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Mucuna Species: Recent Advances in Application of Biotechnology

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ABSTRACT

Biotechnology techniques have been widely used for legumes – important crops with excellent nutritional characteristics and soil improvement qualities. Limited work has been carried out with underutilized legume crops such as velvet bean (*Mucuna* spp.), which have great potential for multiple uses. In recent decades, there has been increased interest in the potential of *Mucuna* spp. as a cover crop and green manure for tropical and subtropical regions. *Mucuna* is also used as a minor food crop in many countries and interest in it as livestock feed – a common use for it in the early 1900s in the USA and elsewhere – is growing. Other minor uses exist, such as roasting the seeds as a coffee substitute. More importantly, L-dopa, extracted from *Mucuna* bean seeds and plants, is used for symptomatic relief of Parkinson's disease. Despite these numerous qualities, some constraints have limited its adoption. Biotechnology techniques can provide a window of opportunity for new or expanding products of *Mucuna*. Earlier biotechnology work with *Mucuna* was mostly related to its medicinal uses and focused on the mechanism of L-dopa production. More recently, biotechnology has also been applied to identify the major virus diseases affecting *Mucuna*, to develop new diagnostic methods for early virus indexing of *in vitro* plants and to clean virus diseases using meristem and thermotherapy techniques, as well as to study genetic diversity through the use of molecular tools. There are still niches to be explored such as the numerous phytochemical qualities of *Mucuna* that can be used to benefit human and animal nutrition and health as well as the environment through use of these compounds in natural weed and pest control management.

"There are few other crops that can be put to so many uses and give such satisfactory results"
John Scott, Florida Agricultural Experiment Station, 1911

Keywords: ELISA, feed, forage, green manure, L-dopa, molecular tools, TBIA, thermotherapy, tissue culture, viruses

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INTRODUCTION

Biotechnology can be defined as a technique that uses parts of or whole living organisms to make/modify a product to improve plants or animals or to develop micro-organisms for specific uses. It is a promising tool that can be used to solve specific problems in agriculture (Persley and Doyle 2001). Biotechnology can improve the productivity and nut-

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Invited Review

rient value of crops. It can also help to reduce the use of toxic agricultural pesticides and to improve the efficiency of fertilizers and improve other soil aspects (FAO 2004). The increasing growth of the world population as well as the drastic degradation of the natural resources (5-7 million ha of agricultural land are lost every year [Steiner 1996]) emphasises the need to look for more economical, viable and environmentally friendly alternatives to increase food production for the near future.

Legumes are increasingly important in efforts to increase food production. They are a low cost source of complementary proteins to the diet of both people and, comprising many types of forage, also livestock. Due to their nitrogen fixing capability, they also contribute to soil N, thereby improving the natural resource base and reducing the need for costly inorganic fertilizers (Jaiwal and Singh 2003a). Biotechnology techniques have been widely used for legumes such as *Glycine*, *Arabidopsis*, *Medicago*, *Vigna* or *Lotus* (Jaiwal and Singh 2003a, 2003b), which are often used as model crops. Limited work has also been carried out in under-utilized legume crops.

Velvet bean (*Mucuna* spp.) is one of these crops that have great potential for multiple uses but at the moment, it is still under-utilized. Despite previous research and extension efforts, *Mucuna* is not currently adopted by farmers on a scale that one would expect based on its production potential and in general, high nutritional quality. This is possibly due to a range of factors such as poor understanding of its diversity, content of anti-nutritional factors that limit its food/feed usage as well as the itchiness of the bristles on the leaves and pods of most species, although some glabrous genotypes occur naturally (the species name '*pruriens*' in Latin means 'itching sensation'). There is little exact knowledge on the total production of *Mucuna* worldwide. Some figures have been estimated in different locations: about 900,000 ha of *Mucuna* were cultivated annually worldwide (Duke 1981); more than 30,000 small-scale farmers in the hillsides of Central America were cultivating *Mucuna* as a green manure and cover crop (Flores 1997; Buckles *et al*. 1998); and 14,000 farmers were growing *Mucuna* as a cover crop in Benin (Honlonkou and Manyong 1999). Surveys sometimes consider cover crops as a whole: more than 125,000 farmers in southern Brazil and others in neighbouring countries (Calegari *et al*. 1997) and over 200,000 farmers in Central America (Flores 1997) have been using cover crops. Therefore, there is a relative paucity of information on *Mucuna*'s adoption in comparison to a relatively large number of scientific reports on *Mucuna* (many of them using modern technologies), demonstrating the need to further study and share information on the issue.

This overview of uses of *Mucuna* gives a general update about research in general, focusing on the use of biotechnology tools that may enhance the use of *Mucuna*, either improving the quality of the starting materials (seeds) or the yields of the final product (plant exudates *in vitro* to extract 3-(3,4-dihydroxyphenyl)-L-alanine [L-dopa]). These studies can contribute to increasing exciting, and sometimes unexpected, knowledge of the *Mucuna* plant, opening new perspectives and niches for better uses.

MAJOR AGRICULTURAL USES

Historical overview

Mucuna has a long and diverse history as a cultivated crop. It has been used in India for many centuries as a remedy in the ancient Ayurvedic system (traditional medicinal system in India [Zandu 2007]) to relieve symptoms of Parkinson's disease and in numerous countries various medicinal uses have been reported. Hundreds of references witness the widespread use, and intensive studies conducted on the feed, forage, and soil fertility improving qualities, in the southern states of the USA between the 1890s and 1940s. After the Second World War, its use quickly declined due to the decreased price of fertilizer and greater cultivation of soybean (Buckles 1995; Eilittä and Sollenberger 2002), and, perhaps more importantly, rapidly increasing labour prices and a significant shift from small family farms with mixed crop-livestock farming to large-scale operations that were focused either on crop or animal production (Eilittä and Sollenberger 2002). Similarly, green manure crops such as *Mucuna* and *Crotalaria* covered 240,000 ha in Zimbabwe, annually, in the 1920s to 1950s, with some use as livestock feed (Rattray and Ellis 1952). The 1990s witnessed an increased interest in the potential of cover crops and green manures among those interested in improving agricultural productivity in developing countries, and *Mucuna* was one of the most popular of those cover crops. Numerous extension efforts focused solely on *Mucuna* uses as a soil improver and weed suppressor, but *Mucuna* use has now become localized and limited, suggesting constraints to its wider adoption. When intercropped, competition with the main crop, most commonly maize, can occur if *Mucuna* is planted too early (typically before 40 days after sowing maize or other similar crop), and therefore low productivity and poor adoption can be expected in areas with a short rainy season. An adoption constraint reported in southern Veracruz, Mexico and elsewhere has been the poor knowledge of *Mucuna*'s uses beyond cover cropping, and therefore lack of farmer-use and markets for *Mucuna* products (Eilittä 1998). Constraints reported from West Africa include difficulties in planting on time (for adequate biomass to accumulate during intercropping), losses of biomass due to bushfires and animals during the dry season, as well as snakes under the *M. pruriens* var. *utilis* canopy (Galiba *et al*. 1998). It is unclear whether *Mucuna*'s L-dopa (an uncommon substance in plants which is also found in far smaller quantities in faba beans, *Vicia faba* L.) content (and consequent risk of poisoning), palatability, or other reasons have caused *Mucuna* to remain a minor food crop despite the fact that its proximate composition is similar to common beans and other grain legumes.

The potential to improve *Mucuna*'s utilization as a feed and food led, in the year 2000, to a coordinated research project on its utilization as a food and feed with scientists participating in Africa, Latin America, USA, and India, and with funding from the Rockefeller Foundation (Flores *et al*. 2002; Eilittä *et al*. 2003a). Since then, increasing attention has been given to the production of new and alternative crops and their bio-products for industrial or pharmaceutical uses (Morris 1999). A review of the nutritional potential of *M. pruriens* var. *utilis* was also done by Pugalenthi *et al.* (2005). Although most of the L-dopa (a non-protein amino acid neurotransmitter precursor) used for medicinal purposes is synthetic, there seems to be an increased demand for natural L-dopa production. According to some studies, the seed powder of *M. pruriens* showed faster anti-Parkinsonian activity than synthetic L-dopa (Mahajani *et al*. 1996; Rajendran *et al*. 1996; Hussain and Manyam 1997; Manyam *et al*. 2004a, 2004b), creating an increasing demand for natural plant extracts. In addition, powder made from *M. pruriens* cotyledons gave superior results when compared to synthetic L-dopa, which can induce DNA damage after several years of use (Tharakan *et al.* 2007). Misra and Wagner (2007) suggested that some components other than L-dopa might be responsible for the anti-Parkinson properties of *Mucuna* seeds. Interest in using vegetable dyes and tannins instead of synthetic alternatives is also growing because of concerns for environmental and human health (PROSEA 2003). As a consequence, although *Mucuna* is an under-utilised crop, there are increasing numbers of references to it in published works worldwide. The development of new and more accessible biotechnology techniques can catalyze the use of the available genetic resources by facilitating the identification of promising genetic traits and increasing their value for industrial and pharmaceutical use. This will allow sustainable conservation and rational use of biodiversity.

Soil improvement qualities (cover crop, green manure)

Mucuna's characteristics as a cover crop have been extensively studied. It is fast-growing, able to cover ground within 60-90 days (Carsky and Ndikawa 1998; Tarawali *et al*. 1999) in West Africa. It produces a great deal of biomass, and commonly in comparison of cover crops, *Mucuna*'s biomass production is the highest.

Extremely high biomass production has been documented in favourable conditions. In the TROPSOILS program trials in Brazil, *Mucuna* grown during the rainy season produced up to 8.5 t ha^{-1} of above-ground dry matter, containing 252 kg of N (Lathwell 1990); the following year maize yields in unfertilized plots in these fields were 4900 kg ha^{-1}. In Honduras, Triomphe (1996) found high average biomass in farmer fields of about 11.7 t ha^{-1}, when *Mucuna* was intercropped with maize in one season, and left to grow alone for the next. *Mucuna* biomass varied greatly with the system in an on-farm study in the humid zone of southern Veracruz, Mexico by Eilittä *et al*. (2003b). When *Mucuna* was grown as a sole crop during the main rainy season it generated a higher amount of biomass (7.3 t ha^{-1}, 147 kg N ha^{-1}) than when intercropped with first season maize (planted 40 days after maize) and left to grow alone in the second season (5.1 t ha^{-1}, 101 kg N ha^{-1}) or when intercropped as above, but slashed prior to the second season maize cultivation (2.8 t ha^{-1}, 50 kg N ha^{-1}).

Mucuna's popularity as a cover crop can partly be attributed to its wide adaptability. It was found to have the fastest growth amongst several legumes when grown for two years as a soil restorer on an eroded Alfisol (Wilson and Akapa 1983), and, in a pot experiment, *Mucuna* had the highest total biomass production of 12 legumes grown in high and low fertility soils with and without NPK fertilizer (Tian and Kang 1998). On depleted sites, *M. pruriens* has been reported to produce biomass yields of over 4 t ha^{-1} (Carsky and Ndikawa 1998) and *M. pruriens* var. *utilis* over 6 t ha^{-1} (Jiri 2004) in Zimbabwe. On acid P-deficient soils, productivity was increased by an average of over 30% with application of 16 kg ha^{-1} P and by over 50% with the addition of 500 kg ha^{-1} lime (Jiri 2004). Without P application the removal of *Mucuna* biomass as hay decreased maize yields the following year as compared to its retention; however, when *M. pruriens* var. *utilis* had been fertilized with P there was no significant difference in the *Mucuna* rotational effect when used as either hay or green manure (Jiri 2004). *M. pruriens* var. *utilis* and *M. deeringiana* were found quite tolerant to soil acidity and associated high aluminium levels in studies by Yost *et al*. (1985), Hairiah *et al*. (1991, 1993) and Hairiah (1992).

There have been relatively few studies on the impact of management on *Mucuna* productivity, with *Mucuna* planting density, planting date, and weeding typically determined by those of the main crop (commonly maize) within the intercropping systems, which have received most attention in the research and development efforts to date.

Mucuna as food and feed

Most commonly throughout Asia and Africa, the beans of *Mucuna* are used as a food. In India, various ethnic groups use *Mucuna* beans as a food; commonly, *M. utilis* is used (Janardhanan and Lakshmanan 1985; Rajaram and Janardhanan 1991) as well as *M. pruriens* (Mary Josephine and Janardhanan 1992; Mohan and Janardhanan 1993). Use of *Mucuna* beans has also been reported in Sri Lanka (Ravindran and Ravindran 1988), the Philippines (Laurena *et al*. 1994) and in Indonesia (K. Hairiah, University of Brawijaya, pers. comm.). In Sub-Saharan Africa, use as food has been reported at least in eastern Nigeria (several species), Ghana, Mozambique, Malawi, and Zambia (Onweluzo and Eilittä 2003). In most areas of Africa, *Mucuna* is a minor food crop and is considered a poor man's crop; its food use seems to be in decline at least in eastern Nigeria (Onweluzo and Eilittä 2003). Despite introduction as a cover crop, *Mucuna* has not found food uses in Latin America. Instead, it has been reported to be consumed as a coffee substitute in Guatemala (Buckles 1995) and southern Mexico (Buckles 1995; Eilittä 1998). Interestingly, interest in pursuing research on *Mucuna*'s food uses continues, particularly in eastern Nigeria, where numerous researchers have been conducting research over the past decades, and in Kenya, where two food science PhD studies are ongoing on *Mucuna*.

Studies on patterns of human food uses in countries where *Mucuna* is used as a traditional food or where it has been introduced as a cover crop, suggest that a temporary poisoning – gastrointestinal and neurological symptoms – does occur if consumed in large quantities (Piper and Tracy 1910; Miller *et al*. 1925; Bunch 2002; Price 2002). An outbreak of acute psychosis attributed to *M. pruriens* when the seeds were not properly processed (e.g., the water was not removed after boiling) was reported in Mozambique during a serious drought period (Infante *et al*. 1990). A survey of its use in eastern Nigeria revealed that *Mucuna* is incorporated in numerous dishes and quantities consumed can be relatively large (Ene-Obong and Carnovale 1992; Ezueh 1977; Ukachukwu and Obioha 1997). Whether those consuming *Mucuna* beans as a food can develop a certain tolerance to L-dopa (commonly seen in patients with Parkinson's disease) is not known. Several studies (Bressani *et al*. 2003; Diallo and Berhe 2003; Egounlety 2003; Wanjekeche *et al*. 2003) have documented the impact of processing on L-dopa content of *M. pruriens*. In short, these studies have confirmed the following methods as having potential in reducing L-dopa content: breaking or grinding of beans, followed by soaking (water to bean ratio as much as 40:1 as well as frequent water changes are often required) in room temperature water; boiling for long periods; utilization of acid or alkaline boiling medium; and fermentation (Eilittä *et al*. 2003a). Periods of long boiling and soaking may reduce nutrient content, however (Pugalenthi and Vadivel 2007).

Historically, *Mucuna* has also found widespread use as a livestock feed. Prior to the beginning of the 20th century, it has been reported to have been used as a fodder for cattle in Mauritius (Piper and Tracy 1910, citing Voigt 1845). Its most widespread use as animal feed was, however, in the United States where it was presumably accidentally introduced (Buckles 1995) and became quickly known as a promising feed when it was discovered by researchers at the Florida Agricultural Experimental Station in 1895 (Clute 1896). Already in 1902, its pods were marketed as cattle, poultry and pig feed (Miller 1902). As short-season varieties were discovered, *Mucuna* quickly spread from the southernmost states and by the late 1910s, it was grown on over 1.5 million hectares (Buckles 1995). Grown most commonly intercropped with maize, for dual functions of soil fertility improvement and feed, numerous systems of utilizing it as a feed developed. The most important of them was winter grazing by cattle and pigs, typically after the maize harvest and a frost. *Mucuna* beans and pods were also fed to cattle and pigs. Hay and silage were only infrequently made from *Mucuna*. At this time, *Mucuna*'s utilization as a feed also spread to southern Africa and South and Central America. Less common feed uses of *Mucuna* have been reported, such as consumption by the migratory elephants in the wilderness of Bengal in India (Santra *et al*. 2006). In southern USA, *Mucuna* has been mentioned as one of the potential forages for feeding wild deer (Cook and Gray 2005).

Studies on non-ruminant animals have been conducted mainly on poultry and have been recently reviewed by Carew and Gernat (2006). They have generally shown extremely poor tolerance to raw *Mucuna*, although lack of use of standard processing methods makes interpretation difficult. Toasting and wet heating of the beans do seem to decrease the adverse effects of *Mucuna*. Various changes in organ size and blood chemistry result from feeding *Mucuna* to poultry, but only some of them are linked to L-dopa. Little research on *Mucuna* as a pig feed has been

done in recent years, although in the first half of the 20th century, pigs commonly grazed on fields with *Mucuna*, or they were fed *Mucuna* pods or beans.

Numerous studies on ruminants showed no negative impacts on animal performance and health with consumption of *Mucuna* grain or foliage. Ayala-Burgos *et al*. (2003) and Sandoval-Castro *et al*. (2003) in examining whether the anti-nutritional compounds present in *Mucuna* (unspecified unknown cultivar produced locally) may depress the degradation activity of rumen micro-organisms, found high digestibility not only of seed dry matter but also of husk (the outer shell of the pod), with no negative impacts of anti-nutritional factors found *in vivo* with cows or *in vitro*. The husk had higher digestibility than many grasses used for ruminant nutrition in tropical Mexico. Similarly, in an *in vitro* study, Adesogan *et al*. (2004) found higher *in vitro* DM digestibility with *M. pruriens* than with soybean. Adding L-dopa to soybean changed the fermentation acid profile but did not affect the extent of digestion or gas production. *M. pruriens* fresh herbage supplementation enabled similar production of milk as *Gliricidia* supplementation in a 24-week study of Muinga *et al*. (2003) in Kenya. Chikagwa-Malunga *et al*. (2005), in determining the effect of replacing *M. pruriens* with soybean meal at four levels on lambs in Florida, found no differences in body weight gain. Matenga *et al*. (2003) found ensiling to lower L-dopa content in *Mucuna* seed by 10-47% in 100-30% crushed *Mucuna* with maize grain. Palatability to lactating goats and intake were increased with ensiling and doe weight loss decreased with a 50:50 mixture, but commercial dairy meal (16% crude protein) was preferred and resulted in better doe performance when fed iso-energetically. Mbuthia and Gachuiri (2003), when examining the integration of *M. pruriens* foliage in Napier grass-based silage in Kenya, found increased protein content and nitrogen balance in sheep. However, although *Mucuna* biomass inclusion at 50% by volume in maize silage increased the crude protein content to 13.6% (7.7% in pure maize silage), it did not ensile as well as many other legume forages (pH of 6.8; pure maize 3.9). Neither was its nutritive value as good with a digestibility of 46% compared to over 50% for all other silages, and the energy content was marginal (Maasdorp and Titterton 1997). No study mentioned any impacts on animal behaviour (seen in non-ruminants), even when this was a specific component of the study (Castillo-Caamal *et al*. 2003a, 2003b; Pérez-Hernandez *et al*. 2003) and even when growing lambs were offered only *Mucuna* for 10 days (Pérez-Hernandez *et al*. 2003).

Pests and diseases affecting *Mucuna*

Although *Mucuna* is generally known for its resistance to pests and diseases (Rich *et al*. 2003), possibly due to the high L-dopa content (Bell and Janzen 1971), there are a few reports of damage caused by pests and diseases. Rani and Sridhar (2004) reported the spotted pod borer (*Maruca testulalis*) as the major economically important pest that attacks the crop in India, amongst the ten arthropod pests tested. The *Mucuna* caterpillar (*Anticarsia gemmatalis*) has been reported as a major pest for *Mucuna*, soybeans and other legume crops (Rich *et al*. 2003) and was considered a major pest of *Mucuna* in the first half of the 20th century in the USA where it was extensively cultivated (Buckles 1995; Eilittä and Sollenberger 2002). Reports from Nigeria (Berner *et al*. 1992) associated necrotic crowns and numerous necrotic lesions observed along the roots and runners of *M. pruriens* var. *utilis* with *Macrophomina phaseolina* and considered that the disease can be a serious threat for *Mucuna*. Foliar fungal diseases (*Cercospora* leaf spot and angular leaf spot, *Phaeoisariopsis griseola*) and an unidentified viral disease were also reported in the USA as substantially reducing the biomass of *Mucuna* (Keinath *et al*. 2003). Brunt *et al*. (1996), in a compilation of previous work, reported the positive identification of one virus infecting *M. pruriens* (Sunnhemp mosaic tobamovirus - SHMV) and two in *M. deeringianum* (*Bean pod mottle comovirus* – BPMV and *Cowpea mosaic comovirus* – CPMV) (although taxonomists consider these two *Mucuna* species similar). *M. pruriens* sap reacted positively to several cowpea virus antisera (using ELISA test kits) in field surveys in Togo: the *Tobacco mosaic virus*-cowpea strain (TMV-CS) (Gumedzoe 1993a, 1993b) and *Bean Common Mosaic potyVirus* (BCMV), *Bean Yellow Mosaic potyVirus* (BYMV), *Cowpea Aphid Borne Mosaic potyVirus* (CABMV), *Cowpea Mottle carmoVirus* (CPMoV), *Cowpea Mosaic comoVirus* (CPMV), *Cowpea Severe Mosaic comoVirus* (CPSMV) and *Cucumber Mosaic cucumoVirus* (CMV) in routine tests of germplasm samples in Ethiopia (Proud *et al*. unpublished).

Mucuna seeds in storage can also contain a wide variety of moulds with aflatoxin and ochratoxin contamination that can pose potential health risks for its consumption. Recently, surface sterilization of the *M. pruriens* seeds using ionizing gamma irradiation decreased the microflora (Bhat *et al*. 2007a).

DISTRIBUTION, ECOLOGY AND GENETIC DIVERSITY

Mucuna originated in southern Asia, possibly India, Malaysia or southern China (Burkill 1966; Duke 1981; Wilmot Dear 1984). It was introduced in the USA in the late 1800s and from there re-introduced to tropical and subtropical areas (Duke 1981). Precipitation requirements are 650-2500 mm $year^{-1}$ (Skerman 1977) with seed multiplication difficult in semi-arid zones for most varieties due to the shortness of the growing season (Carsky *et al*. 1998). It is susceptible to water logging but tolerant to drought (Carsky *et al*. 1998). It grows well at altitudes of 0-2100 m above sea level, with an optimum temperature range of 19-27°C (Skerman 1977). It is sensitive to frost (Skerman 1977). Its life cycle is 100-290 days, with poorly understood photoperiodic effects (Keatinge *et al*. 1996). It tolerates a wide range of soils from sands to clays (Skerman 1977) and the optimal soil pH for its growth is 5-7 (Kiff *et al*. 1996; Weber *et al*. 1997). *Mucuna* can yield up to 12 t ha^{-1} of dry matter (Carsky *et al*. 1998). Although there are more than 100 species in the *Mucuna* genus only very few are cultivated (Duke 1981) and some (*M. gigantea*) are even in danger of extinction (Rajaram and Janardhanan 1993; Siddhuraju *et al*. 1993) due to overexploitation, indicating a need to conserve and study wild types. A compilation of the number of *Mucuna* accessions available in some of the larger genebanks is shown in **Table 1**. The largest collections are from Asia, most of the accessions being from *M. pruriens* or *Mucuna* sp.

There are a few reports on diversity studies in *Mucuna* species (Capo-chichi *et al*. 2001, 2003a, 2003b, 2004; Krishnamurthy *et al*. 2005; Kumar *et al*. 2006; Padmesh *et al*. 2006; Gupta and Kak 2007), although there is a conflicting taxonomic classification of the hundred *Mucuna* species (Buckles 1995) identified. Little is currently known about the diversity in species and there are still new species being discovered such as *M. japira*, recently described in south eastern Brazil (Tozzi *et al*. 2005), that differs from *M. sloanei* due to the presence of pseudoracemose inflorescences and the larger size of the standard petals. There are often several synonyms both at the generic and at the species level (Duke 1981; Buckles 1995) as well as local names that relate to the places where the plants are grown (Capo-chichi *et al*. 2003a) or where they originate from, or to their seed colour (Capo-chichi *et al*. 2001). Extensive and unsystematic germplasm exchange over the years probably contributed to different names being given to the same cultivar.

In addition to this confusion, different genera names were given to *Mucuna* in the past 200 years. It was incorrectly named *Dolichos multiforus* by McCarthy (Bort 1909), re-named as *M. pruriens* var. *utilis* by Bailey (1947) and simultaneously also named *Stizolobium deeringianum* (first reported in Jamaica by Browne in 1736 [Piper and Tracy

Table 1 Number of *Mucuna* accessions conserved *ex situ*, grouped by species from each genebank (that had more than four accessions of *Mucuna*).

Species	Institute														
	Africa					America		Australia		Asia					
	ARC	IITA	ILRI	KARI	MMARS	CENARGEN	CIAT	ATCFGR	CSIRO	AVRDC	CAAS	IPB	NBI	NBPGR	NPGRL
M. bennettii			1												
M. bracteata														1	
M. capitata														1	
M. curranii												6			
M. gigantea			1											1	
M. holtonii							1								
M. monosperma														2	
M. mutisiana							1								
M. nigricans														2	
M. platyphylla															
M. poggei			1	1											
M. rostrata			1												
M. sloanei							1								
M. urens							1								
M. pruriens	4	29	7	13		4	28	16		4	44		55	182	
M. sp	1		7	49	33	1	40	3	49	4				32	29
Total	5	29	18	63	33	5	72	19	49	8	44	6	55	221	29

Indirect sources: www.bioversityinternational.org/Themes/Genebanks/Germplasm_Collection_Directory/report.asp; www.ars-grin.gov/cgi/npgs/swish
ARC - Agriculture Research Council, Forage Genebank, Animal Production Institute Range and Forage - Lynn East, South Africa
IITA - International Institute of Tropical Agriculture, Genebank - Ibadan, Nigeria
ILRI - International Livestock Research Institute, Genebank - Addis Ababa, Ethiopia
KARI - National Genebank of Kenya, KARI - Muguga, Kenya
MMARS - Mount Makulu Agricultural Research Station - Lusaka, Zambia
CENARGEN - EMBRAPA - Brasilia, Brasil
CIAT - Centro Interancional de Agricultura Tropical - Cali, Colombia
USDA - Agricultural Research Service, National Plant Germplasm System, USA
ATCFGR - Australian Tropical Crops & Forages Genetic Resources - Biloela, Queensland, Australia
CSIRO - Townsville Division of Tropical Crops and Pastures - Townsville, Queensland, Australia
AVRDC - The World Vegetable Center, Taiwan
CAAS - Institute of Crop Science, Institute of Crop Science - Beijing, China
IPB - Institute of Plant Breeding, College of Agriculture, University of the Philippines - College, Laguna, Philippines
NBI - National Biological Institute, Centre for Plant Conservation - Bogor, Indonesia
NBPGR - National Bureau of Plant Genetic Resources, New Delhi, India
NPGRL - National Plant Genetic Resources Laboratory - Laguna, Philippines

1910]). *Stizolobium* genus was initially used to distinguish *Mucuna* from the perennial *Mucuna* sp., but later (Bailey 1947; Burkill 1966) all species were classified in the genus *Mucuna*. At the present time, according to the International Legume Database and Information Service (ILDIS 2005) many of the previously different species are considered varieties of *M. pruriens* (for example *M. aterrima*, *M. cochinchinensis*, *M. nivea*, *M. deeringiana*). Some authors, especially those from Asia, still use the old name of *Stizolobium hassjoo* (Obata-Sasamoto *et al*. 1981; Teramoto and Komamine 1988; Huang and Chen 1998; Sung and Huang 2000). This confusion emphasizes the urgent need to assess the genetic diversity of *Mucuna* and the possible relationships between and within species and within the existing accessions maintained in genebanks.

The first diversity study on *Mucuna* species evaluated 40 accessions (Capo-chichi *et al*. 2001) donated from the United States Department of Agriculture (USDA), Centre d'information et d'echange sur les Plantes de Couverture en Afrique (CIEPCA) and from Auburn University (UA) but originating only from 10 countries (USA, one from Singapore, and from numerous countries of Central and South America and Africa). All accessions belonged to either *M. pruriens* or *Mucuna* sp. Amplified Fragment Length Polymorphism (AFLP) techniques were used and the results were classified into two main clusters that related to phenological differences with respect to maturity. Capo-chichi *et al*. (2001) reported more heterogeneity amongst the exotic lines, compared to the USA landraces, a fact that is possibly related to their geographical origins. As a follow-up, Capo-chichi *et al*. (2003a) reported a second and more complete study also using AFLP, evaluating a total of 64 accessions, including all the previous 40, mainly from additional countries of Central and South America, but also one each from Thailand and China. This second study involved twice as many species (*Mucuna* sp., *M. pruriens*, *M. sloanei* and *M. mutisiana*) and showed a clear distinction between different taxa as well as considerable variation within the geographical range evaluated.

A third study was later conducted (Capo-chichi *et al*. 2004), where an intraspecific genetic map of *Mucuna* sp. was constructed based on AFLP markers to identify potential molecular markers linked to important traits. Seed coat colour, pod colour and pod pubescence were among the most important traits observed to differentiate *Mucuna* accessions. Colour varied from white to marbled brown or black and was either uniform or mottled. Pod pubescence was very variable and has been previously reported (Lubis *et al.* 1979) as a way to distinguish cultivated (less pubescence) and wild (dense, dark, and often itchy) species. In this study, Capo-chichi *et al*. (2004) suggested further studies using different AFLP and other primers to increase the resolution of the genetic maps. This AFLP linkage map provides future opportunities for selection and detection of loci controlling morphologically important traits (Capo-chichi *et al*. 2004).

The next study evaluated 19 diverse germplasm samples of *M. pruriens* collected in India (Krishnamurthy *et al*. 2005) for seed yield and associated agronomic characters. The presence of itchy trichomes were associated with higher levels of L-dopa, therefore accessions with non-itchy trichomes were selected for improved agronomic studies.

Padmesh *et al*. (2006) carried out another study with 15 Random Amplified Polymorphic DNA (RAPD) primers

using six accessions each of *M. pruriens* var. *pruriens* and var. *utilis* and a hybrid of both, all collected in southern India. Their results showed var. *pruriens* as being more genetically diverse than var. *utilis*. Similarly to Capo-chichi *et al*. (2001), they detected the formation of two major clusters and accessions within the var. *pruriens* grouping according to their original geographic location. They suggested that *M. pruriens* accessions may have acquired adaptability to changing environmental conditions. Unlike numerous other studies, including that by Capo-chichi *et al*. (2001), they found a uniform and relatively low distribution of the L-dopa content in most accessions, except for the hybrid that had a much lower content. They also concluded that although the favoured mode of reproduction of *Mucuna* is self-pollination, the potential benefits of hybrids are promising for both food and feed purposes, where low L-dopa content is desirable.

Recent studies evaluated the genetic diversity of rhizobia isolated from *M. pruriens*, using amplified 16S rDNA restriction analysis and found homogenous results within the five root-nodule isolates (Kumar *et al*. 2006). Genetic diversity for seed protein of 20 accessions of *M. pruriens* germplasm were analysed using SDS-poly-acrylamide gel electrophoresis (PAGE) (Gupta and Kak 2007). This study showed a range of 8 to 114 micro g/g seed within the accessions evaluated, with one accession clearly distinct from the others.

These molecular studies are important precedents for further work on the selection or improvement of genotypes for anti-nutritional factors, to either lower L-dopa content that could improve the utilization of *Mucuna* as a food or feed crop or to increase L-dopa content that would increase the potential of *Mucuna* for medicinal purposes. More characterization studies are needed in the future, involving as many *Mucuna* species as possible, both cultivated and wild, and should include species from the locations where *Mucuna* originates; India, Malaysia and China.

BIOTECHNOLOGY APPLICATIONS WITH *MUCUNA*

Biotechnology applications to research on *Mucuna* experienced a boom in the last few years due to wide interest in its use as a natural medicine, natural agro-chemical and pesticide and its allelopathic properties.

Chemical characteristics

The nutritional quality of *Mucuna* beans has been studied by numerous authors. Bressani (2002) compared the nutritional quality of *Mucuna* beans to common beans (*Phaseolus vulgaris*) and concluded that the proximate composition, amino acid and micronutrient content, anti-physiological substances, and protein quality and digestibility of *Mucuna* are in general similar to those of common beans and other edible grain legumes (**Table 2**). In a similar vein, *Mucuna* foliage has been reported to be a good quality fodder, with high crude protein and digestibility (**Table 3**). Although numerous indolic alkaloids have been reported present in *Mucuna*, results by Szabo (2003), using various samples of local cultivars of *M. pruriens* from Malawi, Benin, Nigeria and USA indicate that they are present only at low levels, unlikely to affect human or animal health.

M. pruriens bean protein concentrates (starch) have been shown to be good emulsifiers that could have good applications in the food system industry (Tolmasquim *et al*. 1970; Lawal and Adebowale 2004). *M. pruriens* has also been shown to be an excellent source of starch with many potential applications in food products requiring high temperature processing such as jams, jellies and canned products (Betancur-Ancona *et al*. 2002); its starch qualities have at times exceeded those of the commercial corn starch. The acetylated starch has high viscosity and good stability. Oxidised *M. pruriens* starch can also have good application in the production of lemon curd, salad cream and mayonnaise where low viscosity is required (Adebowale and Lawal 2003). Polysaccharide gums from *M. flagellipes* seeds can be used as additives in white bread to increase yield and loaf volume as well as to improve texture and delay hardening in stored bread (Onweluzo *et al*. 1999). The same type of gums also proved to be effective stabilizers in beef burgers without adverse impact on the quality of the product (Onweluzo *et al*. 2004).

Oil extracts from *M. sloanei* have been reported (Ajiwe *et al*. 1997) to have multiple potential uses for the preparation of resin, paint, polish, wood varnish, skin cream and liquid soap.

Antioxidant characteristics of *Mucuna*

Antioxidants are important chemicals in the prevention of human diseases (Rajeshwar *et al*. 2005a). They are also often used in the manufacture of oils and fatty foods to retard their autooxidation. The demand for natural antioxidants has increased recently due to the fear of synthetic antioxidants having carcinogenic characteristics. Methanol extracts of *M. pruriens* seeds showed strong antioxidant activity and also contained a noticeable amount of the total phenols that play a major role in controlling antioxidants (Tripathi and Upadhyay 2001). They can therefore be used as easily accessible sources of natural antioxidants, possibly as food supplements, and in the pharmaceutical industry (Rajeshwar *et al*. 2005a). Recent studies obtained significant *in vitro* lipid peroxidation and antimicrobial activity with these methanol extracts, showing a broad spectrum of activity against *Staphylococcus*, *Bacillus*, *Escherichia coli* and *Vibria cholera* (Rajeshwar *et al*. 2005b).

Table 2 A comparison of the nutritional quality of *Mucuna* beans and common grain legumes.

Nutritional factor	*Phaseolus vulgaris*	*Mucuna*
Amino acids and protein		
Protein (%)	22	23-35
Lysine (mg gN^{-1})	464	327-412
Total sulfur amino acids (mg gN^{-1})	125	116-132
Minerals (mg $100g^{-1}$)		
Sodium	40-210	4.1-70.0
Potassium	1320-1780	1110.0-2537.0
Calcium	70-210	247.0-518.3
Magnesium	160-230	72.4-506.5
Phosphorus	380-570	194.3-459.0
Manganese	1.0-2.0	0.31-2.36
Iron	3.34-8.00	1.30-9.42
Zinc	1.4-6.5	1.00-8.24
Fiber fractions (g 100 g^{-1})	***Cajanus cajan***	***Mucuna***
Acid detergent fiber (ADF)	9.1	8.9-10.4
Neutral detergent fiber (NDF)	15.1	14.7-20.4
Hemicellulose	6	5.8-10.0
Cellulose	6.9	7.1-9.3
Lignin	2.2	0.8-1.8

Adapted from Bressani 2002.

Table 3 A comparison of the nutritional quality of *Mucuna* foliage in three studies.

	Foliage (g kg^{-1})			
	Crude Protein	**Neutral Detergent Fiber**	**Acid Detergent Fiber**	**Tannin**
Muinga *et al.* 2003	100	530	109	290
Mbuthia and Gachuiri 2003	166	497	389	
Nyambati *et al.* 2003	115-179*	374-419*		

* From different seasons and experiments.

Potential use of biotechnology to enhance medicinal compounds in *Mucuna*

Numerous benefits of *Mucuna* are cited for a wide range and variety of diseases and conditions including Parkinson's disease (Gourie-Devi *et al*. 1991; Hussain and Manyam 1997; Vanisree *et al*. 2004; Mercuri and Bernardi 2005), diabetes (Akhtar *et al*. 1990; Rathi *et al*. 2002a, 2002b; Kar *et al*. 2003; Negri 2005), cancer (Jain and Tarafder 1970; Gupta *et al*. 1997), epilepsia (Gupta *et al*. 1997; Rajeshwar *et al*. 2005b), blood cholesterol (Iauk *et al*. 1989), poor male virility (Amin *et al*. 1996; Siddhuraju *et al*. 1996; Lorenzetti *et al*. 1998; Miocinovic *et al*. 2005; Guo *et al*. 2007), abortion (Casey 1960; Nath *et al*. 1992), syphilis, diarrhoea (Girach *et al*. 1994), intestinal parasites (Joshi *et al*. 1980; Weniger *et al*. 1986; Vitalyos 1979; Mors *et al*. 2001), snake bite (Houghton and Skari 1994; Aguiyi *et al*. 1999), kidney stones (Pushpangadan and Atal 1984), and tooth ache (Iauk *et al*. 1993). *Mucuna* has also been used as an aphrodisiac (Amico 1977; Bhattarai 1992; Bhandary *et al*. 1995) and for memory retrieval and to improve learning (Poornachandra *et al*. 2005). Some scientific results support these popular beliefs, with findings from ethnobotany giving credit and scientific support to the often ancient knowledge on the plant.

L-dopa was discovered to have a therapeutic effect on Parkinson's disease in 1961, after a severe brain dopamine deficit was discovered in patients with Parkinson's disease (Barbeau and McDowell 1970; Hornykiewicz 2002). A great deal of interest in natural sources for L-dopa resulted (Daxenbichler *et al*. 1971), but *Mucuna* was the only species of more than 1000 species surveyed from 135 plant families with sufficient L-dopa quantities to justify its further use for commercial production (Daxenbichler *et al*. 1971). Natural L-dopa continues to be used in India and for natural medicines, promoting the use of biotechnology for L-dopa production in *Mucuna*.

Potential use of biotechnology for manipulation of L-dopa content

Mucuna has a variable concentration of L-dopa within parts of the plant, being minimal in dried leaves and pods (0.15%), low in fresh leaves (0.21-0.23%) and dried roots (0.27%), intermediate in stems (0.26-0.34% fresh and 0.49% dried), and maximal in raw seeds (ranging between 1.9 and 9.0% dry matter) (Bell and Janzen 1971; Duke 1981; St.-Laurent *et al*. 2002; Szabo and Tebbett 2002). L-dopa concentration was initially considered to be both environmentally and genetically controlled (Lorenzetti *et al*. 1998; St.-Laurent *et al*. 2002), with production highest near the equator with high light intensity. A recent study contradicted the importance of latitude on the content of L-dopa (Capo-chichi *et al*. 2003b). However, early maturing types had low levels of L-dopa and late maturing types had maximal values of L-dopa (Capo-chichi *et al*. 2003b). The colour of the seeds was also often related to the content of L-dopa; darker seeds having more L-dopa than light coloured seeds (Siddhuraju *el al*. 2000; Capo-chichi *et al*. 2003b). Mamatha *et al*. (2006), in an evaluation of 13 genotypes of *Mucuna* in India, suggested a positive relationship between nitrogen fixation, nodule numbers and biomass, and the L-dopa content in seeds.

Recent research also examined *M. pruriens* seeds by Electron Spin Resonance (ESR) spectroscopy detecting free radicals naturally present or produced after radiation (Bhat *et al*. 2007b), suggesting possible uses of gamma irradiation on *Mucuna* seeds for quarantine purposes. The same authors (Bhat *et al*. 2007c) also found that various doses of this ionizing radiation can reduce phenolics, tannins, phytic acid and L-dopa, emerging as an important technique to improve nutritional or pharmaceutical qualities of *Mucuna*.

Veterinary uses of *Mucuna*

Trials in India found that trichomes from *M. pruriens* were as effective as commercial antihelminthics at reducing parasite loads of pregnant goats. This treatment significantly reduced kid mortality, increased kid growth rates and shortened kidding intervals (Conroy and Thakur 2005). The treatment is traditionally used by buffalo herders for their animals and was more popular with the goat farmers than the commercial product, due to its availability and cheapness.

Crude extracts of *M. pruriens* leaves reduced the parasite-induced fish mortality of goldfish (*Carassius auratus auratus*) demonstrating potential for the effective control of *Ichthyophthirius multifiliis*, one of the most pathogenic parasites in aquaculture (Ekanem *et al*. 2004).

Using phytotoxins for weed control

There is a great potential for the use of natural phyto-toxins, including allelochemicals, to develop new tools for weed and pest management. In the future, biotechnology might be applied in this area to produce these natural phyto-toxins for wider use in agricultural systems.

Mucuna has been known to efficiently control or smother notorious weeds such as *Cyperus* sp., *Imperata cylindrica* (Fujii *et al*. 1991), *Rottboellia cochinchinensis* (Valverde *et al*. 1995; Dominguez Monge *et al*. 2004), as well as *Amaranthus spinosus*, *A. hybridus*, *Cenchrus insertus* and *Parthenium hysterophorus* (Caamal-Maldonado *et al*. 2001) and *Cyperus rotundus* (Carvalho *et al*. 2002). The L-dopa content in the leaves (relatively low) and seeds (ten times more than the leaves) act as an allelochemical (Fujii *et al*. 1991; Fujii 2003) inhibiting the growth of susceptible species. Allelopathy results from the presence of natural organic chemicals in plant tissue (Fujii 2003). L-dopa has been found to be one of the most active allelochemicals present in plants. Species screened for susceptibility showed distinct responses, with root elongation being more suppressed than shoot growth (Hachinohe *et al*. 2004). Leaf extracts of *M. pruriens* have also been reported to affect the root and aerial growth of *Sorghum halepense* (Dominguez Monge *et al*. 2004), being therefore prejudicial if intercropped together. *M. pruriens* extracts at 3 and 4% completely inhibit seed germination of the weeds *Imperata brasiliensis* and *Ageratum conyzoids* (Casini 2004). Lettuce, *Lactuca sativa* (a typical species used to test allelopathic properties [Hachinohe *et al*. 2004; Nishihara *et al*. 2004, 2005]) showed high susceptibility to L-dopa, especially in the roots, while *Echinochloa crus-galli* showed tolerance (Hachinohe *et al*. 2004). The species- selective phytotoxicity to L-dopa was at least partially due to plant metabolism and not to absorption or translocation of L-dopa. It has also been shown (Fujii 2003; Hachinohe *et al*. 2004; Nishihara *et al*. 2004, 2005; Ooi 2005) that in addition to the L-dopa content of the leaves and seeds, the roots of *M. pruriens* also contain and exude L-dopa, the concentration varying with the *Mucuna* cultivar (Nishihara *et al*. 2004, 2005). L-dopa can also be leached out of the leaves by rain (Fujii 2003). L-dopa seems to suppress growth of some broad leaf weeds but little effect has been shown in Poaceae (*Gramineae*) (Fujii *et al*. 1991; Nishihara *et al*. 2004; Ooi 2005) and Fabaceae (*Leguminosae*) (Fujii *et al*. 1991) families, although Hachinohe *et al*. (2004) reported that susceptibility to L-dopa was not correlated with any type of species (monocotyledonous vs. dicotyledonous and C_3 vs. C_4 species). Furthermore, Batish and Gupta (2006) reported reduced root numbers and length on the rooting potential of mung bean (*Phaseolus aureus*) due to high levels of L-dopa application. They also studied the effect of L-dopa on the mitotic index in onion (*Allium cepa*) and found out that L-dopa completely inhibited the cell division in the root tips. The effect of L-dopa on radicle and hypocotyl growth of nearly 70 species has been reported (Fujii *et al*. 1991; Hachinohe *et al*. 2004; Nishihara *et al*. 2004, 2005; Batish and

Table 4 Species susceptible to L-dopa for radicle growth.

Family	Scientific name	L-dopa ($\mu g\ ml^{-1}$)	Susceptibility	Reference
Caryophyllaceae	*Cerastium glomeratum*	50	++	Fujii *et al.* 1991
Caryophyllaceae	*Spergula arvensis*	50	++	Fujii *et al.* 1991
Compositae	*Chrysanthemum coronarium*	1.97	++	Hachinohe *et al.* 2004
Compositae	*Cosmos bipinnatus*	1.97	+	Hachinohe *et al.* 2004
Compositae	*Lactuca sativa*	50	++	Fujii *et al.* 1991
Compositae	*Lactuca sativa*	1.97	++	Hachinohe *et al.* 2004
Compositae	*Lactuca sativa*	250	++	Nishihara *et al.* 2004
Compositae	*Lactuca sativa*	not specified	++	Zasada *et al.* 2006
Compositae	*Solidago altissima*	50	+	Fujii *et al.* 1991
Cruciferae	*Brassica oleracea*	1.97	++	Hachinohe *et al.* 2004
Cruciferae	*Brassica pekinensis*	1.97	+	Hachinohe *et al.* 2004
Hydrophyllaceae	*Phacelia campanularia*	250	++	Nishihara *et al.* 2004
Leguminosae	*Astragalus sinicus*	250	+	Nishihara *et al.* 2004
Leguminosae	*Glycine max*	19.7-197	+	Soares *et al.* 2007
Leguminosae	*Medicago sativa*	250	+	Nishihara *et al.* 2004
Leguminosae	*Trifolium pratense*	250	+	Nishihara *et al.* 2004
Leguminosae	*Trifolium repens*	250	++	Nishihara *et al.* 2004
Leguminosae	*Vigna radiata*	1970	+	Batish and Gupta 2006
Liliaceae	*Allium fistulosum*	1.97	+	Hachinohe *et al.* 2004
Liliaceae	*Allium cepa*	1970	+	Batish and Gupta 2006
Linaceae	*Linum usitatissimum*	50	++	Fujii *et al.* 1991
Solanaceae	*Lycopersicum esculentum*	not specified	+	Zasada *et al.* 2006

++ Radicle length <30% of the control
+ Radicle length <50% and > 30% of the control

Gupta 2006) and a summary of the species with either strong or moderate susceptibility responses to the L-dopa application 3-5 days after germination is shown in **Table 4**.

Using phytotoxins for pest control

In addition to weed control, *Mucuna* has also been reported to control the survival of nematodes (Vargas-Ayala *et al.* 2000; Lopes *et al*. 2005) and perhaps also insects (Caamal-Maldonado *et al*. 2001). Leachates of both *Mucuna* and jackbean (*Canavalia ensiformis*) had nematotoxic effects. Their dry leaves incorporated as mulch into the soil significantly reduced the development of phytopathogenic nematodes in the roots of tomato. Dried powders of *Mucuna deeringiana* leaves and stems added to soil reduced *Rhizoctonia solani*-induced disease on soybeans (Blum and Rodriguez-Kabana 2006). Vargas-Ayala *et al*. (2000) investigated the population and species diversity of bacteria and fungi in soils and rhizosphere at the end of a *M. deeringiana* cropping cycle. They concluded that the use of *Mucuna* in the cropping system altered the microbial communities of the soil and rhizosphere, resulting in nematode control. However, it was recently reported that the natural L-dopa transformation reaction was accelerated in soils with high pH values (Furubayashi *et al*. 2005, 2007). Roots and lower stems of various legumes (including *M. aterrima*) were very compatible with the growth of the pathogen *Fusarium oxysporum* (Dhingra and Netto 2001), and therefore these legumes were not advisable for rotation in infested bean fields.

In vitro techniques for production of L-dopa

The increasing demand for natural L-dopa and the high doses required for medical treatment justified further studies on using biotechnology to improve production. Plant tissue culture shows major potential for the industrial production of bioactive plant metabolites (Giri *et al*. 2001; Rao and Ravishankar 2002). Large-scale plant tissue culture can be an attractive alternative approach to traditional agriculture, offering a controlled and independent biochemical supply from a sterile environment. Biotechnology has also been used to understand the mechanisms of L-dopa biosynthesis and suppression of L-dopa accumulation (Obata-Sasamoto *et al*. 1981).

In vitro techniques for *Mucuna* have focussed on quantification of L-dopa content and its properties, not only for seeds but also for other plant parts and plant exudates. The first reports on plant cells exudates from *M. pruriens* are from Brain (1976), who found that inclusion of di-chlorophenoxy acetic acid (2,4-D) in the tissue culture media increased the amount of L-dopa that was secreted into the medium in concentrations of about 1%. In contrast, Wichers *et al*. (1985) found that 2,4-D inhibited cell growth of *M. pruriens* as well as L-dopa production. No clear reason was found for this discrepancy with the earlier reports of Brain (1976). Another contrast was also reported: cell cultures of *M. deeringiana* (Remmen and Ellis 1980) did not accumulate L-dopa, catabolizing this product into stizolobic acid and carbon dioxide. Similarly, Obata-Sasamoto *et al*. (1981) studied the mechanism of suppression of L-dopa accumulation in callus cultures of *S. hassjoo*, encountering lower values of L-dopa in callus than in the intact plant. Huizing *et al*. (1985) demonstrated the presence of L-dopa in cell suspension cultures of *M. pruriens* using TLC (thin layer chromatography) and HPLC (high pressure liquid chromatography), where L-dopa accumulated largely within the cells without the browning of the medium. They reported, however, browning of the tissues and cessation of growth after six months of culture, recommending using callus stock cultures after a relatively short time after their initiation. Teramoto and Komamine (1988) induced callus of the three species *M. hassjoo*, *M. pruriens* and *M. deeringiana* and optimized the culture conditions, maximizing the L-dopa content to 8% of fresh weight in *M. hassjoo*.

The adequate balance of the media composition as well as the environment appears crucial for maximal L-dopa production. Both Huizing *et al*. (1985) and Pras *et al*. (1993) obtained maximal accumulation of L-dopa in cell suspension cultures (2% and 6% of dry weight, respectively) of *M. pruriens* derived from callus, under 1000 Luminous flux density at a surface (lux) and continuous light, respectively. Interesting results were obtained from detailed studies (Wichers *et al*. 1993) comparing the development of L-dopa content of roots, stems and leaves of *M. pruriens* seedlings with the cell suspension cultures of the same species *in vitro*. They found not only L-dopa (in roots, stems and leaves), but also dopamine (only in the leaves). They also found dopamine in the cell suspension cultures. Dopamine concentration increased with the addition of 2,4-D, which suppressed both cell growth and L-dopa accumulation. The ad-

dition of salt (NaCl) to the medium did not affect the dopamine but increased the release of L-dopa into the medium. Chattopadhyay *et al*. (1994) optimized the nutritional requirements for maximum cell growth and L-dopa production in a cell suspension culture of *M. pruriens f. pruriens* using a two-stage culture system for 30 days. They obtained maximal L-dopa production (4% of dry weight) under continuous illumination and 4% sucrose using half strength MS (Murashige and Skoog 1962) media, 0.5 mg l^{-1} kinetin and 42.5 mg l^{-1} KH_2PO_4. However, they recommended further validation of the method on a larger scale using bioreactors. Detailed studies with bioreactors varied the gaseous composition (Huang and Chou 2000), aeration and agitation rates (Huang *et al*. 2002), nutrient feed modes, root tip density and oxygen tension (Huang *et al*. 2004) as well as nutrients and nitrogen sources (Huang and Chou 2005) to maximize L-dopa metabolite production using *S. hassjoo* plant materials.

A study in China (Huang and Chen 1998) reformulated the MS media to suit L-dopa production, increasing by 10 and 20% the calcium and phosphate and eliminating the zinc and organic supplements. These changes, combined with adjustments on the copper, cobalt and indol-3-acetic acid (IAA) content, more than doubled the L-dopa production, using *S. hassjoo* in a two-stage culture system. Altogether, a 4-fold increase of L-dopa was obtained with adequate and controlled aeration and agitation rates using laboratory large scale bioreactors, showing the possibility of efficiently scaling up the system. Similarly, Kavitha and Vadivel (2005) reported higher L-dopa accumulation from *M. pruriens* using an MS based media supplemented with IAA and 6-benzylaminopurine (BAP), compared to MS media with only IAA, BAP, 2,4-D or kinetin.

Gamborg-B5 (Gamborg *et al*. 1968) media, often used to successfully propagate legume cultures (George 1993), was also optimized to promote L-dopa production from root hairs of *S. hassjoo* (Sung and Huang 2000). In just 16 days of culture they obtained a maximal value of L-dopa of 10.8% of dry weight, an almost 3-fold increase compared to control media with using basal Gamborg-B5. More recently, the same authors (Sung and Huang 2006) used an innovative mesh as a penetration barrier to root tips of *S. hassjoo* in order to enhance lateral root bridging growth in a rational design of a root bioreactor, producing a two-fold L-dopa metabolite.

Commercial exploitation of L-dopa production was done by the Zandu Foundation of Healthcare (DBT 2003) through optimising the tissue culture conditions and improving the starting material of *Mucuna* through conventional breeding before transfer to Zandu Pharmaceuticals (Mumbai) together with the appropriate agricultural package.

In vitro techniques for plant propagation

Micro-propagation is used to rapidly multiply plants on a large scale. The high demand for large and consistent amounts of natural L-dopa can increase the market value of *Mucuna* and may create a new promising market. In addition to using cultivated *Mucuna* as a source of L-dopa, pharmaceutical companies have been extracting L-dopa from wild populations of *Mucuna* (Faisal *et al*. 2006a), but their availability is limited. Tissue culture of elite genotypes provides the opportunity for large scale multiplication of healthy plants for field or *in vitro* production throughout the year without any seasonal constraints. Even more important, tissue culture techniques open a window of opportunity for future genetic manipulation studies with *Mucuna* species.

During the optimization of the culture media for L-dopa production, Huang and Chen (1998) reported that the media composition that favours plant cell growth of *Stizolobium hassjoo* may be sub-optimal for the production of secondary metabolites and *vice versa*. This was also observed by Chattopadhyay *et al*. (1995) and by Jorge *et al*. (2006) in their efforts to propagate *Mucuna* using *in vitro* techniques. A wide range of tissue culture conditions have been reported to produce L-dopa and to propagate *Mucuna in vitro* (**Table 5**). Despite this extensive previous work in tissue culture to produce L-dopa, the media had to be adjusted and sometimes even re-invented to regenerate *Mucuna* plants.

Research in India aimed to develop a suitable system to micro-propagate *Mucuna*. Chattopadhyay *et al*. (1995) were the pioneers, using explants (epicotyls, hypocotyls and

Table 5 Major tissue culture conditions used to either produce L-dopa or to propagate *Mucuna pruriens* and *Stizolobium hassjoo in vitro*.

Plant	Growth regulators	Other conditions	Culture type	L-dopa (% of dry weight)	Reference
S. hassjoo	2,4-D/kinetin	MS	Callus	8.0 (% fresh weight)	Teramoto and Komamine 1988
S. hassjoo	2,4-D/kinetin	MS	Callus	0.17	Obata-Sasamoto *et al.* 1981
M. pruriens	2,4-D	MS, 3% suc	Callus	not specified	Kavitha and Vadivel 2005
M. pruriens	2,4-D	not specified	Cells	2.5 (% fresh weight)	Brain 1976
S. hassjoo	IAA	MS	Cells	0.50	Huang and Chen 1998
M. pruriens	kinetin	MS, 2% suc, cont. light	Cell suspension	4.00	Chattopadhyay *et al.* 1994
M. pruriens	not specified	1000lux	Cell suspension	6.00	Pras *et al.* 1993
M. pruriens	IAA/BAP	MS, 4% suc, cont. light, pH 5.9	Cell suspension	1.80	Huizing *et al.* 1985
M. pruriens	2,4-D/IAA/BAP	MS, 3% suc, cont. light (1500 lux)	Cell suspension	3.50	Wichers *et al.* 1985
M. pruriens	2,4-D	MS, 4% suc	Cell suspension	2.10	Wichers *et al.* 1993
M. pruriens	IAA/BAP	MS, 3% suc	Cell suspension	not specified	Kavitha and Vadivel 2005
S. hassjoo	not specified	[O_2=0.3atm; CO_2<5%]**	Cell suspension	not specified	Huang and Chou 2000
S. hassjoo	not specified	[0.06 vvm and 300 rpm]**	Cell suspension	not specified	Huang *et al.* 2002
S. hassjoo	not specified	[intermitent feeding; 40 roots l-1]**	Cell suspension	not specified	Huang *et al.* 2004
S. hassjoo	not specified	[$(NH_4)_2SO_4$; KNO_3; amino acids]**	Cell suspension	not specified	Huang and Chou 2005
M. pruriens	BAP/NAA	B5, 3% suc, pH 5.6	meristem culture	-	Jorge *et al* unpublished
M. pruriens	NAA	RT, 3% suc	rooting	-	Chattopadhyay *et al.* 1995
M. pruriens	IAA	MS, 3% suc, pH 5.8	rooting	-	Faisal et *al.* 2006a, 2006b
S. hassjoo	none	B5, 3% suc	root hairs	10.80	Sung and Huang 2000
S. hassjoo	none	B5, 3% suc, mesh hindrance*	lat. root bridging**	7.70	Sung and Huang 2006
M. pruriens	BAP	leaf extracts, 3% suc, pH 5.6	shoot elongation	-	Jorge *et al.* 2006
M. pruriens	BAP	B5, 3% suc, pH 5.6	shoot regeneration	-	Jorge *et al.* unpublished
M. pruriens	NAA/2iP	RT, 3% suc, pH 5.7	shoot regeneration	-	Chattopadhyay *et al.* 1995
M. pruriens	BAP/NAA	MS, 3% suc, pH 5.8	shoot regeneration	-	Faisal *et al.* 2006a, 2006b

B5 = Gamborg B5 media; MS = Murashige and Skoog media; RT = Revised Tobacco media;
suc. = sucrose; cont. = continuous; lat. =lateral
vvm = Volume of air per volume of culture per minute
rpm = Rotation per minute
mesh hindrance* = Mesh hindrance mist trickling reactor
** = bioreactors

Fig. 1 Micropropagation techniques used for *Mucuna pruriens* at ILRI. (**A**) A sequence of meristems growing into shoots, after the application of themo therapy techniques to obtain virus-free materials. (**B**) Phenolic compounds produced in the tissue culture media. (**C**) Dry *Mucuna* leaves and stems used to replace MS nutrient salts of the media to proliferate *Mucuna in vitro*. (**D**) Fully grown *Mucuna* plant with roots, ready to be acclimatized. (**E**) *Mucuna* plants during acclimatization. (**F**) Green and soft callus tissues of *Mucuna* produced *in vitro*.

hypocotyls plus cotyledons) from one-week old seedlings to initiate *in vitro* cultures. They used either MS or Revised Tobacco media (RT), (Kaul and Staba 1968), with a range of α-naphthaleneacetic acid (NAA), IAA, BAP, 2-isopentenyladenine (2-iP) or kinetin concentrations. Best shoot regeneration was obtained in three weeks with explants extracted from 7-day old seedlings, using hypocotyls with cotyledons in RT media (Kaul and Staba 1968) and 2.7 µM of NAA and 9.8 µM of 2-iP. Kavitha and Vadivel (2005) reported that an MS media supplemented with 2,4-D induced callus in stem, leaves and root segments of *M. pruriens*. Subsequent research (Faisal *et al*. 2006a, 2006b) to develop a rapid *in vitro* regeneration method used nodal explants from 2-week old plantlets germinated *in vitro*. They also tried a range of hormone concentrations with BAP, kinetin and 2-iP either alone or in combination with NAA. They also tested four (full, half, third or fourth strength) concentrations of MS media as well as different pH levels. Half strength MS medium, when supplemented with 1 mg l^{-1} of BAP and 0.1 mg l^{-1} of NAA and pH of 5.8, produced the highest shoot numbers, regeneration and elongation. Optimal rooting was obtained with 0.2 mg l^{-1} of indol-3-butyric acid (IBA) supplemented medium and *M. pruriens* was successfully acclimatized in the greenhouse.

M. pruriens meristem culture and growth of virus free plants has been used to enhance and promote the conservation and availability of clean germplasm of *Mucuna*, mainly for forage purposes (Jorge *et al*. 2006). Tissue culture techniques were used to clean plants of three accessions of *M. pruriens* that were heavily infected with seed transmitted viruses. A rate of 50% clean materials was obtained using a combination of thermotherapy and meristem techniques for all the accessions. The meristems were regenerated into shoots (**Fig. 1A**) using MS medium, BAP, NAA and citric acid so that phenolic compounds (**Fig. 1B**) that compromised growth *in vitro* could be eliminated. A series of medium combinations were then tested to induce shoot multiplication, many of them adapting the best media reported earlier (Chattopadhyay *et al*. 1995; Faisal *et al*. 2006a, 2006b). Amongst the various cytokinins (BAP, Kinetin, 2-iP and thidiazuron [TDZ]) and gibberellins (gibberellic acid [GA_3]) tested, the survival of tissues (both shoots and callus) was found to be effective in BAP combined with an auxin (NAA). The cytokinins BAP (between 0.1 and 0.25 mg l^{-1}) and TDZ (between 0.25 and 0.5 mg l^{-1}) induced multiple shoots. Rooting was induced at the base of the shoots proliferated on media containing lower concentrations of BAP. However, shoots proliferated in TDZ containing media were difficult to grow. An optimal level of 0.1 mg l^{-1}.of BAP supplemented liquid media containing ¼ of Gamborg B5 salts plus 7.5 g l^{-1} sucrose supplemented with 5 mg $l^{-1}$of IBA was effective in root induction. The addition of vitamins (adenine and thiamine) induced initial shoot formation from meristems but at later growth stages, media without vitamins maintained shoots alive and green for a longer period (Beksissa 2006). However, a strong genotype response to media was observed and a distinct medium was developed for each of the three accessions used in the research. A traditional medium was adopted for accession ILRI 14880 (Gamborg-B5 plus 0.1 mg l^{-1} BAP, vitamins [20 mg l^{-1} thiamine and 2mg l^{-1} adenine], 100 mg l^{-1} citric acid, 30 g l^{-1} sucrose and 8 g l^{-1} agar) but this was not adequate for the other two accessions (ILRI 10084 and ILRI 15169) that were particularly difficult to grow *in vitro*. A new medium for tissue culture was developed using leaf extracts of *Mucuna* plants to substitute for nutrients in the medium (**Fig. 1C**). The medium was prepared from ground dry leaves and stems (6.25 g l^{-1}) of *M. pruriens* (accession ILRI 14880) mixed with water and filtered, supplemented with 0.1 mg l^{-1} BAP, 30 g l^{-1} sucrose and 8 g l^{-1} agar. (Jorge *et al*. 2006). This new medium replaced the Gamborg-B5 nutrients and no extra vitamins were required, reducing the cost of the media by half when compared to using the traditional medium. This can be a useful methodology not only to reduce costs of production for general tissue culture but also to solve the problems of regenerating difficult-to-grow cultures *in vitro*. Plants were successfully acclimatized in the greenhouse using a pine bark substrate (**Figs. 1D, 1E**), compared to no survival obtained with a mixture of soil, peat or vermiculite (Jorge *et al*. unpublished).

Practical considerations in disease diagnosis

Mucuna, like many legumes, has a high level of seed-borne virus transmission. Tissue culture has been used extensively to clean plants of diseases, in particular viruses which are systemic and do not generally respond to chemical treatments as bacterial and fungal infections do. In some species and with some viruses, meristem extraction alone is sufficient to obtain clean materials, while with other species and viruses that move rapidly through the plant, thermotherapy before meristem extraction is needed to get clean materials (George 1993). In *M. pruriens* both thermotherapy with meristem extraction were necessary to remove seed-borne viruses (Jorge *et al*. unpublished).

When tissue culture is being used to produce disease free planting materials, it is important to be able to identify clean and infected materials soon after treatment. For many years Enzyme Linked ImmunoAssay (ELISA) has been used for virus detection (Albrechtsen 2006). However, the amount of tissue needed for a standard assay (0.2 g) meant that the cultures had to be quite large to be tested non-destructively (i.e., taking part of the material for testing but leaving sufficient amount for viable regrowth). When tissue culture was first used at the International Livestock Research Institute (ILRI) to clean virus infected plants of *Mucuna*, it was found that it was possible to use smaller cultures for testing since *Mucuna* was infected to a high degree with viruses with strong antigenic properties. Thus the presence of a virus could be detected in samples of just 0.01 g (Proud *et al*. unpublished). When several replicates of the same tissue are available, it is also possible to destructively sample only some replicates. Experiments were also carried out with tissue with different degrees of phenolic oxidation (presumably containing melanin) and it was found that virus infection was as detectable in severely blackened tissue as in healthy tissue. Thus blackened replicates that will be discarded could be tested, leaving good healthy material for regeneration (Proud *et al*. unpublished).

With thermotherapy and meristem extraction the degree of infection could be significantly reduced for all viruses, with some viruses less susceptible (i.e. CPMoV) to the treatment than others. However the overall success at reducing infection and producing clean plants was 50% (Proud *et al*. unpublished).

The Tissue Blot ImmunoAssay (TBIA) method was also used with *Mucuna* tissue culture material with good results. TBIA is fast, economical (Huth 1997) and sensitive (Hull 2002). TBIA is an immunological assay similar to ELISA but using a nitrocellulose membrane to bind the virus antigen from the sample for detection by the virus specific antibody (Lin *et al*. 1990). An insoluble coloured substrate is used to visualise the presence of virus on the membrane, with the degree of infection being scored. Since in this method tissue is blotted onto nitrocellulose membranes, the sample size needs to be large enough and strong enough to be blotted. Blotting callus samples was difficult as the material was soft and friable (**Fig. 1F**). Blotting was easier using differentiated tissue and a shoot that was being trimmed for subculture could be blotted onto the nitrocellulose membrane for virus testing and then transferred to new media in the usual way (Proud *et al*. unpublished). A problem was encountered using the TBIA method due to the phenolics present in *Mucuna*. Phenolics also bind to the membrane during blotting; giving a colour that can be confused with a weak virus infection. With a severe infection there was no difficulty reading the results. This was not seen with the ELISA method where the phenolics do not interfere with the reading of the soluble substrate since the colours are very different and phenolics may not bind to the plate in the same way (Proud *et al*. unpublished). Similar problems were encountered by Hsu and Lawson (1991) with anthocyanins masking the colour. The extent of binding of the phenolics to the nitrocellulose membrane was decreased by soaking the membranes in sodium sulphite, as an antioxidant, before blotting samples with a high phenolic content (Huth 1997).

FUTURE POTENTIAL OF *MUCUNA* AND USE OF BIOTECHNOLOGY

Mucuna produces large amounts of foliage and seed of good nutritional quality and its positive impacts on soil quality and weed suppression have been well documented. However, it still remains an underutilised crop with high future potential for use in agricultural development in developing countries where small farms, mixed cropping systems and reliance of crops for their multiple benefits are important for sustainable livelihoods of the rural poor.

Biotechnology research will continue on L-dopa synthesis mainly due to its potential in the treatment of Parkinson's disease. There is also great potential for further biotechnology work on *Mucuna* to more effectively exploit its useful characteristics as a nematocide and weed suppressant both in smallholder farming in developing countries (as a low-cost alternative to pesticides, for example) and in large-scale farming (in production of natural products for control of pest species). *In vitro* testing provides the opportunity to study its effects on a range of species and to understand the mechanism of action. Similarly its use as an anti-helminthic could be further studied and the active ingredients identified in cultured cells.

Increased utilisation will depend on exploiting the genetic diversity within *Mucuna*. Building on the work of Capochichi *et al*. (2001, 2003a, 2004) with *Mucuna* sp. and Krishnamurthy *et al.* (2005), Kumar *et al.* (2006), Padmesh *et al*. (2006) and Gupta and Kak (2007) with *M. pruriens*, molecular markers linked to important morphological and agronomical traits can be identified and used for marker assisted selection of genotypes with the desired characteristics. Like most forage crops, there has been little work on the basic characterization of the species and varieties used. Diversity studies would also allow the clarification of the taxonomic classification within the species, and particularly for the currently used accessions and varieties.

The use of *Mucuna* as both a green manure and fodder can increase the sustainability of farming systems. A range of *Mucuna* varieties as well as other legume species may be needed in different zones. Incorporation of leguminous forages into these systems will both reduce degradation of the natural resource base and provide a source of fodder for livestock production, allowing income generation even from otherwise unproductive land. Although *Mucuna* has been underutilised for many years, application of biotechnology provides new opportunities to identify and select better adapted genotypes with high yield, improved feed and food value and reduced anti-nutritional factors and, when ultimate use is medicinal, greater L-dopa content. This will increase the importance and use of *Mucuna* in agricultural systems in the tropics.

An important point to consider is that many of the techniques and studies presented here for *Mucuna* may be also of potential use for other useful legumes or even other crops with similar chemical components. Examples of similar components in other genera are: (1) L-Dopa – present also in *Astralagus*, *Baptisis*, *Cytisus*, *Lupinus* and *Vicia*; (2) nicotine – present also in *Acacia*; and (3) Physostigmine – present also in *Dioclea*, *Physostigma* and *Vicia* (Duke 1981). Many of the biotechnology tools described here will have wide applicability in a range of crops to contribute to improving food security worldwide.

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REFERENCES

Adebowale KO, Lawal OS (2003) Functional properties and retrogradation behaviour of native and chemically modified starch of mucuna bean (*Mucuna pruriens*). *Journal of the Science of Food and Agriculture* **83**, 1541-1546

Adesogan AT, Chikagwa-Malunga SK, Salawu MB, Kim SC (2004) The *in vitro* digestibility, gas production and fermentation characteristics of *Mucuna pruiriens*, and soybean meal treated with or without L-dopa. *Journal of Animal Science* **82 (Suppl. 1)**, 212, 215-216

Aguiyi JC, Igweh AC, Egesie UG, Leoncini R (1999) Studies on possible protection against snake venom using *Mucuna pruriens* protein immunization. *Fitoterapia* **70**, 21-24

Ajiwe VIE, Okeke CA, Nnabuike B, Ogunleye GA, 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

Akhtar MS, Qureshi AQ, Iqbal J (1990) Antidiabetic evaluation of *Mucuna pruriens*, Linn seeds. *Journal of Pakistan Medical Association* **40**, 147-150

Albrechtsen SE (2006) *Testing Methods for Seed-Transmitted Viruses: Principles and Protocols*, CABI, Oxfordshire, UK, 268 pp

Amico A (1977) Medicinal plants of Southern Zambesia. *Fitoterapia* **48**, 101-139

Amin K, Khan M, Zillur-Rehman S, Khan N (1996) Sexual function improving effect of *Mucuna pruriens* in sexually normal male rats. *Fitoterapia* **LXVII**, 53-56

Ayala-Burgos A, Herrera-Diaz P, Castillo-Caamal J, Rosado-Rivas C, Osornio-Muños L, Castillo-Caamal A (2003) Rumen degradability and chemical composition of the velvet bean (*Mucuna* spp.) grain and husk. *Tropical and Subtropical Agroecosystems* **1**, 71-76

Bailey LH (1947) *The Standard Encyclopedia of Horticulture*, Macmillan, New York, NY, USA, 600 pp

Barbeau A, McDowell FH (1970) *L-Dopa and Parkinsonism*, F.A. Davis Company, Philadelphia, USA, 403 pp

Batish DR, Gupta P (2006) L-DOPA (L-3,4-dihydroxyphenylalanine) affects rooting potential and associated biochemical changes in hypocotyl of mung bean, and inhibits mitotic activity in onion root tips. *Plant Growth Regulation* **49**, 229-235

Beksissa H (2006) *A study on the Response of Three Genotypes of Mucuna pruriens to Different Growth Media for Tissue Culture Differentiation*, MSc, Addis Ababa University, Addis Ababa, Ethiopia, 62 pp

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

Berner DK, Killani AS, Aigbokhan E, Couper DC (1992) *Macrophomina phaseolina* on the tropical cover crop *Mucuna pruriens* var. *utilis*. *Plant Disease* **76**, 1283

Betancur-Ancona DA, Chel-Guerrero LA, Bello-Pérez LA, Dávila-Ortiz G (2002) Isolation of velvet bean (*Mucuna pruriens*) starch: Physicochemical and functional properties. *Starch-Stärke* **54**, 303-309

Bhandary MJ, Chandrashekar KR, Kaveriappa KM (1995) Medical ethnobotany of the Siddis of Uttara Kannada district, Karnataka, India. *Journal of Ethnopharmacology* **47**, 149-158

Bhat R, Sridhar KR, Velmourougane K (2007a) Microbial quality evaluation of velvet bean seeds (*Mucuna pruriens* L. DC.) exposed to ionizing radiation. *Tropical and Subtropical Agroecosystems* **7**, 29-40

Bhat R, Sridhar KR, Bhushan B (2007b) Free radicals in velvet bean seeds (*Mucuna pruriens* L. DC.) and their status after γ-irradiation and conventional processing. *LWT - Food Science and Technology* **40**, 1570-1577

Bhat R, Sridhar KR, Tomita K (2007c) Effect of ionizing radiation on antinutritional features of velvet bean seeds (*Mucuna pruriens*). *Food Chemistry* **103**, 860-866

Bhattarai NK (1992) Folk use of plants in veterinary medicine in central Nepal. *Fitoterapia* **63**, 497-506

Blum LEB, Rodriguez-Kabana R (2006) Dired powders of velvetbean and pine bark added to soil reduce *Rhizoctonia solani*-induced disease on soybean. *Fitopatologia Brasileira* **31**, 261-269

Bort KS (1909) The Florida velvet bean and its history. *Bureau of Plant Industry Bulletin No* 141, Government Printing Office, Washington DC, USA, pp 25-32

Brain KR (1976) Accumulation of L-DOPA in cultures from *Mucuna pruriens*. *Plant Science Letters* **7**, 157-161

Bressani R (2002) Factors influencing nutritive value in food grain legumes: *Mucuna* compared to other grain legumes. In: Flores M, Eilittä M, Myhrman R, Carew LB, Carsky RJ (Eds) *Food and Feed from Mucuna: Current Uses and the Way Forward, Proceedings of an International Workshop* (Vol 1), CIDICCO, CIEPCA, World Hunger Research Center, Tegucigalpa, Honduras, pp 164-188

Bressani R, Lau M, Vargas S (2003) Protein and cooking quality and residual content of dehydroxyphenylalalnine and of trypsin inhibitors of processed *Mucuna* beans (*Mucuna* spp.). *Tropical and Subtropical Agroecosystems* **1**, 197-212

Brunt AA, Crabtree K, Dallwitz MJ, Gibbs AJ, Watson L, Zurcher EJ (1996) Plant Viruses Online: Descriptions and Lists from the VIDE Database. Version 20th August 1996. Available online: http://biology.anu.edu.au/Groups/MES/vide/

Buckles D (1995) Velvetbean: a 'new' plant with a history. *Economic Botany* **49**, 13-25

Buckles D, Triomphe B, Sain G (1998) *Cover Crops in Hillside Agriculture: Farmer Innovation with Mucuna*, International Maize and Wheat Improvement Center, Ottawa, Canada, 218 pp

Bunch CC (2002) Community-level development of *Mucuna* recipes: The example of *Nutricocina*. In: Flores M, Eilittä M, Myhrman R, Carew LB, Carsky RJ (Eds) *Food and Feed from Mucuna: Current Uses and the Way Forward, Proceedings of an International Workshop*, CIDICCO, CIEPCA, World Hunger Research Center, Tegucigalpa, Honduras, pp 218-226

Burkill IH (1966) *A Dictionary of the Economic Products of the Malay Peninsula*, Ministry of Agriculture and Cooperatives, Kuala Lumpur, Malaysia, 2444 pp

Caamal-Maldonado JA, Jimenez-Osornio JJ, Torres-Barragan A, Anaya AL (2001) The use of allelopathic legume cover and mulch species for weed control in cropping systems. *Agronomy Journal* **93**, 27-36

Calegari A, do Prado Wildner L, de Freitas V (1997) Adubação verde e sistemas de cobertura do solo na região sul do Brasil. In: *International Workshop on Green-Manure Cover Crop Systems for Smallholders in Tropical and Subtropical Regions*, Rural Extension and Agricultural Research Institute of Santa Catarina, Chapeco, Santa Catarina, Brazil, 50 pp

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

Capo-chichi LJA, Weaver DB, Morton CM (2003a) The use of molecular markers to study genetic diversity in *Mucuna*. *Tropical and Subtropical Agroecosystems* **1**, 309-318

Capo-chichi L, Eilittä M, Carsky R, Gilbert R, 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, Weaver DB (2004) An intraspecific genetic map of velvetbean (*Mucuna* sp.) based on AFLP markers. *Theoretical and Applied Genetics* **108**, 814-821

Carew LB, Gernat AG (2006) Use of velvet beans, *Mucuna* spp., as a feed ingredient for poultry: a review. *World's Poultry Science Journal* **62**, 131-143

Carsky RJ, 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, Galiano G (Eds) *Cover Crops in West Africa - Contributing to Sustainable Agriculture*, IDRC, IITA, Sasakawa Global 2000, Otawa, Canada, Ibadan, Nigeria, Cotonou, Benin, pp 179-187

Carsky RJ, Tarawali SA, Becker M, Chikoye D, Tian G, Sanginga N (1998) *Mucuna* – herbaceous cover legume with potential for multiple uses. *Resource and Crop Management Research Monograph, IITA* **25**, 54 pp

Carvalho GJd, Fontanétti A, Cançado CT (2002) Allelopathic potential of the jack bean (*Canavalia ensiformis*) and of the black mucuna (*Stizolobium aterrimum*) for sedge (*Cyperus rotundus*) control. *Ciência e Agrotecnologia* **26**, 647-651

Casey RCD (1960) 298 Alleged infertility plants of India. *Indian Journal of Medical Science* **14**, 590-601

Casini P (2004) Allelopathic evaluation of selected cover crops for weed control in the humid tropics. *Allelopathy Journal* **14**, 51-58

Castillo-Caamal AM, Castillo-Caamal JB, Ayala-Burgos AJ (2003a) Mucuna bean (*Mucuna* spp.) supplementation of growng sheep fed with a basal diet of Napier grass (*Pennisetum purpureum*). *Tropical and Subtropical Agroecosystems* **1**, 107-111

Castillo-Caamal JB, Jimenez-Osornio JJ, Lopez-Perez A (2003b) Feeding *Mucuna* beans to small ruminants of Mayan farmers in the Yucatan peninsula, Mexico. *Tropical and Subtropical Agroecosystems* **1**, 113-117

Chattopadhyay S, Datta SK, Mahato SB (1994) Production of L-DOPA from cell suspension culture of *Mucuna pruriens* f. *pruriens*. *Plant Cell Reports* **13**, 519-522

Chattopadhyay S, Datta SK, Mahato SB (1995) Rapid micropropagation for *Mucuna pruriens* f. *pruriens* L. *Plant Cell Reports* **15**, 271-273

Chikagwa-Malunga S, Adesogan A, Huisden M, Kim S, Krueger N, Dean D, Ososanya T, Arriola K, Phatak S (2005) The effect of replacing soybean meal with *Mucuna pruriens* on the performance of sheep. *Journal of Animal Science* **83 (Suppl. 2)**, Abstract 131, 34

Clute O (1896) Cassava, the velvet bean, prickly comfrey, taro, Chinese yam, canaigre, alfalfa, flat pea, sachaline. *Florida Agricultural Experiment Station Bulletin No* **35**, 327-354

Conroy C, Thakur YA (2005) A local plant for de-worming goats. *LEISA Magazine* **21**, 3

Cook C, Gray B (2005) *Effective Food Plots for White-tailed Deer in Alabama*, Alabama Department of Conservation and Natural Resources, Division of Wildlife and Freshwater Fisheries, Alabama, USA, 48 pp

Daxenbichler ME, Van Etten CH, Hallinan EA, Earle FR, Barclay SA (1971) Seeds as sources of L-Dopa. *Journal of Medicinal Chemistry* **14**, 463-465

DBT (2003) *Technologies transferred to industries during 2002-2003*. Available online: http://dbtindia.nic.in/techtransfer/techtrans2002-03.html

Dhingra OD, Netto Coelho RA (2001) Reservoir and non-reservoir hosts of bean-wilt pathogen, *Fusarium oxysporum* f. sp. *phaseoli*. *Journal of Phytopathology* **149**, 463-467

Diallo OK, Berhe T (2003) Processing of *Mucuna* for human food in the Republic of Guinea. *Tropical and Subtropical Agroecosystems* **1**, 193-196

Dominguez Monge S, Dominguez Valenzuela JA, Cruz Hipolito HE, Medina Pitalua JL (2004) Allelopathic activity of *Mucuna pruriens* var. *utilis* and *Neonotonia wigthii* on germination and growth of seedlings of *Sorghum halepense* and *Rottboelia coochinchinensis*. In: Pedrero GM, Valenzuela JAD, Díaz GM, Ruiz RAO (Eds) *Memoria XVI Congresso Latinoamericano de Malezas y XXIV Congresso Nacional de la Asociación Mexicana de la Ciencia de la Maleza, del 10 al 12 Noviembre de 2003*, Manzanillo, Colima, Mexico, pp 356-362

Duke JA (1981) *Handbook of Legumes of World Economic Importance*, Plenum Press, New York, USA, 345 pp

Egounlety M (2003) Processing of velvet bean (*Mucuna pruriens* var. *utilis*) by fermentation. *Tropical and Subtropical Agroecosystems* **1**, 173-181

Eilittä M (1998) *On-farm assessment of maize-mucuna systems in the Los Tuxtlas region of Southeastern Veracruz*, PhD, University of Florida, Gainesville, Florida, USA, 224 pp

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

Eilittä M, Carsky R, Mureithi J, Szabo N, Bressani R, Myhrman R, Sandoval C, Muinga R, Carew L, Capo-chichi L, Teixeira A (2003a) Future agenda for research and promotion. *Tropical and Subtropical Agroecosystems* **1**, 329-343

Eilittä M, Sollenberger LE, Littell RC, Harrington LW (2003b) On-farm experiments with maize-*Mucuna* systems in the Los Tuxtlas region of Veracruz, Mexico. I. *Mucuna* biomass and maize grain yield. *Experimental Agriculture* **39**, 5-17

Ekanem AP, Obiekezie A, Kloas W, Knopf K (2004) Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite *Ichthyophthirius multifiliis*. *Parasitology Research* **92**, 316-366

Ene-Obong HN, Carnovale E (1992) Nigerian soup condiments: Traditional processing and potential as dietary fiber source. *Food Chemistry* **43**, 29-34

Ezueh MI (1977) Cultivation and utilization of minor food legumes in Nigeria. *Tropical Grain Legumes Bulletin No 10*, IITA, Ibadan, Nigeria, pp 28-32

Faisal M, Siddique I, Anis M (2006a) An efficient plant regeneration system for *Mucuna pruriens* L. (DC.) using cotyledonary node plants. *In Vitro Cellular and Developmental Biology – Plant* **42**, 59-64

Faisal M, Siddique I, Anis M (2006b) *In vitro* rapid regeneration of plantlets from nodal explants of *Mucuna pruriens* – a valuable medicinal plant. *Annals of Applied Biology* **148**, 1-6

FAO (2004) *Biotechnology: meeting the needs of the poor?* Available online: http://www.fao.org/newsroom/en/focus/2004/41655/index.html

Flores M (1997) El uso de cultivos de cobertura en centroamerica: mas alla del entusiasmo: retos y oportunidades. In: *International Workshop on Green-Manure Cover Crop Systems for Smallholders in Tropical and Subtropical Regions*, Rural Extension and Agricultural Research Institute of Santa Catarina, Chapeco, Santa Catarina, Brazil, 51 pp

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

Fujii Y (2003) Allelopathy in the natural and agricultural ecosystems and isolation of potent allelochemicals from velvet bean (*Mucuna pruriens)* and hairy vetch (*Vicia villosa*). *Biological Sciences in Space* **17**, 6-13

Fujii Y, Shibuya T, 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

Furubayashi A, Hiradate S, Fujii Y (2005) Adsorption and transformation reactions of L-dopa in soils. *Soil Science and Plant Nutrition* **51**, 819-825

Furubayashi A, Hiradate S, Fujii Y (2007) Role of catechol structure in the adsorption and transformation reactions of L-dopa in soils. *Journal of Chemical Ecology* **32**, 239-250

Galiba M, Vissoh P, Dagbenonbakin G, Fagbohoun F (1998) Reactions et apprehensions paysanes liees a l'utilisation du poi mascate (*Mucuna pruriens* var. *utilis*). In: Buckles D, Eteka A, Osiname M, Galiba M, Galiano G (Eds) *Cover Crops in West Africa - Contributing to Sustainable Agriculture*, IDRC, IITA, Sasakawa Global 2000, Otawa, Canada; Ibadan, Nigeria; Cotonou, Benin, pp 55-65

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soyabean root cells. *Experimental Cell Research* **50**, 151-158

George EF (1993) *Plant Propagation by Tissue Culture - The Technology*, Exegetics Limited, Edington, UK, 574 pp

Girach RD, Aminuddin, Siddioui PA, Khan SA (1994) Traditional plant remedies among the Kondh of district Dhenkanal (Orissa). *International Journal of Pharmacognosy* **32**, 274-283

Giri A, Dhingra V, Giri CC, Singh A, Ward OP, Narasu ML (2001) Biotransformations using plant cells, organ cultures and enzyme systems: current trends and future prospects. *Biotechnology Advances* **19**, 175

Gourie-Devi M, Ramu MG, Venkataram BS (1991) Treatment of Parkinson's disease in "Ayurveda" (ancient Indian system of medicine): discussion paper. *Journal of the Royal Society of Medicine* **84**, 491-492

Gumedzoe MY (1993a) Major virus diseases of medicinal plants in West Africa. *Acta Horticulturae* **331**, 307-310

Gumedzoe MYD (1993b) *Mucuna* sp. infected with the cowpea strain of tobacco mosaic in Togo. *Cahiers-Agricultures* **2**, 352-355

Guo G, Yuying T, Xie C-W, Lange LA (2007) Dopamine transporter, gender, and number of sexual partners among young adults. *European Journal of Human Genetics* **15**, 279-287

Gupta M, Mazunder UK, Chakraborti S, Rath N, Bhawal SR (1997) Anti-epileptic and anticancer activity of some indigenous plants. *Indian Journal of Physiology and Allied Science* **51**, 53-56

Gupta V, Kak A (2007) Genetic diversity analysis in *Mucuna pruriens* germplasm using SDS-poly-acrylamide gel electrophoresis (PAGE). *International Journal of Plant Sciences* **2**, 124-127

Hachinohe M, Sunohara Y, Matsumoto H (2004) Absorption, translocation and metabolism of L-DOPA in barnyardgrass and lettuce: their involvement in species-selective phytotoxic action. *Plant Growth Regulation* **43**, 237-243

Hairiah K (1992) *Aluminium Tolerance of Mucuna, a Tropical Leguminous Cover Crop*, PhD thesis, University of Groningen, Hanze, The Netherlands, 152 pp

Hairiah K, van Noordwijk M, Setijono S (1991) Tolerance to acid soil conditions of the velvet beans *Mucuna pruriens* var. *utilis* and *M. deeringiana*. *Developments in Plant Soil Science* **45**, 227-237

Hairiah K, van Noordwijk M, Setijono S (1993) Tolerance to acid soil conditions of the velvet beans *Mucuna pruriens* var. *utilis* and *M. deeringiana* II. Above-ground growth and control of *Imperata cylindrica*. *Plant and Soil* **152**, 175-185

Honlonkou AN, Manyong VM (1999) Learning from farmers in a changing environment: the case of *Mucuna* fallow in southern Benin. In: *Regional Workshop on farmers and scientists in a changing environment: Assessing the impact of research in West Africa*, Cotounou, Benin, 23 pp

Hornykiewicz O (2002) L-DOPA: From a biologically inactive amino acid to a successful therapeutic agent Historical review article. *Amino Acids* **23**, 65-70

Houghton PJ, Skari KP (1994) The effect of blood clotting of some West African plants used against snakebite. *Journal of Ethnopharmacology* **44**, 99-108

Hsu H, Lawson R (1991) Direct tissue blotting for the detection of tomato spotted wilt virus in *Impatiens*. *Plant Disease* **75**, 292-295

Huang S-Y, Chen S-Y (1998) Efficient L-DOPA production by *Stizolobium hassjoo* cell culture in a two stage configuration. *Journal of Biotechnology* **62**, 95-103

Huang SY, Chou CJ (2000) Effect of gaseous composition on cell growth and secondary metabolite production in suspension culture of *Stizolobium hassjoo* cells. *Bioprocess Engineering* **23**, 585-593

Huang S-Y, Shen Y-W, Chan H-S (2002) Development of a bioreactor operation strategy for L-DOPA production using *Stizolobium hassjoo* suspension culture. *Enzyme and Microbial Technology* **30**, 779-791

Huang S-Y, Hung C-H, Chou S-N (2004) Innovative strategies for operation of mist trickling reactors for enhanced hairy root proliferation and secondary metabolite productivity. *Enzyme and Microbial Technology* **35**, 22-32

Huang S-Y, Chou S-N (2005) Elucidation of the effects of nitrogen source on proliferation of transformed hairy roots and secondary metabolite productivity in a mist trickling reactor by redox potential measurement. *Enzyme and Microbial Technology* **38**, 803-813

Huizing HJ, Wijnsma R, Batterman S, Malingre TM, Wichers HJ (1985) Production of L-DOPA by cell suspension cultures of *Mucuna pruriens*. I. Initiation and maintenance of cell suspension cultures of *Mucuna pruriens* and identification of L-DOPA. *Plant Cell, Tissue Organ Culture* **4**, 61-73

Hull R (2002) *Matthew's Plant Virology* (4th Edn), Academic Press, London, UK, 1056 pp

Hussain G, Manyam BV (1997) *Mucuna pruriens* proves more effective than L-DOPA in Parkinson's disease animal model. *Phytotherapy Research* **11**, 419-423

Huth W (1997) Tissue print immunoassay - a rapid and reliable method for routine evidence of *Gramineae* viruses. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes* **49**, 185-191

Iauk L, Galati EM, Forestieri AM, Kirjavainen S, Trovato A (1989) *Mucuna pruriens* decoction lowers cholesterol and total lipid plasma levels in the rat. *Phytotherapy Research* **3**, 263-264

Iauk L, Galati EM, Kirjavainen S, Forestieri AM, Trovato A (1993) Analgesic and antipyretic effects of *Mucuna pruriens*. *International Journal of Pharmacognosy* **31**, 213-216

ILDIS (2005) *World Database of Legumes*, Available online: www.ildis.org

Infante ME, Perez AM, Simao MR, Manda F, Baquete EF, Fernandes AM (1990) Outbreak of acute psychosis attributed to *Mucuna pruriens*. *The Lancet* **336**, 1129

Jain SK, Tarafder CR (1970) Medicinal plant-lore of the santals. *Economic Botany* **24**, 241-278

Jaiwal PK, Singh RP (2003a) *Improvement Strategies of Leguminosae Biotechnology*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 411 pp

Jaiwal PK, Singh RP (2003b) *Applied Genetics of Leguminosae Biotechnology*, Kluwer Academic Publishers, Dordrecht, the Netherlands, 330 pp

Janardhanan K, Lakshmanan KK (1985) Studies on the pulse, *Mucuna utilis*: chemical composition and antinutritional factors. *Journal of Food Science*

and Technology **22**, 369-371

Jiri O (2004) *Integration of Forage Legumes into Smallholder Farming Systems, with Emphasis on Velvet Bean (Mucuna pruriens (L) DC var. utilis)*. MPhil Thesis, University of Zimbabwe, Harare, Zimbabwe, 155 pp

Jorge A, Hanson J, Gebremariam G, Proud J (2006) Natural plant extracts for the *in vitro* culture of *Mucuna pruriens*. *Acta Horticulturae* in press

Joshi MC, Patel MB, Mehta PJ (1980) Some folk medicines of Dangs, Gujarat State. *Bulletin of Medical Ethnobotanical Research* **1**, 8-24

Kar A, Choudhary BK, Bandyopadhyay NG (2003) Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. *Journal of Ethnopharmacology* **84**, 105-108

Kaul B, Staba EJ (1968) *Dioscorea* tissue cultures. 1. Biosynthesis and isolation of diesgenin from *Dioscorea deltoidea* callus and suspension cells. *Lloydia* **31**, 171-179

Kavitha C, Vadivel E (2005) *In vitro* production of L-DOPA from *Mucuna pruriens* (L.) D.C. *Biochemical and Cellular Archives* **5**, 161-168

Keatinge JDH, Qi A, Wheeler TR, Ellis RH, Crufurd PQ, Summerfield RJ (1996) Photothermal effects on the phenology of annual legume crops with potential for use as cover crops and green manures in tropical and subtropical hillside environments. *Field Crop Abstracts* **49**, 1119-1130

Keinath AP, Harrison HF, Marino PC, Jackson DM, Pullaro TC (2003) Increase in populations of *Rhizoctonia solani* and wirestem of collard with velvet bean cover crop mulch. *Plant Disease* **87**, 719-725

Kiff L, Pound L, Holdsworth R (1996) *Covercrops: A Review and Database for Field Users*, Natural Resources Institute, Chatham, UK, 180 pp

Krishnamurthy R, Chandorkar, MS, Kalzunkar BG, Desai MRP, Pathak JM, Rajendra Gupta (2005) Diversity evaluation in velvet bean (*Mucuna pruriens*) germplasm for seed yield and associated agronomical traits. *Journal of Medicinal and Aromatic Plant Sciences* **27**, 291-296

Kumar B, Kumar MS, Annapurna K, Maheshwari, DK (2006) Genetic diversity of plant growth-promoting rhizobia isolated from a medicinal legume, *Mucuna pruriens* Linn. *Current Science* **91**, 1524-1529

Lathwell D (1990) Legume green manures: Principles for management-based on recent research. *TropSoils Technical Bulletin No* 90-01, Soil Management Collaborative Research Support Program, Raleigh, North Carolina, USA, 30 pp

Laurena A, Revilleza MJ, Mendoza E (1994) Poplyphenols, phytate, cyanogenic glycosides, and trypsin inhibitor activity of several Philippine indigenous food legumes. *Journal of Food Composition and Analysis* **7**, 194-202

Lawal OS, 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. *Nahrung/Food* **48**, 129-136

Lin J, Hsu Y, Hsu H (1990) Immunological detection of plant viruses and a mycoplasmalike organism by direct blotting on nitrocellulose membrane. *Phytopathology* **80**, 824-828

Lopes EA, Ferraz S, Freitas LG de, Ferreira PA, Amora DX (2005) Effect of aqueous extracts of velvetbean and of basil on *Meloidogyne incognita* and *M. javanica*. *Nematologia Brasileira* **29**, 67-74

Lorenzetti F, MacIsaac S, Arnason JT, Awang DVC, Buckles D (1998) The phytochemistry, toxicology, and food potential of velvetbean (*Mucuna* Adans. spp., Fabaceae). In*:* Buckles D, Eteka A, Osiname M, Galiba M, Galiano G (Eds) *Cover Crops in West Africa - Contributing to Sustainable Agriculture*, IDRC, IITA, Sasakawa Global 2000, Otawa, Canada; Ibadan, Nigeria; Cotonou, Benin, pp 67-84

Lubis SHA, Sastrapradja D, Sastrapradja S (1979) Genetic variation of *Mucuna pruriens* (I.) DC. III. Inheritance of pod hairs. *Annales Bogorienses* **VII**, 1-10

Maasdorp BV, Titterton M (1997) Nutritional improvement of maize silage for dairying: mixed-crop silages from sole and intercropped legumes and a long-season variety of maize. I. Biomass yield and nutritive value. *Animal Feed Science and Technology* **69**, 241-261

Mahajani SS, Doshi VJ, Parikh KM, Manyam BV (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

Mamatha B, Shivananda TN, Siddaramappa R (2006) Nitrogen fixing ability of 13 genotypes of *Mucuna pruriens*. In: *18th World Congress of Soil Science*, Philadelphia, Pennsylvania, USA. Available online: http://a-c-s.confex.com/crops/wc2006/techprogram/P12219.HTM

Manyam BV, Dhanasekaran M, Hare TA (2004a) Effect of antiparkinson drug HP-200 (*Mucuna pruriens*) on the central monoaminergic neurotransmitters. *Phytotherapy Research* **18**, 97-101

Manyam BV, Dhanasekaran M, Hare TA (2004b) Neuroprotective effects of the antiparkinson drug *Mucuna pruriens*. *Phytotherapy Research* **18**, 706-712

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

Matenga V, Ngongoni N, Titterton M, Maasdorp B (2003) *Mucuna* seed as a feed ingredient for small ruminants and effect of ensiling on its nutritive value. *Tropical and Subtropical Agroecosystems* **1**, 97-106

Mbuthia EW, Gachuiri CK (2003) Effect of inclusion of *Mucuna pruriens* and *Dolichos lablab* forage in napier grass silage on silage quality and on voluntary intake and digestibility in sheep. *Tropical and Subtropical Agroecosystems* **1**, 123-128

Mercuri NB, Bernardi G (2005) The 'magic' of L-dopa: why is it the gold standard Parkinson's disease therapy? *Trends in Pharmacological Sciences* **26**, 341-344

Miller HK (1902) *Velvet bean*. Florida Agricultural Experiment Station, Bulletin No **60**, 445-468

Miller EF, Massengale ON, Barnes MA (1925) Some effects resulting from eating velvet beans. *Journal of the American Pharmaceutical Association* **14**, 1113-1114

Miocinovic R, Zafar S, Sferra J, Wainstein M (2005) Priapism caused by 'All Nite Long'. *International Journal of Impotence Research* **17**, 469-470

Misra L, Wagner H (2007) Extraction of bioactive principles from *Mucuna pruriens* seeds. *Indian Journal of Biochemistry and Biophysics* **44**, 56-60

Mohan VR, Janardhanan K (1993) Studies on Indian tribal pulses. In: Udaiyan K, Janardhanan K, Manian S, Reddy VRK (Eds) *Proceedings of the National Seminar on Biodiversity: Strategies for Conservation and Future Challenges*, Department of Botany, Bharathiar University, India, pp 116-125

Morris JB (1999) Legume genetic resources with novel "value added" industrial and pharmaceutical use. In*:* Janick J (Ed) *Perspectives on New Crops and New Uses*, ASHS Press, Alexandria, VA, pp 196-201

Mors WB, Rizzini CT, Pereira NA (2001) Medicinal plants of Brazil. *Journal of Natural Products* **64**, 1258-1259

Muinga RW, Saha HM, Mureithi JG (2003) The effect of mucuna (*Mucuna pruriens*) forage on the performance of lactating cows. *Tropical and Subtropical Agroecosystems* **1**, 87-92

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-497

Nath D, Sethi N, Singh RH, Jain AK (1992) Commonly used indian abortifacient plants with special reference to their teratologic effects in rats. *Journal of Ethnopharmacology* **36**, 147-154

Negri G (2005) Diabetes melito:plantas e principios ativos naturais hipoglicemiantes. *Revista Brasileira de Ciências Farmaceuticas* **41**, 1-29

Nishihara E, Parvez MM, Araya H, Fujii Y (2004) Germination growth response of different plant species to the allelochemical L-3,4-dihydroxyphenylalanine (L-DOPA). *Plant Growth Regulation* **42**, 181-189

Nishihara E, Parvez MM, Araya H, Kawashima S, Fujii Y (2005) L-3-(L-3,4-dihydroxyphenyl) alanine (L-DOPA), an allelochemical exuded from velvet bean (*Mucuna pruriens*) roots. *Plant Growth Regulation* **45**, 113-120

Nyambati EN, Sollenberger LE, Kunkle WE (2003) Feed intake and lactation performance of dairy cows offered Napier grass supplemented with legume hay. *Livestock Production Science* **83**, 179-189

Obata-Sasamoto H, Nishi N, Komamine A (1981) Mechanism of suppression of DOPA accumulation in a callus culture of *Stizolobium hassjoo*. *Plant and Cell Physiology* **22**, 827-835

Onweluzo JC, Leelavathi K, Haridas Rao P (1999) Effect of *Detarium microcarpum* (Dm) and *Mucuna flagellipes* (MF) gums on the quality of white bread. *Plant Foods for Human Nutrition* **54**, 173-182

Onweluzo J, Eilittä M (2003) Surveying *Mucuna*'s utilization as a food in Enugu and Kogi states of Nigeria. *Tropical and Subtropical Agroecosystems* **1**, 71-76

Onweluzo JC, Obanu ZA, Okwandu MC (2004) Potentials of gum from *Detarium microcarpum* (Dm) and *Mucuna flagellipes* (MF) seeds as raw beef burger stabilizers. *Plant Foods for Human Nutrition* **59**, 137-141

Ooi PAC (2005) Some non-pesticide methods for managing crop insect pests – Present status, issues and strategies. In: Ooi PAC (Ed) *Nonpesticide Methods for Controlling Diseases and Insect Pests*, Asian Productivity Organization, Tokyo, Japan, 180 pp

Padmesh P, Reji JV, Jinish Dhar M, Seeni S (2006) Estimation of genetic diversity in varieties of *Mucuna pruriens* using RAPD. *Biologia Plantarum* **50**, 367-372

Pérez-Hernandez F, Ayala-Burgos AJ, Belmar-Casso R (2003) Performance of growing lambs supplemented with *Mucuna pruriens*. *Tropical and Subtropical Agroecosystems* **1**, 119-122

Persley GJ, Doyle JJ (2001) Biotechnology for developing-country agriculture: problems and opportunities. In*:* Pinstrup-Andersen P, Pandya-Lorch R (Eds) *The Unfinished Agenda: Perspectives on Overcoming Hunger, Poverty, and Environmental Degradation*, International Food Policy Research Institute, Washington, USA, pp 239-243

Piper CV, Tracy SM (1910) The Florida velvet bean and related plants. US Department of Agriculture, *Bureau of Plant Industry Bulletin No 179*. Government Printing Office, Washington, DC, USA, 26 pp

Poornachandra MN, Khanam S, Shivananda BG, Shivananda TN, Dris R (2005) *Mucuna pruriens* (L.) DC – A novel drug for learning and memory retrieval. *Journal of Food, Agriculture and Environment* **3**, 13-15

Pras N, Woerdenbag S, Batterman S, Visser S, van Uden JF (1993) *Mucuna pruriens*: improvement of the biotechnological production of the anti-Parkinson drug L-Dopa by plant cell selection. *Pharmacy World and Science* **15**, 263-268

Price M (2002) The L-dopa problem as viewed by community development workers. In: Flores M, Eilittä M, Myhrman R, Carew LB, Carsky RJ (Eds) *Food and Feed from Mucuna: Current Uses and the Way Forward, Proceedings of an International Workshop*, CIDICCO, CIEPCA, World Hunger Research Center, Tegucigalpa, Honduras, pp 111-118

PROSEA (2003) *Plant Resources of South- East Asia - PROSEA 3: Dye and tannin-producing plants*. Available online: http://www.prosea.nl/prosea3.html

Pugalenthi M, Vadivel V (2007) L-Dopa (L-3,4-Dihydroxyphenylalanine): A

non-protein toxic amino acid in *Mucuna pruriens* seeds. *Food* **1**, 322-343

Pugalenthi M, Vadivel V, 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

Pushpangadan P, Atal CK (1984) Ethno-medico-botanical investigations in Kerala I. Some primitive tribals of Western Ghats and their herbal medicine. *Journal of Ethnopharmacology* **11**, 59-77

Rajaram N, Janardhanan K (1991) The biochemical composition and nutritional potential of the tribal pulse, *Mucuna gigantea* (Willd) DC. *Plant Foods for Human Nutrition* **41**, 45-51

Rajaram N, Janardhanan K (1993) *Ex situ* conservation of genetic resources of tribal pulses and their wild related species. *Plant Genetic Resources Newsletter* **91/92**, 29-32

Rajendran V, Joseph T, David J (1996) Reappraisal of dopamineric aspects *Mucuna pruriens* and comparative profile with L-Dopa on cardiovascular and central nervous system in animals. *Indian Drugs* **33**, 465-472

Rajeshwar Y, Kumar GPS, Gupta M, Mazumder UK (2005a) Studies on *in vitro* antioxidant activities of methanol extract of *Mucuna pruriens* (Fabaceae) seeds. *European Bulletin of Drug Research* **13**, 31-39

Rajeshwar Y, Gupta M, Mazumder UK (2005b) *In vitro* lipid peroxidation and antimicrobial activity of *Mucuna pruriens* seeds. *Iranian Journal of Pharmacology and Therapeutics* **4**, 32-35

Rani BJ, Sridhar V (2004) Record of arthropod pests on velvet bean, *Mucuna pruriens* var. *utilis* under Bangalore conditions. *Journal of Medicinal and Aromatic Plant Sciences* **26**, 505-506

Rao R, Ravishankar GA (2002) Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances* **20**, 101-153

Rathi SS, Grover JK, Vikrant V (2002a) The effect of *Momordica charantia* and *Mucuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. *Phytotherapy Research* **16**, 236-243

Rathi SS, Grover JK, Vikrant V, Biswas NR (2002b) Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. *Phytotherapy Research* **16**, 774-777

Rattray AGH, Ellis BS (1952) Maize and green manuring in southern Rhodesia. *Rhodesia Agricultural Journal* **49**, 188-197

Ravindran V, Ravindran G (1988) Nutritional and anti-nutritional characteristics of mucuna (*Mucuna utilis*) bean seeds. *Journal of the Science of Food and Agriculture* **46**, 71-79

Remmen SFA, Ellis BE (1980) DOPA-synthesis in non-producer cultures of *Mucuna deerengiana*. *Phytochemistry* **19**, 1421-1423

Rich J, Wright D, Marois J, Sprenkel D (2003) *Selected Legumes Used As Summer Cover Crops*. Available online: http://edis.ifas.ufl.edu/IN483, 6 pp

Sandoval-Castro CA, Herrera P, Capetillo CM, Ayala-Burgos AJ (2003) *In vitro* gas production and digestibility of *Mucuna* bean. *Tropical and Subtropical Agroecosystems* **1**, 77-80

Santra AK, Pan S, Samanta AK, Maiti SK (2006) Utilization of food plants by migratory elephants in the wilderness of South West Bengal. *Tigerpaper* **33**, 24-26

Scott J (1911) The velvet bean. In*: 11th Biennial Report of the Commissioner of Agriculture of the State of Florida for the Period Beginning January 1, 1909, and Ending December 31, 1910*, Tallahassee, Florida, USA, pp 174-195

Siddhuraju P, Vijayakumari K, Janardhanan K (1993) Genetic resources of tribal pulses. *Plant Genetic Resources Newsletter* **96**, 47-49

Siddhuraju P, Vijayakumari K, Janardhanan K (1996) Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* (L.) DC.). *Journal of Agricultural and Food Chemistry* **44**, 2636-2641

Siddhuraju P, Becker K, 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

Skerman PJ (1977) The pasture legumes – *Mucuna sp*. In*:* Riveros F (Eds) *Tropical Forage Legumes*, FAO, Rome, pp 357-359

Soares AR, Ferrarese Mde L, Siqueira Rde C, Bohm FM, Ferrarese-Filho O (2007) L-dopa increases lignification associated with *Glycine max* root growth-inhibition. *Journal of Chemical Ecology* **33**, 265-275

St.-Laurent L, Livesey J, Arnason JT, Bruneau A (2002) Variation in L-dopa concentration in acessions of *Mucuna pruriens* (L.) DC. and in *Mucuna brachycarpa* Rech. In: Flores M, Eilittä M, Myhrman R, Carew LB, Carsky RJ (Eds) *Food and Feed from Mucuna: Current Uses and the Way Forward, Proceedings of an International Workshop*, CIDICCO, CIEPCA, World Hunger Research Center, Tegucigalpa, Honduras, pp 352-373

Steiner KG (1996) *Causes of Soil Degradation and Approaches to Sustainable Soil Managemen*, Margraf Verlag, Weikersheim, Germany, 50 pp

Sung L-S, Huang S-Y (2000) Medium optimization of transformed root cultures of *Stizolobium hassjoo* producing L-DOPA with response surface methodology. *Biotechnology Progress* **16**, 1135-1140

Sung L-S, Huang S-Y (2006) Lateral root bridging as a strategy to enhance L-DOPA production in *Stizolobium hassjoo* hairy root cultures by using a mesh hindrance mist trickling bioreactor. *Biotechnology and Bioengineering* **94**, 441-447

Szabo NJ (2003) Indolealkylamines in *Mucuna* species. *Tropical and Subtropical Agroecosystems* **1**, 295-307

Szabo NJ, Tebbett IR (2002) The chemistry and toxicity of *Mucuna* species. In: Flores M, Eilittä M, Myhrman R, Carew LB, Carsky RJ (Eds) *Food and Feed from Mucuna: Current Uses and the Way Forward, Proceedings of an International Workshop*, CIDICCO, CIEPCA, World Hunger Research Center, Tegucigalpa, Honduras, pp 120-141

Tarawali G, Manyong VM, Carsky RJ, Vissoh PV, Osei-Bonsu P, Galiba M (1999) Adoption of improved fallows in West Africa: Lessons from mucuna and stylo case studies. *Agroforestry Systems* **47**, 93-122

Teramoto S, Komamine A (1988) Products of interest of industry – pharmaceuticals - Alkaloids. In*:* Bajaj YPS (Eds) *Biotechnology in Agriculture and Forestry. Medicinal and Aromatic Plants IV*, Springer-Verlag, Berlin, pp 209-224

Tian G, Kang BT (1998) Effects of soil fertility and fertilizer application on biomass and chemical compositions of leguminous cover crops. *Nutrient Cycling in Agroecosystems* **51**, 231-238

Tharakan B, Dhanasekaran M, Mize-Berge J, Manyam BV (2007) Anti-Parkinson botanical *Mucuna pruriens* prevents levodopa induced plasmid and genomic DNA damage. *Phytotherapy Research* **21**, 1124-1126

Tolmasquim E, Corrêa AMN, Nakamura T (1970) Studies on new starches. Part 2. A Study of the properties of four varieties of mucuna bean (*Stizolobium*). *Starch - Stärke* **22**, 313-317

Tozzi AMGA, Agostini K, Sazima M (2005) A new species of *Mucuna* Adans. (*Leguminosae*, *Papilionoideae*, *Phaseoleae*) from southeastern Brazil, with a key to Brazilian species. *Taxon* **54**, 451-455

Triomphe BL (1996) *Seasonal Nitrogen Dynamics and Long-Term Changes in Soil Properties under the Mucuna/Maize Cropping System on the Hillsides of Northern Honduras*, PhD Thesis, Cornell University, Ithaca, New York, USA, 217 pp

Tripathi YB, Upadhyay AK (2001) Antioxidant property of *Mucuna pruriens* Linn. *Current Science* **80**, 1377-1378

Ukachukwu SN, Obioha FC (1997) Improving the nutritive value of *Mucuna cochinchinensis* by thermal processing methods. *Journal of Applied Chemistry and Agricultural Research* **4**, 43-48

Valverde BE, Merayo A, Rojas CE, Alvarez T (1995) Interaction between a cover crop (*Mucuna* sp.), a weed (*Rottboellia cochinchinensis*) and a crop (maize). In: British Crop Protection Council (Eds) *Proceedings Brighton Crop Conference Weeds* (Vol 1), pp 197-200

Vanisree M, Lee C-Y, Lo S-F, Nalawade SM, Lin CY, Tsay H-S (2004) Studies on the production of some important secondary metabolites from medicinal plants by plant tissue cultures. *Botanical Bulletin of Academica Sinica* **45**, 1-22

Vargas-Ayala R, Rodriguez-Kabana R, Morgan-Jones G, McInroy JA, Kloepper JW (2000) Shifts in soil microflora induced by velvetbean (*Mucuna deeringiana*) in cropping systems to control root-knot nematodes. *Biological Control* **17**, 11-22

Vitalyos D (1979) *Phytotherapy in domestic traditional medicine in Matoubapapaye* (*Guadaloupe*). PhD Thesis, University of Paris, Paris, France, 110 pp

Wanjekeche E, Wakasa V, Mureithi JG (2003) Effect of germination, alkaline and acid soaking and boiling on the nutritional value of mature and immature *Mucuna* (*Mucuna pruriens*) beans. *Tropical and Subtropical Agroecosystems* **1**, 183-192

Weber GK, Robert ABC, Carsky RJ (1997) *Handbook for use of LEXSYS (Legume Expert SYStem): decision support for integrating herbaceous legumes into farming systems*. International Institute of Tropical Agriculture, Ibadan, Nigeria, 31 pp

Weniger B, Rouzier M, Daguilh R, Henrys D, Henrys JH, Anton R (1986) Popular medicine of the central plateau of Haiti. *Journal of Ethnopharmacology* **17**, 13-30

Wichers HJ, Visser JF, Huizing HJ, Pras N (1993) Occurrence of L-DOPA and dopamine in plants and cell cultures of *Mucuna pruriens* and effects of 2,4-D and NaCl on these compounds. *Plant Cell, Tissue and Organ Culture* **33**, 259-264

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

Wilmot Dear CM (1984) A revision of *Mucuna* (*Leguminosae-Phaseolae*) in China and Japan. *Kew Bulletin* **39**, 23-65

Wilson GF, Akapa KL (1983) Providing mulches for no-tillage cropping in the tropics. In: Akobundu IO, Deutsch AE (Eds) *No-tillage Crop Production in the Tropics: Proceedings of a Symposium*, Oregon State University, Monrovia, Liberia, pp 51-65

Yost RS, Evans DO, Saidy NA (1985) Tropical legumes for N production: Growth and N content in relation to soil pH. *Tropical Agriculture* **62**, 20-24

Zandu (2007) Moving on with Zandu. Available online: http://www.zandu ayurveda.com/moving.htm

Zasada IA, Klassen W, Meyer SLF, Codallo M, Abdul-Baki AA (2006) Velvetbean (*Mucuna pruriens*) extracts: impact on *Meloidogyne incognita* survival and on *Lycopersicon esculentum* and *Lactuca sativa* germination and growth. *Pest Management Science* **62**, 1122-1127