interaction studies, as well as feasibility check on barcoding and phylogenetic analyses based on karyotype and conserved nuclear and chloroplast genes showed promising outcome. Germplasm screening for select biotic abiotic stresses identified resilient genotypes. Advances in use of DNA markers for diversity analysis, linkage map development, tagging of genes/quantitative trait loci for qualitative and quantitative traits, and progress in genomics are presented.

**Keywords:** barcoding, genomics, karyotype, L-Dopa, linkage map, *Mucuna pruriens*, NGS

## Introduction

*Mucuna pruriens* (L.) DC – described as a self-pollinated species is a tropical legume classified within the Phaseoloid clade of Leguminosae, which also include soybean, common bean, mung bean and relatives. Reported to be native of eastern India and southern China (Wilmot-Dear, 1987) – it is now distributed in several parts of the World including tropics exhibiting tremendous morphological and biochemical variability. It grows well under warm, moist conditions at altitudes below 1600 m both in wild and cultivated forms (Buckles, 1995; Pugalenth et al., 2005). Wild varieties are recognizable

by highly itching pod due to which they are known by the name 'cow itch' or 'itching bean', while landraces, commonly termed as 'velvet bean' bear non-itching pods, and are grown in household/home-gardens as green vegetable in parts of central and southern India (Pugalenth and Vadivel, 2007a, b). It has a diploid genome with 22 pairs of chromosomes ( $2n = 2x = 22$ ) (Sastrapradja et al., 1974) and an estimated genome size of 1361 Mbp. The general characteristics of *M. pruriens* are presented in Table 1.

## Agronomic and medicinal benefits

Cultivated velvet bean (*M. pruriens* var. *utilis*) offers promising agronomic benefits (Fig. 1). It produces seed yield of 2000 kg/hectare (Buckles, 1995); performs well under dry farming and low soil fertility conditions (Siddhuraju et al.,

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**Table 1.** General characters of *Mucuna pruriens* (L.) DC

Vegetative characters	
Roots	Fleshly, usually well nodulated and produced near soil surface
Stems	The long trailing stems are rather slender and sparsely pubescent
Growth pattern	Indeterminate
Growth habit	Indeterminate climber
Twining tendency	Pronounced
Leaves	Numerous, alternate, trifoliolate, lanceolate/ovate to lanceolate
Leaf texture	Coriaceous/membranous
Plant hairiness	Glabrous/short appressed hairs/pubescent/hirsute
Floral and fruit characteristics	
Flower colour	Purple or yellowish white
Inflorescence	Long cluster raceme
Pod colour	Dark green
Pod curvature	Slightly curved/curved/'S' shaped
Pod pubescence colour	Golden orange/velvety black/creamish white
Pod sensation	Itching/non-itching
Seed colour	Brown, black, white, dark brown, grey
Seed shape	Oval, cuboid, round
Seed coat patterns	Black/brown mottled/absent
Seed yield	12.83–200.92 g/100 seeds
Chromosome number	
$2n = 2x =$	22

2000); shows resistance against a wide ranging diseases (Eilitta *et al.*, 2002); exhibits allelopathic properties (Fujii *et al.*, 1991) and effective in lowering nematode population (Carsky and Ndikawa, 1998; Queneherve *et al.*, 1998). Its impact as green manure cover crop is documented in a number of earlier reports (Eilitta *et al.*, 2002; Jorge *et al.*, 2007). Fast-growing habit of this plant allows ground-cover in 60–90 d resulting in large biomass accumulation vis-à-vis other cover crops (Tarawali *et al.*, 1999). Due to this, and high N<sub>2</sub> fixing ability, it is regarded as 'featured example of green manures contribution to the sustainable agricultural system' (Buckles, 1995). Besides, the seeds are also traditionally used as minor food by many indigenous communities in Asia and Africa (Iyayi and Egharevba, 1998; Diallo *et al.*, 2002). The seed powder can be beneficially supplemented with the livestock feed (Burgess *et al.*, 2003; Muinga *et al.*, 2003) and is a source high value industrial starch (Betancur-Ancona *et al.*, 2002; Lawal and Adebowale, 2004). Seed oil is used in the preparation of

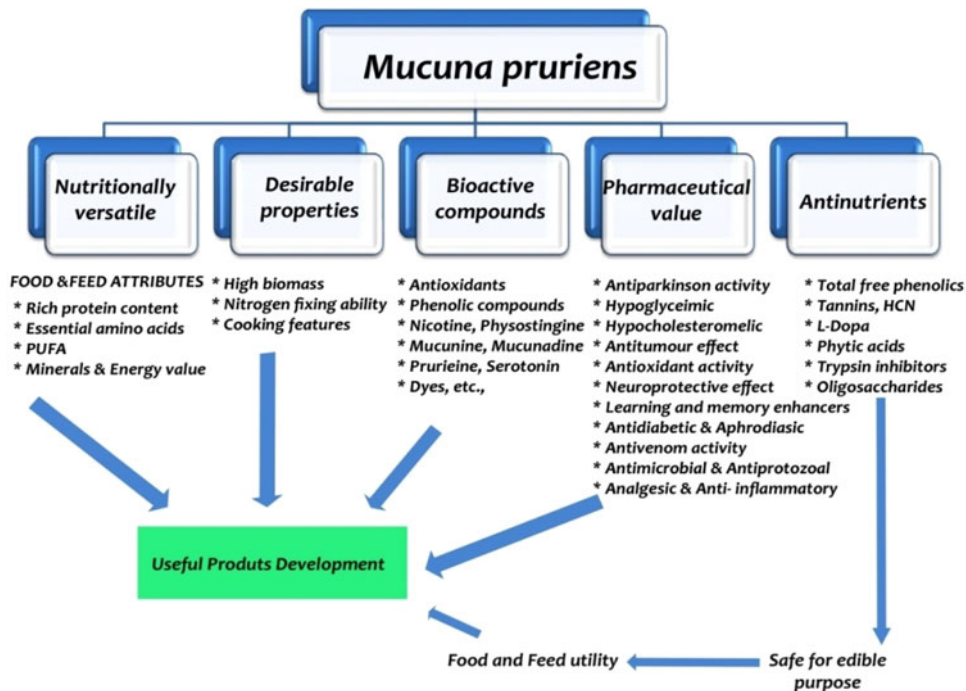
paint, polish, resin, dye, wood varnish, skin cream and soap (Ajiwe *et al.*, 1997).

*M. pruriens* has a rich history in Ayurvedic system of Indian medicine, where the seeds are used as an integral part of over 200 drug formulations. It is reported to possess anti-diabetic, anti-neoplastic, anti-microbial, aphrodisiac and learning and memory enhancing properties (Oudhia, 2002). Importantly, its efficacy in the treatment of Parkinson's disease is well recognized both in the alternative and allopathic system of medicine. When used as drug, 3,4 dihydroxy-L-phenylalanine (L-Dopa), which is copiously present in seeds crosses blood-brain barrier and acts as a precursor for the synthesis of neurotransmitter drug dopamine thus improving the dopamine concentration in Parkinson's patients (Farooqi *et al.*, 1999; Kavitha and Thanagmani, 2014). More recently it has also been shown to promote male fertility by recovering spermatogenic losses in rats (Singh *et al.*, 2013).

## Taxonomy

*Mucuna* Adans. include 100 species of annual and perennial legumes of pantropical distribution (Buckles, 1995). It has been revised by Wilmot-Dear for China and Japan (1984), the Indian subcontinent and Burma (1987), the Pacific (1989), Philippines (1990), Thailand, Indochina and the Malay Peninsula (1991). The genus was earlier subdivided into two subgenera, *Stizolobium* P. Browne and *Mucuna*. *Stizolobium* was first reported by Browne in 1736 to describe the cow-itch plant in Jamaica, commonly known as *M. pruriens* in the USA. However, (Bort, 1909) showed the differences between the two especially in the shape of the hilum. The genus *Stizolobium* was however used to distinguish velvet bean from the perennial *Mucuna* sp., but this distinction was not maintained (Bailey, 1947; Burkill, 1966). As a result, *Stizolobium* was considered a synonym, and all its species were classified in the genus *Mucuna* (Capo-chichi *et al.*, 2001). As a result, *Stizolobium* was considered a synonym, and all its species were classified in the genus *Mucuna* (Capo-chichi *et al.*, 2001). Systematic studies involving *rbcl* and *matK* sequences established genus *Mucuna* as member of sub tribe Erythrinae – within the larger Phaseoloid group at a strong bootstrap support of 93%. Moreover, *Mucuna* (Phaseoleae – Erythrinae), like core Desmodieae taxa, lacked the *rp12* intron (Bailey *et al.*, 1997), and was strongly supported as sister to core Desmodieae in the *rbcl* trees (Doyle *et al.*, 2000; Lee and Hymowitz, 2001). *Mucuna* was created along with Desmodieae in one of the earliest splits in Phaseoloid history around 15 million years ago (Stefanovic *et al.*, 2009).

Notwithstanding this elucidation, considerable taxonomic confusions exist even now within this genus with several



**Fig. 1.** Overview on uses of *M. pruriens* (adopted from Bhat and Karim, 2009).

synonyms reported both at the species and sub-species level (Duke, 1981). Quite a few taxa that were formerly considered separate species such as *Mucuna cochinchinensis*, *Mucuna hassjoo*, *Mucuna nivea* and *Mucuna utilis* are now shown to be merely varieties of *M. pruriens* (Burkill, 1966; Awang et al., 1997). In addition, even within *M. pruriens*, two widely known botanical varieties: var. *pruriens* and var. *utilis*, presence of third group: var. *hirsuta* has been suggested by Wilmot-Dear (1987). Var. *hirsuta* was earlier classified as an independent species (Ellis, 1990; Saldanha, 1996); but subsequent revisions especially by Wilmot-Dear (1987) categorically suggested its inclusion under the botanical varieties of *M. pruriens*. However, literatures continue to treat var. *hirsuta* as an independent species (Rajaram and Janardhanan, 1991). Such problems are even more prevalent in cultivated velvet bean, where extensive exchange of seed materials over the years has led to the emergence of several local names based on cultivation location and/or popular names with which they were introduced. Moreover, presence of several naturalized hybrids has also complicated this problem. Consequently literatures are ambiguous on the description of the species and such anomalies, while predominant in *M. pruriens*, are not uncommon in other taxa of this genus. Because of this and other confusions surrounding the taxonomy, it is necessary to conduct research both at the species and sub-species level to assess the phenetic relationships to place the species in a right taxonomic and phylogenetic perspective. Besides, the ongoing efforts under legume diversity assessment project by Asia-Pacific

Biodiversity Observation Network named, among others, *Mucuna* Adans. as representative genera for genus specific phylogenetic diversity assessment using DNA sequence information. In view of this, the present authors have used karyotype and nrITS and cp-*psbA-trnH* gene sequences for barcoding and phylogenetic studies in *Mucuna* sp. the details of which are given elsewhere in this paper.

## Gene pool collection and evaluation

Germplasm of *M. pruriens* is maintained in several research institutes/organizations across the World. This includes: US Department of Agriculture (USDA); International Institute of Tropical Agriculture, Nigeria; Centro Internacional de Agricultura Tropical (CIAT), Colombia; AVRDC – The World Vegetable Centre, Taiwan; National Biological Institute, Indonesia etc., (Jorge et al., 2007). In India, a few national research organizations viz., National Bureau of Plant Genetic Resources (NBPGR), New Delhi (Jorge et al., 2007; Archana Raina et al., 2012); Indian Institute of Horticultural Research, Bangalore (Mamatha et al., 2010); Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Thiruvananthapuram (Padmesh et al., 2006); Zandu Foundation for health care, Valsad, Gujarat (Krishnamurthy et al., 2005); Arya Vaidya Sala, Kottakkal; Bharathiar University, Coimbatore (Siddhuraju and Becker, 2005) etc. are reportedly maintaining *M. Pruriens* germplasm even though exact number of collections

available with these institutes is not known, except 182 reported in case of NBPGR.

Natural population of *M. pruriens* exhibits significant variations in pod, seed and flower characteristics. Some of the earliest clues on genetics governing them came from the pioneering works of Lubis and co-workers during 1970s and 80s. *M. pruriens* produces two distinct pod hair phenotypes – long rough and short smooth types. Aminah *et al.* (1974) showed that the former is produced only in wild genotypes and the cultivated type possesses smooth ones. Further, it was shown that this character is controlled by two genes *viz.*, R and N and sometime abnormalities and pollen sterility results in recessive alleles either in homozygous or heterozygous condition, which results in alternative phenotypes (Lubis *et al.*, 1979). Similarly, in case of flower colour, of the two – white and purple flower colours produced by the plant, purple is dominant over the white and the genes controlling those lies on one locus (Lubis *et al.*, 1978). In case of seed coat colour, it was demonstrated that the range of phenotypes – from dark black to white including different shades of brown and mottled ones appear due to multigenic factors (Lubis *et al.*, 1980). Several independent evaluation both in India and elsewhere established good diversity for desirable traits in *M. pruriens* germplasm. Bennet-Lartey (1998) found major variability for morphological and phenological traits among velvet bean accessions from Ghana and identified early maturing genotypes. In India, good variability for traits such as days to flowering, fertility index, seed recovery percentage, harvest index etc. have been observed (Gurumoorthi *et al.*, 2003). This led to identification of early and late flowering accessions both in itching and non-itching genotypes in addition to elite lines producing bold seeds, higher seed weight with favourable physico-chemical properties such as swelling capacity, swelling index, hydration capacity and hydration index (Krishnamurthy *et al.*, 2005). Itching trichome lines also possessed higher L-Dopa (4.36–6.12%) content over non-itching ones (2.30–4.18%) possibly due to selection. Besides, nitrogen fixing ability of different *M. pruriens* accessions was found to have positive correlation with nodule number, nodule biomass and content of active principle in seeds (Mamatha *et al.*, 2006). These findings are also corroborated in studies by other workers (Pugalenth and Vadivel, 2007a, b; Mamatha *et al.*, 2010).

### L-Dopa trait: variability in gene pool and genotype and environment (G × E) interaction effects

Of the numerous active principles present in *M. pruriens*, the most intriguing is L-Dopa – which is present in copious quantity in seeds (1.4–9.1%). Biochemically a non-protein

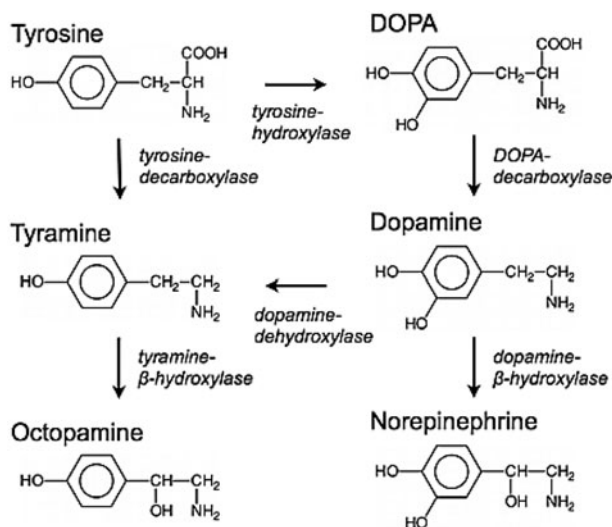


Fig. 2. Biosynthesis of dopamine (courtesy: Barron *et al.*, 2010).

amino acid produced as an intermediary product in the enzymatic synthesis of dopamine from L-tyrosine (Fig. 2) – its therapeutic potential against Parkinson's disease is established beyond uncertainty (Soares *et al.*, 2014). However, the drug is also known to induce severe side effects under non-diseased conditions in human as well as diminish performance and health in livestock (Gray *et al.*, 2013). Due to this, it is regarded as greater risk among all the anti-nutritional substances present in *Mucuna* seeds (Szabo, 2003).

According to Lorenzetti *et al.* (1998), the maximum daily dose of L-Dopa that can be tolerated by an adult individual without any side effects is 1500 mg/d. Therefore, a healthy person should be able to safely consume 500 g of *Mucuna* based food/d with 0.1% L-Dopa; and any dietary prescriptions based on it should strictly adhere to this limit. However, in case of long-term ingestion, or consumption by children, pregnant women and people with medical conditions this dose may vary (Szabo and Tebbett, 2002). Teixeira *et al.* (2003) also confirmed upper limit of L-Dopa for consumption based on their research in Fababean and Broad bean (*Vicia faba*) where it is present in 0.2–0.5% respectively, and consumed safely worldwide.

In this context, assessing the natural variability for L-Dopa content assumes importance for strategizing the breeding programs. Several earlier studies have reported on this aspect in different *Mucuna* sp. (Table 2). Even though different estimation methods have been followed, large number of them has used spectrophotometric methods, except Modi *et al.* (2008) and Raman Singh *et al.* (2010) who used HPTLC and Archana Raina *et al.* (2012), HPLC methods. Recently, a novel approach has been reported by Sampath *et al.* (2013) where methanolic extract

**Table 2.** L-Dopa variability reported in different *Mucuna* species

Sl.No.	<i>Mucuna</i> sp.	L-Dopa (%)	Reference
1	<i>Mucuna andreana</i> Micheli	6.3–8.9	Bell and Janzen (1971)
2	<i>Mucuna aterrima</i> (Piper and Tracy) Holland	3.31–4.2	Daxenbichler <i>et al.</i> (1972); Pieris <i>et al.</i> (1980)
3	<i>Mucuna birdwoodiana</i> Tutcher	9.1	Chem Abstr (1990)
4	<i>Mucuna cochinchinesis</i> (Lour.) A. Chev	2.5–4.2	Prakash and Tewari (1999); Pieris <i>et al.</i> (1980); Chem Abstr (1991)
5	<i>Mucuna deeringiana</i> (Bort.) Merr	2.7–3.13	Miller (1920); Daxenbichler <i>et al.</i> (1972); Pieris <i>et al.</i> (1980)
6	<i>Mucuna gigantea</i> (Willd.) DC	1.50–3.78	Daxenbichler <i>et al.</i> (1972); Rajaram and Janardhanan (1991)
7	<i>Mucuna holtonii</i> (Kuntze) Mold	6.13–7.5	Daxenbichler <i>et al.</i> (1972); Bell and Janzen (1971)
8	<i>Mucuna monosperma</i> DC. Ex Wight	4.24–4.56	Arulmozhi and Janardhanan (1992); Mohan and Janardhanan (1995)
9	<i>Mucuna mutisiana</i> (Kunth.) DC	3.9–6.8	Daxenbichler <i>et al.</i> (1972); Bell and Janzen (1971); Bell <i>et al.</i> (1971)
10	<i>Mucuna pruriens</i> (L.) DC	3.8–9.16	Damodaran and Ramaswamy (1937); Daxenbichler <i>et al.</i> (1972); Chattopadhyay <i>et al.</i> (1988); Mary and Janardhanan (1992); Mahajani <i>et al.</i> (1996); Prakash and Tewari (1999); Padmesh <i>et al.</i> (2006)
		4.36–6.12	Krishnamurthy <i>et al.</i> (2005)
		1.51–6.29	Singh <i>et al.</i> (2008)
		5.6	Modi <i>et al.</i> (2008)
		7.06–8.13	Fathima <i>et al.</i> (2010)
		5.38–6.98	Kalidass and Mohan (2011)
		1.41–6.63	Dhawan <i>et al.</i> (2011)
		3.29–5.44	Archana Raina <i>et al.</i> (2012)
11	<i>Mucuna pruriens</i> f. <i>Hirsuta</i>	1.4–1.5	Chattopadhyay <i>et al.</i> (1988)
12	<i>Mucuna pruriens</i> f. <i>Utilis</i>	1.8	
13	<i>Mucuna pruriens</i>	0.945–3.585	Sathyanarayana <i>et al.</i> (Unpublished data)
14	<i>Mucuna pruriens</i> var. <i>utilis</i> (Wall. Ex Wight) Baker ex Burck	3.6–8.05	Pieris <i>et al.</i> (1980); Janardhanan and Lakshmanan (1985); Mohan and Janardhanan (1995); Prakash and Tewari (1999); Siddhuraju and Becker (2001)
		5.60–6.56	Janardhanan <i>et al.</i> (2003)
		2.30–4.18	Krishnamurthy <i>et al.</i> (2005)
		7.55–7.93	Kala and Mohan (2010)
		4.11–6.61	Mamatha <i>et al.</i> (2010)
		1.58–6.18	Dhawan <i>et al.</i> (2011)
15	<i>Mucuna sloanei</i> Fawcett and Rendle	3.34–9.0	Bell and Janzen (1971); Daxenbichler <i>et al.</i> (1972); Rai and Saidu (1977)
16	<i>Mucuna urens</i> (Linn.) Medik	4.92–7.4	Bell and Janzen (1971); Daxenbichler <i>et al.</i> (1971); Daxenbichler <i>et al.</i> (1972)

of L-Dopa is obtained in semi pure form by chemical fractionation followed by its quantitative analysis using HPTLC.

Besides, little is known on the genetics governing L-Dopa production in *Mucuna* sp.; particularly information on G × E interaction effects lack consensus. Three studies till date have attempted to address this issue. Lorenzetti *et al.* (1998), in their study where latitude was used for environmental factor found both environmental and genotypic factors responsible for L-Dopa production. This was further

supported by St. Laurent *et al.* (2002) who found marginal impact of latitude, but concluded that other factors were influential too. On the contrary, Capo-chichi *et al.* (2003b) found genotype has greater influence on L-Dopa production, whereas, G × E interaction effect was minimal when compared with genotype/accession main effect. In view of these differing viewpoints, our group re-examined the role of G × E interaction on L-Dopa production. It was confirmed that the trait is relatively stable across environments with

preponderance of genotype effect over environmental effects (Mahesh and Sathyanarayana, 2011b). This elucidation will serve as an important clue for devising relevant breeding program for L-Dopa content in *M. pruriens*.

### Screening for resilient genotypes against biotic and abiotic stresses

Growth and yield of crop plants are limited by many biotic and abiotic stresses resulting in appreciable deficit between their realized and expected potential. Bray *et al.* (2000) estimates 51–82% yield loss in annual crops due to different abiotic stresses such as water, heat, salinity, soil, etc. On the other hand, biotic stresses are equally devastating with more than 42% of yield loss attributed to them (Pimentel, 1997). Of the various remedies available, enhancing the genetic resistance/potentials is seen as the most enduring one and has several obvious advantages such as genetic permanency, negligible cost once cultivars are developed and quite high efficiency. However, cost and labour involved in field studies are seen as major deterrent to achieve this. Therefore, screening under-green house and *in vitro* conditions are emerging handy. Accordingly, they have been widely employed in plants like tomato (Frery *et al.*, 2010), peas (Bruggeman *et al.*, 2010), cucumber (Baghbani *et al.*, 2013) and a few legume species (Rai *et al.*, 2011).

Breeding *M. pruriens* varieties for stress tolerance widens its scope for introduction in larger landscape. During the course of field studies for several years now, our group observed greater vulnerability of *M. pruriens* to Fusarium wilt as well as soil salinity. Encouraged by differential response of genotypes in our collection detailed germplasm screenings for genetic response against these stresses were undertaken. The results identified five moderate to highly resistant and six highly susceptible accessions (Fig. 3) to Fusarium wilt (Mahesh and Sathyanarayana, 2011a, b). Further, overlaying the wilt screening results with AFLP marker data identified several pairs of contrasting parents useful for mapping this trait. Likewise in case of salinity, we evaluated 35 accessions using nine indicative parameters. The results of the experiments carried out under controlled conditions revealed different levels of tolerance (Fig. 4) for the selected growth, physiological and biochemical parameters (Mahesh and Sathyanarayana, 2015). Based on this, several contrasting parents were identified for genetic mapping of this phenotype.

### Karyotype and phylogenetic analysis

Conservation of basic chromosome number  $x = 11$  is one of the prevailing features in Phaseoloid legumes. Chromosomal studies even in genus *Mucuna* suggests  $x = 11$  as the base

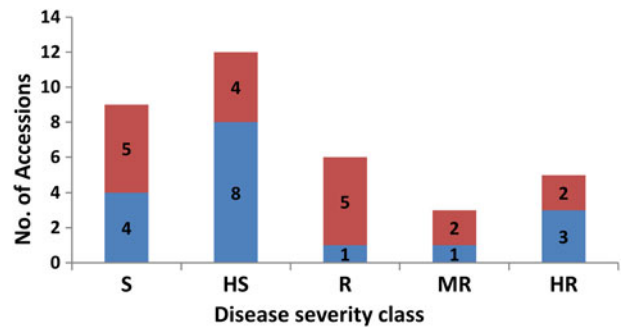


Fig. 3. Distribution of disease severity class for Fusarium wilt in *M. pruriens* germplasm (blue-var. *pruriens* and red-var. *utilis*).

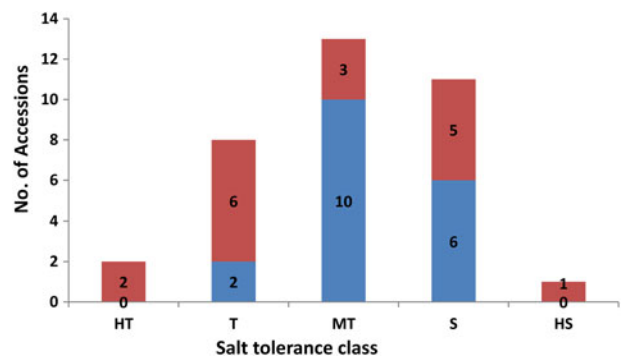
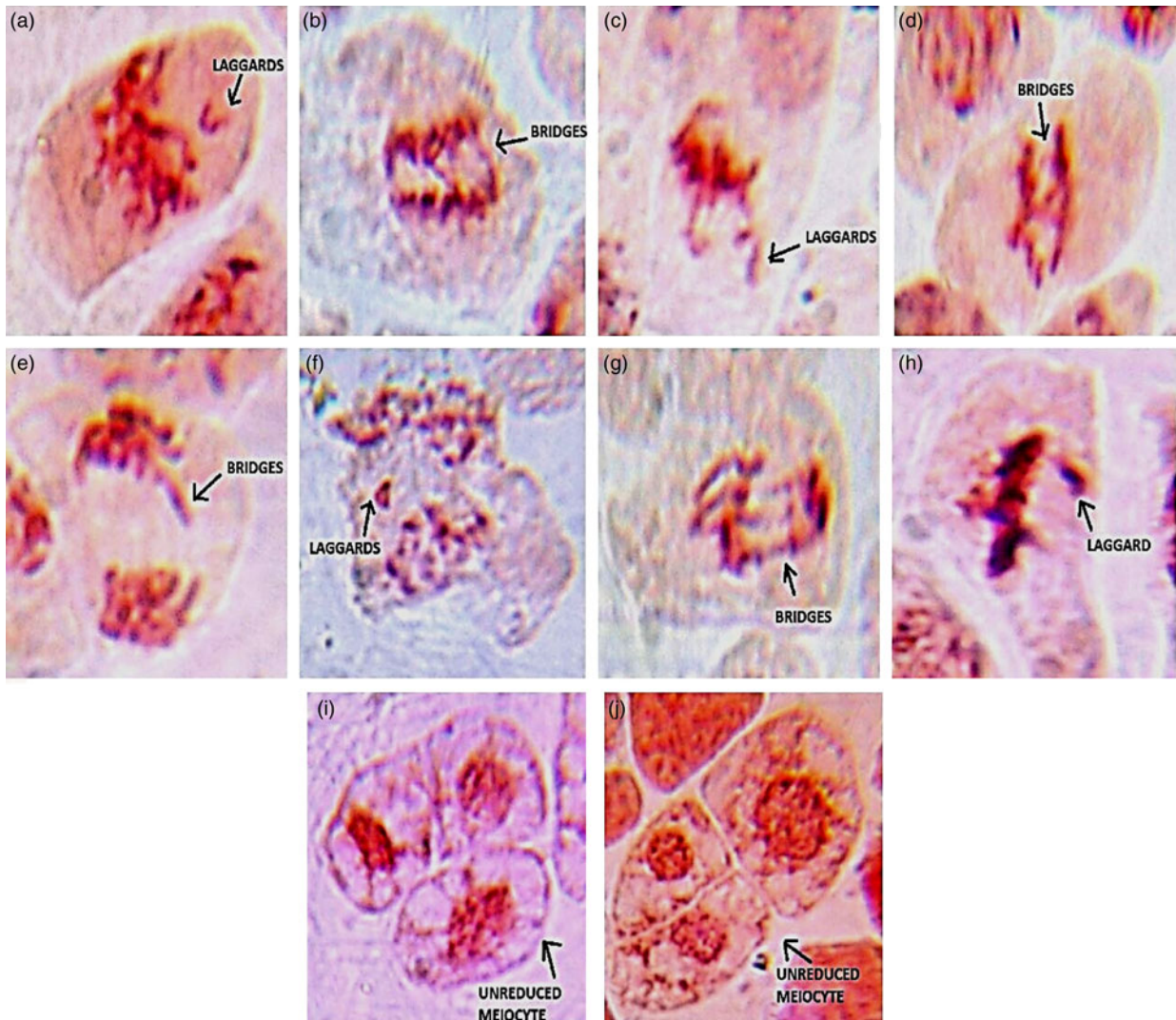


Fig. 4. Distribution of salt tolerance in *M. pruriens* germplasm (blue-var. *pruriens* and red-var. *utilis*).

number even though  $x = 14$  is reported in *Mucuna gigantea* and *Mucuna benettii* (Sastrapradja *et al.*, 1974; Jaheer and Sathyanarayana, 2010). So far, karyotype descriptions are available only for *Mucuna atropurpurea*, *Mucuna monosperma*, *Mucuna nigricans* and *M. pruriens* (Agostini *et al.*, 2009; Jaheer and Sathyanarayana, 2010). Recently our group completed karyotyping of two other Indian species viz., *Mucuna sempervirens*, *Mucuna bracteata*. Overall results show *Mucuna* species vary significantly in their karyotype features. Presence of pair of satellite chromosomes in *M. nigricans* and structural alterations observed at intra-species as well as intra-variety levels in *M. pruriens* (Lahiri *et al.*, 2010) corroborates this viewpoint suggesting their utility as markers in phylogenetic analysis. Besides, our recent studies on meiotic behaviour in some of the species revealed high incidence of chromosome laggards and bridges in *M. gigantea* and *M. atropurpurea*. Another species *M. bracteata* showed formation of uneven pollens and unreduced meiocytes (Fig. 5; Jaheer *et al.*, 2015). The latter perhaps indicate chromosomal instability caused out of tendency towards annual growth habit. Morphologically, *M. bracteata* presents several characters that are transitional between annual and perennial species suggesting possible key role it



**Fig. 5.** Meiotic Chromosome abnormalities in different species: Laggards in *M. gigantea* (a) *M. atropurpurea* (c, f and h). Chromosome bridges in *M. gigantea* (b) and *M. atropurpurea* (d, e and g); unreduced pollens in *M. bracteata* (i and j).

might have played in emergence of annual species including *M. pruriens*. Extensive distribution of *M. bracteata* in Indo-China region – which is also centre of origin of annual *M. pruriens*, substantiates this argument. Nonetheless, detailed analysis is needed to get better insight on this.

Phylogenetic analysis involving nuclear and chloroplast genes have resolved relationship among several legume taxa including Phaseoloid members. In *Mucuna*, however, such efforts for are lacking. So far, it has been only shown to be a member of Erythrinae – a sister tribe to Desmodieae under larger Phaseoloid group (Stefanovic *et al.*, 2009) having plastid genome inversion of 78-kb with a loss of one copy of large inverted repeat (Palmer *et al.*, 1987; Lavin *et al.*, 1990). Given that, our group examined the potential of ITS and *trnH-psbA* sequences as diagnostic markers for species identification and phylogenetic studies in *Mucuna*

species. The results not only revealed these genes to be phylogenetically informative, but also efficacy of combined ITS and *psbA-trnH* sequences for reliable species delineation (Jaheer *et al.*, 2015).

### Molecular markers and genetic diversity

Understanding the pattern of diversity and relationships in a germplasm collection is another important component of breeding programs (Azhaguvel *et al.*, 2006). Diversity based on morphological traits may not be reliable due to likely influence of environment (Tatikonda *et al.*, 2009). In contrast, molecular markers are independent of such effects and can be reliably generated using DNA from any growth stage.

In *Mucuna* sp. earlier studies used random amplification of polymorphic DNA (RAPD) and AFLP markers for this purpose (Capo-chichi *et al.*, 2001; Padmesh *et al.*, 2006; Sathyanarayana *et al.*, 2008). Capo-chichi *et al.* (2001) reported narrow genetic base (3–13%) among the US landraces in their study on 40 *M. pruriens* accessions using AFLP markers. Augmenting this with newer collections from CIAT apparently broadened the genetic base (0–32%) (Capo-chichi *et al.*, 2003a). In case of Indian germplasm, Padmesh *et al.* (2006) first reported good diversity (10–61%) among the accessions collected from the Western Ghats of India using RAPD markers. However, they found narrow genetic base in var. *utilis* (SI-0.82) vis-à-vis var. *pruriens* (SI-0.70). This observation was further corroborated by an extended study by our group on a larger germplasm involving combined morphometric, biochemical, isozyme and RAPD analysis (Leelambika *et al.*, 2010). It was also revealed that a few isozyme markers have diagnostic value in taxon identification (Leelambika and Sathyanarayana, 2011). Revision of genus *Mucuna* by Wilmot-Deary (1987) suggested new variety – var. *hirsuta* along with var. *utilis* and var. *pruriens* under *M. pruriens* indicating that it is distinguishable from var. *pruriens* only in having long crisped indumentum. Molecular data from our studies established its varietal status as against independent species suggested earlier (Baker, 1879; Nair and Henry, 1983; Ellis, 1990; Saldanha, 1996). Further, analysis of representative accessions from all the botanical varieties using AFLP markers confirmed even close genetic similarities between var. *pruriens* and var. *hirsuta* (Leelambika *et al.*, 2010). In view of this, it is suggested to combine all the wild forms of *M. pruriens* viz. var. *pruriens*, var. *hirsuta* and their intermediate types in one subgroup under var. *pruriens* thus allowing only two sub-groups viz., var. *utilis* (cultivated + non-itching trichomes on pod) and var. *pruriens* (wild + itching trichomes on pod) to be recognized under *M. pruriens*. This not only reduces nomenclatural redundancies, but also provides for authentic systematic name to the genotype/variety under consideration in breeding programs.

## Linkage map and quantitative trait loci (QTL) analysis

Genetic linkage maps have emerged as valuable resources to be used as framework for a number of plant breeding applications such as marker assisted selection, map based cloning, physical and comparative mapping etc. (Staub *et al.*, 1996). They are proved to be useful in detection of chromosomal locations and to study individual and interactive effects of genes for complex traits in several important legume species such as *Lotus japonicus* (Hayashi *et al.*, 2001); *Medicago truncatula* (Thoquet *et al.*, 2002);

*Medicago sativa* (Julier *et al.*, 2003); *Phaseolus vulgaris* (Yuste-Lisbona *et al.*, 2012) and *Pisum sativum* (Sun *et al.*, 2014). Recently genetic maps have been successfully developed even for lesser known legume species such as Azuki bean (Han *et al.*, 2005); Bambara groundnut (Ahmad, 2012); Lima bean (Bonifácio *et al.*, 2012); Yardlong bean (Kongjaimun *et al.*, 2012) etc.

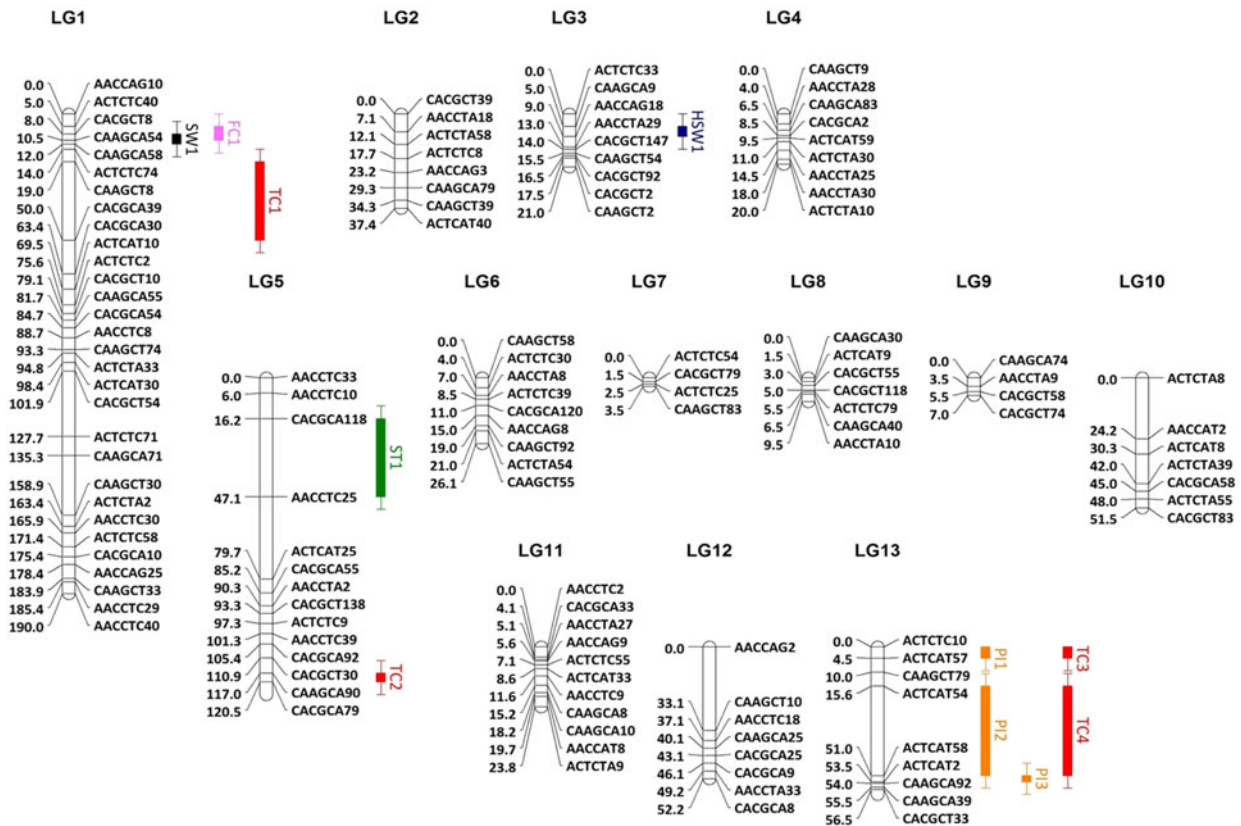
However, little is realized in terms of developing genomic resources for underutilized plant species in general and *M. pruriens* in particular. A lone linkage map using US core collection (Capo-chichi *et al.*, 2004), published earlier demonstrated prospects of good genome coverage for linkage studies with AFLP markers, in addition to segregation of pod colour and pod pubescence in F<sub>2</sub> population. Beyond this, there was no report in the direction of trait-based mapping or QTL studies from this species or any other work from any part of the World, till date. Very recently, the first genetic map from Indian *M. pruriens* (Fig. 6) indicating QTL positions for floral, pod and seed traits using F<sub>2</sub> intraspecific population has been reported by our group (Mahesh *et al.*, accepted publication).

## Comparative genomics and NGS for marker development

Development of codominant markers such as microsatellites and SNPs signifies key milestone in genomic resource development in *M. pruriens*. Microsatellite development based on expressed sequence tags (ESTs) is a promising alternative to cost intensive genomic-SSR for research in underutilized plants. Mining SSRs from the public databases is now sufficiently streamlined to make it cheaper and more efficient (Cordeiro *et al.*, 2001; Kantety *et al.*, 2002; Chen *et al.*, 2006). Also large numbers of processed ESTs are now deposited in public databases. In this backdrop, we explored potential use of public legume EST databases for the development of gene-derived SSR-markers for *M. pruriens*.

Totally, 2,86,488 EST sequences from four legume species *Vigna unguiculata*, *Glycine max*, *Phaseolus vulgaris* and *Cicer arietinum* were analysed, which generated 22,457 SSR containing sequences. From these, 522 primer combinations were designed and 50 were screened against a diverse panel of 25 genotypes, which produced polymorphic profiles with an average PIC of 0.65 (unpublished data). As an extension of this project, currently we have undertaken transcriptome sequencing of the two contrasting parents from our germplasm to generate species specific microsatellite and SNP markers. So far *de novo* assembly has been constructed using *P. vulgaris* as a reference genome. Annotation of some of the gene clusters differentially expressed in different tissues is also in progress. We aim to develop several polymorphic SSR and SNP markers from





**Fig. 6.** Location of quantitative trait loci (QTLs) for three qualitative and three quantitative traits based on combined results of SIM and CIM. The scale on the left side is the genetic distance in centiMorgan (cM), marker designations are given on the left side with distance and marker names spanning on both sides of the linkage group. QTLs are shown at the right side in vertical bars with trait names in different colours for different traits [black – seed width (SW); dark green – seed thickness (ST); blue – hundred seed weight (HSW); pink – flower colour (FC); orange – pod itchiness (PI); red – trichome colour (TC)]. The maps were drawn by the Map Chart 2.2 program. The vertical bar shows the LOD support interval and the line LOD interval of the QTL.

this work in near future, which will greatly boost molecular breeding research in *M. pruriens*.

## Conclusions and future prospects

Significant progress has been made in developing well characterized germplasm for the first time in *M. pruriens* at the laboratory for underutilized legume species at Sikkim University, Gangtok in collaboration with Sir M Visvesvaraya Institute of Technology, Bangalore. The projects currently underway are expected to pave a way for successful integration of molecular markers in breeding starting from development of saturated linkage maps and identification of markers/QTLs linked to L-Dopa and other economic traits. The co-localization of candidate genes with QTLs might even support 'genomics-assisted breeding' for these phenotypes. These advances will accelerate functional genomics or expression studies in near future. The genomic resources so developed will be greatly

useful even in the field of taxonomy and evolutionary studies in genus *Mucuna*.

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## References

- Agostini K, Sazima M, Tozzi AMGA and Forni-Martins ER (2009) Microsporogenesis and pollen morphology of *Mucuna jaspira* Azevedu, Agostini and Sazima and *M urens* (L) Medikus. *Phytomorph* 59: 61–69.

- Ajiwe VIE, Okeke CA, Nnabuike B, Ogunleye GA and Elebo E (1997) Applications of oils extracted from African star apple (*Chrysophyllum africanum*), horse eye bean (*Mucuna sloanei*) and African pear (*Dacryodes edulis*) seeds. *Bioresource Technology* 59: 259–261.
- Ahmad NS (2012) Genetic analysis of plant morphology in Bambara groundnut [*Vigna subterranea* (L.) Verdc.]. BSc Thesis. University of Nottingham.
- Aminah SH, Sastrapradja S, Lubis I, Sastrapradja D and Idris S (1974) Irritant hairs of *Mucuna* species. *Ann Bogoriensis* 5: 179–186.
- Archana Raina P, Tomar JB and Dutta M (2012) Variability in *Mucuna pruriens* L. germplasm for L-Dopa, an anti parkinsonian agent. *Genetic Resources and Crop Evolution* 59: 1207–1212.
- Arulmozhi M and Janardhanan K (1992) The biochemical composition and nutritional potential of the tribal pulse, *Mucuna monosperma* DC. Ex Wight. *Plant foods for Human Nutrition* 42: 45–53.
- Awang D, Buckles D and Arnason JT (1997) The phytochemistry, toxicology and processing potential of the covercrop velvetbean (cow(h)age, cowitch) (*Mucuna* Adans. spp, Fabaceae). International Workshop on Green Manure – Cover Crop Systems for Smallholders in Tropical and Subtropical Regions 6–12 April, Chapeco, Santa Catarina, Brazil. Santa Catarina, Brazil: Rural Extension and Agricultural Research Institute of Santa Catarina.
- Azhaguvel P, Vidya Saraswathi D, Sharma A and Varshney RK (2006) *Methodological Advancement in Molecular Markers to Delimit the Genes for Crop Improvement, Floriculture, Ornamental and Plant Biotechnology, 1*. London: Global Science books, pp. 460–468.
- Baghiani A, Forghani AH and Kadkhodaie A (2013) Study of salinity stress on germination and seedling growth in greenhouse cucumber cultivars. *Journal of Basic and Applied Scientific Research* 3: 1137–1140.
- Bailey LH (1947) *The Standard Cyclopedia of Horticulture*. New York, NY, USA: Macmillan.
- Bailey CD, Doyle JJ, Kajita T, Nemoto T and Ohashi H (1997) The chloroplast *rpl2* intron and ORF184 as phylogenetic markers in legume tribe Desmodieae. *Systematic Botany* 22: 133–138.
- Baker JG (1879) Leguminosae. In: Hooker JD (ed.) *The Flora of British India*. London, UK: Rev and Co, Kent, Vol. 2, pp. 56–306.
- Barron AB, Sovik E and Cornish JL (2010) The roles of Dopamine and related compounds in reward seeking behavior across animal phyla. *Frontiers in Behavioral Neuroscience* 4: 163.
- Bell EA and Janzen DH (1971) Medical and ecological considerations of L-Dopa and 5-HTP in seeds. *Nature* 229: 136–137.
- Bell EA, Nulu JR and Cone C (1971) L-DOPA and l-3-carboxy-6,7-dihydroxy-1,2,3,4 tetrahydroisoquinoline, a new imino acid, from seeds of *Mucuna mutisiana*. *Phytochemistry* 10: 2191–2194.
- Bennett-Lartey SO (1998) Characterization and preliminary evaluation of some accessions of local germplasm of velvet bean (*Mucuna pruriens* L. var. *utilis* Wall) of Ghana. *Ghana Journal of Agricultural Science* 31: 131–135.
- Betancur-Ancona DA, Chel-Guerrero LA, Bello-Pérez LA and Dávila-Ortiz G (2002) Isolation of velvet bean (*Mucuna pruriens*) starch: physicochemical and functional properties. *Starch-Stärke* 54: 303–309.
- Bhat R and Karim AA (2009) Exploring the nutritional potential of wild and underutilized legumes. *Comprehensive Reviews in Food Science and Food Safety* 8: 305–331.
- Bort KS (1909) *The Florida Velvet Beans and its History*. Washington, DC: Bureau of Plant Industry, Bull. 141, USDA, pp. 25–32.
- Bray EA, Bailey-Serres J and Weretilnyk E (2000) Responses to abiotic stresses. In: Grissem W, Buchannan B and Jones R (eds) *Biochemistry and Molecular Biology of Plants*. Rockville: American Society of Plant Physiologists, pp. 1158–1249.
- Bruggeman A, Hamdy A, Karajeh F, Oweis T and Touchan H (2010) Invitro salinity tolerance screening of some legumes and forage cultivars. *Options Méditerranéennes: Série B. Etudes et Recherches* 44: 163–169.
- Bonifácio EM, Fonsêca A, Almeida C, dos Santos KGB and Pedrosa-Harand A (2012) Comparative cytogenetic mapping between the lima bean (*Phaseolus lunatus* L.) and the common bean (*P. vulgaris* L.). *Theoretical and Applied Genetics* 124: 1513–1520.
- Buckles D (1995) *M. pruriens*: A “new” plant with a history. *Economic Botany* 49: 13–25.
- Burgess S, Hemmer A and Myhrman R (2003) Examination of raw and roasted *Mucuna pruriens* for tumerogenic substances. *Tropical and Subtropical Agroecosystems* 1: 287–293.
- Burkill IH (1966) *A Dictionary of the Economic Products of the Malay Peninsula*. Kuala Lumpur, Malaysia: Ministry of Agriculture and Cooperatives.
- Capo-chichi LJA, Weaver DB and Morton CM (2001) AFLP assessment of genetic variability among velvet bean (*Mucuna* sp.) accessions. *Theoretical and Applied Genetics* 103: 1180–1188.
- Capo-chichi LJA, Weaver DB and Morton CM (2003a) The use of molecular markers to study genetic diversity in *Mucuna*. *Tropical and Subtropical Agroecosystems* 1: 309–318.
- Capo-chichi LJA, Eilittä M, Carsky R, Gilbert R and Maasdorp B (2003b) Effect of genotype and environment on L-Dopa concentration in *Mucuna*'s (*Mucuna* sp.) seeds. *Tropical and Subtropical Agroecosystems* 1: 319–328.
- Capo-chichi LJA, Morton CM and Weaver DB (2004) An intraspecific genetic map of velvet bean (*Mucuna* sp.) based on AFLP markers. *Theoretical and Applied Genetics* 108: 814–821.
- Carsky RJ and Ndikawa R (1998) Identification of cover crops for the semi-arid savanna zone of West Africa. In: Buckles D, Eteka A, Osiname M, Galiba M and Galiano G (eds) (2000) *Cover Crops in West Africa - Contributing to Sustainable Agriculture*. Ottawa, Canada: IDRC, IITA, Sasakawa Global, Ibadan, Nigeria, Cotonou, Benin. pp. 179–187.
- Chattopadhyay *et al.* (1988) *Acta horticulturae*. 188: 51.
- Chem Abstr (1990) 113: 29157.
- Chem Abstr (1991) 114: 108913.
- Chen CX, Zhou P, Choi YA, Huang S and Gmitter FG (2006) Mining and characterizing microsatellites from citrus ESTs. *Theoretical and Applied Genetics* 112: 1248–1257.
- Cordeiro GM, Casu R, McIntyre CL, Manners JM and Henry RJ (2001) Microsatellite markers from sugarcane (*Saccharum* spp.) ESTs cross transferable to erianthus and sorghum. *Plant Science* 160: 1115–1123.
- Damodar M and Ramaswamy R (1937) Isolation of L-dopa from the seeds of *Mucuna pruriens*. *Biochemistry* 31: 2149–2151.
- Daxenbichler ME, Van Etten CH, Hallinan EA, Earle FR and Barclay SA (1971) Seeds as sources of L-Dopa. *Journal of Medicinal Chemistry* 14: 463–465.
- Daxenbichler ME, Kleiman R, Weisleder D, Van Etten CH and Carlson KD (1972) Tetrahedron Letters, pp. 1801.
- Dhawan SS, Rai GK, Darokar MP, Lal RK, Misra HO and Khanuja SPS (2011) Comparative genetic analysis of trichome-less and

- normal pod genotypes of *Mucuna pruriens* (Fabaceae). *Genetics and Molecular Research* 10: 2049–2056.
- Diallo OK, Kante S, Myhrman R, Soumah M, Cissé NY and Berhe T (2002) Increasing farmer adoption of *Mucuna pruriens* as human food and animal feed in the Republic of Guinea. In: Flores BM, Eilittä M, Myhrman R, Carew LB and Carsky RJ (eds) *Food and Feed from Mucuna*: Current uses and the Way Forward, Workshop, (April 26–29, 2000). Tegucigalpa, Honduras: CIDICCO, CIEPCA and World Hunger Research Center, pp. 60–72.
- Doyle JJ, Chappill JA, Bailey DC and Kajita T (2000) Towards a comprehensive phytochemistry of legumes: evidence from rbcL sequences and non-molecular data. In: Herendeen PS and Bruneau A (eds) *Advances in Legume System Part 9*. Kew: Royal Botanic Gardens, *Kew Bulletin*. pp. 1–20.
- Duke JA (1981) *Handbook of Legumes of World Economic Importance*. New York, USA: Plenum Press, pp. 345.
- Eilittä M, Bressani R, Carew LB, Carsky RJ, Flores M, Gilbert R, Huyck L, St. Laurent L and Szabo NJ (2002) *Mucuna* as a food and feed crop: An overview. In: Flores BM, Eilittä M, Myhrman R, Carew LB and Carsky RJ (eds) *Food and Feed from Mucuna*: Current uses and the Way Forward, Workshop, (April 26–29, 2000) Tegucigalpa, Honduras: CIDICCO, CIEPCA and World Hunger Research Center, pp. 18–47.
- Ellis JL (1990) *Flora of Nallamalais* 2: 220–490.
- Farooqi AA, Khan MM and Asundhara M (1999) *Production Technology of Medicinal and Aromatic Crops*. Bangalore, India: Natural Remedies Pvt. Ltd., pp. 26–28.
- Fathima KR, Tresina Soris P and Mohan VR (2010) Nutritional and antinutritional assessment of *Mucuna pruriens* (L.) DC var. *pruriens* an underutilized tribal pulse. *Advances in Bioresearch* 1: 79–89.
- Frary A, Göl D, Keleş D, Ökmen B, Pınar H, Şığva HO, Yemencioğlu A and Doğanlar S (2010) Salt tolerance in *Solanum pennellii*: antioxidant response and related QTL. *BMC Plant Biology* 10: 58.
- Fujii Y, Shibuya T and Yasuda T (1991) L-3, 4-Dihydroxyphenylalanine as an allelochemical candidate from *Mucuna pruriens* (L.) DC. var. *utilis*. *Agricultural and Biological Chemistry* 55: 617–618.
- Gray G, Tse MD, Brian B, Kim MD, Aaron M, McMurtray MD and Nakamoto BK (2013) Case of Levodopa toxicity from ingestion of *Mucuna gigantean*. *Hawaii Journal of Medicine and Public Health* 72: 157–160.
- Gurumoorthi P, Senthil Kumar S, Vadivel V and Janardhanan K (2003) Studies on agrobotanical characters of different accessions of velvet bean collected from Western Ghats, South India. *Tropical and Subtropical Agroecosystems* 2: 105–115.
- Han OK, Kaga A, Isemura T, Wang XW, Tomooka N and Vaughan DA (2005) A genetic linkage map for azuki bean [*Vigna angularis* (Wild.) Ohwi and Ohashi]. *Theoretical and Applied Genetics* 111: 1278–1287.
- Hayashi M, Miyahara A, Sato S, Kato T, Yoshikawa M, Taketa M, Hayashi M, Pedrosa A, Onda R, Imaizumi-Anraku H, Bachmair A, Sandal N, Stougaard J, Murooka Y, Tabata S, Kawasaki S, Kawaguchi M and Harada K (2001) Construction of a genetic linkage map of the model legume *Lotus Japonicus* using an Intraspecific F2 population. *DNA Research* 8: 301–310.
- Iyayi EA and Egharevba JI (1998) Biochemical evaluation of seeds of an underutilized legume (*Mucuna utilis*). *Nigerian Journal of Animal Production* 25: 40–45.
- Jaheer M and Sathyanarayana N (2010) Karyomorphological studies in *Mucuna* of India. *Chromosome Botany* 5: 37–41.
- Jaheer M, Chopra R, Kunder KR, Bhat D, Rashmi KV and Sathyanarayana N (2015) Cytogenetic and ITS-psbA-trnH sequence analysis for phylogenetic inference in *Mucuna* sp. of India. *Tropical Plant Biology* 8: 108–116.
- Janardhanan K and Lakshmanan KK (1985) Studies on the pulse, *Mucuna utilis*: chemical composition and antinutritional factors. *Journal of Food Science and Technology* 22: 369–371.
- Janardhanan K, Gurumoorthi P and Pugalenth M (2003) Nutritional potential of five accessions of a South Indian tribal pulse, *Mucuna pruriens* var. *utilis* I, The effect of processing methods on the content of L-Dopa, phytic acid and oligosaccharides. *Tropical and Subtropical Agroecosystems* 1: 141–152.
- Jorge MA, Eilittä M, Proud FJ, Barbara Maasdorp V, Beksissa H, Ashok Sarial K and Hanson J (2007) *Mucuna* species: recent advances in application of biotechnology. *Fruit Vegetable Cereal Science and Biotechnology* 2: 80–94.
- Julier B, Flajoulot S, Barre P, Cardinet G, Santoni S, Huguet T and Huyghe C (2003) Construction of two genetic linkage maps in cultivated tetraploid alfalfa (*Medicago sativa*) using microsatellite and AFLP markers. *BMC Plant Biology* 3: 9.
- Kala BK and Mohan VR (2010) Nutritional and antinutritional potential of three accessions of itching bean (*Mucuna pruriens* (L.) DC var. *pruriens*): an under-utilized tribal pulse. *International Journal of Food Sciences and Nutrition* 61: 497–511.
- Kalidass C and Mohan VR (2011) Nutritional and antinutritional composition of itching bean [*Mucuna pruriens* (L.) DC. var. *pruriens*]. An underutilized tribal pulse in Western Ghats, Tamil Nadu, Trop. *Subtropical Agroecosystems* 14: 279–293.
- Kantety RV, La Rota M, Matthews DE and Sorrells ME (2002) Data mining for simple sequence repeats in expressed sequence tags from barley, maize, rice, sorghum and wheat. *Plant Molecular Biology* 48: 501–510.
- Kavitha C and Thanagmani C (2014) Amazing bean “*Mucuna pruriens*”- a comprehensive review. *Journal of Medicinal Plants Research* 8: 138–143.
- Kongjaimun A, Kaga A, Tomooka N, Somta P, Shimizu T, Shu Y, Isemura T, Vaughan DA and Srinives P (2012) An SSR-based linkage map of yardlong bean (*Vigna unguiculata* (L.) Walp. Sub sp., *unguiculata* Sesquipedalis Group) and QTL analysis of pod length. *Genome* 55: 81–92.
- Krishnamurthy R, Chandrokar MS, Kalzunkar BG, Palsule Desai MR, Pathak JM and Gupta R (2005) Diversity evaluation in velvet bean (*Mucuna pruriens*) germplasm for seed yield and associated agronomic traits. *International Journal of Medicinal and Aromatic Plants* 27: 291–296.
- Lahiri K, Mukhopadhyay MJ and Mukhopadhyay S (2010) Karyotype analysis and in situ 4C nuclear DNA quantification in two varieties of *Mucuna pruriens* L. *Journal of Tropical Medicinal Plants* 11: 219–225.
- Lavin M, Doyle JJ and Palmer JD (1990) Evolutionary significance of the loss of the chloroplast-DNA inverted repeat in the Leguminosae subfamily Papilionoideae. *Evolution* 44: 390–402.
- Lawal OS and Adebowale KO (2004) Effect of acetylation and succinylation on solubility profile, water absorption capacity, oil absorption capacity and emulsifying properties of *Mucuna* bean (*Mucuna pruriens*) protein concentrate. *Nabrun/food* 48: 129–136.
- Lee J and Hymowitz T (2001) A molecular phylogenetic study of the subtribe Glycininae (Leguminosae) derived from the chloroplast DNA rps16 intron sequences. *American Journal of Botany* 88: 2064–2073.

- Leelambika M and Sathyanarayana N (2011) Genetic characterization of Indian *Mucuna* (Leguminosae) species using morphometric and random amplification of polymorphic DNA (RAPD) approaches. *Plant Biosystems* 145: 786–797.
- Leelambika M, Mahesh S, Jaheer M and Sathyanarayana N (2010) Comparative evaluation of genetic diversity among Indian *Mucuna* species using morphometric, biochemical and molecular approaches. *World Journal of Agricultural Sciences* 6: 568–578.
- Lorenzetti F, MacIsaac S, Arnason JT, Awang DVC and Buckles D (1998) The phytochemistry, toxicology, and food potential of *M. pruriens* (*Mucuna* Adans. spp., Fabaceae). In: Buckles D, Eteka A, Osiname M, Galiba M and Galiano G (eds) *Cover crops in West Africa - contributing to sustainable agriculture*. Ottawa, Canada: IDRC, IITA, Sasakawa Global 2000; Ibadan, Nigeria; Cotonou, Benin, pp. 67–84.
- Lubis SHA, Sastrapradja S, Lubis I and Sastrapradja D (1978) Genetic variation of *Mucuna pruriens*. II. Inheritance of flower color. *Ann Bogorieneses* 4: 187–191.
- Lubis SHA, Sastrapradja S, Lubis I and Sastrapradja D (1979) Genetic variation of *Mucuna pruriens* (L.) DC. III inheritance of pod hairs. *Ann Bogorieneses* 1: 1–10.
- Lubis SHA, Sastrapradja S, Lubis I and Sastrapradja D (1980) Genetic variation of *Mucuna pruriens*. IV. Inheritance and genotypes of seed coat colors. *Ann Bogorieneses* 8: 79–87.
- Mahajani SS, Doshi VJ and Parikh KM (1996) Bioavailability of L-Dopa from HP-200- a formulation of seed powder of *Mucuna pruriens* (Bak): a pharmacokinetic and pharmacodynamic study. *Phytotherapy Research* 10: 254–256.
- Mahesh S and Sathyanarayana N (2011a) Identification of contrasting genotypes for Fusarium wilt disease in *M. pruriens* germplasm through combined in vitro screening and AFLP analysis. *Electronic Journal of Plant Breeding* 2: 510–519.
- Mahesh S and Sathyanarayana N (2011b) The genotype environment interaction and stability analysis for L-Dopa trait in *M. pruriens* seeds. *Indian Journal of Genetics* 71: 279–282.
- Mahesh S and Sathyanarayana N (2015) Intra-specific variability for salinity tolerance in Indian *Mucuna pruriens* L. (DC.) Germplasm. *Journal of Crop Science and Biotechnology* 18: 181–194. doi: 10.1007/s12892-015-0019-7.
- Mamatha B, Shivananda TN and Siddaramappa R (2006) *Nitrogen Fixing Ability of 13 Enotypes of Mucuna pruriens*. Philadelphia, Pennsylvania, USA: 18th World Congress of Soil Science, pp. 9–15.
- Mamatha B, Siddaramappa R and Shivananda TN (2010) Evaluation of *Mucuna utilis* germplasm for higher biomass production, active principle and seed yield. *Journal of Medicinal Plant Research* 4: 1297–1300.
- Mary JR 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 Chemistry* 43: 13–18.
- Miller ER (1920) Dihydroxyphenylalanine, a constituent of the velvet bean. *Journal of Biological Chemistry* 44: 481–486.
- Modi KP, Natvarlal Patel NM and Goyal RK (2008) Estimation of L-Dopa from *Mucuna pruriens* Linn and formulations containing *M. pruriens* by HPTLC method. *Chemical and Pharmaceutical Bulletin* 56: 357–359.
- Mohan VR and Janardhanan K (1995) Chemical analysis and nutritional assessment of lesser known pulses of the genus, *Mucuna*. *Food Chemistry* 52: 275–280.
- Muinga RW, Saha HM and Mureithi JG (2003) The effect of *Mucuna* (*Mucuna pruriens*) forage on the performance of lactating cows. *Tropical and Subtropical Agroecosystems* 1: 87–91.
- Nair NC and Henry AN (1983) *Flora of Tamil Nadu, India, Series 1: Analysis*. Coimbatore: Botanical Survey of India, Vol. 1.
- Oudhia P (2002) Kapikachu or Cowhage (*M. pruriens*) *Crop Fact Sheet*. Version of 5–9.
- Padmesh P, Reji JV, Jinish Dhar M and Seeni D (2006) Estimation of genetic diversity in varieties of *M. pruriens* using RAPD. *Biologia Plantarum* 50: 367–372.
- Palmer JD, Nugent JM and Herbon LA (1987) Unusual structure of geranium chloroplast DNA: a triple sized inverted repeat, extensive gene duplications, multiple inversions, and two repeat families. *Proceedings of the National Academy of Sciences of the United States of America* 84: 769–773.
- Pieris N, Jansz ER and Dharmadasa HM (1980) Studies on *Mucuna* species of Sri Lanka 1- The L-Dopa content of seeds. *Journal of Natural Sciences* 8: 35–40.
- Pimentel D (1997) *Techniques for Reducing Pesticides: Environmental and Economic Benefits*. Chichester, UK: John Wiley, p. 444.
- Prakash D and Tewari SK (1999) Variation on L-DOPA content in *Mucuna* species. *Journal of Medicinal and Aromatic Plant Sciences* 21: 343–346.
- Pugalenthi M and Vadivel V (2007a) L-Dopa (L-3, 4-Dihydroxyphenylalanine): non-protein toxic amino acid in *Mucuna pruriens* seeds. *Food* 1: 322–343.
- Pugalenthi M and Vadivel V (2007b) Agro biodiversity of eleven accessions of *Mucuna pruriens* (L.) DC. var. utilis (Wall ex. Wight) Baker ex Burck (velvet bean) collected from four districts of south India. *Genetic Resources and Crop Evolution* 54: 1117–1124.
- Pugalenthi M, Vadivel V and Siddhuraju P (2005) Alternative food/feed perspectives of an underutilized legume *Mucuna pruriens* var. utilis – a review. *Plant Foods and Human Nutrition* 60: 201–218.
- Queneherve P, Topart P and Martiny B (1998) *Mucuna pruriens* and other rotational crops for control of *Meloidogyne incognita* and *Rotylenchulus reniformis* in vegetables in poly-tunnels in Martinique. *Nematotropa* 28: 19–30.
- Rai PP and Saidu M (1977) Characterization of L-DOPA in seeds of *Mucuna sloanei*. *Current Science* 46: 778.
- Rai MK, Kalia RK, Singh R, Gangola MP and Dhawan AK (2011) Developing stress tolerant plants through in vitro selection. An overview of the recent progress. *Environmental and Experimental Botany* 71: 89–98.
- Rajaram N and Janardhanan K (1991) The biochemical composition and nutritional potential of the tribal pulse, *Mucuna gigantea* (Wild) DC. *Plant Foods for Human Nutrition* 41: 45–51.
- Raman Singh M, Pawan Saini K, Satish Mathur C, Gyanendra Singh N and Santosh Kumar (2010) Application of high performance liquid chromatography to the determination and validation of levodopa in the methanolic extract of *Mucuna utilis*. *International Journal of Green Pharmacy*. 156–158.
- Saldanha CJ (1996) *Flora of Karnataka*. ISBN 81-204-1040-8.
- Sampath V, Mohamed Faizal K and Mani Babu N (2013) A novel approach of the isolation of L-Dopa from the methanolic extract of *Mucuna pruriens* seeds and its quantitative analysis by HPTLC. *International Journal of Pharmacognosy and Phytochemistry Research* 5: 259–262.
- Sastrapradja S, Sastrapradja D, Aminah SH, Lubis I and Idris S (1974) Morphological and cytological investigation on some species of *Mucuna* (Papilionaceae). *Ann Bogorieneses* 5: 173–178.
- Sathyanarayana N, Bharath Kumar TN, Vikas PB and Rajesha R (2008) In vitro clonal propagation of *Mucuna pruriens* var.

- utilis and its evaluation of genetic stability through RAPD markers. *African Journal of Biotechnology* 7: 973–980.
- Siddhuraju P and Becker K (2001) Effect of various indigenous processing methods on the  $\alpha$ -galactoside and mono- and disaccharide content of an Indian tribal pulse, *Mucuna pruriens* var. utilis. *Journal of the Science of Food and Agriculture* 81: 718–725.
- Siddhuraju P and Becker K (2005) Nutritional and antinutritional composition, in vitro amino acid availability, starch digestibility and predicted glycemic index of differentially processed *Mucuna beans* (*Mucuna pruriens* var. utilis): an under-utilized legume. *Food Chemistry* 91: 275–286.
- Siddhuraju P, Becker K and Makkar HP (2000) Studies on the nutritional composition and antinutritional factors of three different germplasm seed materials of an under-utilized tropical legume, *Mucuna pruriens* var. utilis. *Journal of Agricultural and Food Chemistry* 48: 6048–6060.
- Singh AK, Malik SS and Tomar YS (2008) Studies on the medicinal compound L-Dopa in *Mucuna pruriens* (Bak.). *Indian Journal of Plant Genetic Resources* 21: 217–220.
- Singh AP, Sarkar S, Tripathi M and Rajender S (2013) *Mucuna pruriens* and its major constituent L-DOPA recover spermatogenic loss by combating ROS, loss of mitochondrial membrane potential and apoptosis. *PLoS ONE* 8: e54655.
- Soares AR, Marchiosi R, Soares RCS, Lima RB, Santos WD and Ferrarese-Filho O (2014) The role of L-Dopa in plants. *Plant Signalling and Behavior* 9: e28275-1–7.
- St. Laurent L, Livesey J, Arnason JT and Bruneau A (2002) Variation in L-dopa concentration in accessions of *M. pruriens* (L.) DC. and in *Mucuna brachycarpa* Rech. In: Flores M, Eilittä M, Myhrman R, Carew LB and Carsky RJ (eds) *Food and Feed from Mucuna: Current uses and the Way Forward, Proceedings of an International Workshop*. Tegucigalpa, Honduras: CIDICCO, CIEPCA, World Hunger Research Center, pp. 352–373.
- Staub JE, Serquen FC and Gupta M (1996) Genetic markers, map construction, and their application in plant breeding. *Horticultural Science* 31: 729–740.
- Stefanovic S, Bernard EP, Jeffrey DP and Jeff JD (2009) Relationship among Phaseoloid legumes based on sequences from eight chloroplast regions. *Systematic Botany* 34: 115–128.
- Sun X, Yang T, Hao J, Zhang X, Ford R, Jiang J, Wang F, Guan J and Zong X (2014) SSR genetic linkage map construction of pea (*Pisum sativum* L.) based on Chinese native varieties. *The Crop Journal* 170–174.
- Szabo NJ (2003) Indolealkylamines in *Mucuna* species. *Tropical and Subtropical Agroecosystems* 1: 295–307.
- Szabo NJ and Tebbett IR (2002) The chemistry and toxicity of *Mucuna* species. In: Flores M, Eilittä M, Myhrman R, Carew LB and Carsky RJ (eds) *Mucuna as a Food and Feed: Current uses and the Way Forward, Workshop held April 26–29, 2000 in Tegucigalpa, Honduras*. Tegucigalpa, Honduras: CIDICCO, CIEPCA, and World Hunger Research Center, CIDICCO, Honduras, pp. 120–141.
- Tarawali G, Manyong VM, Carsky RJ, Vissoh PV, Osei-Bonsu P and Galiba M (1999) Adoption of improved fallows in West Africa: lessons from *Mucuna* and *Stylo* case studies. *Agroforestry Systems* 47: 93–122.
- Tatikonda L, Wani SP, Kannan S, Beerelli N, Sreedevi TK, David A, Hoisington DA, Prathibha Devi P and Varshney RK (2009) AFLP-based molecular characterization of an elite germplasm collection of *Jatropha curcas* L., a biofuel plant. *Plant Science* 176: 505–513.
- Teixeira AA, Rich EC and Szabo NJ (2003) Water extraction of L-Dopa from *Mucuna bean*. *Tropical and Subtropical Agroecosystems* 1: 159–171.
- Thoquet P, Gherardi M, Journet EP, Kereszt A and Ane JM (2002) The molecular genetic linkage map of the model legume *Medicago truncatula*: an essential tool for comparative legume genomics and the isolation of agronomically important genes. *BMC Plant Biology* 2: 1.
- Wilmot-Dear CM (1984) A revision of *Mucuna* (Leguminosae-Phaseoleae) in China and Japan. *Kew Bulletin* 39: 23–65.
- Wilmot-Dear CM (1987) A revision of *Mucuna* (Leguminosae-Phaseoleae) in the Indian Subcontinent and Burma. *Kew Bulletin* 42: 23–46.
- Wilmot-Dear CM (1989) A revision of *Mucuna* (Leguminosae, Phaseoleae) in the Pacific. *Kew Bulletin* 45: 1–35.
- Wilmot-Dear CM (1990) A revision of *Mucuna* (Leguminosae, Phaseoleae) in the Philippines. *Kew Bulletin* 46: 213–251.
- Wilmot-Dear CM (1991) A revision of *Mucuna* (Leguminosae, Phaseoleae) in Thailand, Indochina and the Malay Peninsula. *Kew Bulletin* 47: 203–245.
- Yuste-Lisbona FJ, Santalla M, Capel C, García-Alcázar M, De La Fuente M, Capel J, De Ron AM and Lozano R (2012) Marker-based linkage map of Andean common bean (*Phaseolus vulgaris* L.) and mapping of QTLs underlying popping ability traits. *BMC Plant Biology* 12, 136.