

# 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

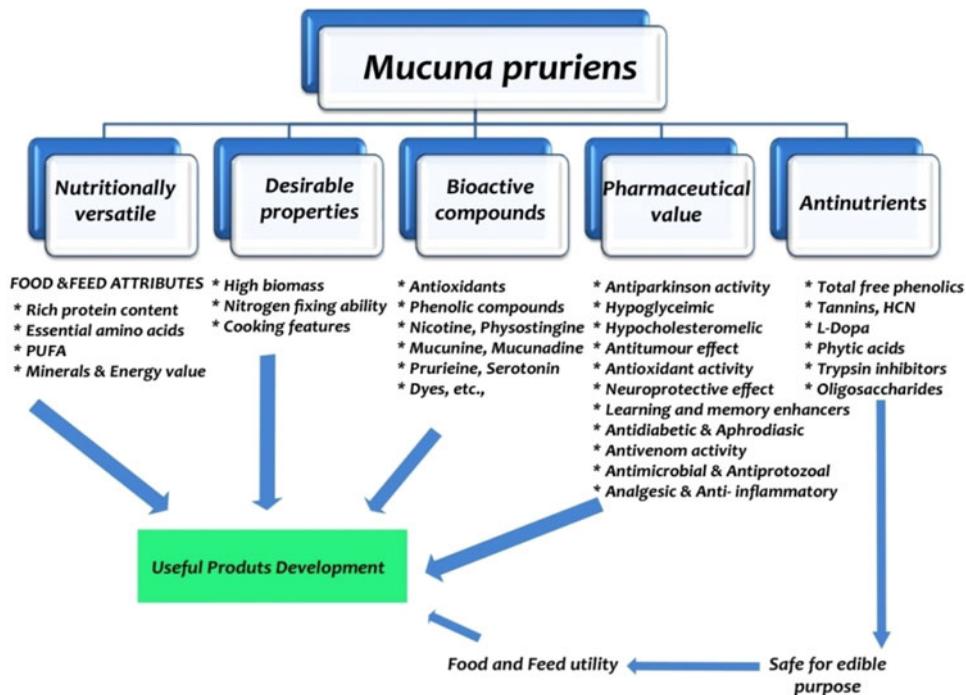
paint, polish, resin, dye, wood varnish, skin cream and soap (Ajiwe *et al.*, 1997).

*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-Deer 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

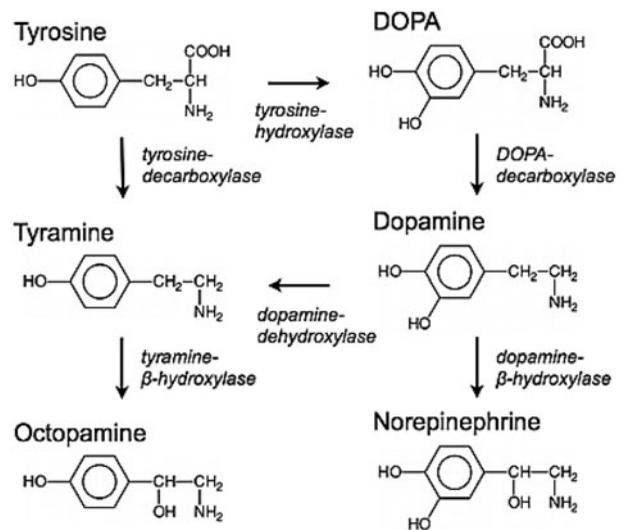
Germplasm of *M. pruriens* is maintained in several research institutes/organizations across the World. This includes: US Department of Agriculture (USDA); International Institute of Tropical Agriculture, Nigeria; Centro Internacional de Agricultura Tropical (CIAT), Colombia; AVRDC – The World Vegetable 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

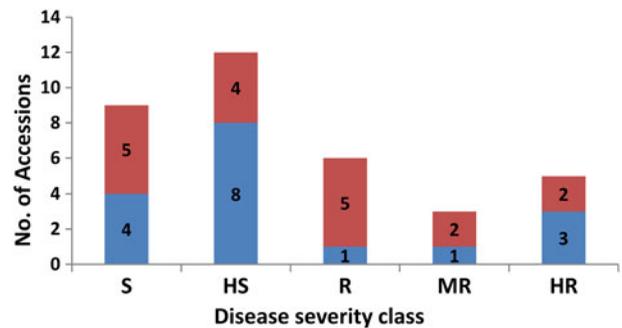


Fig. 3. Distribution of disease severity class for Fusarium wilt in *M. pruriens* germplasm (blue-var. *pruriens* and red-var. *utilis*).

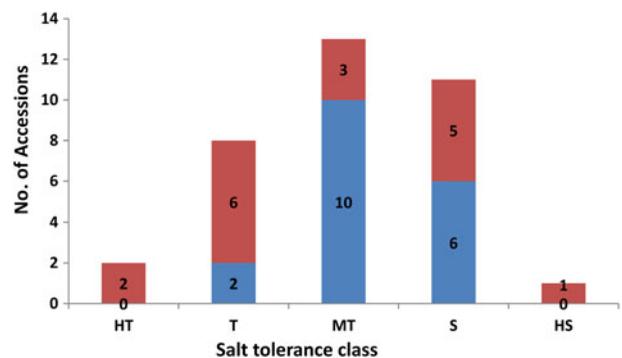
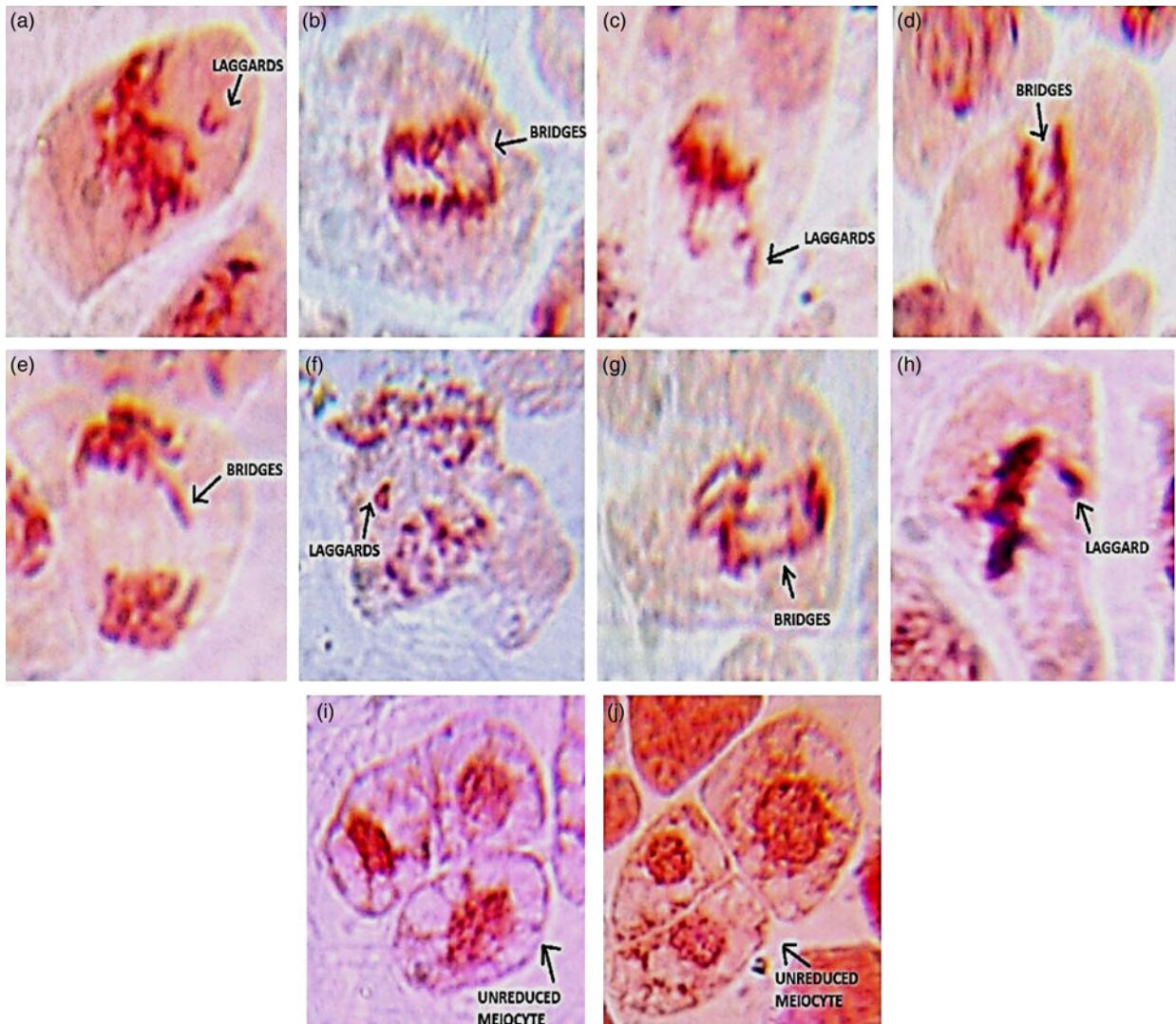


Fig. 4. Distribution of salt tolerance in *M. pruriens* germplasm (blue-var. *pruriens* and red-var. *utilis*).

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.

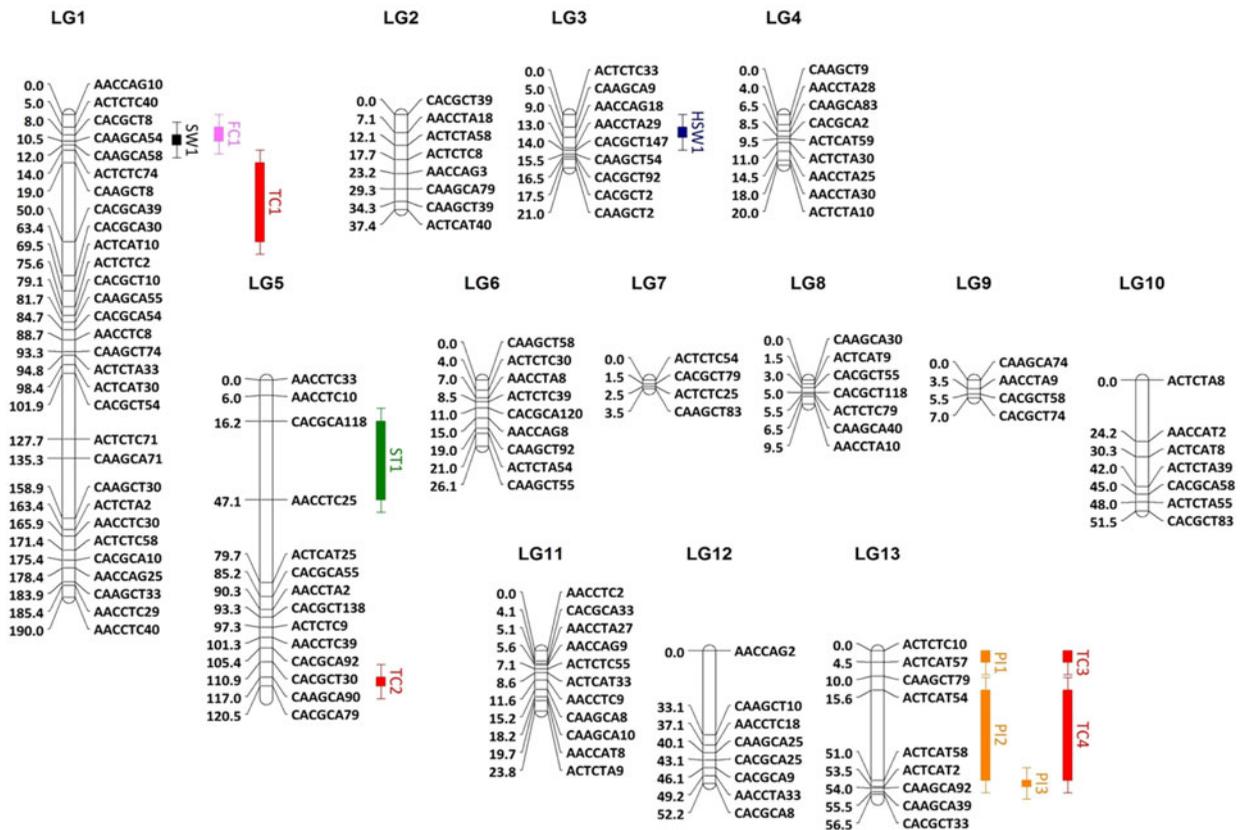
However, little is realized in terms of developing genomic resources for underutilized plant species in general and *M. pruriens* in particular. A lone linkage map using US core collection (Capo-chichi *et al.*, 2004), published earlier demonstrated prospects of good genome coverage for linkage studies 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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