Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden

By

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DECLARATION

I hereby declare that this thesis entitled **"Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden"** is a *bonafide* record of research work done by me, during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Vellayani,
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CERTIFICATE

Certified that this thesis entitled **"Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden"** is a record of research work done independently by **Mrs. ANN SAMUEL** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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Ann Samuel

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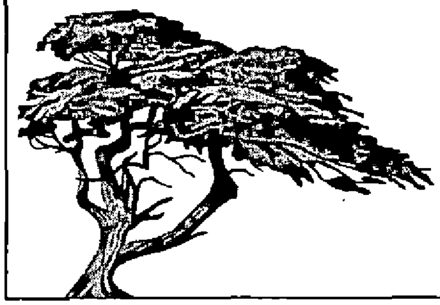
LIST OF ABBREVIATIONS

°C	Degree Celsius
CD	Critical difference
CIAT	Centro Internacional de Agricultura Tropica (International Centre of Tropical Agriculture), Columbia
CIDICCO	Centro Internacional de Informacion sobre cultivas de cobertura (International Centre for Information on Cover Crops), Honduras
CIEPCA	Centre d'Information et d'Echanges sur les Plantes de Couverture en Afrique (Centre for Cover Crop Information and Seed Exchange in Africa), Republic of Benin
CSIR	Council for Scientific and Industrial Research, New Delhi
DC	Deccan
ECHO	Educational Concerns for Hunger Organization, Florida, USA
FAO	Food and Agricultural Organisation of the United Nations, Rome
Fig.	Figure
HCl	Hydrochloric acid
IDRC	International Development Research Centre, Canada
IITA	International Institute of Tropical Agriculture, Ibadan, Nigeria
KFRI	Kerala Forest Research Institute, Peechi, Thrissur, India
kg ha^{-1}	Kilogrammes per hectare
L-DOPA	L-3, 4-dihydroxyphenylalanine
MP	Medicinal Plants
N	Normal
NBPGR	National Bureau of Plant and Genetic Resources, New Delhi and Thrissur, India
nm	nanometer
NPK	Nitrogen, Phosphorus, Potassium
SAA	Sasakawa Association for Africa, Ottawa, Canada
TBGRI	Tropical Botanical Garden and Research Institute, Palode, Thiruvananthapuram, India
t ha^{-1}	tonnes per hecare
WARDA	West African Rice Development Association, Ivory Coast
WP	Wetable powder

Dedicated to my beloved family



A friend loves at all times, and a brother is born for adversity.

Proverbs 17v.17
Holy Bible

CONTENTS

INTRODUCTION

1. INTRODUCTION

India is eulogised as 'the meadow of medicinal plants' with over 7500 species of medicinal plants. Before the dawn of modern era, herbal drugs were the only source available to mankind the world over, for healthcare. Advances in science have led not only to the isolation of many active principles from these herbs in pure form, but also to the production of their synthetic analogues.

However, due to high cost of production, difficulty involved in the synthesis and due to side effects resulting from their continual use, the emphasis is now back on the traditional and indigenous medicinal plants, and their products in pure form.

The World Health Organization has estimated that eighty per cent of the world's population relies upon traditional medicine for its primary health care needs. Hence the goal of "Health for all by the year 2005" can be achieved only through the adoption of a "multiple approach" of health care. This necessitates the merging of indigenous system of medicine with the modern system of medicine.

While the impetus on such research has increased, there has been simultaneously, a reduction in the availability of medicinal plants. This is accounted for by the escalating demand of such raw materials, destruction of natural pockets of medicinal plants and the non-availability of agricultural land.

Among the medicinal plants of India, attention has been drawn to a herbaceous leguminous plant species, *Mucuna pruriens*.

This plant has been mentioned in the treatises of the Charaka Samhiti as well as the Susrutha Samhiti as a potent aphrodisiac, geriatric tonic and cure for various diseases of the nervous system.

Recently, this plant is recognized in the treatment of Parkinson's disease. This disease is now one of the most common neurological ailments and is triggered by the lowered levels of dopamine, a chemical neurotransmitter in the body. The seed of *M. pruriens* is a natural source of L-DOPA, a precursor of dopamine.

In a state like Kerala, where homestead type of cultivation predominates, it is difficult to introduce medicinal plants as pure crops. *Mucuna pruriens* can be easily introduced as an intercrop in plantations of coconut, rubber and oil palm. Besides being able to exploit available resources, this also helps in over coming problems of land scarcity. Being a leguminous plant it enriches the soil and thus can help in eliminating the use of chemical fertilizers. It acts as a cover crop in the vegetative phase and after pod harvest, the biomass can be incorporated to the soil as green manure. Its main advantage lies in providing an additional source of income to the farmer by way of selling seeds, to pharmaceutical companies.

The present study is aimed at the collection of various accessions of *M. pruriens* and their evaluation for seed yield and L-DOPA content. An attempt is also made to estimate the nitrogen fixing efficiency of the accessions.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Medicinal plants are carving a niche for themselves in today's world. New yields to old as scientists all over reverse back to 'Nature', and with her healing powers, 'Nature' makes the finest doctor.

Mucuna pruriens Baker non DC. is one such a medicinal plant, and in addition to its medicinal properties, it provides a host of other benefits to the cultivator.

The present work comprised screening of various accessions of *M. pruriens* for seed yield and content of pharmaceutically valuable L-DOPA. An attempt to estimate nitrogen fixing potential of various accessions is also made.

The following pages provide pertinent literature on the research carried out in this area as well as on related aspects.

2.1 Cultivated species of *Mucuna* and germplasm collection

Mucuna pruriens originated in southern China and eastern India. It is now found all over the world. In India, it is found at the foothills of the Himalayas, plains of West Bengal, Madhya Pradesh, Rajasthan, Gujarat, Karnataka, Kerala and Tamil Nadu (CSIR, 1962).

The genus *Mucuna* consists of about one hundred species of annual and perennial legumes. *Mucuna* is self pollinating and natural out crossing is rare. The dozen or so cultivated species found in the tropics represent a fragment of the Asian cultigen (Duke, 1981).

The most commonly cited species and relevant information on them is given in Table 1.

There are several centres that maintain germplasm collections of *Mucuna spp.*, within the country as well as outside. Relevant information on different centers or sources of germplasm collected is provided in Table 2.

At NBPGR (Issapur farm), eighty-one accessions of indigenous and exotic *Mucuna* germplasm have been collected and placed into evaluation trials for determination of agro-botanical and chemical traits (Chadha, 1995).

A few lines of *M. pruriens* having high L-DOPA content are maintained at Zandu Foundation for Health Care, Gujarat (Pathak, 1997).

There are five accessions of *M. pruriens* maintained at Regional Station, NBPGR, Vellanikkara, Kerala; Table 2.

2.2. Crop production and management

2.2.1 Soil and climate

Mucuna pruriens can be grown on a wide range of soils. Preferred soil texture is sandy to sandy clay. Growth in clay soils may be hindered by water logged conditions (Kay, 1979).

Table 1. Commonly cultivated species of genus *Mucuna* (Kay, 1979)

Sl. No.	Species	Distribution	Flower colour	Seed colour	Pod length	Maturity cycle (days)	Main use
1.	*Mucuna pruriens*	India, Japan, Philippines, Africa, W.Indies, USA	Purple/white	Black, creamy yellow or mottled	9-13 cm	210-240	Medicinal plant, human food crop, cattle fodder
2.	*M. deeringiana*	USA, S.America	Purple	Mottled	5-8 cm	100-150	Cattle fodder, green manure crop
3.	*M. aterrina*	Mauritius, Australia, Brazil, W. Indies	Purple/white	Black	9-13 cm	210-260	Rotation crop, cover crop
4.	*M. utilis*	India, SE Asia	Purple	Mottled	8-13 cm	120-160	Green manure, fodder
5.	*M. hassjoo*	Japan	Purple	Mottled	9-13 cm	150-240	Fodder, cover crop
6.	*M. cochinchinensis*	Philippines, S.E.Asia	Purple	Grey, white or mottled	8-13 cm	120-160	Vegetable crop

Table 2. Sources of germplasm collected

Sl. No.	Sources of germplasm	Location	Purpose of germplasm	Catalogue numbers
1	National Bureau of Plant and Genetic Resources (NBPGR)	Pusa, New Delhi	Research on L-DOPA content and agro-botanical characteristics	IC-25833-1, Selection 3, OUM, IC-15809, IC-127363, IC-1892, IC-83195, V-1833
2	Dr. Abraham, Z., Senior Scientist, Regional Station, NBPGR	Vellanikkara, Thrissur, Kerala	No work carried out so far	TCR-175, TCR-176, TCR-179, TCR-180, TCR-207
3	Tropical Botanical Garden and Research Institute (TBGRI)	Palode, Thiruvananthapuram, Kerala	Medicinal plant exhibit	Nil
4	Dr. Milton Flores, CIDICCO	Honduras	Research on soil improvement, animal feed, human feed	Nil
5	CIAT	Columbia	- do -	Nil
6	FAO	Rome	- do -	Nil
7	IITA	Ibadan, Nigeria	- do -	Nil
8	WARDA	Ivory Coast	- do -	Nil
9	SAA	Ottawa, Canada	- do -	Nil

Contd....

Table 2 (contd....)

Sl. No.	Sources of germplasm	Location	Purpose of germplasm	Catalogue numbers
10	CIEPCA	Republic of Benin	Research on soil improvement, animal feed, human feed	Nil
11	KFRI	Peechi, Thrissur, Kerala	Cover crop exhibit	Nil
12	Dr. Swami Velji	Kottoor, Thiruvananthapuram	Wild cultivation	Nil
13	Pampa Valley forest	Pathanamthitta	Wild cultivation	Nil
14	Dr. P.J. Thomas, Arya Stores	Karugachal, Kottayam	Sale as medicinal plant	Nil
15	Thullapally forest	Wayanad	Wild cultivation	Nil
16	Shri. Mathewkutty, Theruvapuzha	Palai, Kottayam	Private collection for exhibition as medicinal plant	Nil
17	Chengara forest	Pathanamthitta	Wild cultivation	Nil
18	Ayur forest	Thiruvananthapuram	Wild cultivation	Nil
19	Thalachira forest	Pathanamthitta	Wild cultivation	Nil
20	Zandu Foundation for Health Care	Gujarat	Research on L-DOPA content	Nil

Sowing should be done with the onset of monsoon rains so that pods are harvested during January or February. The crop is sensitive to frost and hence frost prone areas should be avoided for cultivation (Chadha, 1995).

The optimum temperature for cultivation of the crop is between 20°C to 30°C (Buckles, 1995).

2.2.2 Land preparation and planting

The land is ploughed two to three times to make a fine seed bed before sowing. A basal dose of 1.0 t ha^{-1} of farm yard manure together with 80 kg ha^{-1} of phosphorous and 40 kg ha^{-1} of potassium is recommended (Chadha, 1995).

Botton (1958) and Kay (1979) reported a seed rate of 11 to 22 kg ha^{-1} for row planting and 45 to 90 kg ha^{-1} when broadcasted. Chadha (1995) suggested a seed rate of 50 kg ha^{-1}.

Versteeg (1998) suggested a seed rate of 11 kg ha^{-1} for intercropping systems.

According to Kay (1979), inter-row spacing of *Mucuna* grown for seed should be 1 to 2 m. When grown for green manure the rows should be 0.5 to 1.0 m apart. Versteeg (1998) found a spacing of 80 x 80 cm to give acceptable ground rows. Chadha (1995) reported a spacing of 60 x 60 cm over rows or ridges.

The crop is given a top dressing of a mixture of 80 kg of nitrogen per hectare, in two equal split doses at 30 and 60 days after sowing (Chadha, 1995).

Kay (1979) reported that application of superphosphate at doses ranging from 75 to 225 kg ha^{-1} favoured crop growth.

2.2.3 Irrigation and inter-culture

The crop can be raised both as irrigated and rainfed crop. The rainfed crop is sown after the onset of monsoon (Chadha, 1995).

When spaced properly and growing normally, *Mucuna* hardly needs to be weeded (Versteeg, 1998).

Mucuna can be intercropped with maize, wheat, oats, rice, banana, orange, sorghum, millet, cassava and other common crops (Becker and Tarawali, 1998).

Staking, or trellising, for support of vines is suggested for increasing seed yield, by Humphreys and Riveros (1986) and Chadha (1995).

2.2.4 Diseases and pests

In general, *Mucuna* crop does not have much fungal or pest problems. It is susceptible to mosaic virus, vine rot disease and may have leaf spot attack towards maturity (Chadha, 1995). It is resistant to root knot nematodes (ECHO, 1998).

2.2.5 Development of pods and harvest

The flowering starts 35 to 75 days after sowing. Pods mature and are harvested with the help of knives or sickles (Becker and Tarawali, 1998).

Mucuna vegetation after harvest of pods can be used as green fodder, or processed as dry season hay (Buckles, 1995).

2.3 Agro-botanical characteristics

Mucuna pruriens belongs to the family *Fabaceae*. The life cycle ranges from 100 to 290 days. It is of a herbaceous twining nature with trifoliate and broadly ovate leaves. The flowers are produced in pendulous clusters and usually purple in colour. *Mucuna* grows vigorously producing suffuse vegetative matter or biomass (Buckles, 1995).

Accordingly to Singh (1957), biomass is mainly a function of the vegetative growth by virtue of the inherent genetic makeup of each legume.

Levels of *Mucuna* biomass range from 6 to 12 t ha^{-1} of dry matter as reported by Chavez (1993).

A maximum dry matter production of 4.5 t ha^{-1} for vegetable cowpea (100 days duration) occurs when irrigation is given at 75 per cent field capacity (Jyothi, 1995).

Mercy (1981) reported a production of 14.1 t ha^{-1} of velvetbean green fodder and 2.3 t ha^{-1} of velvetbean dry matter.

Biomass yield (18.77 t ha^{-1}) of *M. pruriens* when grown under shaded condition is reported to be superior to biomass yield (16.83 t ha^{-1}) when the crop is grown under open condition (Sunitha, 1996).

Mini (1997) found that in summer vegetable cowpea, frequent light irrigation produces maximum dry matter at all stages.

Biomass yield of *Mucuna* varies directly with length of growing season and soil fertility conditions. High biomass accumulation (10 t ha^{-1}) was observed in areas of longer growing season. Variety characteristics were also observed to influence the rate of dry matter production (IITA, 1997).

According to Becker and Johnson (1998) soil phosphorus is an important factor in *Mucuna* biomass accumulation, as legumes require phosphorus for growth and nitrogen fixation. IITA (1998) reported a biomass yield of 2.3 t ha^{-1} in *M. pruriens* grown at a site in Nigeria.

2.3.1 Plant length

Variation in plant length within legumes is purely a function of the genetic make up (Bose, 1963).

Mercy (1981) reported that *Mucuna* vines are longer and more aggressive than cowpea and horsegram, and produce more number of leaves which help the crop for greater utilization of environmental resources.

Veerupakshappa (1982) observed the negative association of plant length with pod length and number of seeds per pod, in cowpea.

An increase in plant length may increase transpirational loss of water due to the increase in vegetative growth. This in turn may hinder reproductive growth and ultimately reduce the yield in legume crops (Anitha, 1989).

A plant height of 3.7 m at three month stage was observed in vegetable cowpea by Jyothi (1995).

According to Mini (1997) plant length in vegetable cowpea is significantly influenced by frequent light irrigation.

Geetha (1999) reported that nitrogen plays a key role in influencing the length of vines in vegetable cowpea.

2.3.2 Number of leaves

According to Nair (1966), the number of leaves per plant like other growth parameters is purely a function of the genetic makeup, under identical conditions of growth.

An average of 79 leaves per plant at 75 DAS and 67 leaves per plant at 90 DAS, (ie. harvest stage) was observed in cowpea. The decrease in the number of leaves is attributed to the phenomenon of 'leaf-shedding' before harvest (Jyothi, 1995).

Frequent light irrigation produced highest number of leaves per plant, in vegetable cowpea (Mini, 1997).

Geetha (1999) reported significant influence of high levels of nitrogen on number of leaves per plant.

2.3.3 Root characters

A well developed root system is characteristic of increased yield in cowpea (Babalola, 1980).

Kavitha (1982) reported a positive association of root length with yield, in blackgram. In contrast Anitha (1989) observed that greengram varieties with high root length and spread were low yielding due to increase of vegetative growth at the expense of reproductive growth.

Rajan (1999) found that length of primary root showed a positive association with weight of nodules, plant length, number of seeds per pod and 100 grain weight, and a negative association with number of pods.

2.3.4 Pod characters

Mucuna pruriens produces pods of around 9 to 13 cm length. They are covered by long stinging hairs which contain alkaloids like *mucunine*, *mucunadine* and *mucunane*. These cause an extremely painful and itchy rash, on contact with human skin. These hairs are beneficial to the plant; they discourage seed predators, hence *Mucuna* pods are fairly free from insect attack (Buckles, 1995).

According to Nair (1966), pod length in cowpea mainly depends on genetic make up of the variety. He also reported that the higher the amount of nitrogen applied to the crop, the more the vegetative growth and lesser the yield of pods.

Jayarani (1993) observed a range of 6.93 to 26.57 number of pods per plant, and a length of 8.62 cm to 26.58 cm podlength in grain cowpea.

Sajikumar (1995) reported a range of 12.33 to 26.34 pod clusters per plant, 23.89 to 78.78 total number of pods per plant, 11.95 to 27.86 g average weight of pods per plant and a range of 3.65 to 4.95 cm pod length in blackgram.

According to Geetha (1999), the maximum yield of green pods and number of pods in cowpea, occur when nitrogen and potassium are given in doses of 20 kg ha^{-1} each.

2.3.5 Seed characters

In *Mucuna pruriens*, there are about 4 to 8 seeds per pod. The seeds are globular or reniform and usually coloured black, white, creamy yellow or may be mottled. Hundred seed weight may vary from 25 to 110 g (Buckles, 1995).

Rajendran (1979) reported in cowpea, a positive association of grain yield with height of plant, number of days to flowering, number of pod clusters per plant and number of seeds per pod.

Misra (1985) observed that the number of pods per plant, 1000 seed weight, number of seeds per pod and length of reproductive period contributed directly to seed yield in greengram.

According to Chikkadyavaiah (1985), in cowpea, seed yield is positively associated with number of branches per plant, number of pods per plant, number of seeds per pod and 100 seed weight.

Kay (1979) summarized *Mucuna* seed yields as ranging from 700 to 1100 kg ha^{-1} in India, 1700 to 2200 kg ha^{-1} in the USA, and the lowest of 600 kg ha^{-1} in Australia.

Jayarani (1993) reported a number of 8.5 to 14.5 seeds per pod in blackgram.

Sajikumar (1995) reported a number of 4.75 to 6.65 seeds per pod, seed weight of 7.89 to 22.95 g per plant and a 100 seed weight of 4.87 to 6.42 g in blackgram.

Support or staking of some kind is generally recommended for improving quantity and quality of legume seed production (Humphreys and Riveros, 1986).

According to Kachalreiss and Tarawali (1994) for climbing legumes, seed yields can be increased at least five times by trailing.

Seed yield from a rainfed crop of *M. pruriens* without staking was 1500 to 1750 kg ha^{-1}. Where stakes are provided, an yield of 3000 to 3750 kg ha^{-1} was obtained, and from a well managed irrigated crop having the support of stakes, a seed yield of 5000 kg ha^{-1} was obtained (Chadha, 1995).

2.3.6 Leaf area parameters and physiological parameters

Russel (1961) reported that an increase in total leaf area which results in a higher leaf area index (LAI), had a profound effect on enhancing photosynthetic activity and hence, the yield of crop.

Mercy (1981) observed the LAI of legumes at three stages ie. 45 days after sowing (DAS), 60 DAS and 90 DAS, to be around 3.34, 5.80 and 9.50 respectively.

According to Maggie (1989), the LAI of cowpea increased at vegetative and flowering stage (0.85 and 1.50 respectively) and decreased at harvest (0.50) due to leaf shedding.

Leaf area index is the primary factor that determines the rate of dry matter production. It is found to lower during reproductive phase, as vegetative growth is reduced at this phase; Pearce and Mitchel (1990).

Sajikumar (1995) reported LAI in the range of 1.95 to 8.19 at harvest, among different genotypes of blackgram.

Frequent irrigations enhanced the LAI in greengram (Pannu and Singh, 1993).

High levels of nitrogen are found to significantly influence the LAI of cowpea (Geetha, 1999).

The net assimilation rate (NAR) is one of the most important growth characteristics describing the net production efficiency of the assimilation apparatus. It reflects the net photosynthetic rate per unit leaf area for a given growth period. As the plant develops and the number of leaves increase, more of them get shaded and this results in decrease of photosynthetic rate, hence the NAR is lowered; Pearce and Mitchel (1990).

Harvest Index (HI) varies among legumes according to its genetic makeup. Different genotypes of cowpea displayed a range of 0.30 to 0.65 in the value of the harvest index (Maggie, 1989).

The crop growth rate (CGR) can be considered as the most meaningful growth analysis term. It is closely related to the LAI. Growth in plants is influenced by the period over which plants maintain their leaf area and its persistance in time; Pearce and Mitchel (1990).

Crop growth rate (CGR) of cowpea at four stages ie 30 DAS, 45 DAS and 90 DAS was observed as 0.6, 0.7 and 0.6 respectively. The decline in CGR is attributed to leaf shedding (Jyothi, 1995).

2.4 Biological nitrogen fixation

Legumes can fix around 50 to 300 kg ha^{-1} of nitrogen, resulting in the addition of more nitrogen to agricultural soil than all other fertilizing practices combined (Venkataraman and Tilak, 1990).

The encouraging results of three decades of cover legume research in the tropics, has resulted in the promotion and increased use of legumes for the improvement of soil fertility (IITA, 1993).

Hartwell and Pember (1911) credited an annual average gain of 65 kg ha^{-1} of nitrogen to fields cultivated with legumes.

Greaves and Jones (1950) reported that returning the legume crop to the soil after cultivation, significantly increased the soil nitrogen content.

Sen and Rao (1953) quantified nitrogen fixed by legumes in the soil as 130 kg ha^{-1} per year.

According to Khan (1953), the quantity of nitrogen fixed varies with each legume and ranges from 15 to 70 kg ha^{-1} generally. The amount of nitrogen fixed is the sum total of nitrogen derived from the atmosphere and nitrogen derived from the soil, and comprises around two thirds and one-third amount, respectively.

Russel (1961) observed that all the nitrogen fixed is transferred to the plant tops and seeds, hence not much increase may be seen in the soil nitrogen.

Under Kerala conditions, loss of nitrogen in cultivated fields is very high due to high temperature and excessive rainfall. Hence growing legumes is a cheap source of maintaining soil fertility. Cowpea is observed to fix about 201.80 kg ha^{-1}, *Sesbania aculeata* about 179.55 kg ha^{-1}, *S.speciosa* about 23.95 kg ha^{-1} and *Arachis hypogoea* about 90.10 kg ha^{-1} of soil nitrogen (Bose, 1963).

Mucuna was tested extensively in Nigeria for soil fertility maintenance (Vine, 1953). Botton (1958) recommended it for Ivory Coast.

An estimated amount of 155 to 200 kg ha^{-1} of nitrogen was found in the leaves, pods and roots of well grown sole crop *Mucuna* without mineral fertilization. In general, *Mucuna* may fix anywhere between 70 and 130 kg ha^{-1} of nitrogen. An analysis on the long term changes in soil properties showed an overall increase of 30 per cent in nitrogen content after ten years of growing *Mucuna* (Sanchez, 1993).

According to Becker and Johnson (1998), nitrogen accumulation in *Mucuna* varied between 30 to 257 kg ha^{-1} and nitrogen derived from atmosphere varied from 30 to 90 kg ha^{-1}. These benefits were found to be significantly superior to other legumes like cowpea, stylosanthes, calapogonium and centrosema.

In Vellayani conditions (Trivandrum, Kerala) *M. pruriens* was found to increase the soil nitrogen content by 60 kg ha^{-1} (Sunitha, 1996).

Nodulation is the main index of symbiotic nitrogen fixing efficiency in legumes. Atmospheric nitrogen fixed by the legumes returns to the soil through nodule sloughings or excretion of fixed nitrogen. The plant that records the maximum yield has significantly more number of nodules and weight at all stages of observations (Venkataram and Tilak, 1990).

According to Bose (1963), legumes with larger number of nodules fix higher amounts of soil nitrogen; a good part of the nodules are returned in the organic form, to soil. *Sesbania aculeata* was observed to have

110.13 nodules at 60 DAS compared to *S. speciosa* (26.88), *Arachis hypogoea* (39.62) and *Vigna sinensis* (3.75).

Sunitha (1996) reported that the number of nodules per plant in *M. pruriens* was higher under shaded condition (113.5) than under open condition (105.35).

Rajan (1999) recorded a range of 14.40 to 140.00 mg in nodule weight per plant, at 50 per cent flowering in greengram.

The application of calcium acetate to soybeans was observed to increase the nodulation by ten times (Scalan, 1928).

Whyte and Trumble (1953) reported on the importance of calcium for nutrition of the legume plant, as well as nitrogen fixing bacteria.

According to Fred and Graw (1942), phosphorous plays a dynamic role in symbiotic nitrogen fixation, as it has a stimulatory effect on movement of rhizobia towards the root system.

Sen and Rao (1953) reported on the marked response to application of phosphorous, on the extent of nodulation and nitrogen fixation in legumes.

The application of phosphorous was found to significantly increase the number of nodules per plant in cowpea, and gave a maximum of 27.83 nodules per plant under trial (Nair, 1966).

Rajashree (1994) reported that high doses of both phosphorous and potassium increased nodulation in greengram.

2.5 Role of nutrients

2.5.1 Nitrogen

Excessive vegetative growth is induced by readily available nitrogen (Singh, 1957).

According to Mercy (1981) nitrogen uptake in legumes varied between 15 to 60 kg ha^{-1}.

A starter dose of nitrogen was found to enhance the early growth and establishment of cowpea (Geetha, 1999).

2.5.2 Phosphorous

Phosphorous has a beneficial effect on root growth, flowering, pod formation and seed setting in legumes. It also helps in the uptake of nitrogen and other nutrients (Robert and Olsen, 1944).

Phosphorous is essential in the formation of fruits and seeds (Nair, 1966).

Mercy (1981) observed an uptake of phosphorous in the range 2.5 to 5.7 kg ha^{-1}, during growth of legumes.

Rajashree (1994) reported that phosphorous influences the growth and development of roots in legumes. A better root system is observed to enhance the rate of nitrogen fixation which in turn, increases the plant herbage yield.

Legumes require phosphorus for growth and nitrogen fixation, and soil phosphorus is an important factor in *Mucuna* biomass accumulation (Becker and Johnson, 1998).

2.5.3 Potassium

Mercy (1981) reported an uptake of around 8.45 to 21.5 kg ha^{-1} of potassium during growth of legumes.

According to Yahiya (1996) grain legumes in general required a high quantity of potassium for normal growth and development, and for enhancing nodulation and nitrogen fixation.

Geetha (1999) found that potassium helps in effective use of irrigation water and overcoming summer stress in vegetable cowpea.

2.6 Medicinal value and L-DOPA content

2.6.1. Medicinal value

Mucuna pruriens is a plant of immense medicinal value. Its properties are put to use in the treatment of a vast number of disorders.

The seeds can be used as a nervine tonic; Aiyar and Kolammal (1962), antidiabetic; Dhar and Dhawan (1968) and Lal (1990), to reduce levels of cholesterol; Pant (1978), to enhance spermatogenesis; Misra and Shukla (1984) and Manyam and Ramos (1999). They can serve as a very potent aphrodisiac; Saksena and Dixit (1987), Ananthakumar *et al.* (1994) and Amin (1996). The leaves are used in the treatment of syphilis and sores; Atal *et al.* (1986), in reducing hypertension; Mogra (1987), in the treatment of cancer; Panikkar and Pillai (1988) and snakebite; Houghton (1994). The roots can be used as an analgesic; Yang (1985) and as an emollient; Aruna (1998). The hairs present on the pods are a good vermifuge when administered along with honey; Wallis (1985).

Yet the most outstanding use of *M. pruriens*, is in the treatment of Parkinson's disease.

Parkinson's disease, a progressive neuro-degenerative disorder which causes degeneration and gradual loss of motor activity, is of world wide occurrence and life long presence. It is caused due to the reduction in levels of dopamine; a neuro transmitter produced naturally by the body. This affects the motor activities and functioning of the nervous system resulting in jerky or spasmodic movements (Parikh and Manyan, 1999).

Several scientists have reported on the healing powers of *M. pruriens* seed in Parkinson's disease. This treatment is claimed to be safe from side effects, efficient and economical (Vaidya and Khan, 1978);

Heidel (1987); Dan (1990); Taiyab and Khan, (1991); Kurien (1995) and Altern (1995).

2.6.2. L-DOPA content, factors influencing its synthesis, extraction methods and utilization

The seeds of *M. pruriens* contain the aminoacid L-DOPA (L-3,4-dihydroxyphenylalanine) a precursor of dopamine. This makes the plant valuable in the treatment of Parkinson's disease (Dymock *et al.*, 1980).

Ramaswamy (1957) reported on the isolation of L-DOPA (1.5 per cent on dry weight basis) from *Mucuna* seeds.

Considering the importance of L-DOPA, attempts have been made throughout the world to screen and identify high L-DOPA yielding lines for commercial cultivation (Pieris and Janz, 1980).

Scientists at NBPGR (Issapur farm) have reported on a range of 1.80 to 3.32 per cent L-DOPA in the accessions collected, and have obtained fifteen promising accessions of *M. pruriens* having over 3.0 per cent content of L-DOPA (Chadha, 1995).

Versteeg (1993) observed an amount of 1.5 to 4.8 per cent L-DOPA on dry weight basis from *Mucuna* seeds.

According to Prakash and Tewari (1999) the highest content of L-DOPA can be acquired from half mature (6 weeks old) seeds rather than from mature seeds.

Climatic factors are observed to have no direct effect on L-DOPA content in *M. pruriens* while plants supported by stakes have higher L-DOPA content (Pieris and Janz, 1980).

Sunitha (1996) reported that there was no significant difference in the content of L-DOPA when *M. pruriens* was grown under shade or under open conditions.

Application of plant nutrients appear to affect the quantity of plant metabolites produced.

Chevelier (1911) reported that *Atropa belladona* yielded high amount of alkaloid when nitrogenous fertilizers were applied.

According to Prasad (1944), alkaloid content in *Datura alba* increased on application of higher doses of potassium and nitrogen.

Winters and Loustalot (1952) recorded higher content of total alkaloids and quinine, in *Cinchona ledgeriana*, with higher doses of nitrogen.

According to Bhaskaran (1966), nitrogen and potassium had significant effect in enhancing alkaloid content in roots of *Rauvolfia serpentina* by 56 per cent.

There are various methods of extraction of L-DOPA. They include paper chromatography (Kidwai, 1989), High Particular Liquid

Chromatography (Parikh and Doshi, 1990) and column chromatography (Jingzian, 1994).

Scientists at Zandu Pharmaceutical Works, Mumbai, reported on the formulation of a commercial product (HP 200) which has as lead component, levodopa, present in *M. pruriens*. HP 200 is claimed to be very effective against Parkinson's disease (Parikh and Manyam, 1999).

To accomodate the huge demand for L-DOPA, *in vitro* production of the drug using cell cultures, is now practised extensively. Huizing (1986) reported on the synthesis of L-DOPA, upto 9.0 per cent, from *M. pruriens* cultures, whereas Woerdenbag (1993) reported only 2.0 per cent.

2.7 *Mucuna pruriens* - agricultural uses

2.7.1. As an intercrop in coconut garden

Several reports are available on the successful intercropping of medicinal plants in coconut garden (Singh *et al*., 1990; Nair *et al*., 1991).

One of the prominent alternatives to existing cropping systems is the introduction of leguminous intercrops (Balasubramaniyam, 1991).

Soil improvement is a major factor in increasing cereal yields after *Mucuna* cultivation, due to fixation of nitrogen (Lathwell, 1991).

According to Sanchez (1993) when *Mucuna* was intercropped with maize, the nitrogen released by *Mucuna* (70 to 115 kg ha^{-1}) reached a peak about 30 days after slashing. High levels of nitrogen (60 to 70 kg ha^{-1}) were found in the soil before slashing, indicating the active decomposition of litter biomass, caused by leaf shedding and consequent release of nitrogen.

Van Noordwjk (1995) estimated that 83 per cent of nitrogen contained in a *Mucuna* crop was available to a subsequent maize crop.

Legumes can potentially sustain gains in productivity in intensified systems (Wagger, 1996).

According to Becker and Johnson (1998), use of *Mucuna* as dry season fallow increased upland rice yields by around 500 kg ha^{-1}, which corresponds to a mineral fertilizer substitution of about 50 kg ha^{-1} of nitrogen, as urea.

The net increase in inorganic nitrogen resulting from mineralization, after cultivation of *Mucuna* varied from 60 to 165 kg ha^{-1} (Flores, 1999).

2.7.2 As a cover crop

In recent years, covercrops have received considerable attention as means to increasing the productivity and sustainability, of agricultural systems. *Mucuna* is prominent among the cover crops studied and promoted (Bunch, 1990).

Mucuna can achieve nearly 100 per cent ground cover in two months if soil fertility is adequate. This rapid ground cover can help to physically protect soil from raindrop impact and soil erosion. This helps to maintain the physical and chemical properties of soil. *Mucuna* leaf litter as mulch, protects the soil and builds up organic matter content. It also moderates soil temperature, and alleviates soil compaction (Becker and Tarawali, 1998; IITA, 1998).

According to Peoples and Grey (1995) the mean nitrogen accumulation in leguminous cover crops, is high; about 100 kg ha^{-1}.

Mucuna bracteata has been introduced as a cover crop in rubber plantations (KAU, 1996).

2.7.3 As a means to control weeds

Botton (1958) reported that *M. pruriens* was effective in controlling both nutgrass (*Cyperus rotundus*) and speargrass (*Imperata cylindrica*), two of the most difficult to control weeds in the tropics. This was in congruence with the findings of Poku (1985) and Thurston (1997).

Continuous cropping of *M. pruriens* was found to reduce the weed population by 75 per cent. The crop was also reported to possess allelopathic properties. The harmful substance was identified as L-DOPA which had a suppressing effect on *Asystasia intrusa*, *Paspalus conjugatus* and *Phaseolus vulgaris* (Fujii and Yasuda, 1991).

In Costa Rica, Valverde and Rojas (1995) found that *Mucuna* reduced the biomass of itchgrass (*Rottboellia cochinchinesis*) by 75 to 95 per cent.

Becker and Johnson (1998) observed that *Mucuna* completely controlled *Imperata cylindrica* and *Chromolaena odorata.*

2.7.4 As a means to control nematodes

Reddy *et al.* (1986) reported that *Mucuna* was effective in reducing soil population of root knot nematodes.

Caveness (1993) and Thurston (1997) reported on the suppression of root lesion nematodes like *Pratylenchus spp.* by *Mucuna.*

2.7.5 As a fodder and food crop

According to Botton (1958) and Buckles (1995), *Mucuna* has good forage quality and can be given along with other fodder, in fresh or dry form.

Mucuna grain has good potential as an animal feed. It contains upto 29 per cent protein (Buckles, 1995).

Mucuna beans are described as extremely good, fairly palatable, high protein feed for most livestock (ECHO, 1998).

M. pruriens is also considered as a human food legume (Kay, 1979). *Mucuna* seeds have been regularly consumed by tribals of Andhra Pradesh (Pant, 1992).

According to Rajyalakshmi (1994), the seeds contain upto 29 per cent protein and traces of calcium, phosphorous, iron and niacin. As the seeds contain traces of anti-nutritional factors too, they have to be treated beforehand by keeping them in boiling water for 40 minutes and then discarding the water.

The seeds of *Mucuna* are roasted and ground to be used as a coffee substitute in Gautemala. Hence they are known by the name 'nescao' or 'nescofi'. Currently there is sale of seeds under commercial brand names like 'Nutricoffee' (ECHO, 1998).

In Brazil, flour is prepared from both the seeds and roots of *M. pruriens*. *Mucuna* beans are a good choice as famine foods, as they are easy to cultivate and are produced abundantly (Flores, 1999).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The study titled 'Evaluation of genetic stock of *Mucuna puriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden' was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani during the period May 1999 to January 2000. The details of the materials used and the methods adopted during the course of investigation are presented in this chapter. The work was carried out in four phases *viz.*,

1. Collection of accessions of *Mucuna pruriens*
2. Seed characterization and germination studies
3. Cultural trial of selected accessions under shade in coconut garden
4. Phytochemical analysis

3.1 PHASE I : Collection of accessions of *Mucuna pruriens*

Seeds of twenty five different accessions of *Mucuna pruriens* were collected from inside and outside the state. The accessions were duly registered in the accession register for medicinal and aromatic plants of the Department of Plantation Crops and Spices, College of Agriculture, Vellayani. The details of accessions collected are recorded in Table 3.

Table 3. List of *Mucuna pruriens* accessions collected and their sources

Sl. No.	Accession Number	Cultivated/ Wild	Source of collection
1	MP-44	Cultivated	Dr. Z. Abraham, Senior Scientist, NBPGR Regional Station, Thrissur
2	MP-45	Cultivated	
3	MP-46	Cultivated	
4	MP-47	Cultivated	
5	MP-48	Cultivated	
6	MP-49	Wild	TBGRI, Palode, Thiruvananthapuram
7	MP-50	Cultivated	NBPGR, Pusa, New Delhi
8	MP-51	Cultivated	NBPGR, Pusa, New Delhi
9	MP-52	Cultivated	NBPGR, Pusa, New Delhi
10	MP-53	Cultivated	NBPGR, Pusa, New Delhi
11	MP-54	Cultivated	NBPGR, Pusa, New Delhi
12	MP-55	Cultivated	NBPGR, Pusa, New Delhi
13	MP-56	Cultivated	NBPGR, Pusa, New Delhi
14	MP-57	Cultivated	NBPGR, Pusa, New Delhi
15	MP-58	Wild	Dr. Swami Velji, Kottoor, Thiruvananthapuram
16	MP-59	Wild	Mr. Mathewkutty Theruvapuzha, Palai, Kottayam
17	MP-60	Wild	Plapally, Pampa Valley, Pathanamthitta
18	MP-61	Wild	Thullapally, Wynad
19	MP-62	Wild	Dr. P.J. Thomas, Arya Stores, Karugachal, Kottayam
20	MP-63	Wild	R.S. Traders, Vennikulam, Thiruvalla
21	MP-64	Wild	Chengara forest areas, Pathanamthitta
22	MP-65	Wild	K.F.R.I., Peechi, Thrissur
23	MP-66	Wild	Ayur forest areas, Pathanamthitta
24	MP-67	Wild	Thalachira forest areas, Pathanamthitta
25	MP-68	Wild	Mr. P.S. Thomas, Kumplampoika, Pathanamthitta

3.2 PHASE II : Seed characterization and germination studies

Observations such as weight of 100 seeds, seed colour and description of external appearance of the seeds were made on each accession.

Seed germination study to assess viability of seeds was done as described below.

Each accession collected was represented by three replicates of twenty seeds each. The seeds were surface sterilized using 0.1 per cent mercuric chloride for one minute, followed by washing thrice with distilled water.

As the seed coat was hard and highly impervious to moisture, the seeds were subjected to hot water treatment. This was carried out by dipping seeds in boiling water for two minutes, after wrapping them in muslin cloth. The seeds were then kept in cold water, overnight.

The seeds were placed on moistened filter paper in petridishes, and covered to ensure high moisture inside.

Seeds were considered to have germinated when the greenish structure of the radicle emerged out of the seed coat. Seed germination count was taken from the second day and the percentage of germination of each accession was determined. The number of days taken for 50 per cent seed germination in each accession was also recorded.

3.3 PHASE III : Cultural trial of selected accessions under shade in coconut garden

3.3.1 Experimental site

The experiment was laid out at the College of Agriculture, Vellayani from May 1999 to January 2000.

The site was located at 8°5' North latitude, 77°1' East latitude and at an altitude of 29 m, above mean sea level. Soil of the experimental site is red loam belonging to Vellayani series. The area enjoys a humid tropical climate with an average annual precipitation of 3000 mm. The meterological data for this period is given in Appendix 1.

3.3.2 Field preparation and sowing

The land was thoroughly prepared by digging and levelling. Plots of dimension 6.0 m x 5.4 m were made. Dried and powdered cowdung was incorporated at the rate of 3 kg per m^2. Seeds were sown at a spacing of 60 x 60 cm.

3.3.3. Experimental design and layout

The experiment was laid out in randomized block design with three replications. Based on germination studies 10 accessions were selected for cultural trial. They were, MP-44, MP-45, MP-46, MP-57, MP-58, MP-60, MP-62, MP-64, MP-66 and MP-67. Ninety plants of each accession were maintained in each plot, at a spacing of 60 x 60 cm. Each plot measured 6.0 m x 5.4 m. The vines were allowed to trail up with the help of *Casuarina* poles, G.I. wire and coir.

Destructive sampling was done at pre-flowering (60 DAS), flowering (120 DAS), seeding (180 DAS) and seed maturation (240 DAS) stage. Four plants per plot per replication were utilized for the periodic sampling. Altogether, there were three replications. After taking biometric observations, samples were partitioned to shoots, roots, leaves and pods for growth and yield analysis.

3.3.4 Growth parameters

The following biometric observations were taken at four growth stages, *viz.*, pre-flowering stage (60 DAS), flowering stage (120 DAS) seeding stage (180 DAS) and seed maturation stage (240 DAS).

3.3.4.1 Plant length (m)

Each sample plant was uprooted. The branches were alligned together and measurement was taken from the collar region to the farther most growing tip of the plant. The mean value was worked out and recorded.

3.3.4.2 Number of leaves

The number of leaves present in each sample plant was recorded and the mean value worked out and recorded.

3.3.4.3 Leaf area (cm^2)

From each sample plant, 25 leaves were taken at random. The leaf area of each was worked out, by making use of graph paper.

The mean value was worked out and recorded.

3.3.4.4 Root length (m)

Each sample plant was uprooted. The tap roots and lateral roots were alligned together. Measurement was taken from the collar region to the farthermost tip of the root system.

The mean value was worked out and recorded.

3.3.4.5 Root girth (cm)

The girth of root at collar region, was measured in each sample plant, making use of thread and measuring tape.

The mean value was worked out and recorded.

3.3.4.6 Number of pod clusters per plant

The number of pod clusters present in each sample plant was taken and the mean value worked out.

3.3.4.7 Number of pods per cluster

The number of pods per cluster in each sample plant was recorded and the mean value was worked out.

3.3.4.8 Length of pods (cm)

The length of pods was measured in each sample plant with the help of a measuring scale. The mean value was worked out.

3.3.4.9 Number of seeds per pod

The number of seeds present in each pod of the sample plant was recorded and the mean value worked out.

3.3.5 Yield attributes

3.3.5.1 Fresh and dry weight of leaves (g)

The leaves of each sample plant was packed separately in a brown paper cover and the fresh weight was taken using a Digiweigh (IPA scale) Model ITB 22/01 electric balance. The mean value was worked out and recorded.

The samples were then dried in a KEMI hot air oven at 60°C for five days until constant dry weights were observed. The mean dry weight of leaves per plant was recorded.

3.3.5.2 Fresh and dry wieght of shoot (g)

After removing the leaves, the shoot portion of each sample plant was packed in a brown paper cover and the freshweight was taken using a Digiweigh (IPA scale) Model ITB 22/01 electric balance. The mean fresh weight of shoot per plant was recorded. The shoot samples were then dried in a KEMI hot air oven at 60°C for five days until constant dry weights were observed. The mean dry weight was measured.

3.3.5.3 Fresh and dry wieght of roots (g)

The roots of each sample plant was packed separately in a brown paper bag and the fresh weight of each was taken using a Digiweigh (IPA scale) Model ITB 22/01 electric balance. The mean fresh weight was worked out.

The samples then were dried in a hot air oven at 60°C until constant dry weights were obtained. The mean dry weight of roots per plant was worked out and recorded.

3.3.5.4 Fresh and dry weight of pods (g)

The pods of each sample plant were placed together in a brown paper bag and fresh weight taken. The pods were dried in a hot air oven at 60°C for five days until constant dry weights were obtained.

The mean fresh weight and dry weight of pods per plant were worked out and recorded.

3.3.5.5. Weight of seeds at harvest (240 DAS) (g)

The pods of each sample plant were collected and their pod shells were cracked open using a light mallet. The seeds were extracted from the pods and cleared of the pod shell remains. The mean weight of seeds per plant were worked out and recorded.

3.3.6 Root nodules

3.3.6.1 Number of root nodules

The nodules were detached from the root system of the sample plants. The total count of root nodules for plant was made and the mean value worked out and recorded.

3.3.6.2 Number of effective root nodules

With the help of a sharp blade, each nodule was dissected cross-sectionally.

Nodules which were firm and pocessed a bright red centre were considered as effective root nodules.

The count of effective nodules per sample plant was taken, and the mean value worked out.

3.3.6.3 Fresh and dry weights of nodules (g)

The nodules obtained from each sample were placed into small brown paper covers. The fresh weight of each was taken using a Sartorius analytic electronic balance and the mean fresh weight of nodules per plant was worked out.

The samples were then dried in a hot air oven at 60°C for 3 days until constant dry weights were obtained. The mean value of dry weight of nodules per plant was worked out and recorded.

3.3.7 Leaf area parameters

3.3.7.1 Leaf area index (LAI)

The area of leaf was measured using graph paper. The LAI was worked out based on the method suggested by Williams (1946).

$$\text{LAI} = \frac{\text{Total leaf area of the plant } (m^2)}{\text{Area of land covered by the plant } (m^2)}$$

3.3.7.2 Leaf area duration (LAD) (days or weeks or months)

The leaf area duration was calculated using the formula given by Watson (1958).

$$\text{LAD} = \frac{(L1 + L2)}{2} \times T_2 - T_1$$

L_1 = LAI at time T_1
L_2 = LAI at time T_2
T_2-T_1 = Time interval in days or weeks or months

3.3.8. Physiological parameters

3.3.8.1 Net assimilation rate (NAR) (g day^{-1})

The method proposed by Williams (1946) was employed for calculating the NAR on leaf dry weight basis.

$$NAR = \frac{(W_2 - W_1)}{(T_2 - T_1)} \times \frac{\log_e W_2 - \log_e W_1}{L_2 - L_1}$$

where:

W_1, W_2 : dry weights of whole plants at time T_1 and T_2 respectively

L_1,L_2 : dry weight of leaf at time T_1 and T_2 respectively

T_2-T_1 : time interval in days

3.3.8.2 Crop growth rate (CGR) (g m^{-2} day^{-1})

The CGR was worked out by using the formula of Watson (1958).

$$CGR = \frac{W_2 - W_1}{P\,(T_2 - T_1)}$$

W_1, W_2 : whole plant dry weights at time T_1 and T_2
T_2 - T_1 : time interval in days
P : ground area on which W_1 and W_2 are estimated

3.3.8.3 Relative growth rate (RGR) (g day^{-1})

The RGR was determined by utilizing the formula given by Blackman (1919).

$$RGR = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

W_1, W_2 : plant dry weights at time T_1 and T_2
T_2 - T_1 : time interval in days

3.3.8.4 Absolute growth rate (AGR) (g day^{-1})

The AGR was determined by utilizing the formula given by Watson (1958).

$$AGR = \frac{W_2 - W_1}{T_2 - T_1}$$

W_1, W_2 : plant dry weights at time T_1 and T_2
$T_2 - T_1$: time interval in days

3.3.8.5 Harvest index (HI)

Harvest Index was calculated in terms of pod yield.

It was worked out from the data of pod yield and biological yield (total plant dry matter)

$$HI = \frac{\text{Economic yield}}{\text{Biological yield}}$$

3.3.9 Harvest

The pods were harvested at the seed maturation stage, ie. 240 DAS.

The pods were harvested making use of paper bags and a pair of scissors.

After necessary observations were taken, the pods were cracked open with the help of a light mallet and the seeds were extracted.

The seeds were cleared free of the pod shell remains and stored in brown paper covers, in a cool dry place, before extraction and estimation of aminoacid was carried out.

3.4 PHASE IV Phytochemical analysis

3.4.1 Soil analysis before and after the experiment for N, P and K

Soil samples were drawn individually from every plot, before the commencement of the experiment, and after the harvest of the crop.

Samples were taken at 0-15 cm depth, air-dried and passed through a 2 mm sieve.

The status of available nitrogen was estimated using the alkaline potassium permanganate method (Subbiah and Asija, 1956), and expressed in kg ha^{-1}.

The available phosphorous content was estimated using Bray's colourimetric method (Jackson, 1973) and expressed in kg ha^{-1}.

The ammonium acetate method given by Jackson (1973) was used to estimate the available potassium and expressed in kg ha^{-1}.

3.4.2 Estimation of L-DOPA content in seeds

The L-DOPA content of the seeds of various accessions was estimated using modified Kidwai's process (Kidwai, 1989).

Ten grams of seeds from each accession was weighed and ground finely. It was transferred into 250 ml round bottom flasks. To each was added 25 ml of 0.1 N alcoholic hydrochloric acid. It was refluxed for four hours. The resultant solution was cooled, decanted and filtered into 100 ml volumetric flasks. The solvent 0.1 N alcoholic HCL (25 ml) was added again to the residue and the process repeated four times. The final volume of filtrate was made up to 100 ml using the same solvent.

Liquid chromatography tanks were prepared. Into each was poured 250 ml of the solvent Butanol, Acetic acid and water in the ratio of 4:1:5.

Chromatography papers of dimensions 9 inches breadth and 12 inches length were taken. They were each spotted with 0.5 ml of the sample filtrate and 0.5 ml of pure standard L-DOPA solution. One such chromatography sheet was placed in each tank. The solvent was allowed to ascend eleven inches before the sheets were removed.

The chromatography sheets were air-dried for 24 hours.

The sheets were then sprayed with Ninhydrin reagent and dried in a hot air oven at 100°C for 10 minutes.

The portions corresponding to the purple coloured band of the standard L-DOPA region were cut out.

Due to the unstablity of colour when exposed to light, the cut out portion was quickly eluted with 5 ml alcohol into a 10 ml volumetric flask, and 2 ml ninhydrin reagent was added to it. It was heated in boiling waterbath for 5-7 minutes.

The colour developed was read in an electronic colourimeter at 490 nm.

The content of L-DOPA in the extract was computed as follows.

L-DOPA content (expressed in percentage of seed weight)

$$= \frac{\text{weight of standard}}{500} \times \frac{0.5}{\text{reading of standard}} \times \frac{\text{reading of sample}}{0.5} \times \frac{100}{\text{weight of sample}} \times 100$$

3.5 Incidence of pests and diseases

Throughout the duration of the crop, periodical surveilance was carried out for the detection of pests and diseases. Curative measures were undertaken to counteract the diseases.

3.6 Statistical analysis

Qualitative as well as quantitative parameters of the ten accessions under trial, were recorded from twelve sample plants at four different stages of growth.

Analysis of variance was worked out for all the traits to find out whether there was significant difference between the accessions in respect of various traits.

For the computation of analysis of variance, procedure proposed by Panse and Sukhatme (1978) was used. The significance of the computed values for 'F' was tested with reference to the F-table.

3.7 Selection index

Selection indices were worked out through the application of discriminant function proposed by Smith (1936). The characters used for the construction of selection index were biomass yield, root yield seed yield, nodule characters (effective nodule count and dry weight of nodules at seeding) and content of L-DOPA.

Seperate selection indices were worked out based on the following characters :

a) Seed yield

b) Seed yield and L-DOPA content

c) Seed yield and biomass yield

d) Seed yield, biomass yield, root yield and root nodule characters.

RESULTS

4. RESULTS

The results of the study on 'Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden' are presented in this chapter phase wise. Phase I consists of collection of accessions of *Mucuna pruriens*. Phase II consists of the seed characterization and germination studies. Phase III comprises cultural trial of selected accessions under shade in coconut garden and Phase IV comprises the phytochemical analysis.

4.1. Phase I Collection of accessions of *M. pruriens*

Seeds of various accessions of *M. pruriens* were collected from inside and outside the state. List of collected accessions with their sources is given in Table 3.

4.2. Phase II - Seed characterization and germination studies

Seeds of the twenty five accessions collected were subjected to seed germination tests and the result is given in Table 4.

Observations on the weight of hundred seeds, hundred seed volume, seed length and seed colour were recorded for each accession.

Table 4. Seed characteristics of the accessions of *Mucuna pruriens*

Sl. No.	Accession No.	100 seed weight (g)	100 seed volume (ml)	No. of days to 50% seed germination	Seed germination (%)	Seed length (cm)	Seed colour
*1	MP-44	52	35	6	95	1.3	Mottled (light brown with dark brown streaks)
*2	MP-45	68	50	5	95	1.5	Black, distinct white hilum
*3	MP-46	66	48	5	97	1.4	Black, distinct white hilum
4	MP-47	49	83	pg	12	0.9	Mottled
5	MP-48	50	34	pg	9	1.0	Mottled
6	MP-49	78	58	ng	0	1.5	Light brown
7	MP-50	82	65	ng	0	1.9	Black and brown patches
8	MP-51	77	59	ng	0	1.6	Creamy yellow
9	MP-52	76	59	ng	0	1.5	Creamy yellow
10	MP-53	81	62	ng	0	1.8	Black and brown patches
11	MP-54	81	62	ng	0	1.8	Black and brown patches
12	MP-55	75	58	ng	0	1.5	Black and brown patches
13	MP-56	85	68	ng	0	2.0	Creamy yellow

Table 4. (Contd....)

Sl. No.	Accession No.	100 seed weight (g)	100 seed volume (ml)	No. of days to 50% seed germination	Seed germination (%)	Seed length (cm)	Seed colour
*14	MP-57	51	45	6	98	1.2	Black, distinct white hilum
*15	MP-58	50	32	7	97	1.1	Mottled
16	MP-59	52	34	ng	0	1.2	Black, distinct white hilum
*17	MP-60	51	31	6	95	1.1	Mottled
18	MP-61	54	36	ng	0	1.3	Mottled
*19	MP-62	51	30	6	98	1.2	Mottled
20	MP-63	53	35	ng	0	1.2	Black, distinct white hilum
*21	MP-64	50	31	7	97	1.1	Mottled
22	MP-65	55	37	ng	0	1.3	Black, distinct white hilum
*23	MP-66	51	31	7	95	1.1	Mottled
*24	MP-67	52	32	6	97	1.2	Mottled
25	MP-68	54	36	ng	0	1.3	Mottled

* Accessions selected based on high germination percentage
pg - poor germination ng = no germination

Based on the results of seed germination test, ten accessions which recorded higher percentage of germination were selected for further evaluation in field. Accessions selected were MP-44, MP-45, MP-46, MP-57, MP-58, MP-60, MP-62, MP-64, MP-66 and MP-67 (Table 5).

4.3. Phase III Cultural trial of selected accessions under shade in coconut garden

Ten accessions selected based on seed germination test were raised under shade in a coconut garden. Observations were made at four stages of growth viz., pre-flowering, flowering, seeding and seed maturation stage. The results of the experiments carried out are presented in the following pages.

4.3.1. Growth parameters

The data on growth parameters such as plant length, number of leaves per plant, leaf area, root length, root girth, number of pod clusters per plant, number of pods per cluster, length of pod and number of seeds per pod are given in Table 6, Table 7, Table 8, Table 9 and Table 10.

4.3.1.1. Plant Length

The plant length varied significantly among the ten accessions (Table 6) at all stages of plant growth.

Table 5. List of accessions selected on the basis of germination percentage

Sl. No.	Accession number	Source of collection
1.	MP-57	NBPGR, New Delhi
2.	MP-44	NBPGR, Thrissur
3.	MP-58	Dr. Swami Velji, Kottoor, Thiruvananthapuram
4.	MP-67	Thalachira forest areas, Pathanamthitta
5.	MP-62	Dr. P. J. Thomas, Karugachal, Kottayam
6.	MP-60	Pampa Valley, Pathanamthitta
7.	MP-45	NBPGR, Thrissur
8.	MP-66	Ayur forest areas, Pathanamthitta
9.	MP-46	NBPGR, Thrissur
10.	MP-64	Chengara forest areas, Pathanamthitta

Table 6. Growth parameters of *Mucuna pruriens* at four stages of plant growth : plant length (m)

Sl. No.	Accession Number	Pre-flowering (60 DAS)	Flowering (120 DAS)	Seeding (180 DAS)	Seed maturation (240 DAS)
1.	MP-57	1.81	6.44	9.02	9.63
2.	MP-44	2.13	7.11	9.42	9.80
3.	MP-58	1.30	5.39	8.57	9.50
4.	MP-67	0.97	4.16	7.21	7.96
5.	MP-62	2.22	5.45	9.37	10.05
6.	MP-60	1.30	5.07	7.47	8.41
7.	MP-45	1.68	5.25	7.16	8.25
8.	MP-66	0.90	4.33	7.21	8.38
9.	MP-46	1.84	4.42	8.18	9.05
10.	MP-64	0.66	4.36	5.58	7.24
	F value	114.128**	53.231**	240.442**	178.683**
	CD	0.15	0.40	0.23	0.20

** Significant at 1 per cent level

DAS : Days after sowing *in situ*


1


2


3

At pre-flowering stage, maximum plant length was recorded by MP-62 (2.22 m), followed by MP-44 (2.13 m) and the least by MP-64 (0.66 m).

At flowering stage, MP-44 recorded the longest plant length of 7.11 m, followed by MP-57 (6.44 m) and was least for MP-67 (4.16 m).

At seeding stage, plant length was maximum for MP-44 (9.42 m) which was on par with MP-62 (9.37 m). It was least for MP-64 (5.58 m).

At seed maturation stage, MP-62 recorded the longest plant length (10.05 m), followed by MP-44 (9.80 m) and MP-57 (9.63 m) which were on par with MP-58 (9.50 m). Plant length was least for MP-64 (7.24 m).

4.3.1.2 Number of leaves

The number of leaves per plant differed significantly among the accessions, at all stages of plant growth (Table 7).

At pre-flowering stage the greatest number of leaves per plant was recorded by MP-46 (54.88), followed by MP-44 (43.38) and MP-62 (36.50). Least number of leaves was recorded by MP-64 (17.25). MP-45, MP-57 and MP-58 were on par with 35.75, 34.88 and 34.13 number of leaves per plant respectively.

At flowering stage, MP-44 recorded the maximum number of leaves (186.5) followed by MP-62 and MP-57 which were on par with

Table 7. Growth parameters of *Mucuna pruriens* at four stages of plant growth : number of leaves per plant and leaf area (cm^2)

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)	
		No. of leaves	Leaf area (cm^2)	No. of leaves	Leaf area (cm^2)	No. of leaves	Leaf area (cm^2)	No. of leaves	Leaf area (cm^2)
1.	MP-57	34.88	18.13	151.88	53.15	178.50	88.40	169.25	75.75
2.	MP-44	43.38	19.46	186.50	53.25	224.75	87.70	162.25	77.75
3.	MP-58	34.13	21.50	139.25	49.05	187.25	88.80	148.00	72.09
4.	MP-67	23.25	20.75	110.50	51.48	133.38	79.67	105.50	68.92
5.	MP-62	36.50	19.92	159.63	56.10	198.00	89.67	177.00	77.80
6.	MP-60	33.75	21.96	103.75	48.13	152.63	81.75	104.75	73.59
7.	MP-45	35.75	20.42	137.50	55.70	148.50	81.84	110.13	74.58
8.	MP-66	31.88	19.63	81.88	47.20	129.13	80.45	86.25	67.17
9.	MP-46	54.88	19.50	105.13	51.63	140.50	86.42	114.63	74.25
10.	MP-64	17.25	14.46	91.88	48.50	119.13	72.14	85.38	71.42
	F value	189.407**	61.365**	129.146**	4.667**	210.084**	5.916**	214.239**	2.200
	CD	2.20	0.80	8.73	4.33	7.04	6.75	7.02	7.02

** Significant at 1 per cent level DAS - Days after sowing *in situ*

159.63 and 151.88 number of leaves per plant respectively. MP-66 had the minimum number of leaves (81.88).

At seeding stage, the highest number of leaves were recorded by MP-44 (224.75) followed by MP-62 (198.00), MP-58 (187.25) and MP-57 (178.50). The least number of leaves were recorded by MP-64 (119.13), preceded by MP-66 (129.13) and MP-67 (133.38) which were on par.

At seed maturation stage, MP-62 recorded the maximum number of leaves (177.00), followed by MP-57 (169.25) and MP-44 (162.25) which were on par, and MP-58 (148.00). The least number of leaves was recorded by MP-64 (85.38) which was on par with MP-66 (86.25). This was preceded by MP-45, MP-67 and MP-60 which were on par with 110.13, 105.50 and 104.75 number of leaves per plant respectively.

4.3.1.3 Leaf Area

The leaf area varied significantly among the accessions (Table 7) at all stagess of plant growth.

At pre-flowering MP-60 had maximum individual leaf area of 21.96 cm^2. This was on par with MP-58 (21.50 cm^2). Next in the order were MP-67 (20.75 cm^2) and MP-45 (20.42 cm^2) which were on par. This was followed by MP-62 (19.92 cm^2), MP-66 (19.63 cm^2), MP-46 (19.50 cm^2) and MP-44 (19.46 cm^2) which were on par. The least leaf area was recorded by MP-64 (14.46 cm^2).

At flowering, MP-62 recorded the largest leaf area of 56.10 cm^2. This was followed by MP-45 (55.70 cm^2), MP-44 (53.25 cm^2), MP-57 (53.15 cm^2) and MP-46 (51.63 cm^2) which were on par. Least leaf area was observed for MP-66 (47.20 cm^2) which was on par with MP-67 (51.48 cm^2), MP-58 (49.05 cm^2), MP-64 (48.50 cm^2) and MP-60 (48.13 cm^2).

At seeding stage, maximum leaf area (89.67 cm^2) was recorded by MP-62 and this was on par with MP-58 (88.80 cm^2), MP-57 (88.40 cm^2) and MP-44 (87.70 cm^2). Least leaf area was for MP-64 (72.14 cm^2) preceded by MP-46 (86.42 cm^2), MP-45 (81.84 cm^2), MP-60 (81.75 cm^2), MP-66 (80.45 cm^2) and MP-67 (79.67 cm^2) which were on par.

At seed maturation stage there was no significant difference in leaf area among the accessions. The highest leaf area recorded was 77.8 cm^2 for MP-62 and lowest was 67.17 cm^2 for MP-66.

4.3.1.4 Root Length

Root length differed significantly among the accessions at all stages of plant growth (Table 8).

At pre-flowering stage, root length was maximum for MP-44 (0.27 m) which was on par with MP-57 (0.25 m) and MP-58 (0.25 m) respectively. Least root length was observed for MP-64 (0.18 m) which was on par with MP-60 (0.20 m) and MP-66 (0.20 m).

Table 8. Growth parameters of *Mucuna pruriens* at four stages of plant growth : root length (m) and root girth (cm)

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)	
		Root length	Root girth	Root length	Root girth	Root length	Root girth	Root length	Root girth
1.	MP-57	0.25	1.36	1.22	2.43	1.36	2.75	1.51	3.73
2.	MP-44	0.27	1.18	1.16	2.31	1.25	2.73	1.40	3.64
3.	MP-58	0.25	1.21	0.77	1.87	1.13	2.82	1.40	3.67
4.	MP-67	0.23	1.18	0.85	2.12	1.06	2.28	1.26	3.45
5.	MP-62	0.21	1.19	1.06	2.30	1.33	2.63	1.49	3.82
6.	MP-60	0.20	1.09	0.70	2.07	1.06	2.69	1.43	3.61
7.	MP-45	0.21	1.22	0.80	2.31	1.25	2.61	1.37	3.39
8.	MP-66	0.20	1.12	0.59	1.86	0.97	2.43	1.30	3.17
9.	MP-46	0.22	1.06	1.05	2.41	1.20	2.78	1.26	3.51
10.	MP-64	0.18	1.00	0.82	1.74	0.90	1.86	1.20	2.68
	F value	10.269**	15.779**	74.679**	27.381**	8.754**	17.619**	48.605**	42.616**
	CD	0.03	0.07	0.07	0.14	0.11	0.14	0.10	0.14

** Significant at 1 per cent level   DAS - Days after sowing *in situ*

At flowering stage the longest root length was recorded by MP-57 (1.22 m) which was on par with MP-44 (1.16 m). This was followed by MP-62 (1.06 m) which was on par with MP-46 (1.05 m). Least length was recorded as 0.59 m for MP-66.

MP-57 showed the maximum root length of 1.36 m which was on par with MP-62 (1.33 m) at seeding stage. It was followed by MP-44 and MP-45 which were on par, having 1.25 m each. MP-64 recorded the least root length of 0.90 m which was on par with MP-66 (0.97 m).

At seed maturation stage, the highest root length was recorded for MP-57 (1.51 m) which was on par with MP-62 (1.49 m). Next in the order was MP-60 (1.43m) which was on par with MP-44 (1.40 m), MP-58 (1.40 m) and MP-45 (1.37 m). The least root length was for MP-64 (1.20 m) which was on par with MP-67 (1.26 m), MP-46 (1.26 m) and MP-66 (1.30 m).

4.3.1.5 Root girth

Root girth differed significantly at all stages of plant growth among the accessions (Table 8).

At pre-flowering stage, the highest root girth (1.36 cm) was recorded for MP-57. The accessions MP-45, MP-58, MP-62, MP-67, MP-44 and MP-66 were on par with 1.22 cm, 1.21 cm, 1.19 cm, 1.18

cm, 1.18 cm and 1.12 cm respectively. MP-64 and MP-46 recorded least root girth (1.00 cm and 1.06 cm respectively).

At flowering stage MP-57, MP-46 (2.41 cm), MP-45 (2.31 cm), MP-44 (2.31 cm) and MP-62 (2.30 cm) were on par, with maximum root girth of 2.43 cm for MP-57. Least root girth measurement was for MP-64 (1.74 cm) preceded by MP-58 (1.87 cm) and MP-66 (1.86 cm) which were on par.

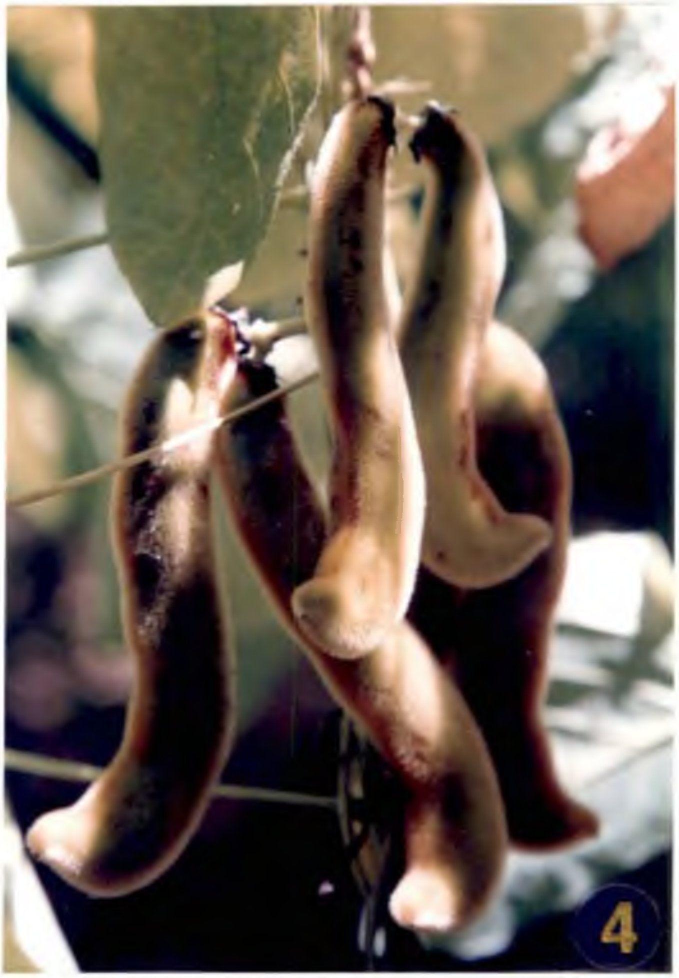
At seeding stage, maximum root girth was recorded by MP-58 (2.82 cm) which was on par with MP-46 (2.78 cm) and MP-57 (2.75 cm). The lowest root girth was for MP-64 (1.86 cm) preceded by MP-67 (2.28 cm) and MP-66 (2.43 cm).

At seed maturation stage, MP-62 had the highest root girth (3.82 cm). It was followed by MP-57 (3.73 cm) which was on par with MP-58 (3.67 cm) and MP-44 (3.64 cm). The lowest root girth (2.68 cm) was recorded by MP-64.

4.3.1.6. Number of pod clusters per plant

The number of pod clusters per plant differed significantly among the accessions (Table 9) at seeding and seed maturation stage.

At seeding stage, MP-60 recorded the maximum number of pod clusters per plant (7.38) and was on par with MP-62 (7.25). Next in the order was MP-58 (7.00) which was on par with MP-57 (6.63). The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accessions MP-67, MP-44 and MP-66 were on par with 6.38, 6.25 and 5.88 pod clusters per plant respectively. The least number of pod clusters per plant were recorded by MP-45 (5.13), MP-64 (5.13) and MP-46 (4.88).

At seed maturation stage, MP-60 had maximum number of pod clusters per plant (10.88), followed by MP-62 (7.88). The accessions MP-58 (7.65), MP-67 (7.63), MP-44 (7.63), MP-57 (7.63) and MP-66 (7.50) were on par. Least number of pod clusters were recorded by MP-64 (5.38) preceded by MP-45 (6.00) and MP-46 (6.38).

4.3.1.7. Number of pods per cluster

The number of pods per cluster differed significantly among the accessions (Table 9) at seeding and seed maturation stage.

At seeding stage, MP-62 had maximum number of pods per cluster (6.63), followed by MP-58 (6.25) which were on par with MP-67 (6.13), MP-60 (6.13) and MP-44 (6.00). Least number of pods per cluster was recorded by MP-45 and MP-46 which had 3.63 pods per cluster each.

At seed maturation stage, MP-62 recorded the highest number of pods per cluster (6.25) followed by MP-67 (6.00) which were on par with MP-58 (5.88) and MP-60 (5.88). The accessions MP-66 and MP-44 were on par with 5.50 and 5.38 pods per cluster. Least number of pods per cluster were observed for MP-46 and MP-45 as 3.63 and 3.50 respectively and they were on par.

4.3.1.8. Length of pods

The length of pods differed significantly among the accessions (Table 10) at seeding and seed maturation stage.

At seeding stage, MP-45 recorded the greatest pod length of 7.31 cm which was on par with MP-46 having 7.25 cm. MP-60 recorded the next highest pod length of 6.58 cm. This was followed by MP-44 (6.47 cm). Least pod length was for MP-64 (5.06 cm).

At seed maturation stage, maximum pod length was recorded by MP-45 (11.07 cm), followed by MP-46 (10.96 cm) and both were on par. The accessions MP-44, MP-67, MP-66 and MP-60 followed with pod lengths of 8.66 cm, 8.58 cm, 8.52cm and 8.48 cm respectively and they were on par. MP-64 had the least pod length of 7.73 cm.

4.3.1.9. Number of seeds per pod

The number of seeds per pod differed significantly among the accessions (Table 10) at seeding and seed maturation stage.

At seeding stage, MP-66 recorded the maximum number of seeds per pod (6.38). This was followed by MP-58 (5.88) and MP-64 (5.88). Next in the order was MP-67 with 5.75 seeds per pod, and this was on par with MP-62 (5.63) and MP-57 (5.63). The least number of seeds per pod were recorded as 4.38 each by MP-45 and MP-46, and they were on par.

Table 10. Growth parameters of *Mucuna pruriens* at seeding stage and seed maturation stage : length of pod (cm) and number of seeds per pod

Sl. No.	Accession Number	Seeding (180 DAS)		Seed maturation (240 DAS)	
		Length of pod	No. of seeds per pod	Length of pod	No. of seeds per pod
1.	MP-57	6.30	5.63	8.34	6.75
2.	MP-44	6.47	5.25	8.66	6.00
3.	MP-58	6.14	5.88	8.42	6.50
4.	MP-67	6.37	5.75	8.58	6.50
5.	MP-62	6.08	5.63	8.24	6.38
6.	MP-60	6.58	5.38	8.48	6.38
7.	MP-45	7.31	4.38	11.07	5.88
8.	MP-66	5.24	6.38	8.52	6.50
9.	MP-46	7.25	4.38	10.96	5.75
10.	MP-64	5.06	5.88	7.73	6.38
	F value	476.851**	59.604**	253.265**	8.104**
	CD	0.10	0.25	0.21	0.33

** Significant at 1 per cent level

DAS - Days after sowing *in situ*


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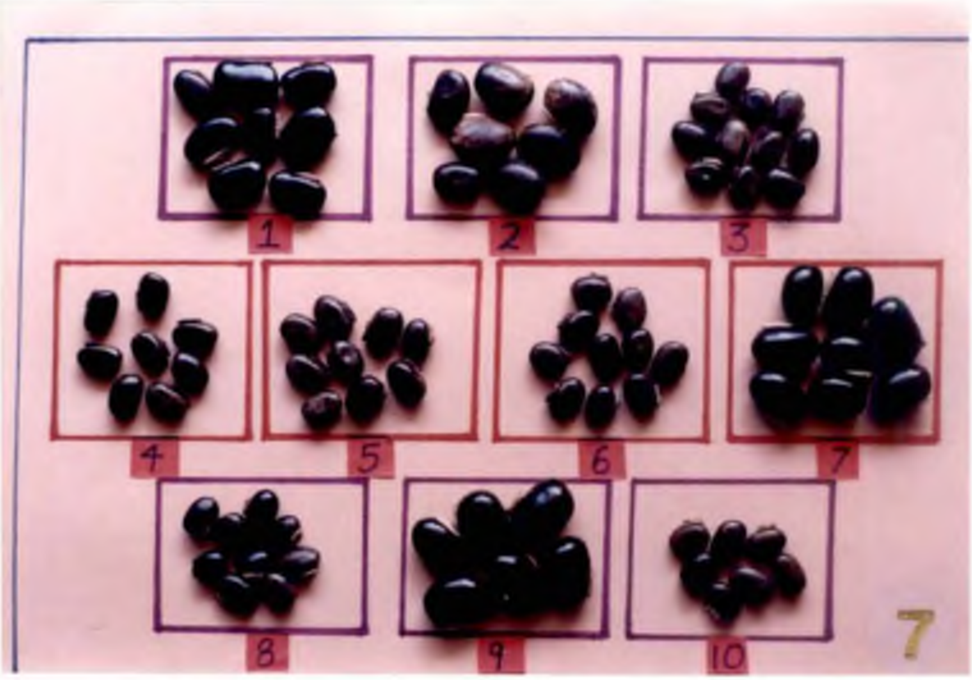
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At seed maturation stage, MP-57 was observed to have the highest number of seeds per pod (6.75). This was followed by MP-58 (6.50) and was on par with MP-67 (6.50), MP-66 (6.50), MP-62 (6.38), MP-60 (6.38) and MP-64 (6.38). The accessions MP-45 and MP-46 had 5.88 and 5.75 seeds per pod respectively and were on par. They recorded the least number of seeds per pod.

4.3.2. Yield parameters

4.3.2.1. Fresh and dry weight of leaves

The fresh and dry weight of leaves per plant differed significantly among the accessions at all stages of plant growth (Table 11).

At pre-flowering stage, MP-46 recorded maximum fresh weight of leaves per plant (26.97 g) and was on par with MP-44 (24.61g). This was followed by MP-45 (20.92 g) which was on par with MP-62 (18.91 g) and MP-60 (18.47 g). Least fresh weight was recorded by MP-66 (9.68 g) which was on par with MP-64 (12.12 g).

Leaf dry weight was recorded as highest for MP-46 (5.33 g) followed by MP-44 (4.48 g). MP-45 (4.02 g), MP-67 (4.02 g) and MP-58 (4.01g) were next in the order and on par. Least value was recorded by MP-66 (1.98 g).

At flowering, highest fresh leaf weight was recorded by MP-62 (356.72 g) followed by MP-44 (251.82 g). Least fresh weight was

Table 11. Yield parameters of *Mucuna pruriens* at four stages of plant growth : fresh weight and dry weight of leaves per plant (g)

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
1.	MP-57	16.86	3.76	239.59	48.40	437.67	91.45	330.62	69.19
2.	MP-44	24.61	4.48	251.82	49.82	410.87	83.93	371.76	73.10
3.	MP-58	16.20	4.02	221.67	44.61	400.93	80.40	327.80	63.34
4.	MP-67	15.00	4.01	154.94	31.57	276.49	57.26	196.06	38.21
5.	MP-62	18.91	3.56	356.72	69.42	481.94	102.34	391.76	76.48
6.	MP-60	18.47	3.51	168.27	28.21	327.23	66.42	322.17	46.10
7.	MP-45	20.92	4.02	169.07	33.06	318.33	64.30	254.32	47.12
8.	MP-66	9.68	1.98	164.58	30.96	278.45	56.33	242.04	47.26
9.	MP-46	26.97	5.33	209.14	42.43	356.66	76.20	237.69	52.48
10.	MP-64	12.12	2.84	125.41	24.12	262.69	53.38	195.12	38.87
	F value	36.609**	86.109**	100.667**	292.614**	467.872**	153.23**	180.389**	203.468**
	CD	2.59	0.29	6.28	2.35	10.32	3.93	15.93	2.95

** Significant at 1 per cent level

DAS - Days after sowing *in situ*

recorded by MP-64 (125.41 g) preceded by MP-67 (154.94 g). Leaf dry weight was highest for MP-62 (69.42 g), followed by MP-44 (49.82 g) and MP-57 (48.40 g), which were on par. MP-58 and MP-46 were on par having 44.61 g and 42.43 g respectively. Lowest leaf dry weight was recorded by MP-64 (24.12 g) followed by MP-60 (28.21 g).

At seeding, MP-62 was recorded as having highest leaf fresh weight of 481.94 g, followed by MP-57 (437.67 g) and MP-44 (410.87 g) which was on par with MP-58 (400.93 g). Least leaf fresh weight was recorded by MP-64 (262.69 g). It was preceded by MP-66 (278.45 g) and MP-67 (276.49 g) which were on par. Leaf dry weight was highest for MP-62 (102.34 g), followed by MP-57 (91.45 g). It was recorded as least by MP-67 (57.26 g), followed by MP-66 (56.33 g) and MP-64 (53.38 g) and all three were on par.

At seed maturation stage, leaf fresh weight was highest for MP-62 (391.76 g), followed by MP-44 (371.76 g). The accessions MP-58 and MP-60 were next in the order and were on par having 327.80 g and 322.17 g respectively. Least value was recorded by MP-64 (195.12 g) which was on par with MP-67 (196.06 g). Dry leaf weight was highest (76.48 g) for MP-62, followed by MP-44 (73.10 g) and MP-57 (69.19 g). Least leaf dry weight was recorded by MP-64 (38.87 g) and MP-67 (38.21 g).

4.3.2.2. Fresh and dry weight of shoot

The fresh and dry weights of shoot differed significantly among the accessions (Table 12) at all stages of plant growth.

Table 12. Yield parameters of *Mucuna pruriens* at four stages of plant growth : fresh weight and dry weight of shoot per plant (g), biomass yield per plant (g) and per hectare (t ha^{-1})

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)		Biomass yield (240 DAS)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Per Plant	*Per hectare
1.	MP-57	19.70	4.54	158.37	29.80	192.07	36.64	218.77	46.68	119.66	1.01
2.	MP-44	21.45	4.70	144.58	27.28	209.71	40.35	231.47	50.01	117.31	0.98
3.	MP-58	17.54	4.03	120.99	24.93	157.06	33.58	203.64	43.16	109.49	0.91
4.	MP-67	14.93	3.95	105.83	22.27	152.71	32.83	181.40	38.45	86.57	0.72
5.	MP-62	22.40	5.06	156.79	28.43	204.43	39.59	227.14	49.37	125.85	1.05
6.	MP-60	15.52	3.89	120.40	24.94	162.07	34.40	189.95	44.67	88.77	0.74
7.	MP-45	15.47	3.99	120.14	24.31	156.98	32.71	192.74	45.61	89.73	0.75
8.	MP-66	16.14	4.33	106.74	21.54	151.81	30.59	182.50	41.31	83.66	0.70
9.	MP-46	15.85	4.47	107.65	23.46	162.24	33.94	195.98	46.71	90.18	0.75
10.	MP-64	14.70	3.65	108.41	18.24	148.90	28.44	185.03	41.19	80.06	0.67
	F value	87.631**	5.592**	133.422**	66.482**	299.055**	12.753**	87.028**	6.397**	107.638**	–
	CD	0.89	0.55	5.32	1.25	3.96	3.08	5.94	4.37	4.88	–

** Significant at 1 per cent level DAS - Days after sowing *in situ*

* Calculated per hectare of land under coconut cultivation, utilizing thirty per cent of land available for intercropping at a spacing of 0.60 x 0.60 m for *M. pruriens*

At pre-flowering stage, MP-62 was recorded as having the maximum fresh weight of shoot (22.40 g). This was followed by MP-44 (21.45 g), MP-57 (19.70 g) and MP-58 (17.54g). The accession MP-64 recorded least fresh shoot weight (14.70 g) and was on par with MP-60 (15.52 g), MP-45 (15.47 g) and MP-67 (14.93 g). The maximum shoot dry weight (5.06 g) was recorded by MP-62 followed by MP-44 (4.70 g), MP-57 (4.54 g) and MP-66 (4.33 g), which were on par. Least shoot dry weight was recorded by MP-64 (3.65 g) which was on par with MP-60, MP-67, MP-45 and MP-58 having 3.89 g, 3.95 g, 3.99 g and 4.03 g respectively.

At flowering stage MP-57 had maximum shoot fresh weight of 158.37 g which was on par with MP-62 (156.79 g). The accession MP-44 followed with 144.58 g. MP-58 (120.99 g), MP-60 (120.40 g) and MP-45 (120.14 g) were next in the order and were on par. Shoot dry weight was maximum for MP-57 with 29.80 g. This was followed by MP-62 (28.43 g) and MP-44 (27.28 g) which were on par. Least shoot dry weight was for MP-64 (18.24 g).

At seeding stage, MP-44 had maximum shoot fresh weight of 209.71 g followed by MP-62 with 204.43 g. MP-57 was next in the order (192.07 g) followed by MP-46 (162.24 g) and MP-60 (162.07 g) which were on par. Lowest shoot fresh weight (148.90 g) was recorded by MP-64 followed by MP-66 (151.81 g) and MP-67 (152.71 g) and they were on par. Shoot dry weight for MP-44 recorded the highest value of 40.35 g. This was followed by MP-62 (39.59 g) and MP-57 (36.64 g) which were on par. The rest of the accessions were on par with each other, recording lowest weights of 28.44 g for MP-64 and 30.59 g for MP-66.

At seed maturation stage MP-44 had maximum fresh weight of 231.47 g which was on par with MP-62 (227.14 g). This was followed by MP-57 (218.77 g), MP-58 (203.64 g) and MP-46 (195.98 g). MP-45 (192.74 g) and MP-60 (189.95 g) followed and were on par. Least fresh weight was recorded by MP-67 (181.40 g) and this was on par with MP-64 (185.03 g) and MP-66 (182.50 g). The highest shoot dry weight was recorded by MP-44 (50.01 g) and this was on par with MP-62 (49.37 g), MP-46 (46.71 g), MP-57 (46.68 g) and MP-45 (45.61 g). Lowest dry weight was recorded by MP-67 (38.45 g).

4.3.2.3. Fresh and dry weights of roots

The fresh and dry weights of roots differed significantly among the accessions at different stages of plant growth (Table 13).

At pre-flowering stage, the fresh weights of roots were on par. MP-62 had maximum fresh weight of 4.56 g and MP-64 had minimum root fresh weight of 3.42 g. Root dry weight was maximum for MP-62 (1.64 g), followed by MP-44 (1.56 g) and MP-67 (1.47 g) and all three were on par. Least root dry weight was recorded by MP-64 (1.22 g).

At flowering stage, MP-57 had highest root fresh weight of 7.68 g, followed by MP-44 (6.26 g) and MP-62 (5.40 g). Least fresh weight

Table 13. Yield parameters of *Mucuna pruriens* at four stages of plant growth : fresh weight and dry weight of roots per plant (g)

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
1.	MP-57	3.84	1.42	7.68	3.80	14.43	4.55	16.67	5.37
2.	MP-44	4.23	1.56	6.26	3.30	12.72	4.02	16.05	4.99
3.	MP-58	3.50	1.25	4.64	2.57	13.78	4.20	16.11	4.80
4.	MP-67	4.26	1.47	4.36	1.79	8.98	2.69	10.84	3.16
5.	MP-62	4.56	1.64	5.40	2.71	15.45	4.72	19.14	5.74
6.	MP-60	3.64	1.25	4.44	2.40	10.59	3.46	13.58	3.67
7.	MP-45	3.85	1.30	4.10	2.21	10.36	3.20	13.82	3.41
8.	MP-66	3.92	1.34	3.98	1.97	7.99	2.34	10.72	3.15
9.	MP-46	3.56	1.27	4.51	2.47	11.33	3.80	13.83	4.00
10.	MP-64	3.42	1.22	1.75	1.61	7.70	2.23	11.17	3.21
	F value	1.385	4.649**	48.966**	7.652**	48.631**	20.267**	352.460**	244.462**
	CD	0.94	0.20	0.66	0.72	1.15	0.59	0.44	0.19

** Significant at 1 per cent level

DAS - Days after sowing *in situ*

was recorded by MP-64 (1.75 g). Highest root dry weight was recorded by MP-57 (3.80 g), which was on par with MP-44 (3.30 g). The accessions that followed were MP-62 (2.71 g), MP-58 (2.57 g), MP-45 (2.21 g) and MP-60 (2.40 g) which were on par. Least root dry weight was recorded for MP-64 (1.61 g) preceded by MP-67 (1.79 g), MP-66 (1.97 g) and MP-45 (2.21 g), and they were on par.

At seeding stage, the greatest fresh root weight was recorded by MP-62 (15.45 g) which was on par with MP-57 (14.43 g). MP-58 (13.78 g) and MP-44 (12.72 g) were next. This was followed by MP-46 (11.33 g), MP-60 (10.59 g) and MP-45 (10.36 g) which were on par. Least root fresh weight was recorded by MP-64 (7.70 g) which was on par with MP-67 (8.98 g) and MP-66 (7.99 g). Highest root dry weight was recorded by MP-62 (4.72 g) which was on par with MP-57 (4.55 g). This was followed by MP-58 (4.20 g), MP-44 (4.02 g), MP-46 (3.80 g) and MP-45 (3.20 g) which were on par. Least root dry weight was recorded by MP-64 (2.23 g).

At seed maturation stage, MP-62 had maximum root fresh weight (19.14 g), followed by MP-57 (16.67 g). This was followed by MP-58 (16.11 g) and MP-44 (16.05 g) which were on par. Least fresh weight was recorded by MP-67 (10.84 g) and MP-66 (10.72 g) which were on par. The highest root dry weight was obser ed for MP-62 (5.74 g). This was followed by MP-57 (5.37 g), MP-44 (4.99 g) and MP-58 (4.80 g). The lowest root dry weight was recorded for MP-66 (3.15 g) and it was on par with MP-67 (3.16 g) and MP-64 (3.21 g).

4.3.2.4. Fresh and dry weights of pods

There was significant difference in fresh and dry weights of pods among the accessions (Table 14) at seeding and seed maturation stage.

At seeding stage MP-60 had the maximum fresh weight of pods (542.58 g). It was on par with MP-58 (515.41 g), MP-62 (512.08 g) and MP-57 (494.21 g). This was followed by MP-67 (459.35 g), MP-66 (436.09 g) and MP-44 (390.96 g) which were on par. Least fresh weight of pods was recorded by MP-46 (199.43 g) which was on par with MP-64 (210.96 g) and MP-45 (244.38 g). The pod dry weight was maximum for MP-60 (215.37 g), followed by MP-67 (213.13 g). This was followed by MP-62 (211.66 g) and MP-58 (206.87 g) which were on par. Least pod dry weight was for MP-46 (73.55 g).

At seed maturation stage the highest fresh pod weight was observed for MP-60 (426.93 g), followed by MP-58 (369.46 g) , MP-62 (359.25 g) and MP-67 (344.65 g) which were on par. The least pod fresh weight was for MP-64 (103.97 g) which was on par with MP-46 (108.91 g). Maximum pod dry weight was recorded by MP-60 (423.81 g), followed by MP-62 (328.25 g). This was followed by MP-67 (312.23 g) and MP-58 (310.55 g) which were on par. Lowest pod dry weight was recorded by MP-46 (100.33 g), preceded by MP-64 (102.35 g).

4.3.2.5. Weight of seeds at harvest

There was significant difference in the weight of seeds among accessions (Table 14) at seed maturation stage.

Table 14. Yield parameters of *Mucuna pruriens* at seeding and seed maturation stage : fresh weight and dry weight of pods per plant (g), weight of seeds per plant (g) and per hectare (kg ha^{-1}) at seed maturation

Sl. No.	Accession Number	Seeding (180 DAS)		Seed maturation (240 DAS)		Weight of seeds per plant	*Weight of seeds per hectare
		Fresh weight of pods	Dry weight of pods	Fresh weight of pods	Dry weight of pods		
1.	MP-57	494.21	117.85	259.90	240.52	139.59	1163.25
2.	MP-44	390.96	140.96	261.73	230.26	117.67	980.58
3.	MP-58	515.41	206.87	369.46	310.55	156.81	1306.75
4.	MP-67	459.35	213.13	344.65	312.23	151.81	1265.08
5.	MP-62	512.08	211.66	359.25	328.25	169.32	1411.00
6.	MP-60	542.58	215.37	426.93	423.81	214.64	1788.67
7.	MP-45	244.38	106.76	140.25	122.87	88.53	737.75
8.	MP-66	436.09	168.02	230.27	228.92	139.52	1162.67
9.	MP-46	199.43	73.55	108.91	100.33	86.41	720.08
10.	MP-64	210.96	80.75	103.97	102.35	80.15	667.92
	F value	21.127**	1074.883**	138.679**	931.885**	59.540**	-
	CD	86.24	5.16	29.04	3.29	3.05	

** Significant at 1 per cent level DAS - Days after sowing *in situ*

* Calculated per hectare of land under coconut cultivation, utilizing thirty per cent of land available for intercropping with a spacing of 0.60 x 0.60 m for *M. pruriens*

Fig. 1. Seed yield at harvest (240 DAS) of best six accessions

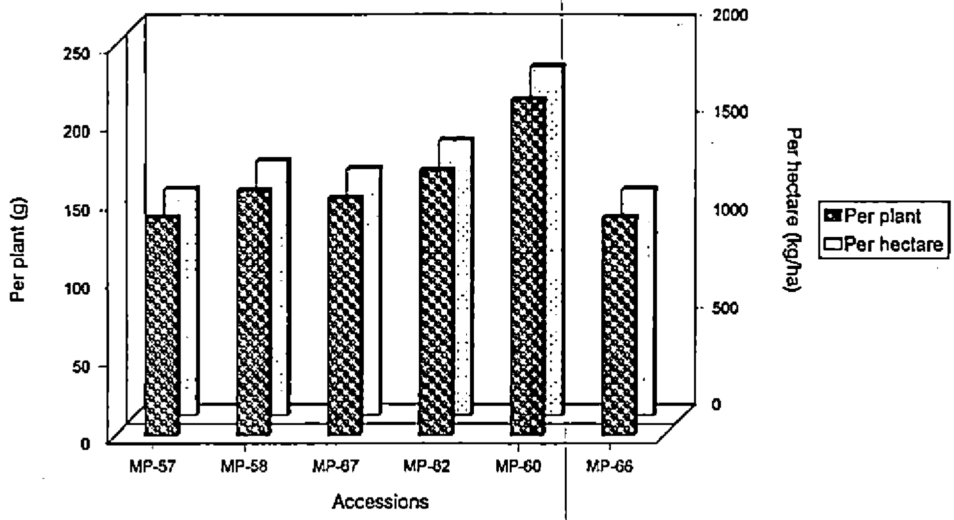
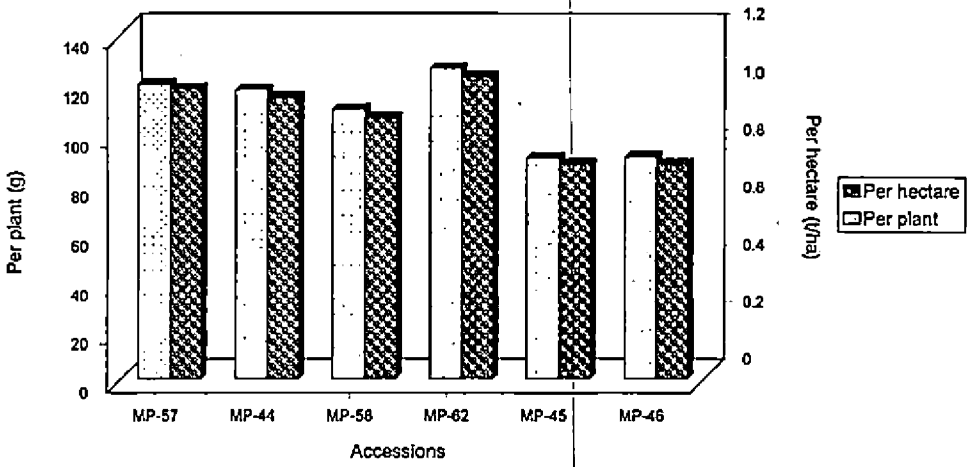


Fig. 2. Biomass yield at harvest (240 DAS) of best six accessions



At harvest (240 DAS), MP-60 had the greatest weight of seeds per plant (214.64 g). This was followed by MP-58 (156.81 g) and MP-67 (151.81 g). The accessions MP-57 and MP-66 were next in the order and on par, having 139.59 g and 139.52 g respectively. The lowest seed weight per plant was recorded by MP-64 (80.15 g) preceded by MP-46 (86.41 g).

4.3.2.6. Biomass yield at harvest

The biomass yield was found to vary significantly among the accessions (Table 12) at harvest (240 DAS). It was obtained by recording the sum of leaf and shoot dry weight at seed maturation stage or harvest.

The accession MP-62 recorded maximum biomass yield of 125.85 g per plant. This was followed by MP-57 (119.66 g) and MP-44 (117.31 g) which were on par. Next in the order was MP-58 (109.49 g). Least biomass yield was recorded by MP-64 (80.06 g) preceded by MP-66 (83.66 g) and they both were on par.

4.3.3. Root Nodules

4.3.3.1. Number of root nodules

There was significant difference in the number of root nodules among the accessions at all stages of plant growth (Table 15).

Table 15. Root nodules of *Mucuna pruriens* : total number of nodules per plant and number of effective nodules per plant at four stages of plant growth

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)	
		Total number of nodules	No. of effective nodules	Total number of nodules	No. of effective nodules	Total number of nodules	No. of effective nodules	Total number of nodules	No. of effective nodules
1.	MP-57	8.88	3.75	93.50	60.00	145.88	62.25	29.25	6.50
2.	MP-44	6.88	3.75	104.00	78.75	120.13	71.88	26.88	6.88
3.	MP-58	9.63	5.00	90.25	57.50	108.75	53.38	25.75	4.75
4.	MP-67	7.13	3.63	71.88	37.00	51.38	30.50	15.88	4.25
5.	MP-62	9.25	4.88	114.88	86.88	140.13	66.50	32.88	7.00
6.	MP-60	5.75	2.88	79.88	65.88	52.63	32.00	17.50	4.88
7.	MP-45	8.50	2.50	71.50	33.63	81.38	40.88	19.38	4.25
8.	MP-66	4.50	2.25	62.63	43.50	43.50	19.38	14.25	4.50
9.	MP-46	7.50	3.38	84.38	65.13	51.13	36.50	19.25	5.88
10.	MP-64	6.50	2.50	52.25	20.13	32.38	19.75	12.00	3.38
	F value	120.238**	53.190**	267.869**	120.839**	51.904**	124.186**	106.470**	150.553**
	CD	0.44	0.49	3.45	5.67	17.56	5.10	2.00	0.30

** Significant at 1 per cent level

DAS - Days after sowing *in situ*

At pre-flowering stage, MP-58 and MP-62 were having highest number of nodules per plant (9.63 and 9.25 respectively) and were on par. MP-57 (8.88) and MP-45 (8.50) followed and were on par. MP-46 (7.50) and MP-67 (7.13) were next in the order and were on par. Least number of nodules per plant were recorded by MP-66 (4.50) preceded by MP-60 (5.75) and MP-64 (6.50).

MP-62 had maximum number of nodules per plant (114.88) at flowering. This was followed by MP-44 (104.00). Next in the order was MP-57 (93.50) and MP-58 (90.25) which were on par. It was least for MP-64 (52.25) and was preceded by MP-66 (62.63).

At seeding stage MP-57, had maximum number of nodules per plant (145.88), followed by MP-62 (140.13) and were on par. Next in the order was MP-44 (120.13) and MP-58 (108.75) which were on par. The accessions MP-60 (52.63), MP-67 (51.38) and MP-46 (51.13) were on par. Least number of nodules per plant were recorded by MP-64 (32.38).

At seed maturation stage, MP-62 had maximum number of nodules per plant (32.88) followed by MP-57 (29.25). MP-44 (26.88) and MP-58 (25.75) were next in the order and on par. This was followed by MP-45 (19.38) and MP-46 (19.25) which were on par. Least number of nodules per plant were recorded by MP-64 (12.00).

4.3.3.2. Number of effective root nodules

The number of effective root nodules varied significantly at all stages of plant growth among the different accessions (Tables 15).

At pre-flowering stage, MP-58 had maximum number of effective nodules per plant (5.00), which was on par with MP-62 (4.88). Next in the order was MP-57 (3.75), MP-44 (3.75), MP-67 (3.63) and MP-46 (3.38) which were on par. The least number of effective nodules per plant (2.25) was recorded by MP-66, which was on par with MP-45 (2.50) and MP-64 (2.50).

At flowering stage MP-62 had maximum number of effective nodules per plant (86.88). This was followed by MP-44 (78.75). Next in the order was MP-60 (65.88) and MP-46 (65.13), which were on par. This was followed by MP-57 (60.00), MP-58 (57.50) and MP-66 (43.50). Least value of effective nodules was recorded by MP-64 (20.13) preceded by MP-45 (33.63) and MP-67 (37.00) which were on par.

At seeding stage, MP-44 had maximum number of effective nodules per plant (71.88). This was followed by MP-62 (66.50) and MP-57 (66.25) and were on par. Next in the order was MP-58 (53.38), MP-45 (40.88) and MP-46 (36.50) which were on par. Least value was recorded by MP-66 (19.38) which was on par with MP-64 (19.75).

At seed maturation stage, MP-62 had the maximum number of effective nodules per plant (7.00), followed by MP-44 (6.88) and MP-

Fig. 3. Effective nodule dry weight and nodule count at seeding of best six accessions

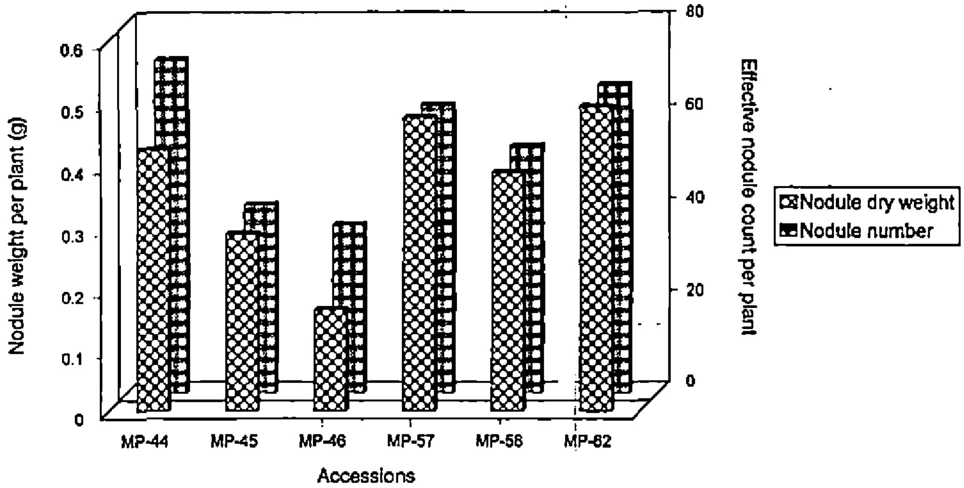
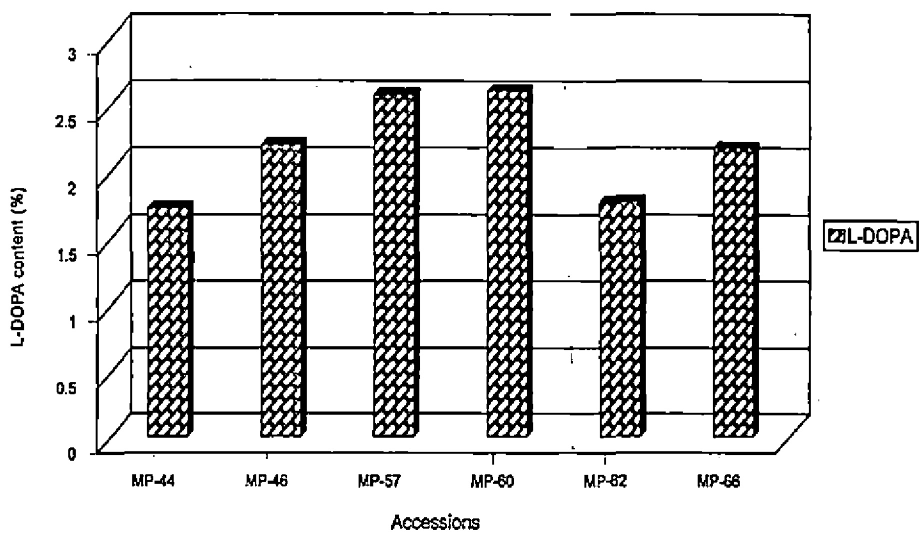


Fig. 4. L-DOPA content (%) of best six accessions



57 (6.50). This was followed by MP-46 (5.88) and then by MP-60 (4.88), and MP-58 (4.75) which were on par. Least number of effective nodules per plant was recorded by MP-64 (3.38) preceded by MP-66 (4.50), MP-45 (4.25), and MP-67 (4.25) which were on par.

4.3.3.3. Fresh and dry weight of root nodules

The fresh and dry weight of nodules varied significantly among the accession (Table 16) at all stages of plant growth.

Att pre-flowering stage, MP-58 recorded the highest nodule fresh weight of 0.125 g per plant. This was on par with MP-62 (0.120 g), MP-57 (0.115 g), MP-45 (0.111 g), MP-46 (0.105 g) and MP-67 (0.099 g). Lowest nodule fresh weight of 0.059 g per plant was recorded by MP-66 and it was preceded by MP-60 (0.075 g). The highest nodule dry weight (0.031 g) was recorded by MP-58, and was on par with MP-62 (0.031 g), MP-57 (0.029 g), MP-45 (0.028 g), MP-46 (0.027 g) and MP-67 (0.026 g). Least nodule dry weight was recorded by MP-66 (0.015 g).

At flowering, maximum nodule fresh weight was recorded for MP-62 (1.608 g) and was on par with MP-44 (1.560 g). The accessions MP-57 (1.403 g), MP-58 (1.354 g), MP-46 (1.266 g) and MP-60 (1.198 g) followed and were on par. Lowest nodule fresh weight was recorded by MP-64 (0.784 g). Maximum nodule dry weight (0.403 g) was recorded by MP-62. This was followed by MP-44 (0.393 g) and MP-57 (0.353 g) and were on par. Next in the order were MP-58 (0.341 g), MP-46 (0.316 g), MP-60 (0.308 g) and MP-67 (0.273 g) which were on par. Least

Table 16. Root nodules of *Mucuna pruriens* : fresh weight and dry weight of root nodules per plant (g) at four stages of plant growth

Sl. No.	Accession Number	Pre-flowering (60 DAS)		Flowering (120 DAS)		Seeding (180 DAS)		Seed maturation (240 DAS)	
		Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
1.	MP-57	0.115	0.029	1.403	0.353	1.896	0.476	0.383	0.094
2.	MP-44	0.083	0.020	1.560	0.393	1.682	0.425	0.345	0.086
3.	MP-58	0.125	0.031	1.354	0.031	1.523	0.388	0.335	0.084
4.	MP-67	0.099	0.026	1.078	0.273	0.771	0.194	0.205	0.053
5.	MP-62	0.120	0.031	1.608	0.403	1.963	0.494	0.419	0.106
6.	MP-60	0.075	0.018	1.198	0.308	0.739	0.189	0.235	0.061
7.	MP-45	0.111	0.028	1.073	0.270	1.142	0.290	0.265	0.064
8.	MP-66	0.059	0.015	1.002	0.258	0.658	0.162	0.186	0.047
9.	MP-46	0.105	0.027	1.266	0.316	0.653	0.166	0.250	0.065
10.	MP-64	0.085	0.021	0.784	0.197	0.458	0.117	0.165	0.042
	F value	5.388**	5.034**	6.414**	6.299**	17.005**	16.058**	42.982**	52.014**
	CD	0.028	0.008	0.302	0.076	0.410	0.106	0.039	0.009

** Significant at 1 per cent level   DAS - Days after sowing *in situ*

nodule dry weight was recorded by MP-64 (0.197 g) which was on par with MP-66 (0.258 g) and MP-45 (0.270 g).

At seeding stage, the highest nodule fresh weight was recorded by MP-62 (1.963 g per plant) followed by MP-57 (1.896 g) and MP-44 (1.682 g) which were on par. The least nodule fresh weight, 0.458 g per plant was recorded by MP-64 and was on par with MP-46 (0.653 g), MP-66 (0.658 g), MP-60 (0.739 g) and MP-67 (0.771 g). The maximum nodule dry weight was observed for MP-62 (0.494 g), followed by MP-57 (0.476 g), MP-44 (0.425 g) which were on par. Lowest nodule dry weight was recorded by MP-64 (0.117 g) which was on par with MP-66 (0.162 g), MP-46 (0.166 g), MP-60 (0.189 g) and MP-67 (0.194 g).

At seed maturation stage, the largest nodule fresh weight was 0.419 g for MP-62 which was on par with MP-57 (0.383 g). This was followed by MP-58 (0.335 g). Least nodule fresh weight was recorded by MP-64 (0.165 g) which was on par with MP-66 (0.186 g). Maximum nodule dry weight was recorded by MP-62 (0.106 g). This was followed by MP-57 (0.094 g). Lowest nodule dry weight was for MP-64 (0.042 g) which was on par with MP-66 (0.047 g), MP-67 (0.053 g) and MP-60 (0.061 g).

4.3.4. Leaf area parameters

4.3.4.1. Leaf area index (LAI)

The LAI varied significantly among the accessions at all stages of plant growth (Table 17).

Table 17. Leaf area index at four stages of plant growth and leaf area duration (months) of *Mucuna pruriens* for three periods of plant growth

Sl. No.	Accession Number	Leaf area index				Leaf area duration		
		Pre-flowering (60 DAS)	Flowering (120 DAS)	Seeding (180 DAS)	Seed maturation (240 DAS)	Period 1 (60-120 DAS)	Period 2 (120-180 DAS)	Period 3 (180-240 DAS)
1.	MP-57	0.20	2.14	4.82	3.65	2.34	6.96	8.47
2.	MP-44	0.22	2.71	4.59	3.33	2.46	6.95	7.92
3.	MP-58	0.19	2.14	4.05	3.20	2.33	6.19	7.25
4.	MP-67	0.18	1.36	3.03	2.18	1.53	4.39	5.21
5.	MP-62	0.23	2.25	4.50	3.55	2.69	6.74	8.04
6.	MP-60	0.20	1.72	3.32	2.17	1.91	5.03	5.49
7.	MP-45	0.20	2.00	3.50	2.06	1.61	4.91	5.56
8.	MP-66	0.14	1.41	3.10	1.82	1.62	4.59	4.92
9.	MP-46	0.31	1.49	3.40	2.27	2.30	5.39	5.67
10.	MP-64	0.07	1.12	2.94	1.64	1.19	4.06	4.58
	F value	209.361**	59.702**	36.201**	62.757**	38.097**	45.747**	59.564**
	CD	0.01	0.19	0.35	0.28	0.24	0.48	0.56

** Significant at 1 per cent level

DAS - Days after sowing *in situ*

At pre-flowering stage, the LAI was highest for MP-46 with a value of 0.31. This was followed by MP-62 (0.23) and MP-44 (0.22), which were on par. The least LAI was recorded by MP-64 (0.07) which was preceded by MP-66 (0.14).

At flowering stage, MP-44 had the highest LAI (2.71), followed by MP-62 (2.25) and MP-58 (2.14) which were on par with MP-57 (2.14) and MP-45 (2.00). The least LAI was recorded by MP-64 (1.12), which was preceded by MP-67 (1.36) and this was on par with MP-46 (0.31) and MP-66 (0.14).

At seeding stage, MP-57 recorded the highest LAI (4.82), followed by MP-44 (4.59) and MP-62 (4.50) and all the three were on par. Next in the order was MP-58 with a LAI of 4.05. MP-64 had the least LAI (2.94) which was on par with MP-66 (3.10) and MP-67 (3.03).

At seed maturation stage, MP-57 recorded the maximum LAI (3.65) which was on par with MP-62 (3.55). This was followed by MP-44 (3.33) and MP-58 (3.20) which were on par. The least LAI was recorded by MP-64 (1.64), and this was on par with MP-66 (1.82).

4.3.4.2. Leaf area duration (LAD)

The LAD varied significantly among the accessions at all periods of plant growth (Table 17).

The leaf area duration was calculated for each period of two months taken between each stage. The values were expressed in unit 'months'. For the period 60-120 DAS, the highest LAD was recorded as 2.69 months for MP-62. This was followed by MP-44 (2.46 months), which were on par with MP-57, MP-58 and MP-46 with 2.34, 2.33 and 2.30 months respectively and these were on par. Least LAD was recorded by MP-64 (1.19 months). It was preceded by MP-66 (1.62 months), MP-45 (1.61 months) and MP-66 (1.62 months) and they were on par.

For the period 120-180 DAS, the maximum LAD was recorded by MP-57 (6.96 months) which was on par with MP-44 (6.95 months) and MP-62 (6.74 months). This was followed by MP-58 (6.19 months) and MP-46 (5.39 months). The least LAD was recorded by MP-64 (4.06 months) and this was on par with MP-67 (4.39 months).

For the period 180-240 DAS, the maximum LAD was recorded by MP-57 (8.47 months) which was on par with MP-62 (8.04 months) and MP-44 (7.92 months). The least LAD was recorded by MP-64 (4.58 months) and this was on par with MP-66 (4.92 months).

4.3.5. Physiological parameters

4.3.5.1. Net assimilation rate (NAR) (g day^{-1})

The NAR varied significantly among the accessions at different periods of plant growth (Table 18).

The NAR for the period 60-120 DAS recorded the highest value of 0.059 for MP-60. This was followed by MP-57 (0.057) and MP-44

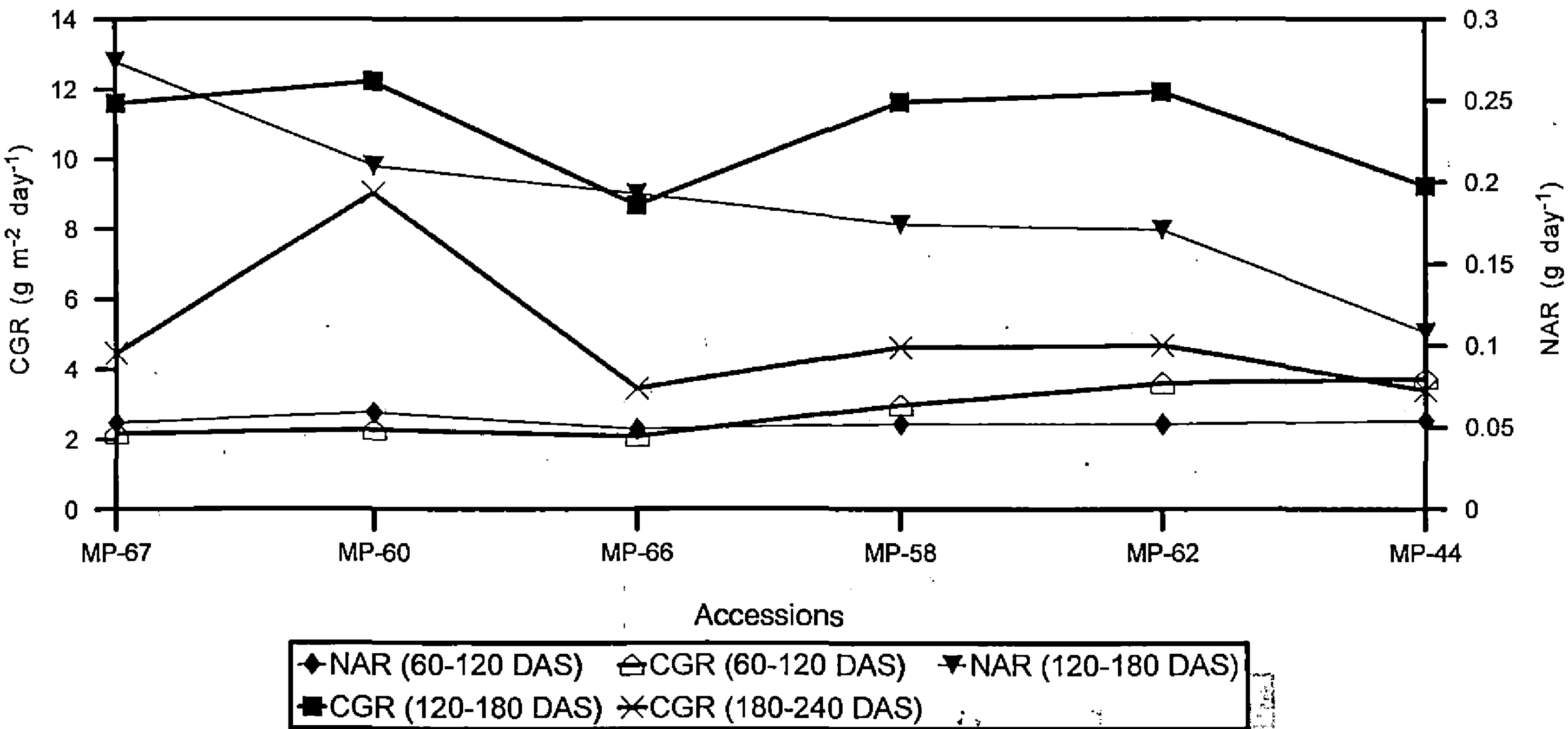
Table 18. Physiological parameters of *Mucuna pruriens* : net assimilation rate (NAR) (g day^{-1}) for two periods, crop growth rate (CGR) (g m^{-2} day^{-1}) and absolute growth rate (AGR) (g day^{-1}) for three periods of plant growth

Sl. No.	Accession number	Period 1 (60-120 DAS)			Period 2 (120-180 DAS)			Period 3 (180-240 DAS)	
		NAR	CGR	AGR	NAR	CGR	AGR	CGR	AGR
1.	MP-57	0.057	3.41	1.23	0.077	7.87	2.84	5.19	1.87
2.	MP-44	0.054	3.72	1.37	0.108	9.22	3.32	3.38	1.22
3.	MP-58	0.052	2.96	1.07	0.174	11.62	4.19	4.61	1.66
4.	MP-67	0.053	2.17	0.78	0.274	11.59	4.18	4.45	1.57
5.	MP-62	0.052	3.60	1.30	0.171	11.92	4.29	4.67	1.68
6.	MP-60	0.059	2.26	0.82	0.210	12.21	4.40	9.02	3.40
7.	MP-45	0.051	2.21	0.80	0.098	6.96	2.51	0.41	0.15
8.	MP-66	0.049	2.08	0.75	0.193	8.68	3.18	3.45	1.24
9.	MP-46	0.052	2.77	0.57	0.058	5.42	1.95	0.58	0.21
10.	MP-64	0.050	1.67	0.60	0.097	5.79	2.09	0.92	0.33
	F value	4.740**	24.342**	9.408**	60.961**	274.435**	340.808	254.530**	201.025**
	CD	0.004	0.43	0.28	0.026	0.47	0.15	0.49	0.20

** Significant at 1 per cent level

DAS - Days after sowing *in situ*

Fig. 5. Physiological parameters (NAR and CGR) during crop growth for six accessions



(0.054), which were on par. The rest of the accessions were on par and least NAR value of 0.049 was recorded by MP-66.

For the period 120-180 DAS, the maximum NAR value of 0.274 was observed for MP-67, followed by MP-60 (0.20). The least NAR value was recorded as 0.058 for MP-46, preceded by 0.077 for MP-57.

The NAR for the period of 180-240 DAS could not be calculated because of recurring negative values, which were caused by lowered leaf weights due to 'leaf shedding' phenomenon.

4.3.5.2. Crop growth rate (CGR) (g m^{-2} day^{-1})

The CGR varied significantly at all periods of plant growth (Table 18).

During the period 60-120 DAS, maximum CGR of 3.72 was recorded for MP-44. This was on par with MP-62 (3.60) and MP-57 (3.41). This was followed by MP-58 (2.96) and MP-46 (2.77). The least value of 1.67 was recorded for MP-64 and was on par with MP-66 (2.08).

For the period 120-180 DAS, the highest CGR of 12.21 was observed for MP-60. This was followed by MP-62 (11.92), MP-58 (11.62) and MP-67 (11.59). MP-44 (9.22), MP-66 (8.68) and MP-57 (7.87) followed. It was least for MP-46 (5.42) which was on par with MP-64 (5.79).

During the period 180-240 DAS, the maximum CGR of 9.02 was recorded by MP-60. This was followed by MP-57 (5.19). The accessions that followed next were MP-62 (4.67), MP-58 (4.61) and MP-67 (4.45) which were on par. Least CGR was recorded by MP-45 as 0.41 and it was on par with MP-46 (0.58).

4.3.5.3. Absolute growth rate (AGR) (g day^{-1})

The AGR varied significantly among the accessions at all periods of plant growth (Table 18).

For the period 60-120 DAS, MP-44 had the maximum AGR of 1.37. This was followed by MP-62 (1.30), MP-57 (1.23) and MP-58 (1.07) which were on par. The rest of the accessions were on par with MP-46 recording the lowest AGR of 0.57.

During the period 120-180 DAS, MP-60 had a maximum AGR of 4.40. This was followed by MP-62 (4.29), MP-58 (4.19) and MP-67 (4.18), which were on par. Next in the order was MP-44 (3.32) and MP-66 (3.18) which was on par. The least AGR was recorded as 1.95 for MP-46 and was on par with MP-64 (2.09).

For the period 180-240 DAS, the highest AGR was observed as 3.40 for MP-60, followed by MP-57 (1.87). The accessions MP-62 (1.68), MP-58 (1.66) and MP-67 (1.57) were next in the order and they were on par. The least AGR value was for MP-45 (0.15) and was on par with MP-64 (0.33) and MP-46 (0.21).

4.3.5.4. Relative growth rate (RGR) (g day^{-1})

The RGR varied significantly among all accessions at the different periods of plant growth (Table 19).

The accession MP-62 recorded the maximum RGR of 0.037 for the period 60-120 DAS. This was followed by MP-57 (0.036) and MP-44 (0.034) which were on par. The accessions MP-60 (0.032), MP-67 (0.031), MP-66 (0.031) and MP-45 (0.031) were on par. MP-64 had least RGR of 0.030.

For the period 120-180 DAS, MP-67 had the maximum RGR of 0.029 and it was on par with MP-60 (0.029). This was followed by MP-66 (0.026) and MP-58 (0.025). The least RGR (0.017) was recorded by MP-46.

During period 180-240 DAS, MP-60 had maximum RGR of 0.008. This was followed by MP-57 (0.006), MP-44 (0.006), MP-67 (0.005), MP-62 (0.005), MP-58 (0.005) and MP-66 (0.005) which were on par. Least RGR was recorded as 0.001 by MP-45 and was on par with MP-46 (0.002).

4.3.5.5. Harvest index (HI)

The harvest index varied significantly among the accessions (Table 19).

Table 19. Physiological parameters of *Mucuna pruriens* : relative growth rate (RGR) (g day^{-1}) for three periods of plant growth and harvest index

Sl. No.	Accession Number	Period 1 (60-120 DAS)	Period 2 (120-180 DAS)	Period 3 (180-240 DAS)	Harvest index (HI)
1.	MP-57	0.036	0.019	0.006	0.66
2.	MP-44	0.034	0.019	0.006	0.66
3.	MP-58	0.033	0.025	0.005	0.73
4.	MP-67	0.031	0.029	0.005	0.78
5.	MP-62	0.037	0.021	0.005	0.72
6.	MP-60	0.032	0.029	0.008	0.82
7.	MP-45	0.031	0.022	0.001	0.57
8.	MP-66	0.031	0.026	0.005	0.73
9.	MP-46	0.033	0.017	0.002	0.50
10.	MP-64	0.030	0.023	0.003	0.56
	F value	11.138**	12.839**	15.364**	68.307**
	CD	0.002	0.002	0.002	0.012

** Significant at 1 per cent level DAS - Days after sowing *in situ*

MP-60 recorded the maximum harvest index of 0.82. This was followed by MP-67 (0.78) then by MP-58 and MP-66 which were on par and having HI of 0.73 each, and then by MP-62 (0.72). The least HI was recorded by MP-46 (0.50) and was preceded by MP-64 (0.56).

4.4. Phase IV Phytochemical analysis

4.4.1. Soil NPK content before and after the experiment

4.4.1.1. Soil nitrogen (kg ha^{-1})

Soil analysis carried out before the commencement of experiment revealed a value of 230.55 kg ha^{-1} of soil nitrogen.

The soil nitrogen content after the experiment varied significantly in each plot where the accessions were raised (Table 20).

The plot where the accession MP-62 was grown, recorded maximum soil N content of 312.04 which was on par with MP-57 (305.76). The accessions MP-67 (297.92), MP-44 (296.36), MP-58 (296.35) and MP-60 (293.22) followed. Lowest soil N content was observed for MP-64 as 257.16.

4.4.1.2. Soil phosphorous (kg ha^{-1})

Soil analysis carried out before the commencement of the experiment revealed a value at 31.50 kg ha^{-1} as the soil phosphorus content.

Table 20. Nitrogen, phosphorus and potassium status in soil after harvest (240 DAS) of *Mucuna pruriens*

Sl. No.	Accession Number	Nitrogen ($kg\ ha^{-1}$)	Phosphorus ($kg\ ha^{-1}$)	Potassium ($kg\ ha^{-1}$)
1.	MP-57	305.76	18.45	40.78
2.	MP-44	296.36	21.27	45.98
3.	MP-58	296.35	19.34	40.39
4.	MP-67	297.92	24.29	68.75
5.	MP-62	312.04	22.30	28.64
6.	MP-60	293.22	18.56	53.96
7.	MP-45	283.81	22.99	62.95
8.	MP-66	280.68	25.24	50.87
9.	MP-46	278.80	24.11	63.23
10.	MP-64	257.16	19.54	57.65
	F value	32.482**	1.217	34.089**
	CD	8.18	6.83	6.35

** Significant at 1 per cent level

The soil phosphorous content after the experiment did not vary significantly among the accessions (Table 20).

4.4.1.3. Soil potassium (kg ha^{-1})

The soil analysis carried out before the start of the experiment revealed the potassium content as 90.35 kg ha^{-1}.

Soil potassium values were found to differ significantly among the plots where various accessions were grown (Table 20).

After the experiment the plot where MP-67 was grown was observed as having the highest soil K content of 68.75. This was followed by MP-46 (63.23) which was on par. MP-62 was observed as having the least soil K content of 28.64.

4.4.2. L-DOPA (L-3,4-dihydroxyphenylalanine) content

The content of amino acid, L-DOPA, varied significantly among the accessions under evaluation (Table 21).

On phytochemical analysis it was observed that MP-60 had the maximum L-DOPA content (2.60 per cent). This was on par with MP-57 (2.57 per cent). MP-46 was next in the order with 2.19 per cent and was on par with MP-66 (2.17 per cent). MP-62 recorded 1.77 per cent L-DOPA. The accessions MP-44 (1.72 per cent), MP-58 (1.68 per cent), MP-45 (1.62), MP-67 (1.58) and MP-64 (1.51 per cent) were on par. MP-64 had least L-DOPA content of 1.51 per cent.

Table 21. L-DOPA content in the seeds of *Mucuna pruriens*

Sl. No.	Accession number	L-DOPA content in seeds (%)
1.	MP-57	2.57
2.	MP-44	1.72
3.	MP-58	1.68
4.	MP-67	1.58
5.	MP-62	1.77
6.	MP-60	2.60
7.	MP-45	1.62
8.	MP-66	2.17
9.	MP-46	2.19
10.	MP-64	1.51
	F value	28.556**
	CD	0.23

** Significant at 1 per cent level

4.5. Selection index

Selection index based on the following characters viz., seed yield, L-DOPA content, biomass yield, root yield and root nodule characters, (number of effective nodules at seeding, fresh and dry weight of root nodules at seeding) was worked out to identify superior genotypes.

As per the results obtained, the accessions were ranked in descending order of genotype superiority, under the following sections (Table 22).

a) Seed yield : MP-60, MP-62, MP-58, MP-67, MP-57, MP-66, MP-44, MP-46, MP-45 and MP-64.

b) Seed yield and L-DOPA content : MP-60, MP-62, MP-58, MP-67, MP-57, MP-66, MP-44, MP-46, MP-45 and MP-64

c) Seed yield and biomass yield : MP-60, MP-62, MP-58, MP-67, MP-57, MP-44, MP-66, MP-45, MP-46 and MP-64.

d) Seed yield, biomass yield, root yield and root nodule characters : MP-62, MP-60, MP-58, MP-57, MP-44, MP-67, MP-66, MP-45, MP-46 and MP-64.

4.6. Pest and diseases

During the entire period of crop growth, frequent surveillance for the occurrence of pests and diseases was carried out.


8


MP-44
9


MP-57
10

Table 22. Ranking of accessions of *Mucuna pruriens* based on yield, L-DOPA content and root nodule characters

Sl. No.	Accession	Sy* Rank	Sy and Dy*		Sy and By*		Sy,Dy,By,Ry*,Nc* and Nw*	
			Selection Index	Rank	Selection Index	Rank	Selection Index	Rank
1.	MP-57	5	424.14	5	1515.51	5	2628.23	4
2.	MP-44	7	356.55	7	1409.94	6	2500.21	5
3.	MP-58	3	473.81	3	1745.15	3	2721.83	3
4.	MP-67	4	458.61	4	1661.91	4	2309.52	6
5.	MP-62	2	511.51	2	1887.62	2	3038.55	1
6.	MP-60	1	649.22	1	2195.94	1	2869.79	2
7.	MP-45	9	268.97	9	914.82	8	1671.13	8
8.	MP-66	6	423.06	6	1377.66	7	1940.15	7
9.	MP-46	8	299.86	8	896.98	9	1594.49	9
10.	MP-64	10	243.62	10	807.69	10	1365.57	10
b-coefficient			Sy* = 0.999 Dy* = 7.33		Sy* = 1.042 By* = 0.985		Sy* 1.0726 Dy* = 293.1357 By* = 0.8071 Ry* = 7.4770 Nc* = 1.0106 Nw* = 1074.753	

Sy - Seed yield
Ry - Root yield
Dy - L-DOPA content
Nc - Effective nodule count at seeding
By - Biomass yield
Nw - Effective nodules dry weight at seeding


KOTOOR
11


PAMPA
VALLEY
12


13

After flowering, incidence of bacterial blight was observed in a few plants at random. Preventive and curative measures were carried out by drenching the soil with 0.2 per cent copper oxychloride (Cupramar 50WP).

Towards the seeding stage of the crop, a few plants were infected with cowpea mosaic virus. Infected plants were uprooted and burned. Random visitings of pea-aphids and mealy bugs on tender pods as well as leaf cutters on some plants were recorded.

DISCUSSION

5. DISCUSSION

The present study titled 'Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden' was carried out at the College of Agriculture, Vellayani from May 1999 to January 2000. The study aimed at selecting promising accessions from the genetic stock of *M. pruriens*.

The results of the study are discussed in this chapter.

5.1 Seed characterization and germination

Out of twenty five accessions of *M. pruriens,* only ten accessions recorded more than 90 per cent seed germination, under laboratory conditions (Table 4). Accessions MP-57 and MP-62 recorded the maximum seed germination percentage of 98. These accessions could be genetically more vigorous than the others.

Accessions MP-45 and MP-46 took the least number of days (5 days) to record 50 per cent seed germination. Accessions MP-47 and MP-48 exhibited very poor rate of germination; 12 per cent and 9 per cent respectively.

Thirteen accessions did not germinate at all (Table 4), despite providing all favourable conditions for germination. This may be due to seed dormancy factors, such as presence of germination inhibitors or rudimentary embryos. Detailed investigation is needed before arriving at a conclusion, and precise standardisation of seed treatment may be required.

With regard to hundred seed weight it was highest for MP-45 (68 g) followed by MP-46 (66 g). These two accessions also recorded the greatest seed length (1.5 cm and 1.4 cm respectively).

The selected accessions were either black seeded or light brown seeded with dark brown streaks, on the surface.

The variation in seed characteristics may be attributed to genetic variation among the accessions.

5.2 Growth parameters

5.2.1 Shoot characters

The data available on shoot characters viz. plant length, number of leaves per plant and leaf area revealed that it differed significantly among the accessions at all stages of plant growth (Table 6 and Table 7).

Increment of plant length was observed to be greatest between pre-flowering and flowering stages, and was vividly superior to the subsequent periods of plant growth. It is evident that this being the main vegetative phase, nutrients were directed towards enhancing the vegetative characters.

Among the accessions, those that revealed a trend to dominate in plant length were MP-57, MP-44, MP-58 and MP-62 (which recorded the maximum plant length ie., 10.05 m, at seed maturation). It could be deduced that these accessions were genetically more vigorous than the others and also, more shade tolerant.

Sunitha (1996) reported a plant length of 7.25 m in *M. pruriens* grown under coconut shade. Excepting accessions MP-67 and MP-64, all other selected accessions recorded greater plant length.

Sunitha (1996) reported that the primary factors likely to limit growth and productivity of crop under shade, in coconut garden, was the low light intensity, if water and nutrients are available in adequate quantities.

When compared to other legumes, *Mucuna* vines are far superior in plant length. This observation was reiterated by Mercy (1981) who commented on the length and aggresiveness in growth of *Mucuna* vines.

The variation in plant length among the accessions can be judged to be purely a function of the genetic make up, as reported by Bose (1963) in leguminous crops and Buckles (1995) in *Mucuna*.

Study of data available on the number of leaves per plant revealed that like plant length, the greatest increment was between the period of pre-flowering and flowering. On the contrary, the leaf number per plant was found to decrease after seeding, which may be accounted to leaf shedding (estimated to be around 20 to 30 per cent) due to senescence. Jyothi (1999) had made a similar observation in vegetable cowpea.

Among the accessions, those that displayed superior leaf number were MP-44, MP-57, MP-58 and MP-62 (which recorded a maximum number of 117.00 leaves per plant at seed maturation).

Since accessions that recorded greater plant length also displayed superior leaf production, these growth parameters can be judged to be positively associated with each other.

As Nair (1966) reported that number of leaves per plant are a function of the genetic make up under identical conditions of growth, the genetic superiority of these accessions can be emphasized. It can also be reasoned that greater plant length would have helped in better utilization of environmental resources, and thus helped to increase the number of leaves.

Similar findings were reported by Mercy (1981) in legume-maize mixtures.

On the other hand, leaf area did not reveal any positive association with plant length or leaf number. Though it cannot be decided upon, it could be so, as the leaves of each accession were in various stages of development and value recorded was the mean value.

Accessions that generally maintained a steady superiority of leaf area were MP-57, MP-58 and MP-62. This factor is beneficial to the plant as a larger leaf surface serves to capture more solar radiation and hence, increase photosynthetic rates. Besides, leaf area index and leaf area duration are functions of the total plant leaf area and are also factors that influence the plant dry matter production.

The mean leaf size declined at seed maturation and this may be accounted due to the shedding of mature and largest sized leaves. At seeding stage MP-62 recorded maximum leaf area of 89.67 cm^2. There was no significant difference in leaf area among the accessions at seed maturation.

5.2.2 Root characters

The data on root length and root girth revealed that they differed significantly among the accessions at all stages of plant growth (Table 8).

Like the shoot characters, root length and root girth recorded the highest increment between pre-flowering and flowering period. This could be so, as the plants were in the vegetative phase and nutrients absorbed and assimilated were directed towards increasing vegetative characters. Another observation was that those accessions which dominated in plant length and leaf production were found to lead in root length measurements. These accessions could be genetically more vigorous. The positive association arrived at between root length and shoot characters, is further supported by the findings of Babalola (1980) and Rajan (1999) that in legumes, a positive association is usually seen for root length with plant length and leaf production.

Sunitha (1996) had reported in *M. pruriens* a root length of 0.83 m, when grown under coconut shade. Among the accessions, MP-44, MP-57, MP-58 and MP-62 displayed superior root length, with MP-57 recording the maximum (1.51 m) at seed maturation stage.

It could be noted that accessions having comparatively lesser root length like MP-64, MP-67, MP-46 and MP-66 had lesser vegetative growth parameters too, which led to comparatively lower yields. This could be so, as lesser root length would have limited the root surface area for absorbtion of nutrients, as well as the root nodule count. This conforms to the observations reported by Babalola (1980), Kavitha (1982) and Rajan (1999) that a well developed root system is positively associated with yield and nodule weight.

Though a definite positive association could not be deduced between root length and root girth, it was evident that some accessions viz. MP-57, MP-58 and MP-62, which displayed more or less steady trend of superiority in root girth, had been noted for dominance in vegetative characters. Accession MP-62 had recorded the highest root girth of 3.82 cm at seed maturation stage.

Sunitha (1996) reported a root girth of 5.37 cm in *M. pruriens* grown under coconut shade.

It could be concluded that ultimately, alike other growth parameters, root length and root girth varied according to individual genetic constitution of the accessions.

5.2.3 Pod characters

The pod characters viz. number of pod clusters per plant, number of pods per cluster, pod length and number of seeds per pod were found to differ significantly among the accessions at seeding and seed maturation stages (Table 9 and 10).

The number of pod clusters per plant were found to increase after seeding, probably due to staggered flowering. Hence harvesting had to be carried out two to three times, at an interval of 3 to 4 days.

The accession MP-60 was noted for its much superior pod production of 10.88 pod clusters per plant, at seed maturation stage. Other such accessions were MP-62, MP-58, MP-67, MP-66, MP-57 and MP-44. These accessions would be of importance if the crop was raised for seed.

The data on number of pods per cluster revealed a slight decrease at seed maturation stage in some accessions. This decline was attributed to loss of tender pods, through fungal rot or attack of pea aphids, mealy bugs and red ants. The occurrence of rain during seeding stage coupled with conditions of shade of coconut canopy, would have created a microclimate condusive to the incidence of pest and diseases.

Accessions having higher number of pods per cluster were MP-62 (recording a maximum of 6.25), MP-67, MP-58 and MP-60.

No positive association could be judged between both characters, though pod clusters per plant and number of pods per cluster contribute to final pod yield per plant.

Another observation was that some accessions with comparatively poorer vegetative growth parameters viz. MP-60, MP-66 and MP-67 displayed high pod yield. This may be because the nutrients absorbed and assimilated, would have been directed towards enhancing reproductive yield, rather than vegetative yield.

Similar findings were reported by Nair (1966) and Anitha (1989) that in legumes, vegetative growth increased at the expense of reproductive growth, and by Rajan (1999) that length of root (a vegetative growth parameter) showed negative association with number of pods.

The data on pod length and number of seeds per pod did not reveal any positive association between them.

Pod length varied among the accessions in accordance with characteristic genetic make up. This is in justification with the reports of Nair (1966) regarding pod length in cowpea.

The accession MP-45 and MP-46 though having maximum pod length did not show maximum seed number, due to the large size of individual seeds. MP-57 recorded maximum seed number of 6.75.

Buckles (1995) had reported a pod length of 9 to 13 cm and with a range of 4 to 8 seeds per pod in *Mucuna pruriens*.

Taking into account the pharmaceutical importance of the crop, the valuable accessions would be those with superior number of pod clusters per plant, pods per cluster and seeds per pod, ie. all characters which contribute to final seed yield. Such accessions could be listed as MP-60, MP-62, MP-58, MP-67, MP-57 and MP-66.

Taking the growth parameters on a whole, they can be judged to vary according to genetic make up of the accessions.

Similar inferences were made by Nair (1966) that growth parameters are purely a function of individual genetic make up under identical conditions of growth.

5.3 Yield and yield attributes

5.3.1 Shoot yield

The yield of shoot characters, ie. leaf yield and shoot yield, was found to vary significantly among the accessions at all stages of plant growth (Table 11 and 12).

Leaf yield increment was highest between pre-flowering and flowering stages, and the leaf yield was found to decrease at the last stage. This trend in variation was found to be similar to that of leaf number and can be explained by the production of leaves at all stages, and shedding of leaves at last stage, due to senescence.

MP-62 recorded maximum leaf dry weight of 76.48 g and other such superior accessions were MP-57, MP-58, MP-44 and MP-46.

Shoot yield is dependent on plant length and number of branches and similar to the trend in variation of plant length, it had highest increment between pre-flowering and flowering stage, and increased at all stages due to continual growth.

A definite positive association can be made between plant length and shoot yield as those accessions which had higher plant lengths recorded higher shoot yield too. Greater plant length would have resulted in more number of leaves which would have helped in greater utilization of available resources, increasing the number of branches and total shoot weight.

A similar report by Mercy (1981) in legumes was that the number of branches (which contributes to shoot yield) was usually related to the height of plants.

5.3.2 Root yield

Upon analysis of the data it was evident that root yield differed significantly at all stages of plant growth (Table 13).

The increase in root yield at every stage can be attributed to the continued increase in root length and root girth, and similar to them, the increment of root yield was highest between pre-flowering and flowering stages.

It is evident that the high root yield recorded by accessions MP-62, MP-44, MP-57 and MP-58 was associated with their high shoot yield as well.

It could be reasoned that accessions with higher root yield had a better and more efficient root system and root surface area that served to absorb more nutrients for crop growth and development, thus increasing vegetative yields.

Similar findings were reported by Babalola (1980) that a well developed root system is characteristic of increased yield in vegetable cowpea. Anitha (1989) has also reported that higher root mass inclines the plant towards greater vegetative yield. It may also be suggested that higher root yield which is resultant of better root length and spread, may accomodate more nodules, which in turn would contribute to higher vegetative yield.

5.3.3 Pod yield

Pod yield was found to differ significantly among the accessions (Table 14).

The data revealed that it varied according to the number of pods per plant.

At seeding stage, due to succulent and heavier nature of the pods, fresh weight of pods was higher than that at seed maturation. On the other hand, pod dry weight was highest at seed maturation due to increase in the number of pods at this stage.

The variation in pod mass among the accessions was in relation to the total number of pods per plant and this in turn was a function of the number of pod clusters per plant and the number of pods per cluster. Hence pod mass could be judged to differ according to individual genetic constitution.

Accessions possessing superior pod mass were MP-60, MP-62, MP-67 and MP-58. Accession MP-60 was noted in particular for high podmass of 423.81 g per plant. There accessions are favourable in view of their pharmaceutical importance.

Analysis of the data revealed that accessions with comparatively poorer vegetative yield viz. MP-60, MP-66 and MP-67 had good pod yields. This could be so, as plant assimilates had been directed to reproductive growth and development, rather than vegetative growth. This is in conformity with the report of Anitha (1989) in legumes, that vegetative growth increases at the expense of reproductive growth.

5.3.5 Seed yield

Analysis of data available on seed yield revealed that it differed significantly among the accessions at harvest (Table 12).

More than half of the accessions were statistically on par with respect to the number of seeds per pod, and seed yield. Seed yield was observed to have a positive association with number of pod clusters per plant and number of pods per cluster, but not with pod length.

Accession MP-60 had recorded maximum seed yield of 214.64 g per plant ie 1789 kg ha^{-1}. Other such accessions were MP-58, MP-67, MP-57 and MP-66. These accessions would be of importance to cultivators growing *M. pruriens* for pharmaceutical value.

Chadha (1995) had reported a seed yield of 5000 kg ha^{-1} from a well managed, irrigated sole crop of *M. pruriens* supported with stakes. Difference in seed yield from that of the present study, can be acounted to the difference in accessions used, variation caused by genotype environment interaction, and the effect of varying seasons of planting. It can be noted that the seed yield can serve as an additional source of income to the coconut farmers.

5.4 Biomass yield

The biomass yield at harvest was found to differ significantly among the accessions (Table 12).

According to Singh (1957) biomass is mainly a function of the vegetative growth by virtue of the inherent genetic make up of each legume.

Biomass yields ranging from 80.06 g per plant (0.67 t ha^{-1}) to 125.85 g per plant (1.05 t ha^{-1}) were recorded. The trend in variation among the accessions were similar to that of leaf and shoot yield, as biomass is measured as vegetative dry matter produced by each accession.

Mercy (1981) reported a production of 2.3 t ha^{-1} biomass yield for *Mucuna* in legume-maize mixtures. Sunitha (1996) reported a biomass yield of 18.77 t ha^{-1} of *M. pruriens* grown under coconut shade. Chavez (1993) observed a range of 6 to 12 t ha^{-1} of *Mucuna* dry matter production when grown as a pure crop. A biomass yield of 2.3 t ha^{-1} was reported from a crop of *M. pruriens* grown in Nigeria (IITA, 1998).

The data led to the deduction that biomass yield of the accessions varied according to characteristic genetic consitution. It could also be suggested that low soil phosphorous content (31.50 kg ha^{-1}) and the acidic P fixing nature of the experimental site soil may have limited biomass yields.

These findings were supported by the reports of Becker and Johnson (1998) that soil P is an important factor in *Mucuna* biomass accumulation, as legumes require phosphorous for growth and nitrogen fixation, and by the observations of IITA (1997) that variety characteristics were observed to influence the rate of dry matter production.

5.5 Root nodule characters

From the data on nodule count and nodule weight, it was evident that it differed significantly among the accessions at all stages of plant growth (Table 15 and Table 16).

Both nodule count and weight was found to be least at pre-flowering stage. It increased at flowering and seeding stage and then decreased at seed maturation stage, probably due to sloughing of nodules after their life span. Similar findings were reported by Venkataraman and Tilak (1990).

In almost all accessions (excepting MP-57 and MP-45) the effective nodule count was highest at flowering stage. This could be the reason why increment of vegetative yield was highest between pre-flowering and flowering stages, as higher nodule count and weight would have favoured more nitrogen fixation and hence higher yields.

It could also be observed that those accessions which displayed superior vegetative yields viz. MP-62, MP-44, MP-57 and MP-58 revealed superior nodule count and weight at all stages. A definite association can hence be made between these characters.

The findings and conclusions are supported by the report of Venkataraman and Tilak (1990) that vegetative yield is enhanced by increase in nodule count and nodule weight at all stages of growth.

5.6 Leaf area parameters

Both leaf area parameters ie leaf area index (LAI) and leaf area duration (LAD) were found to differ significantly among the accessions (Table 17).

The LAI was found to be least at pre-flowering stage. It increased at flowering stage, was maximum at seeding stage and decreased afterwards. This decline can be attributed to leaf shedding caused by senescence which reduced the leaf number, and hence total leaf area. The trend of LAI variation across the stages was also observed to be similar to that of leaf number and leaf yield production, as LAI was a function of these characters. As the trend was also similar to that of shoot yield, it may be rightly deduced that LAI is positively associated with dry matter production. This could be as higher LAI is indicative of larger areas of leaf exposed to solar radiation, hence resulting in enhanced rates of photosynthesis.

Pearce and Mitchel (1990) reported that LAI is the primary factor that determines the rate of dry matter production, and it reflects the actual productive surface area.

The LAD was observed to be least between pre-flowering and flowering stages. It increased subsequently and attained maximum between seeding and seed maturation stages. This could be so, as LAD being a function of the mean LAI of the two stages under consideration, and the mean LAI of the last period recording the highest value, the LAD of this period was maximum.

From the data it was evident that those accessions having higher LAD were those with higher vegetative yield. This could be reasoned with the findings of Pearce and Mitchel (1990) that the growth in plants is influenced by the period over which plants maintain their leaf area and its persistence in time.

5.7 Physiological parameters

The physiological parameters viz. net assimilation rate (NAR), crop growth rate (CGR), absolute growth rate(AGR), relative growth rate (RGR) and harvest index were found to vary significantly among the accessions at different periods of plant growth (Table 18 and Table 19).

The NAR was calculated for the first two periods and could not be calculated for the third period (180-240 DAS) as negative values in leaf weight difference occured, due to leaf shedding.

The NAR was found to be higher at second period (120-180 DAS) due to increase in whole plant dry matter at seeding, mainly due to pod production.

The accessions that showed higher values of NAR were MP-67, MP-60 and MP-66. This was so despite comparitively lesser leaf yield of these accessions. These accessions must had more photosynthetically efficient leaves, which resulted in higher dry matter production. This finding was supported by Pearce and Mitchel (1990) that NAR is one of the most important growth characteristics, describing the net production efficiency of the assimilation apparatus, and it reflects the net photosynthetic rate per unit leaf area for a given growth period.

Crop growth rate (CGR) and absolute growth rate (AGR) displayed similar trends in variation among the periods of growth. This could be so, as CGR is a function of AGR and both took the whole plant dry matter into consideration. The values of CGR and AGR was least during 60-120 DAS, maximum during 120-180 DAS and decreased subsequently. This decline can be attributed to lowering of whole plant dry matter at seed maturation, due to leaf shedding and maturing or drying of pods. This decrease in growth rate also corresponds with reduction in LAI, nodule count and nodule weight during the same period of plant growth.

Similar observations that CGR was closely related to the LAI and that it could be considered as the most meaningful growth analysis term, was made by Pearce and Mitchel (1990).

It can be deduced finally that the accessions displaying higher growth rates produced more plant dry matter and can be said to be genetically more vigorous, since all accessions were provided initially with identical growth and environmental conditions.

Like CGR and AGR, the relative growth rate (RGR) also took the whole plant dry matter into consideration and its trend of variation among the accessions were similar to that of CGR and AGR. But unlike them, the RGR was found to be maximum during the first period of plant growth ie. between pre-flowering and flowering stages and decreased subsequently. This was probably because of the increment of plant dry matter yield was greatest during this period and it decreased in subsequent periods.

In general the accessions which recorded high measures of CGR, AGR and RGR were MP-60, MP-57, MP-58, MP-62 and MP-67.

The harvest index (HI) represents the amount of biomass partitioned to the economically important plant parts out of the total biomass of the plant, and can be observed to vary according to genetic constitution of the accession. Similar findings were reported by Maggie (1989) in legumes.

Accessions recording high HI are economically more important than the others as their pod yield is high, in comparison with their biomass, and hence beneficial to cultivators interested in the pods.

Accessions recording high HI were MP-60, MP-67, MP-58, MP-66 and MP-62, and the HI was observed to range from 0.82 to 0.50 among all accessions.

Maggie (1989) reported a range of 0.30 to 0.65 in the value of HI for different genotypes of cowpea.

5.8 Phytochemical analysis in soil and plant

The soil nitrogen content in the plots where the accessions were grown were found to differ significantly (Table 20).

The initial soil N content was recorded as 230.55 kg ha^{-1} and analysis of the data revealed that a range of 26.61 kg ha^{-1} to 81.44 kg ha^{-1} of soil N had been fixed.

Venkataraman and Tilak (1990) reported that legumes in general can fix about 50 to 300 kg ha^{-1} of soil N, while Sanchez (1993) observed *Mucuna* to fix about 70 to 130 kg ha^{-1} of soil N. Sunitha (1996) had reported on 60 kg ha^{-1} of soil N fixed by *M. pruriens* grown under shade.

It was evident that nodulation was the main index of nitrogen fixation in legumes, as accessions having superior nodule count and weight were observed to have fixed higher levels of soil N. This was in congruence with the report of Bose (1963) that legumes with large amount of nodules fixed higher amounts of soil nitrogen.

Another observation deduced from the available data was that accession displaying superior efficiency in nitrogen fixation had also recorded high vegetative yield. This could be due to the promoting effect of nitrogen on vegetative growth of the crop. Similar findings were reported by Singh (1957) that excessive vegetative growth is induced by readily available nitrogen.

The accessions MP-62, MP-57, MP-67, MP-44, MP-58 and MP-60 were observed to have fixed comparitively higher levels of soil N, though MP-67 and MP-60 had recorded comparatively lower nodule count and nodule weight. The reason was not evident from the study, though it could be that speedier rates of decomposition of shedded leaves and senescent nodules of these accessions, would have returned more organic nitrogen to the soil. Another observation made was that higher nodule count and weight which favoured the plant towards more luxuriant vegetative growth, was not a desirable feature when the crop was grown for pod and seed production. The accession MP-44 which had recorded high vegetative growth and yield measures, was found to have comparatively lower seed yield.

Yet when the crop is raised for fodder purpose, nitrogen fixation efficiency of the accessions is to be given more importance, as the data revealed a definite and positive association with plant drymatter production.

Initial soil phosphorous content was 31.50 kg ha^{-1} and can be classified as low. After plant growth, there was no significant difference in soil P content among the plots (Table 20).

Rajashree (1994) reported that phosphorous influences the growth and development of root system in legumes, which in turn increases nodule count and rate of nitrogen fixed, leading to enhanced plant herbage. Study of the data revealed that no such findings could be deduced in this experiment.

The plant uptake of soil P may have been hindered by the acidic and P fixing nature of the soil (Vellayani red loam series) used for cultivation. This in turn may have limited plant growth. Becker and Johnson (1998) found soil P to be an important factor in *Mucuna* biomass accumulations.

On the other hand the soil potassium level of the plots differed significantly among them (Table 20).

Soil analysis revealed an initial soil K content of 90.35 kg ha^{-1} and an uptake of 21.6 kg ha^{-1} to 61.71 kg ha^{-1} of soil K after the experiment.

It was evident from the study that those accessions which had absorbed more soil K, displayed higher vegetative yield and comparatively good seed yield.

A similar observation was made by Yahiya (1996) that grain legumes in general required a high quantity of potassium for normal growth and development and for enhancing nodulation and nitrogen fixation.

The L-DOPA (L-3,4-dihydroxyphenylalanine) content varied significantly among the accessions (Table 21).

Accessions having comparatively higher content of L-DOPA (two per cent and more) were MP-60, MP-57, MP-46 and MP-66. L-DOPA content ranged from 1.51 per cent to 2.60 per cent among the ten accessions.

Ramaswamy (1957) had reported 1.5 per cent L-DOPA (dry weight basis) in the seeds of *M. pruriens*. Versteeg (1993) observed a range of 1.5 to 4.8 per cent L-DOPA in *Mucuna* seeds. Chadha (1995) reported a range of 1.30 to 3.32 per cent L-DOPA in the accessions maintained at NBPGR, Issapur farm.

The present study did not reveal any association for L-DOPA content with other characters under observation. The content of L-DOPA being an amino acid and a primary metabolite, could be judged to have varied according to characteristic genetic make up of the accessions.

The accessions which recorded high L-DOPA content as well as high seed yield, would be of much importance to cultivators aiming at sale of seeds to pharamceutical companies.

5.9 Selection index

The selection indices based on seed yield, root yield, biomass yield, L-DOPA content and root nodule characters can be used to identify superior genotypes according to their purpose of maintenance (Table 22).

When the cultivators are interested in either production of seeds alone for various commercial purposes, or for its medicinal value as well, the six best accessions would be MP-60, MP-62, MP-58, MP-67, MP-57 and MP-66.

Some cultivators would be interested in maintaining the crop for green fodder, as livestock feed, in addition to selling the seed. Here the superior six accessions would be MP-60, MP-62, MP-58, MP-67, MP-57 and MP-44.

When the crop is raised for seeds and roots in view of their medicinal value, green fodder production, as well as improving soil fertility conditions, the best six accessions would be MP-62, MP-60, MP-58, MP-57, MP-44 and MP-67.

5.10 Pest and diseases

Surveillance for the occurrence of pests and diseases revealed that certain accessions were susceptible to cowpea mosaic and bacterial blight. Though, the intensity of disease attack was not severe, control measures were taken up in both cases.

The attack of pea aphids and mealy bugs on tender pods could be of economical importance when the crop is maintained for pod and seed yield, as they pave the way for fungal rot causing tender pod drop. In the present sudy although the attack of pea aphids and mealy bugs were observed, no control measures were adopted as intensity of their attack was negligible.

Presence of leaf cutters were noted but did not call for much concern as they are commonly seen in legume crops and do not cause any economical damage.

SUMMARY

6. SUMMARY

A study on 'Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden' was carried out at the College of Agriculture, Vellayani from May 1999 to January 2000. The objectives of the study were to identify genotypes of *M. pruriens* having superior seed yield, biomass yield, L-DOPA content and nitrogen fixation potential.

The experiment was carried out in four phases viz., collection of germplasm, seed characterization and germination studies, cultural trial of accessions under coconut shade and phytochemical analysis. The salient features of the investigation are summarized below:

Phase I

Twenty five accessions were collected from various sources such as authentic centres of germplasm collection as well as areas where the species was found growing wild.

Phase II

The seeds of the collected accessions were subjected to seed characterization and germination studies. On the basis of high germination

percentage, ten accessions were selected viz., MP-57, MP-44, MP-58, MP-67, MP-62, MP-60, MP-45, MP-66, MP-46 and MP-64. Out of these, two accessions viz., MP-62 and MP-57 recorded the highest germination percentage of 98.

Phase III

The ten accessions selected were placed under a cultural trial and pertinent observations were recorded. Analysis of the results revealed the following findings:

1. Vegetative growth and yield prarameters such as plant length, leaf number, leaf area, root length, root girth, shoot yield, leaf yield, root yield and biomass yield at harvest were noted to be generally superior for accessions MP-62, MP-44, MP-57, MP-58 and MP-46.

2. Pod characters such as number of pod clusters per plant, number of pods per cluster, number of seeds per pod, pod yield and seed yield were constantly recorded as high for accessions MP-60, MP-62, MP-58, MP-67 and MP-66. No positive association was noted between pod characters and vegetative growth and yield parameters.

3. The root nodule characters such as effective root nodule count and nodule weight was found to be superior for the accessions MP-57, MP-44, MP-62 and MP-58. The accessions were also noted for their high rate of nitrogen fixation.

Detailed study of the results revealed a positive association between root nodule characters and vegetable growth and yield parameters.

4. Physiological parameters including the net assimilation rate that is indicative of photosynthetic efficiency, and the crop growth rate that measures the rate of dry matter production were estimated using standard formulae. It was deduced that accessions MP-67, MP-60, MP-66, MP-58 and MP-62 had the most photosynthetically efficient leaves while the accessions MP-60, MP-62, MP-58, MP-67 and MP-44 were noted for higher rates of dry matter production.

Phase IV

Phytochemical analysis for L-DOPA in the seeds of the ten accessions revealed high estimates (2.0 per cent and above) for MP-60, MP-57, MP-46 and MP-66.

Selection indices were worked out and accessions ranked on the basis of seed yield, root yield, biomass yield, L-DOPA content and root nodule characters.

The following conclusions could be drawn based on the results of the study :

1. If the selected accessions are to be maintained for seed yield alone, the six best accessions would be MP-60, MP-62, MP-58, MP-67, MP-57 and MP-66.

2. If the purpose of cultivation is for its medicinally valuable seeds as well as for its green matter to be used as a fodder, the six best accessions would be MP-60, MP-62, MP-58, MP-67, MP-57 and MP-44.

3. If the accessions are to be cultivated for its seeds and roots, use as green fodder and for improving the fertility status of the soil, the six best accessions would be MP-62, MP-60, MP-58, MP-57, MP-44 and MP-67.

The study conducted has led to the identification of promising genotypes of *M. pruriens* which can be raised as intercrops in coconut garden.

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APPENDIX

Appendix I

Weather data for the crop period - May 1999 to January 2000

Sl. No	Month and year	Temperature (^oC)		Relative Humidity (%)	Total rainfall (mm)	No. of rainy days
		Max.	Min.			
1	May 1999	30.17	23.75	85.35	9.8	6
2	June 1999	29.48	23.91	85.25	397.4	18
3	July 1999	29.00	23.45	84.09	164.2	21
4	August 1999	29.69	22.95	83.23	108.2	16
5	September 1999	31.12	23.89	78.58	10.6	8
6	October 1999	28.96	23.26	86.80	374.9	21
7	November 1999	29.79	23.17	81.78	156.8	13
8	December 1999	30.71	21.67	81.77	6.6	4
9	January 2000	30.92	21.71	77.95	18.4	4

Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden

By

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ABSTRACT OF THE THESIS
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ABSTRACT

The present study titled "Evaluation of genetic stock of *Mucuna pruriens* Baker non DC. for yield, L-DOPA content and nitrogen fixing potential in coconut garden" was carried out at the Department of Plantation Crops and Spices, College of Agriculture, Vellayani from May 1999 to January 2000.

Seeds of twenty five different accessions were collected from inside and outside the state. They were subjected to seed characterization and seed germination studies.

Based on the germination rate, ten accessions with high germination percentage were selected. These were raised under coconut shade.

Various biometric observations were taken at four different stages, viz., pre-flowering, flowering, seeding and seed maturation state. These observations included biomass yield, root yield and seed yield.

Phytochemical analysis was carried after crop harvest to estimate the quantity of L-DOPA present in the seeds of each accession. Soil tests were carried out to determine the amount of nitrogen fixed in the soil by the accessions.

After analysis of the results it was evident that the accessions MP-62, MP-57, MP-44, MP-58, MP-46 and MP-45 had superior biomass yields compared to the others. The accessions showing superior seed yield were MP-60, MP-62, MP-58, MP-67, MP-57 and MP-66.

The content of soil nitrogen fixed varied significantly among the plots of individual accessions. The accessions MP-62, MP-57, MP-67, MP-44, MP-58 and MP-60 were observed to have fixed higher amounts of nitrogen.

After phytochemical tests, the accessions with significantly superior L-DOPA content (ie., greater than two per cent) were MP-60, MP-57, MP-46 and MP-66.

From the study conducted, the most promising accessions, ie., those having superior biomass, root and seed yield, as well as superior L-DOPA content and nitrogen-fixation potential could be identified. They are MP-62, MP-60, MP-58, MP-57, MP-44 and MP-67.

It can be reiterated that growing such accessions of this plant species would be of much benefit to the cultivator enabling him to utilize existing areas under cultivation, enrich its soil fertility status as well as provide him with an additional source of income by way of selling seeds; the medicinally valuable plant part.