STUDIES IN THREE CANAVALIA SPECIES

THESIS SUBMITTED TO THE GOA UNIVERSITY
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BOTANY

BY

BERNARD F. RODRIGUES
M. Sc.

GUIDE:

Dr. S. G. TORNE
M. Sc., Ph.D.

DEPARTMENT OF BOTANY
S. P. CHOWGULE COLLEGE, MARGAO-GOA

1990

TO

MY PARENTS

A C K N O W L E D G E M E N T S

I wish to express my sincere gratitude to my guide, Dr. S.G. Torne, Professor and Head, Department of Botany, Smt. Parvatibai Chowgule College, Margao, Goa, for suggesting this problem and his valuable guidance and encouragementthroughout the course of investigation.

I am also grateful to Principal, Shri V.R. Shirgurkar and Vice-Principal Shri R.R. Ghantwal, for providing research facilities. I am thankful to CSIR for award of Fellowship.

I am also thankful to Dr. B.C. Sutton, Mr. P.M. Kirk, Dr. D. Brayford, Dr. Z. Lawrence and Dr. D.W. Minter of the Commonwealth Mycological Institute (CMI), Kew England, for their kind help in identifying the fungal specimens.

I also like to thank Prof. D.J. Bagyaraj, University of Agricultural Sciences, GKVK, Banglore, for supplying the mycorrhizal cultures and for his valuable suggestions in mycorrhizal studies.

I also like to thank Dr(Miss) S. Mavinkurve, Professor and Head of the Microbiology Department (Goa University) and Dr. A.D. Agate, Head, microbiology department, M.A.C.S., Research Institute, Pune, for their valuable suggestions in microbiology.

I also like to thank Dr. A.B. Wagh Department of Biofouling and Biodeteriorating N.I.O. DOna Paula (Goa) for his encouragement throughout the research period. My sincere thanks are also due to Dr. R.V. Gaonkar, Department of Botany, S.P. Chowgule college, for valuable suggestions.

I also wish to express my thanks to all my teachers, Research Scholars and the non-teaching staff of the department of Botany for their kind help and constant encouragement in my work.

CERTIFICATE

As required under R.No. 0.19. 8 (vi) of the Goa University, I certify that the thesis entitled "Studies in three Canavalia species" submitted by Mr. B.F. Rodrigues for the award of degree of Doctor of Philosophy is a record of research work done by the candidate during the period of study under my guidance.

(S. G. Torne)

Signature of the guide.

DR. S. G. TORNE,
M. Sc., Ph. D.
S. P. CHOWGULE COLLEGE.
MARGAO, GOA.

STATEMENT

As required under R.No. 0.19.8 (ii), I state that the research work entitled "Studies in three *Canavalia* species" is my original contribution and the same has not been submitted for any degree of this or any other University on any previous occasion.

To the best of my knowledge the present study is the first of its kind.

The research work comprising in this thesis is my original contribution of such type.

1. The microcharacters like stomata, epidermal hairs, idioblast crystals, starch grains and pollen grains provide an important tool in classifying genera and species.

2. Screening an effective native rhizobia for *C. ensiformis* plant suggest that not all the isolated native strains were effective. Certain effective strains can improve the crop yield. Screening of such effective strains in other legumes for inoculation on a mass scale can play an important role in maintaining nitrogen balance.

3. VA mycorrhizal studies in *Canavalia* suggests that VAM fungi-improves nutrient uptake, growth and total biomass. Similar attempts should be made in other crops for improving the productivity with

the association of VAM fungus.

4. Canavanine studies in Canavalia species indicates that C. virosa should be exploited for extraction of canavanine since it is non-edible.

5. Lectins present in Canavalia species have the capacity of agglutinating specifically red blood cells of different blood groups and hence can be used as reagents for blood typing.

The sources on which the present work is based are indicated in "Literature Cited".

The observations and discussions are entirely original and are based on the research carried out by me.

(B.F. Rodrigues)
Signature of the Student

(S.G. Torne)
Signature of the Guide.

DR. S. G. TORNE,
M. Sc., Ph. D
S. P. CHOWGULE COLLEGE
MARGAO, GOA

CONTENTS

INTRODUCTION

INTRODUCTION

In most developing countries, people mainly use cereals as food which are rich in starch but poor in protein content. This protein deficient nutrition of millions of people living in the regions of hot climate has become one of today's most acute problem. Solving this problem largely depends on the identification of the little known and under exploited legumes, their selection, and yield improvement shows great promise in the near future.

Many of the legumes have capacity to provide their own nitrogenous fertilizer through bacteria that live in nodules on their roots. This bacteria chemically converts nitrogen from the air into soluble compounds that the plant can absorb and utilized. As a result, legumes generally require no additional nitrogenous fertilizer for normal growth. This is advantageous because commercial nitrogenous fertilizers are now extremely expensive for marginal farmers.

Today, of the thousands of known legume species, less than 20 are extensively used. The genus Canavalia is one of the legumes among these neglected and under exploited ones. This genus includes 51 species widely distributed throughout the tropics and subtropics of the Old World and the New World and the islands of the West Indies and South Pacific. They inhabit sandy and rocky coastal seashores, woodlands, and thickets (Allen & Allen, 1981).

Sauer (1964) expressed the opinion that this genus probably diverged from other Phaseoleae during the Cretaceous period.

In Goa, three species of Canavalia are found. They include C. ensiformis (L) DC., C. gladiata (Jacq.) DC., and C. virosa (Roxb.) Wight et Arn.

The two species extensively cultivated include C. ensiformis (Jackbean) and C. gladiata (Swordbean). These are robust, high yielding, closely related species which produce nutritious pods and high-protein seeds. These plants can grow under extremely difficult conditions and thus offers a means of extending protein production to marginal areas, particularly to tropical lowlands with depleted soils and to areas with unpredictable climate or varying soil types, high altitudes, and pest infestations.

Canavalia virosa is found growing wild and is not edible. However, the seeds are at times used by village people as narcotic. Also, they contain good amount of urease and lectins.

The hardiness of all these plants is shown by the range of conditions under which they grow.

* Temperature range: Plants of C. ensiformis have been grown successfully where average annual temperature range from 14^{o}C to 27^{o}C from warmer parts of the temperate zone to hot, tropical rainforest areas.

* Rainfall: Plants of C. ensiformis have been reported to grow well where rainfall is as high as 4,200 mm and as low as 700mm. A deep root system allows these plants, once established, to draw on stored soil moisture and to survive dry conditions.

* Sunlight: Although these plants are photophilous, they can also be Sciophilous.

* Soils: These plants tolerate a wide range of soil textures and fertility. They can grow on the highly leached, nutrient-depleted, lowland tropical soils. They grow well on acid soils (pH 4.3-6.8) and they are far less affected by waterlogging and salinity than other pulse crops.

* Altitude : Although generally grown in the lowlands, they can also be grown at elevations at least as high as 1,800m.

In addition to their uncommon adaptability, these plants are relatively fast growing, usually producing a crop in 3-4 months.

Yound seeds and pods of C. ensiformis and C. gladiata are cooked and eaten as vegetables, but once they have matured and become hard, they are not delectable. This is mainly because of the presence of growth inhibiting compounds. These are the proteins Canavalin, Concanavalin A and B, the enzyme urease, and the amino acid Canavanine. However the toxicity in C. ensiformis and C. gladiata seems largely due to Con A, which binds to the mucosal cells living in the intestine and thus reduces the body's ability to absorb nutrients from the intestine.

Considering the present problem, these legumes have a great role to play in uplifting the nutritional economy and hence one cannot afford to ignore these legumes. Elimination/or reduction of these factors would make these legumes more acceptable as source of inexpensive nutritious proteins and maximize their utilization for human food. Therefore a world-wide collection of the germ plasm is needed, which is to be followed by mass screening for types free for Con A and other toxic constituents.

The present thesis comprises of five parts. The first part deals with mitosis and karyotype analysis. epidermal features, starch grain studies and, palynology and pollen physiology. The second part deals with nodulation studies in C. ensiformis. The third part deals with agronomic studies in Canavalia species. The fourth part deals with mycoflora and diseases. The fifth part deals with toxicology and chemical analysis.

The available relevant literature on the subject has been reviewed under the title "Previous work" at the beginning of the thesis followed by morphological description of the plants studied under the heading "Taxonomy".

PREVIOUS WORK

PREVIOUS WORK

CYTOLOGY

A search through available literature indicates that very little cytological work has been done in *Canavalia* species. Out of a total of 51 *Canavalia* species known, only 8 have received cytological attention.

Canavalia species	somatic chromosome number	Reference
C. africana Dunn	22	Miége 1960
C. ensiformis (L.) DC.	22	Kawakami 1930 Simmonds 1954 Shibata 1962 (I.1963)
C. gladiata (Jacq.) DC.	22	Yexob, Kaprameba 1932, 6 Poucques 1945
	22,44	Covas 1949
	44	Shibata 1962 (I.1963)
C. lineata DC.	22	Jinno 1956
C. obtusifolia DC.	22	Frahm-Leliveld 1960
C. plagiosperma Piper	22	Simmonds 1954
C. rosea (Sw.) DC.	22	Miége 1960
C. virosa (Roxb.)Wight et Arn	22	Kedharnath 1950 Riley H.P. 1960

TAXONOMY

Chatterjee (1948) classified different species of *Canavalia* on the basis of leaflets, calyx, inflorescence, pods and seeds. Purseglove (1968) has classified *Canavalia* species on the basis of habit, pods and seeds. Saldanha

(1984) has classified Canavalia species on the basis of leaflet morphology.

EPIDERMAL FEATURES

Wellendorf (1966) studied the structure of starch grains in C. ensiformis.

Frank & Jensen (1970) studied the pattern of formation of Idioblast crystals in C. ensiformis. Horner & Zindler-Frank (1982) studied the location and morphology of crystals in the leaves of C. ensiformis.

NODULATION STUDIES

Narayana & Gothwal (1964) made a detail study of nodule morphogenesis and development in C. gladiata.

PHYSIOLOGY AND AGRONOMY

Fors (1955) has studied the use of C. ensiformis as a soil improver in cane fields.

PATHOLOGY

Raj & Jose (1969) reported Collar rot of C. ensiformis caused by the fungus Sclerotium rolfsii Sacc. Laximinarayana & Reddy (1976) reported fruit rot disease in C. ensiformis caused by Coleophoma empetri (Rostr.) Patrak. Singh & Shrivastava (1979) reported fruit rot in C. ensiformis caused by Trichothecium roseum (Pers.).

TOXICOLOGY AND CHEMICAL ANALYSIS :

Canavanine was originally discovered by Kitagawa & Tomiyama (1929) in C. ensiformis; Kitagawa (1937) in C. lineata, and Damodaran & Narayanan (1939) in C. obtusifolia. Birdsong et al., (1960) reported the presence of canavanine in four Canavalia species viz., C. ensiformis, C. gladiata, C. lineata and C. maritima. Later, Turner & Harborne (1967) detected canavanine in five Canavalia species, but did not specify the names of the species studied by them. Naylor (1966) studied the synthesis and degradation of canavanine in C. ensiformis. Rosenthal (1970) studied the canavanine utilization and biosynthesis in the developing plant of C. ensiformis. Later in 1971, he studied the ontogeny of canavanine formation in the fruit of C. ensiformis. Downum et al., (1983) reported Arginine and L-canavanine metabolism in C. ensiformis and Glycine max.

The lectin, Con A was first isolated from the jackbean by Sumner (1919), and was subsequently shown to agglutinate erythrocyte (Sumner & Howell, 1936), Schertz et al., (1960) observed seed extracts of 311 species of plants for agglutinating activity with human blood cells in an attempt to find plant sources of naturally occurring hemagglutinins for specific blood types. Liener (1962) studied hemagglutinins in a few legume seeds and proposed a mechanism explaining the nature of the interactions of hemagglutinins with red blood cells, and their possible function in the plant. Huprikar & Sohonie (1961) detected hemagglutinins in C. ensiformis and studied the effect of autoclaving

and germination on hemagglutinating activity. Sehgal & Naylor (1966) made an ontogenetic study of urease in C. ensiformis. Smith et al., (1982) studied the biochemical characterization of canavalin. Sammour et al., (1984) studied the homology of canavalin, a major storage protein of C. ensiformis to pea vicilin and its separation from ∝- mannosidase. Herman & Shannon (1984) made studies on the immunocytochemical localization of concanavalin A in developing jack bean cotyledons. Ghosh et al., (1985) studied lectin concanavalin A distribution at different stages in the tissues of C. gladiata. Daisuke & Minamikawa (1986) made in vivo studies on protein synthesis in developing seeds of C. gladiata. Yamauchi & Minamikawa (1987) studied the synthesis of canavalin and concanavalin A in maturing seeds of C. gladiata.

Molina & Bressani (1974) studied the protein-starch extraction and nutritive value of seeds and protein isolate of C. ensiformis.

TAXONOMY

Brief description of Family leguminosae (Pea or Pulse family).

Origin and distribution

The oldest agricultural records available indicate that leguminous crops have been cultivated for centuries and that they were valued for food and soil enrichment long before their ability to work symbiotically with bacteria was understood. Fossils of leguminosae have been traced back to the Cretaceous or last division of the secondary or Mesozoic era, 95-120 million years ago (Fred et al., 1932).

The leguminosae form one of the largest families of flowering plants, ranking third in terms of world-wide occurrence with about 600 genera and 18,000 species.

Taxonomic characters

Herbs, shrubs and trees, many of which show climbing habits. Leaves mostly alternate, rarely opposite, compound (mostly pinnately so, but sometimes palmately compound) or simple by supression of leaflets, stipulate or infrequently exstipulate; Flowers rarely solitary, usually in axillary or terminal clusters; commonly in recemes, panicles, spikes or heads, occasionally in umbels and rarely in cymes, often subtended by bracts and bractlets. Flowers almost always bisexual, actinomorphic (regular) or more commonly zygomorphic (irregular) and usually with complete perianth, typically hypogynous or somewhat perigynous. Calyx usually gamosepalous, usually 5-parted, often unequal, sometimes combined in 2 lips. Corolla almost, always present, rarely lacking or reduced to a single

petal, typically 5-parted, distinct or the lower 2 partly or completely adherent along one side or all gamopetalous, imbricate or valvate. Stamens usually 10, occasionally 5 or rarely even fewer, but sometimes numerous, distinct, monadelphous or diadelphous; anthers usually 2-celled, usually dehiscing by longitudinal slits or less commonly by apical pores. Pistil 1 (very rarely 2-15), 1-carpellate, usually 1-loculate, the placentation parietal along the ventral suture; the ovary superior, sessile or stalked. Fruit usually a legume dehiscing along both sutures (or rarely follicular and then splitting only along one side) or variously modified - sometimes a loment (the fruit constricted between the seeds and breaking into 1-seeded indehiscent segments) and sometimes fleshy or membranous and occasionally indehiscent. Seeds usually with little or no endosperm, usually exalbuminous; Testa usually hard or leathery, occasionally strophiolate; Cotyledons fleshly or leafy; the radicle straight or accumbent.

Economic importance of Family leguminosae

Economically, the leguminosae is one of the most important family of flowering plants. Legumes are cultivated to produce food-seeds of high protein content, as well as valuable fodder. They are also a source from which secondary plant products like dyes, gums, resins and oils are extracted. Many of them are grown domestically as ornamentals. Legumes can help spearhead the fight to stop the erosion now prevalent in the tropics and can help to rebuild the already damaged and degraded soils. Economically important plants of this family can be grouped as per their use.

Food value: Common bean (Phaseolus vulgaris). Lima bean (Phaseolus lunatus), Cowpea (Vigna unguiculata), Lentils (Lens esculenta), Pigeon pea (Cajanus cajan), Chick-pea (Cicer arietinum), Soya bean (Glycine max), Green gram (Phaseolus aureus) Black gram (Phaseolus mungo).

Cover crop, Green manure and Fodder:

Calopogonium mucunoides, Centrosema plumieri, C. pubescens, Pueraria phaseoloides, P. thunbergiana, Stizolobium aterrimum, S. deeringianum, Medicago sativa, Sesbania spp., Trifolium spp., Alysicarpus vaginalis, Clitoria laurifolia, Crotalaria spp., Moghania macrophylla, Flemingia congesta, Lupinus spp., Stylosanthes erecta, Tephrosia.

Oils:

Groundnut (Arachis hypogaea and Soya bean (Glycine max).

Dyes:

Indigofera arrecta, I. sumatrana and I. tinctoria.

Ornamentals:

The golden shower (Cassia fistula), The pink-and-white shower Cassia nodosa), Pride of Barbados (Caesalpinia pulcherrima), Orchid trees (Bauhinia spp.), Cock's comb coral tree (Erthrina crista-galli), Raintree (Samanea saman), Sweet pea (Lathyrus odoratus), Wisteria (Wisteria spp), Laburnum (Laburnum spp.) and Butterfly pea (Clitoria ternatea).

Other products:

Sunnhemp (Crotalaria juncea) is an important fibre crop in India. Derris spp. and Lonchocarpus spp. are a source of rotenone and are used as insecticides and as fish poisons. Tonka bean (Dipteryx spp.) is used for flavouring. Liquorice is obtained from the roots of Glycyrrhiza glabra and gumtragacanth from Astragalus gummifer. Seeds of Trigonella foenumgraecum are used in curries in India. Senna pods from Cassia angustifolia are used for their laxative properties.

Brief description of Subfamily Papilionoideae

Origin and distribution

The subfamily papilionoideae is the largest, heterogenous with members equally distributed in tropical and temperate zones. The most primitive, woody genera are found mostly in the tropics while the herbaceous more advanced ones occur in temperate region. About 482 genera and 12,000 species are distributed throughout the world (Hutchinson, 1964).

Taxonomic characters

Herbs, shrubs, trees or climbers. Leaves alternate, simple or digitately or pinnately compound, rarely bipinnate, sometimes ending in tendrils. Flowers typically perfect and zygomorphic. Hypanthium very small. Calyx gamosepalous, 5-toothed or-lobed or the upper lobes more or less connate, or bilabiate the 2 upper opposed to the 3 lower, rarely apathaceous. Petals typically 5, unequal, imbricate, papilionaceous (i.e. the uppermost or odd petal (the

"standard") the largest and external in bud, the two lateral (the "wings") exterior to the two lowermost (the "Keel") which are weakly are coherent and usually enclose the stamens and pistil), borne on the upper rim of the very short hypanthium. Stamens usually 10, their filaments seperate or more typically with their filaments united into a sheath partially enveloping the pistil , most commonly diadelphous or monadelphous; anthers typically opening by a lateral silt. Ovary s essile or stipitate, typically 1-celled or occasionally partially or completely 2-celled through the intrusion of the sutures or transversely segmented and with 1 to many ovules. Endosperm absent, cotyledons accumbent, embryo with an inflexed radicle.

Taxomonic history of Canavalia

The genus Canavalia belongs to subfamily Papilionoideae, of the family leguminosae. The generic name Canavalia is the Latinized version of the word "Canavali ", which was used by Rheede (1688) as one of the vernacular Malabar name. Rheede's name was validated as a genus by Adanson (1763) who supplied a Latin diagnosis based on Rheede's plant. Some authors therefore preferred to use the generic name Canavali Adanson. It was De Candolle (1935), who actually used the word Canavalia as against Canavali of Adanson.

From Paleobotanical studies, Sauer (1964) expressed the opinion that this genus probably diverged from other phaseoleae during the Cretaceous period. The Wilcox flora, which grew on Eocene sea beaches on the Tennessee-Mississippi region, included plants whose fossile leaves are strikingly like those of Canavalia maritima, the pantropical beach species that grow

on the Gulf Coast today. These fossils were named C. eocenia (Berry, 1916) and Leguminosites andiraformis (Berry, 1930); the difference in leaf shape between the two forms is within the range of variation of the living species. A very similar form from the Miocene of Trinidad, which also may be inseparable from C. maritima, was named L. canavaliformis (Berry, 1925).

The wilcox flora includes other leaves that may represent Eocene members of a different subgenus, Wenderothia, one form not separately named by Berry, resembles the living C. altipendula of Jamaica (Berry, 1930). Another form that resembles the living C. dura of Mexico and C. picta of South America was named C. acuminata (Berry, 1916). Unfortunately, this name is a later homonym of C. acuminata Rose, belonging to a different, living species.

A third subgenus, Catodonia, is evidently represented by fossils from the Miocene of Trinidad, named Canavalia miocenica (Berry, 1925). These leaflets are an excellent match for the living C. nitida, not known from modern Trinidad but widespread elsewhere in the West Indies.

The fourth subgenus, Maunaloa, endemic to Hawaii, is not represented by any known fossils.

The only known report of fossil Canavalia material from outside the Americas is a statement by Menzel (1920) that leaves of C. ensiformis were found in tuff of uncertain geologic age on the coast of Cameroons.

Sauer (1964) suggested that Wenderothia is the most primitive subgenus, where it includes the species, such as C. dura, which are morphologically closest to the other genera of Diocleinae. From the Wenderothia stock, the subgenera Catodonia and Canavalia may have independently initiated and Maunaloa, an endemic to the Hawaiian islands may be comparatively late off-shoot of the last.

Brief description of Genus Canavalia

Origin and distribution

The genus Canavalia includes about 50 species widely distributed throughout the tropics and subtropics of the Old World and the New World and the islands of the West Indies and South Pacific. They inhabit sandy and rocky coastal seashores woodlands, and thickets (Allen & Allen, 1981). According to Guppy (1906, 1917), Canavalia and certain other tropical legumes might have evolved from widespread sea dispersed littoral species. This genus actually originated in America, spread around the tropics of the world spawing a large number of similar but discrete species some of which are endemic to oceanic islands and have no adaptation for long range dispersal (Sauer, 1964).

Taxonomic characters

Herbs, twining, or prostrate, slender, annual or stout, tall, woody, perennial climbers. Leaves pinnately 3-foliate; leaflets variously shaped, mostly ovate and acuminate, often large, entire, papery or leathery, undersurfaces usually hairy; stipels generally minute, caducous; stipules

small, wart like or inconspicuous. Flowers generally purple to violet, varying from reddish to bluish and from dark to pale, sometimes white, with yellow markings near the petal bases, often resupinate, 2-6 pedicelled at swollen nodes on short or long peduncles in axillary thyrses; bracts small; bracteoles 2, caducous, subtending each flower. Calyx campanulate, tubular at the base, bilibiate, upper lip large, truncate or 2-lobed, lower lip smaller, minutely 3-toothed or entire; standard large, broad-obovate, reflexed, calloused along the midvein above the claw; wings free, narrow, subtwisted or falcate, eared above the claw; keel wider than the wings, incurved, free at the base, bluntly beaked, sometimes gibbous, eared above the claw. Stamens monadelphous or vexillar stamen free at the base and connate with the other in the middle; anthers uniform. Ovary sessile, usually hairy, many-ovuled; style incurved or folded with keel, beardless; stigma small, capitate, terminal. Pod broad-linear, flattened, sometimes swollen, compressed between the seeds, with a prominent ridge or rib along the upper suture, endocarp papery, white, separating, 2-valved, valves leathery, often thinly filled between the seeds, dehiscent, sometimes explosively, or indehiscent. Seeds 4-20, large, ovate or orbicular, oblong to elliptic, strongly to moderately compressed, usually various shades of reddish brown, mettled, hilum linear.

Economic importance of genus canavalia

The genus Canavalia includes 51 species distributed in the tropics and subtropics of both hemispheres (Allen & Allen, 1981). Among these species, C. ensiformis (L.) DC., C. gladiata (Jacq.)DC. and C. plagio-

sperma Piper are grown as pulse crops. Young seeds and immature pods of these species are cooked and eaten as vegetables, but once they are matured and become hard, they are not delactable.

Nutritionally, the foliage appears acceptable, but livestock eat them with reluctance as they find it coarse and unpalatable. Young immature leaves of Canavalia species are a source of gibberellins.

Members of the genus Canavalia are grown as green manure and as cover and rotation crops favored for their rapid and heavy growth, semi-drought-resistance, deep-rooted habit, and shade tolerance. All species are excellent soil-binders and soil-improvement plants. C. maritima (Aubl.) thou. is a common pantropic strand plant.

The genus Canavalia has also a great pharmacological importance. C. ensiformis has a cooling denulcent, antibilious and cordial action, while C. virosa (Roxb.) Wight et Arn. has a narcotic action. Decoction of leaves is taken for nervous disorders in Honduras (Lentz, 1986).

The genus Canavalia is a potential source of Phytohaemagglutinins such as enzyme Urease (Summer, 1919), Concanavalin A, Concanavalin B (Summer & Howell, 1936) and Canavalin (Summer, 1919). Other nitrogenous constituents present are Chloline, Trigonelline, Betonicine, Caneine and Ketogine. The occurrence of Canavanine $C_5H_{12}N_4O_3$, an amino acid (Kitagawa & Tomiyama, 1929 in the seeds of Canavalia species is of related interest as it is extensively used as a research biochemical.

Key to the Canavalia species studied (Fig. 2)

I. Pods 20 - 35 cm. long:

II. Seeds white; hilum 8mm. long .. .. .. C. ensiformis

II. Seeds red; hilum 15 - 20 mm. long .. .. C. gladiata

I. Pods 10 - 15 cm. long:

III. Leaflet ovate to oblong-ovate, acute to subacute; Calyx distinctly pubescent; receme many flowered (12 - 20); endosperm not separating. .. .. C. virosa

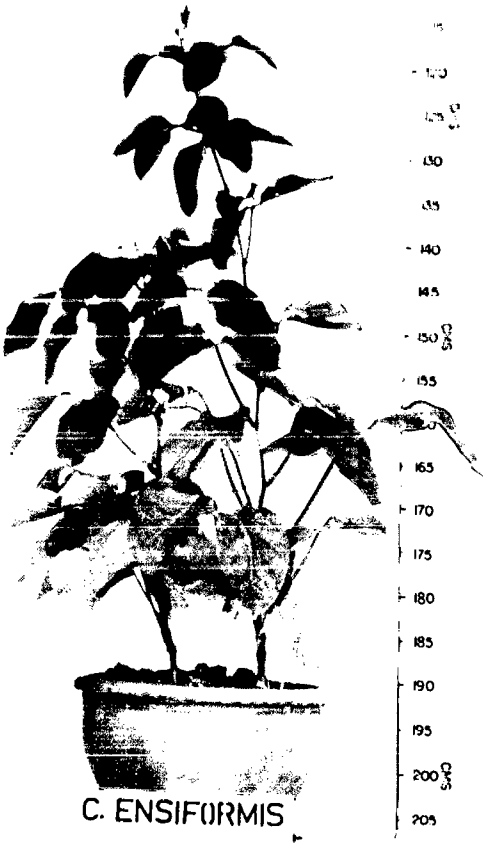

C. ENSIFORMIS
130
135
140
145
150
155
165
170
175
180
185
190
195
200
205

Fig 1. (a)

Fig. 1. (b)

Canavalia ensiformis (L.) DC. (Fig. 1a)

Synonyms:

Dolichos ensiformis L.

Dolichos acinaciformis Jacq.

Dolichos pugioniformis Gmel.

Malocchia ensiformis (L) Savi

Canavalia ensiformis (L.) DC.

Canavalia gladiata (Jacq.) DC.

Origin and distribution

The Jackbean originated in the West Indies, whence it was brought to Central and South America. After Christopher Columbus's voyage this crop was introduced into Africa, India, China and Burma (Ustimenko-Bakumovsky, 1983).

Taxonomic characters

Annual, usually bushy and erect, 1-2m. high; stem stout, terete, sparsely strigillose; petiole usually longer than the leaflets; stipules lanceolate; stipels minute, subulate. Leaflets oval to ovate, obtuse to acute, 6-12 cm. long, about 7.5 cm. wide, strigillose at first on both surfaces, later becoming glabrescent, peduncle 10-20 flowered; bracteoles orbicular. Calyx campanulate, 14-16 mm. long, upper lip emarginate, lower lip 3-lobed, lobes triangular, acute. Corolla 15mm. long, rose coloured, fading to white towards the base; standard oblong-orbicular, notched at

the apex; claw 5 mm. long; wings oblong, obtuse basal auricle inflexed; keel as long as the wings, united except at base. Stamens monadelphous, the vexillar one free near the base; all others free for the terminal one-sixth; style glabrous; stigma capitate. Pods linear, gently curved, beaked at the tip, 25-30 cm. long, 2-3.5 cm. wide, 12-20 seeded; each valve with three longitudinal ridges, one near each suture, the third, 4 mm. From the ventral suture; inner layer thin white, papery, separating. Seeds ellipsoid, compressed, shiny white, 22 mm. long, 14 mm. wide, about 8 mm thick; hilum 8 mm. long, greyish, about one-seventh the circumference, surrounded by an orange-brown border.

Canavalia gladiata (Jacq.) DC. (Fig.1b)

Synonyms:

Dolichos incurvus Thunb.

Dolichos gladiatus Jacq.

Canavali maxima Thouars

Canavali incurva Thouars

Malocchia gladiata (Jacq.) Savi

Canavalia incurva (Thunb.) DC.

Canavalia gladiata (Jacq.) DC.

Canavalia loureirii G. Don

Canavalia machaeroides (DC) Steud.

Canavalia lunareti Carriere

Canavalia ensiformis (L.) DC.

Bara - mareca Rheede

Lobus machaeroides Rumph.

Origin and distribution

Mostly found in cultivation in India, Burma, Srilanka and Malaysia. It is cultivated in tropical Africa and the U.S.A.. The native country is difficult to ascertain with precision but it is generally agreed that the plant is a native of the Old World tropics. Ramchandran et al., (1980) believes that this plant belongs to the Hindustan centre of origin.

Taxonomic characters

Annual or biennial climber, climbing to a height of several meters; stem terete, glabrous. Petioles usually shorter than or equalling the leaflets. Leaflets ovate, or broadly ovate, acute, sub-acute, or shortly acuminate, base sub-acute, or shortly acuminate, base sub-truncate, 10-18 cm. long, 7-12 cm. wide, both surfaces glabrous; petiolule 8-10 mm. long, slightly flattened and ridged on two sides; stipules lanceolate; stipels subulate, deciduous; peduncle 10-25 cm. long, 12-16 flowered; flowers usually borne singly and arranged laxly on the peduncle; bracteoles minute ovate, caducous. Calyx campanulate, 15-20 mm. long, upper lip slightly shorter than the tube, with two rounded lobes, hardly emarginate, distinctly reticulate veined; lower lip 3-lobed, lobes 2-3 mm. long, broadly ovate, acute. Corolla usually lilac, or pale pink, 20-25 mm. long; standard orbicular, emarginate, with two small semi-lunar processes near the base; wings about 3 cm. long, oblong-spatulate, slightly curved, auricled at base; claw 14 mm. long with a rounded protruding appendage on dorsal edge; keel as long as wings, blong, obtuse, falcate limb united near the tip, auricled

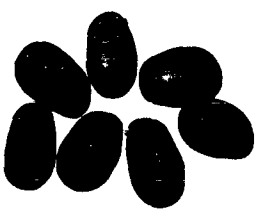
EXPLANATION OF PLATE - 2

Fig. 1 (c) *Canavalia virosa* (Roxb.) Wight et Arn.

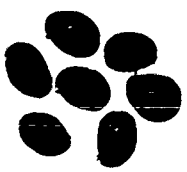
Fig. 2 Seeds of three *Canavalia* species.

Fig 1. (c)

C. ENSIFORMIS



C. GLADIATA



C. VIROSA

Fig. 2

near the base; claw about 7 mm. Stamens monadelphous; ovary linear, pubescent, 15 mm. long, style 12-13 mm. long; stigma capitate. Pod linear-oblong, compreeed, slightly curved, beaked, straw-coloured when mature, 20-35 cm. long, 2.5-4 cm (rarely 5 cm) wide, 8-10 seeded, each valve with a longitudinal ridge close to each suture; and a third, prominent one, about 4-7 mm. from the ventral suture; inner layer thin papery, white separating. Seeds ellipsoid, compressed, 22-35 mm. long, 16-20 mm. wide, 5-6 mm. thick, red or dull red; hilum 15-20 mm. about one-third of the circumference.

Canavalia virosa (Roxb.) Wight et Arn. (Fig. 1c)

Synonyms:

Dolichos polystachios Forsk.

Dolichos virosus Roxb.

Canavalia polystachya (Forsk.) Schweing.

Canavalia ensiformis (L.) DC.

Canavalia africana Dunn

Canavalia ferruginea Piper

Canavalia mollis. wall. ex wight et Arnott

Dolichos cienkowskii Schweinf.

Katu - Baramareca Rheede

Origin and distribution

It is believed to be a native of India, from which the cultivated species may have been derived (Chatterjee, 1948). It is also found in China, Siam, Mauritius, Tropical Africa, Madagascar and Arabia.

Taxonomic characters

A large biennial climber with glabrous or pubescent leaflets. Petiole longer than the leaflets with a few irregular longitudinal streaks. Leaflets membranous, ovate or broadly-ovate, sometimes orbicular, subulate or obtuse, with a very short mucronate tip; base usually cuneate or sub-truncate, 8-14 cm. long, 5-9 cm. wide, glabrescent to hirsute on both surfaces; petiolule 6-8 mm. long, pubescent; stipules and stipels subulate, caducous; peduncle very variable, 20-40 cm. long, with 10-20 flowers borne usually in pairs, are closely aggregated in the upper third or upper fourth. Calyx narrowly campanulate, 15 mm. long, upper lip of two rounded slightly overlapping lobes, about as long as the tube, lower lip of three short ovate-acute lobes, central tube longer than the lateral pair. Corolla rose-purple, or lilac, about 3 cm. long; standard obcordate, with two thick callosites near the base, 3 cm. long, 2 cm. wide, appendage short and obtuse, claw 5-7 mm. long; wings falcate, obtuse, with two appendages on the dorsal side, claw filiform, about 7 mm. long; keel as long as the wings, but broader, gently curved, with a sharp acute base appendage, 2.8-3 cm. long, including 7-8 mm. long claw. Stamens monadelphous, 2.5-3 cm. long; ovary linear, 2 cm. long, strigose; style glabrous, 5-6 mm. long; stigma capitate. Pod elliptic-elongate compressed, sparsely hairy or glabrous, 4-8 seeded, 10-14.5 cm. long, 2-2.5 cm. wide, beak short. Seed ovoid or ellipsoid, 1.8-2 cm. long, 10-11 mm thick, amber with gamboge streaks; hilum 10 mm. long, broader near the micropyle.

PART I

A. MITOSIS AND KARYOLOGICAL STUDIES.

B. EPIDERMAL FEATURES.

C. STARCH GRAINS .

D. PALYNOLOGY AND POLLEN PHYSIOLOGY.

A. MITOSIS AND KARYOTYPE ANALYSIS OF THREE CANAVALIA SPECIES

In the study of evolution, phylogeny and classification of plants, the importance of cytological study has been widely appreciated. The cytological data provides an important tool in classifying the genera and species; the number, form and size of their chromosomes being the major features.

The Russian school of cytologists, headed by S. Navaschin, developed the fundamentals of the Karyotype concept from their observations that most species of living organisms show a distinct and constant individuality of their somatic chromosomes and that closely related species have more similar chromosomes than those of more distantly related ones.

The karyotype was first defined by Delaunay (1926) as a group of species resembling each other in the morphology and number of their chromosomes. However Levitsky (1924, 1931), on the basis of records indicating that the evolution of karyotype in many genera takes place through a series of alterations in chromosome morphology, gave a new definition for karyotype. According to him, karyotype is the phenotypic appearance of the somatic chromosomes, in contrast to their genotype.

As regards to the Phaseoleae, chromosome counts of a large number of species of this tribe have been reported. Sinha & Kumar (1971),

Joseph & Bouwkamp (1978), Goswani (1979), Sarbhoy (1978, 1980), Sharma & Gupta (1982).

Unfortunately, due to difficulties in cytological preparation no significant work has been done on a critical analysis of the Karyotype of Canavalia species. The cytological information that is available on the genus Canavalia is almost negligible, which has been already reviewed under 'Previous work'.

MATERIALS AND METHODS

Seeds of all the three Canavalia species were collected from different places (Margao, Colva, Macasana & Kolem) of Goa. Seeds were germinated in pots containing vermiculite. Healthy root-tips were excised, washed and pretreated with saturated aqueous solution of p-dichlorobenzene for 2-3 hours at 10°C. Pre-treated root-tips were washed thoroughly and fixed in modified Carnoy's fluid (6 Absolute alcohol : 3 Chloroform : 1 Acetic acid) for 5-8 minutes. Fixation was followed by hydrolysis at 60°C in 2N HCl and then staining with 2% aceto-orcein which gave satisfactory staining. Slides were made permanent by using N-Butyl alcohol - Acetic acid series and mounted in Euparal.

For determining the length of the chromosomes, about 50 well spread metaphase plates were studied and the average length of each individual chromosome was calculated from the data obtained. The symbols for the karyotype descriptions are based on those of Battaglia (1955) and Sinoto

(1943) as m and sm representing chromosomes with median and submedian centromeres respectively. TF% was calculated as given by Huziwara (1962) where,

$$TF\% = \frac{\text{Total sum of short arm length}}{\text{Total sum of chromosome length}}$$

For the karyotype analysis the method of Levan et al., (1964) was followed.

Important plates were drawn at table level with the aid of Carl Zeiss drawing apparatus using Ergaval microscope with 20 x periplan ocular, a 100 x oil immersion and a 1.4 N.A. aplanatic condenser. Photomicrographs were taken with Asahi Pentax camera with the help of microscope adapter.

OBSERVATIONS

Chromosomes were observed at somatic metaphase with regard to their number, size and length. In all the three species, the somatic chromosome number has been found to be 2n=22.

The process of mitosis in all the three Canavalia species studied, is found to be more or less similar. The resting nucleus has a granular appearance and has generally one nucleolus. At the commencement of the prophase all the chromosomes are in the form of fine threads, while at the end they have got their individuality with a prominent pair of chromatids. The metaphase chromosomes attain their greatest contraction

and were lying along the equator. Further stages are found to be usual.

Chromosomes are characterised in having mostly median to submedian primary constrictions and on its basis three general classes can be recognised, viz, long, medium and short chromosomes. The following general morphological types of chromosomes have been recorded.

Type A - Very long chromosomes with submedian centromere.
Type A_1- Long chromosomes with median centromere.
Type B - Long chromosomes with submedian centromere.
Type C - Medium chromosomes with median centromere.
Type C_1- Medium chromosomes with submedian centromere.
Type D - Short chromosomes with median centromere.
Type D_1- Short chromosomes with submedian centromere.
Type E - Very short chromosomes with median centromere.

For the karyotype analysis d, r, and i values have been calculated. The total length of chromosome was denoted by 'C' and the length of long and short arm as 'l' and 's' respectively. The location of the centromere was expressed as a difference d=l-s. The ratio between the arms was calculated as centromeric index (i) = $\frac{100s}{C}$.

KARYOTYPES:

Canavalia ensiformis (L.) DC.

The normal somatic chromosome number in C. ensiformis was found

Table 1 Measurement and position of centromere of somatic chromosomes in C. ensiformis.

Chromsome pairs	Length of long arm in μ (l)		Length of short arm in μ (s)		Total length in μ (C)		d=l-s	r=l/s	i=100s/C	centromeric position
	Mean	s.d.	Mean	s.d.	Mean	s.d.				
I	1.02	0.16	0.97	0.14	1.99	0.30	0.05	1.05	48.74	m
II	1.07	0.14	0.66	0.13	1.73	0.27	0.41	1.62	38.15	sm
III	0.97	0.13	0.66	0.13	1.63	0.26	0.31	1.47	40.49	sm
IV	1.02	0.15	0.56	0.13	1.58	0.28	0.46	1.82	35.44	sm
V	0.82	0.14	0.71	0.12	1.53	0.26	0.11	1.15	46.40	m
VI	0.82	0.12	0.66	0.12	1.48	0.24	0.16	1.24	44.59	sm
VII	0.82	0.12	0.56	0.13	1.38	0.25	0.26	1.46	40.58	sm
VIII	0.61	0.12	0.61	0.11	1.22	0.23	0.00	1.00	50.00	M
IX	0.71	0.71	0.11	0.51	0.10	1.22	0.21	1.39	41.80	sm
X	0.71	0.11	0.46	0.11	1.17	0.22	0.25	1.54	39.32	sm
XI	0.61	0.11	0.51	0.10	1.12	0.21	0.10	1.20	45.54	m

T.F.% = 42.82

Total chromatin length: 32.10 ± 5.46μ.

MITOSIS AND KARYOTYPE OF C. ENSIFORMIS (DIPLOID PLANT)

EXPLANATION OF PLATE - 3

Fig 3.(a) Somatic Chromosomes from root tip cell of C. ensiformis (x2000).

Fig 3.(b) Idiogram of the Chromosomes.

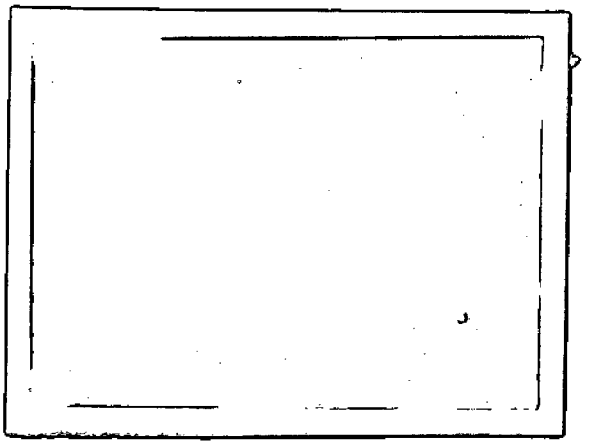


Fig. 3. (a)

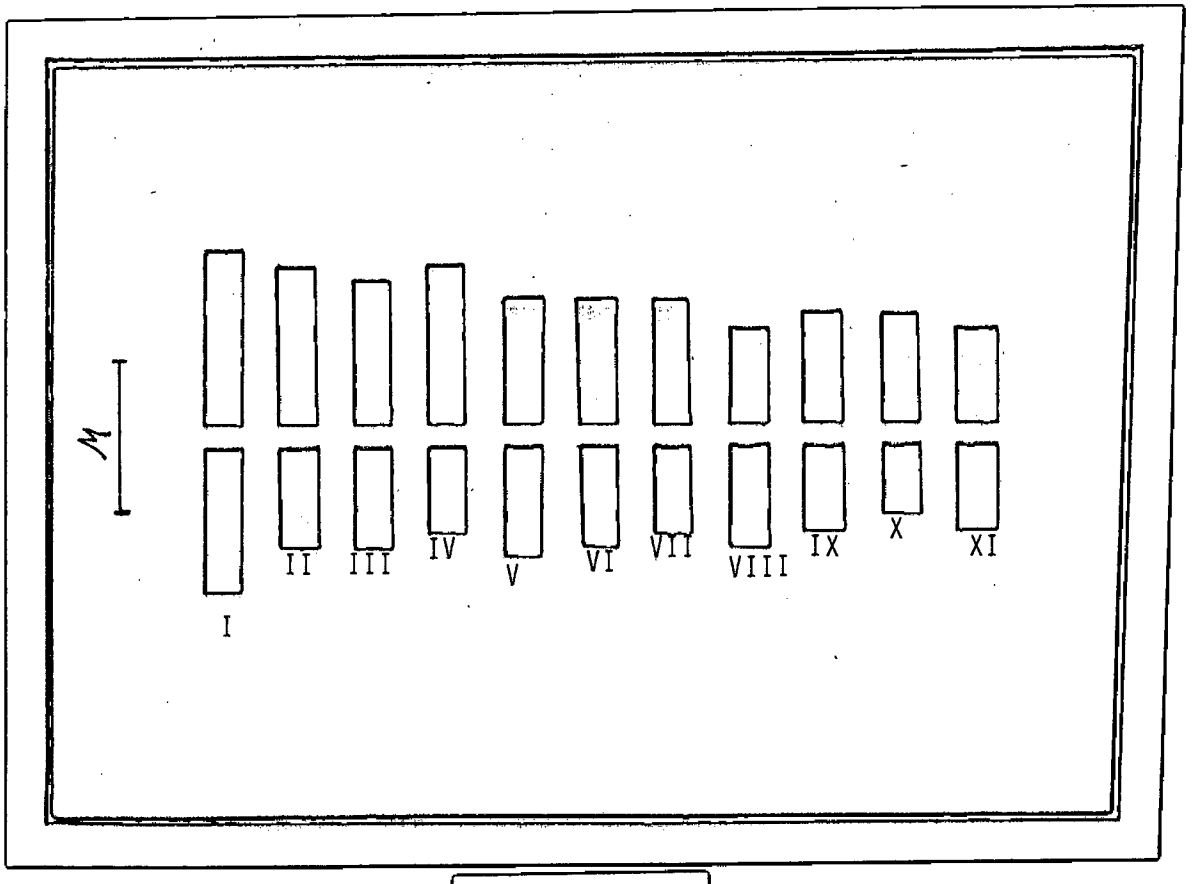


Fig. 3. (b)

to be 2n=22 (Fig. 3a). The length of the chromosomes range from 1.12 μ to 1.99 μ (Table 1) The Chromosomes are Idiogrammed in Fig. 3b. The chromosomes could be classified as follows:

Type A_1 - (Chromosome I) one pair of long chromosomes (1.99±0.30 μ) with median centromere.

Type B - (Chromosome II-IV) Three pairs of long chromosomes (1.58±0.28μ 1.73±0.27μ) with submedian centromere.

Type C - (Chromosome V) One pair of medium chromosome (1.53±0.26 μ) with median centromere.

Type C_1 - (Chromosome VI-VII) Two pairs of medium chromosomes (1.38±0.25μ - 1.48±0.24 μ) with submedian centromere.

Type D - (Chromosome VIII & XI) Two pairs of short chromosome (1.12±0.21 μ - 1.22±0.23 μ) with median centromere.

Type D_1 - (Chromosome IX-X) Two pairs of short chromosomes (1.17±0.22 μ - 1.22±0.21 μ) with submedian centromere.

The Karyotype formula for Canavalia ensiformis can, therefore be represented as :

$$K(2n): 22 : 2A_1^{m} + 6B^{sm} + 2C^{m} + 4C_1^{sm} + 4D^{m} + 4D_1^{sm}$$

Canavalia gladiata (Jacq) DC.

The normal somatic chromosome number in C. gladiata was found to be 2n=22 (Fig.4a). The length of the chromosomes range from 1.40 μ - 3.07 μ (Table 2). The chromosomes are Idiogrammed in

Table 2 Measurement and position of centromere of somatic chromosomes in C. gladiata.

Chromosome pairs	Length of long arm in μ (l)		length of short arm in μ (s)		total length d=l-s r=l/s i=100s/C					Centromeric position
	Mean	s.d.	Mean	s.d.	Mean	s.d.				
I	1.67	0.22	1.40	0.29	3.07	0.51	0.27	1.19	45.60	sm
II	1.41	0.19	1.09	0.13	2.50	0.32	0.32	1.29	43.60	sm
III	0.93	0.16	0.97	0.11	2.46	0.27	0.52	1.54	39.43	sm
	±0.56									
IV	1.32	0.16	1.01	0.15	2.33	0.31	0.31	1.31	43.35	sm
V	1.10	0.15	0.96	0.12	2.06	0.27	0.14	1.15	46.60	sm
VI	1.09	0.18	0.91	0.17	2.00	0.35	0.18	1.20	45.50	sm
VII	1.07	0.18	0.82	0.13	0.89	0.31	0.25	1.31	43.39	sm
VIII	0.99	0.14	0.79	0.13	1.78	0.27	0.20	1.25	44.38	sm
IX	0.93	0.19	0.75	0.09	1.68	0.28	0.18	1.24	44.64	sm
X	0.93	0.12	0.71	0.13	1.58	0.25	0.16	1.23	44.94	sm
XI	0.77	0.11	0.63	0.09	1.40	0.20	0.14	1.22	45.00	sm

MITOSIS AND KARYOTYPE OF C. GLADIATA (DIPLOID PLANT).

EXPLANATION OF PLATE - 4

Fig 4 (a) Somatic Chromosomes from root tip cell of C. gladiata (x2000).

Fig 4 (b) Idiogram of the Chromosomes.

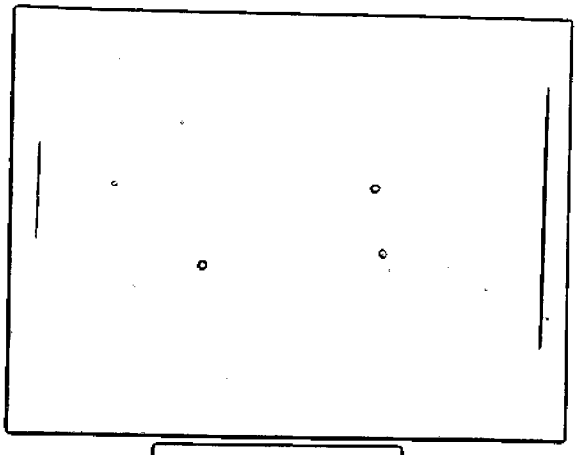


Fig. 4. (a)

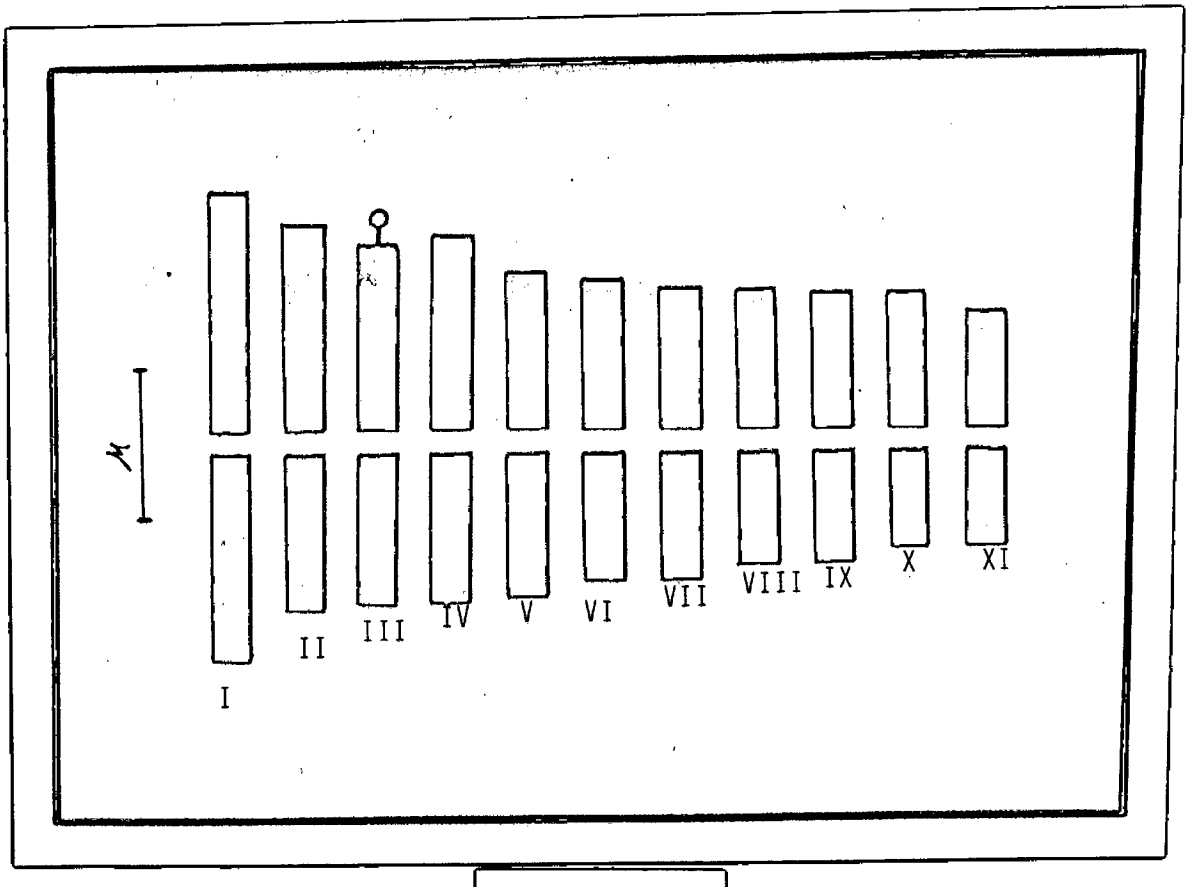


Fig. 4. (b)

Fig. 4b. The chromosomes could be classified as follows:

Type A - (Chromosome I) One pair of very long chromosomes (3.07±0.51μ) with submedian centromere.

Type B - (Chromosome II-IV) Three pairs of long chromosomes (2.33±0.31 μ - 2.50±0.32 μ) with submedian centromere.

Type C_1 - (Chromosome V-IX) Five pairs of medium chromosomes (1.68±0.28 μ) with submedian centromere.

Type D_1 - (Chromosome X-IX) Two pairs of short chromosomes (1.40±0.20 μ - 1.58±0.25 μ) with submedian centromere.

The karyotype formula for Canavalia gladiata can, therefore be represented as :

$$K(2n):22:\ 2A^{sm} + 6B^{sm} + 10C_1^{sm} + 4D_1^{sm}$$

Canavalia virosa (Roxb) Wight et arn.

The normal somatic chromosome number in C. virosa was found to be 2n=22 (Fig. 5a). The length of the chromosomes range from 0.79 μ to 1.51μ(Table 3). The chromosomes are Idiogrammed in Fig. 5b. The Chromosomes could be classified as follows:

Type B - (Chromosome I-II) Two pairs of long chromosomes (1.48±0.07 μ - 1.51±0.04 μ) with submedian centromere.

Type C - (Chromosome III-VI) Four pairs of medium chromosomes (1.23± 0.08 μ - 1.35±0.04 μ) with median centromere.

Table 3 Measurement and position of somatic chromosome in C. virosa.

Chromosome pairs	Length of long arm in μ (l)		Length of short arm in μ (s)		Total length in μ (C)		d=l-s	r=l/s	i=100s/C	Centromeric position
	Mean	s.d.	Mean	s.d.	Mean	s.d.				
I	0.87	0.00	0.64	0.04	1.51	0.04	0.23	1.36	42.28	sm
II	0.82	0.00	0.66	0.07	1.48	0.07	0.16	1.24	44.60	sm
III	0.74	0.04	0.61	0.00	1.35	0.04	0.13	1.21	45.19	m
IV	0.74	0.04	0.59	0.04	1.33	0.08	0.24	1.25	44.36	m
V	0.69	0.04	0.56	0.00	1.25	0.04	0.13	1.23	44.80	m
VI	0.69	0.04	0.54	0.04	1.23	0.08	0.15	1.28	43.90	m
VII	0.61	0.00	0.51	0.00	1.12	0.00	0.10	1.20	45.54	m
VIII	0.59	0.04	0.51	0.00	1.10	0.04	0.08	1.16	46.36	m
IX	0.51	0.07	0.43	0.04	0.94	0.11	0.08	1.19	45.75	m
X	0.48	0.04	0.43	0.11	0.91	0.51	0.05	1.12	47.25	m
XI	0.43	0.11	0.36	0.02	0.79	0.31	0.07	1.19	45.57	m

T.F% = 45.06

Total chromatin length: 26.02±1.56μ.

MITOSIS AND KARYOTYPE OF C. VIROSA (DIPLOID PLANT).

EXPLANATION OF PLATE - 5

Fig 5 (a) Somatic Chromosomes from root tip cell of C. virosa (x1000).

Fig 5 (b) Idiogram of the Chromosomes.

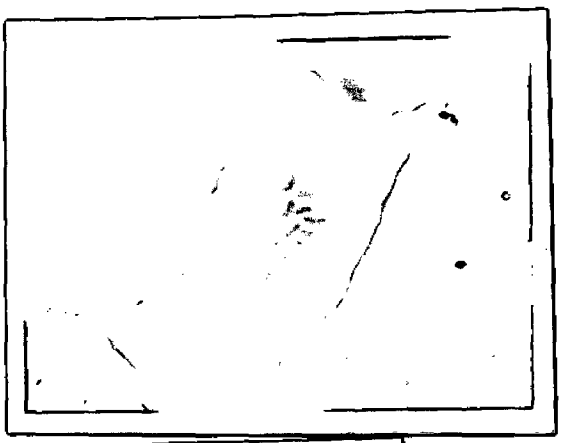


Fig. 5. (a)

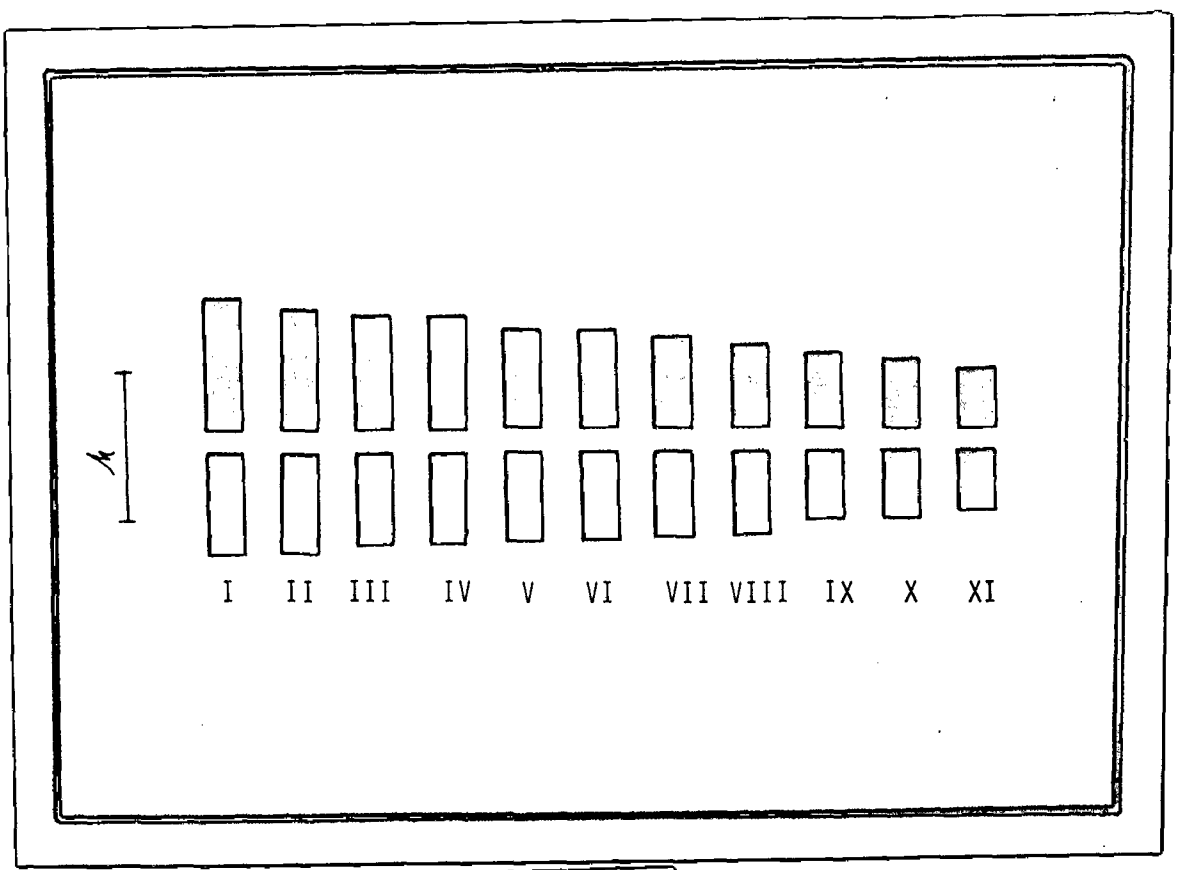


Fig. 5. (b)

MITOSIS AND KARYOTYPE OF *CANAVALIA* SPECIES

EXPLANATION OF PLATE - 6

Fig. 6 Histogram showing total Chromatin length in *C. ensiformis*, *C. gladiata* and *C. virosa*.

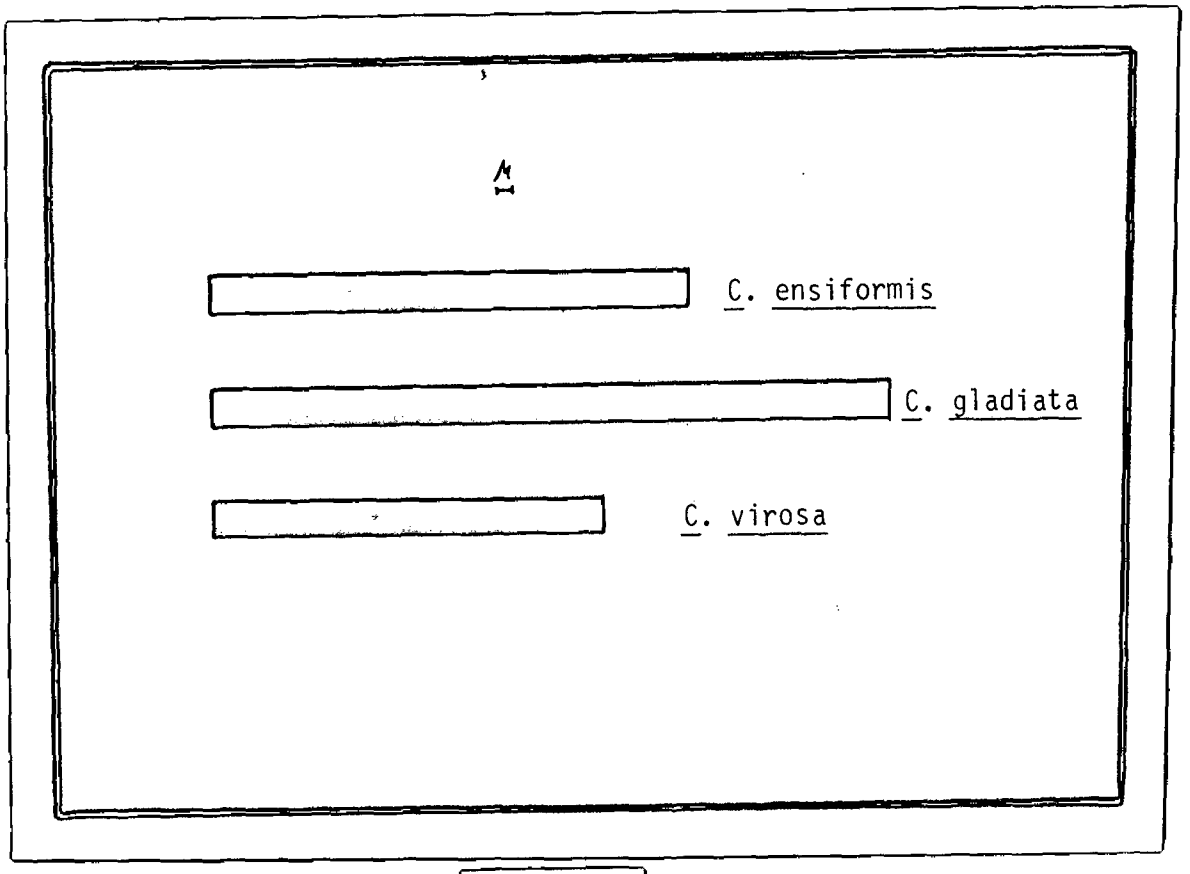


Fig. 6.

Type D - (Chromosome VII-X) Four pairs of short chromosomes (0.94±0.11 μ - 1.12±0.00 μ) with median centromere.

Type E - (Chromosome XI) One pair of very short chromosomes (0.79±0.13 μ) with median centromere.

The karyotype formula for Canavalia virosa can, therefore be represented as:

$$K(2n):22:4B^{sm} + 8C^{m} + 8D^{m} + 2E^{m}$$

Total chromatin length was maximum in C. gladiata (45.50 μ), followed by C. ensiformis (32.10 μ) and C. virosa (26.02 μ). Histogram showing total chromatin length in three species of Canavalia is depicted in Fig.6.

D I S C U S S I O N

The present study reveal that the somatic chromosomes number in all the three Canavalia species is identical and was found to be 2n=22. This finding is in agreement with the earlier reports in C. ensiformis (Kawakami, 1930 ; Simmonds, 1954 ; Shibata, 1962) ; C. gladiata (Poucques, 1945a ; Covas, 1949b) and C. virosa (Kedarnath, 1950 ; Miège, 1960a ; Riley, 1960).

It is observed that although the chromosome number is the same in all the three speceis so far, yet they differed markdly in their chromosome morphology and total chromatin length. The karyotype analysis of the three Canavalia species reveal the presence of 4-6 types of chromosomes, which are differentiated on the basis of their length and centromeric position. The chromosomes are accordingly classified into types ranging

from A-E and have been grouped on the basis of chromosome length and centromeric position. In C. ensiformis, 4 pairs of long chromosomes, 3 pairs of medium chromosomes and 4 pairs of short chromosomes were found. In C. gladiata, 1 pair of very long chromosomes, 3 pairs of long chromosomes, 5 pairs of medium chromosomes and 2 pairs of short chromosomes were present, while in C. virosa, 2 pairs of long chromosomes, 4 pairs of medium chromosomes, 4 pairs of short chromosomes and 1 pair of very short chromosomes were observed. Thus all the three species contained long, medium and short chromosomes.

It was observed that C. virosa has the highest TF% value (45.06) followed by C. gladiata (44.68) and C. ensiformis (42.82).

All the three species studied showed asymmetrical karyotypes. According to Stebbins (1950), asymmetrical karyotypes are supposed to be more advanced than symmetrical ones. According to this hypothesis chromosome complements with more of medium chromosomes should be considered primitive. Keeping this in view, C. virosa appears to be more primitive with 16 median chromosomes, where as C. ensiformis showed 8 median chromosomes and C. gladiata, which appeared to be the most advanced with no median chromosomes.

But such a categorisation cannot be fully applicable here because according to Babcock & Cameron (1934), the decrease in chromatin length may be considered as one of the evolutionary tendencies. According to this hypothesis, C. virosa, with a chromatin length of 26.02±1.56 μ appears

to be most advanced, closely followed by C. ensiformis (32.10±5.46 μ), while C. gladiata (45.50±6.68 μ) comes last in the evolutionary series among these three species.

After taking into consideration all the above factors together it appears that no clear cut opinion can be formed regarding the position of these three species in the evolutionary series, although clear out differences in all the three species at the cytological level have been obtained.

One pair of chromosome in C. gladiata appeared to be satellited with satellite on the long arm. However, no satellites were recorded in either C. ensiformis or C. virosa. Stebbins (1950) stated that in most of the diploid plants only one pair of satellite chromosome is found.

Thus, from the present study, it can be concluded that all the Canavalia species investigated so far, are characterised by a common chromosome number of 2n =22 as evidenced from the present study as well as the previous reports.

B EPIDERMAL FEATURES IN THREE *CANAVALIA* SPECIES

INTRODUCTION

The present level of our knowledge of plant taxonomy and phylogeny based largely on gross morphology, leaves much to be desired and is totally inadequate. The recent flow of data from Cytology, Embryology, Palynology, Anatomy, etc. has served to accentuate the concept of using an assemblage of knowledge gathered from diverse fields of study. Consequent to the enlightened approach to the problem of plant taxonomy, due attention is now being paid to the study of foliar epidermal characters as revealed by the cuticle. Besides their significance as an important tool in taxonomic and phylogenetic researches, the epidermal characters of plants have proved to be immensely useful to the palaeobotanists in the study and identification of fossil leaf impressions and to pharmacognocists in the identification of plants used in herbal medicine and in checking the adulteration of genuine foliar drugs with the cheap and spurious ones.

Very little work has been done on the epidermal features of *Canavalia* species with regards to Stomata, Trichomes and Idioblast crystals which has already been reviewed under "Previous work". Hence, the present investigation was carried out to study the type of Stomata, Trichomes and Idioblast crystals found in the three *Canavalia* species.

MATERIALS AND METHODS

Plants of all the three *Canavalia* species viz., *C. ensiformis*, *C.*

gladiata and C. virosa growing in the botanical gardens of S.P. Chowgule College were used. Fresh epidermal peelings from leaves, pods and anthers were mounted directly in 1% Glycerine and observed under Ergavel (C.Z.) microscope.

For stomatal studies, stomatal frequency and its index, size of the stomata and the pore size were calculated from both the upper and the lower epidermal surfaces of the leaf. Stomata were tested for Ion-adsorbent substomatal structures by using the method of Stevens & Martin (1977a).

Morphology of the Idioblast crystals was studied in the leaves, anthers and pods of all the three species. Number of crystals per unit area, size of epidermal cell containing the crystals and the crystal size was also studied. Various stages towards the development of Idioblast crystals have been traced.

Trichome types, their length and breadth were studied from the leaf material in all the three species.

RESULTS

It is observed that the leaves in all the three species of Canavalia are amphistomatic, the stomata being predominantly rubiaceous (paracytic) associated with anisocytic type (Fig.7). Stomata are with well developed guard cells. The chloroplasts were observed on the guard cell and were found to be arranged in an irregular pattern. They showed a wide range of variation in size and number. The guard cells are surrounded by either 3,4 or 5 epidermal cells, which have U-V shaped sinuous cell walls. The size of the epidermal cells on the upper epidermis was found to be larger than the ones found on the lower epidermis. It was observed that the stomata are sparse on the upper epidermis, while showed a higher frequency on the lower epidermis in all the

EPIDERMAL FEATURES IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 7

Fig. 7 Stomatal types in Canavalia species.

Fig 7a Paracytic type of stomata. (x450).

Fig.7b Anisocytic type of stomata. (x450).

Fig. 8 Trichomes in Canavalia species.

Fig. 8a Young developing trichomes. (x 100)

Fig. 8b A developed trichome. (x 100).

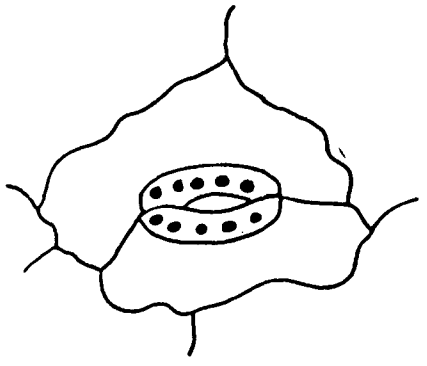


Fig. 7a

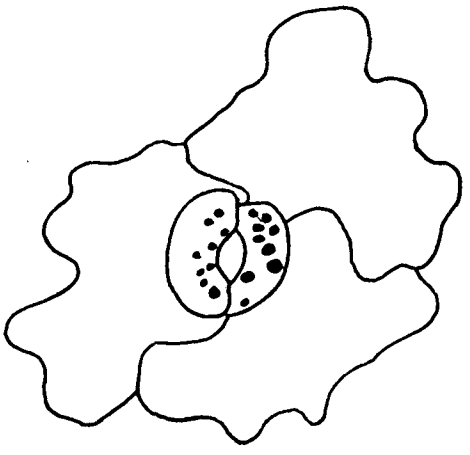


Fig 7b

Fig. 7

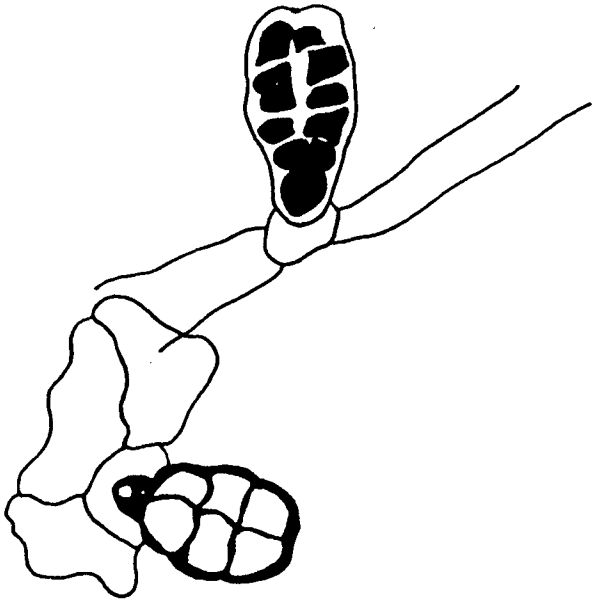


Fig. 8a

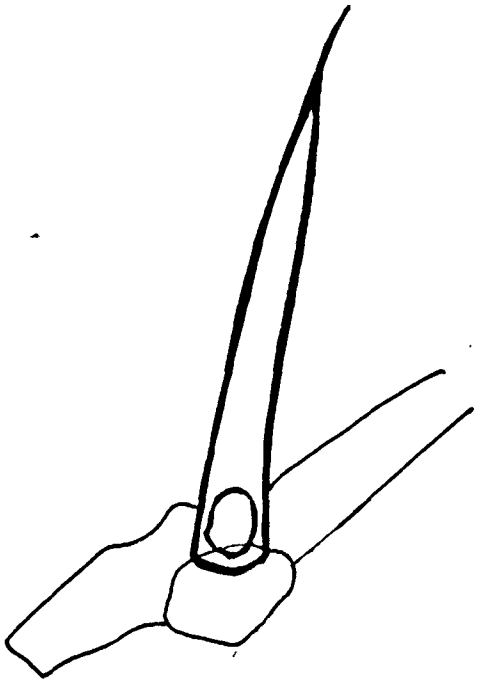


Fig.8b

Fig. 8

Table 4. Stomatal features in three Canavalia species.

species	surface	stomatal index	stomatal frequency	stomatal size in μm		Pore size in μm	
				Length	breadth	Length	breadth
	upper	17.0	3.1	28.8 ±2.5	18.0 ±2.8	16.6 ±2.5	4.0 ±1.3
C. ensiformis							
	lower	24.9	7.8	28.1 ±2.7	15.8 ±1.7	17.4 ±1.8	4.8 ±1.7
	upper	9.8	2.7	27.9 ±3.3	17.7 ±1.9	12.9 ±2.4	3.4 ±0.0
C. gladiata							
	lower	17.8	6.8	23.0 ±1.9	14.3 ±1.4	18.6 ±3.2	4.5 ±1.6
	upper	10.9	2.3	28.7 ±2.3	20.1 ±2.0	14.2 ±2.1	4.4 ±1.6
C. virosa							
	lower	19.4	7.8	25.8 ±2.2	18.1 ±2.5	20.1 ±2.7	6.5 ±1.4

± Indicates standard deviation.

Mean of 50 readings.

three Canavalia species. It was also observed that the stomatal size was more on the upper epidermal surface as compared to those found on the lower epidermis (Table 4). No Ion-adsorbent substomatal bodies were observed in any of the three Canavalia species studied.

The Crystalliferous cells containing two crystals separated by a median wall (rarely three) usually occur singly among the epidermal cells. However, in very few cases two crystalliferous cells are found to be either oppositely or diagonally placed to each other. Very rarely the two crystalliferous cells are joined by a central epidermal cell. The different arrangement of the crystalliferous cells encountered in the three Canavalia species have been depicted in Fig.9.

In the leaves, the frequency of the idioblast crystals was observed to be higher along the main vein and veinlets, where they were arranged almost in rows, while the frequency in the rest of the region was found to be quite low. Again, the frequency of the crystalliferous cells was more on the lower epidermis, while the size of the crystalliferous cells and the crystals was larger on the ones found on the upper epidermis. However, the frequency of the crystals was much lesser in the fruit wall and the anther wall in all the three Canavalia species (Table 5). In the leaves, the crystalliferous cells are surrounded by 4-5 epidermal cells, while in the fruit wall, they are surrounded by 6-8 cells, which were much smaller and polygonal in shape.

Various stages leading towards the development of Idioblast crystals have been traced in Canavalia species (Fig.10). The mother cell undergoes

a division forming two daughter cells. Later each of the daughter cell develops a crystal initial, which later forms into an Idioblast crystal.

The trichomes found on the leaf surface in Canavalia species were of non glandular type with a pointed tip (Fig.8).The basal cell of the trichome is broad and is surrounded by 4-8 epidermal cells. In all the Canavalia species, the trichomes on the leaves showed size variations (Table 6).

DISCUSSION

Epidermal features have a particularly high taxonomic value when they are found to be more stable, unambiguous and not easily changeable. They are a common feature in the legumes and occur in all the three sub-families of Leguminosae viz. Mimosaceae, Caesalpiniaceae and Papilionaceae.

The present study showed that Canavalia species showed two distinct types of stomata viz. paracytic and anisocytic. It is seen that the stomatal size is proportional to the size of the epidermal cell. Therefore, usually the larger epidermal cells have bigger stomata than the small size epidermal cells. Stace (1965) considers the stomatal type to be of considerable taxonomic significance. Stomatal size can sometimes be used as a dignostic character at the species level and some species of a genus can be seperated from the other on the basis of their average stomatal size, among other characters (Ahmad, 1979).

In the present investigation, no Ion-adsorbent structures were seen. Stomatal Ion-absorbent structures were reported earlier in the members of Polypodiaceae and Commelinaceae (Stevens & Martin, 1977a, b). The Ion-adsorbent bodies are believed to be acting as the immediate sink/source of potassium ions involved in stomatal closing and opening (Macallum, 1905). According to Stevens

EPIDERMAL FEATURES IN CANAVALIA SPECIES.

EXPLANATION OF PLATE - 8

Fig. 9. Types of Idioblast crystals encountered in Canavalia species. (x 450).

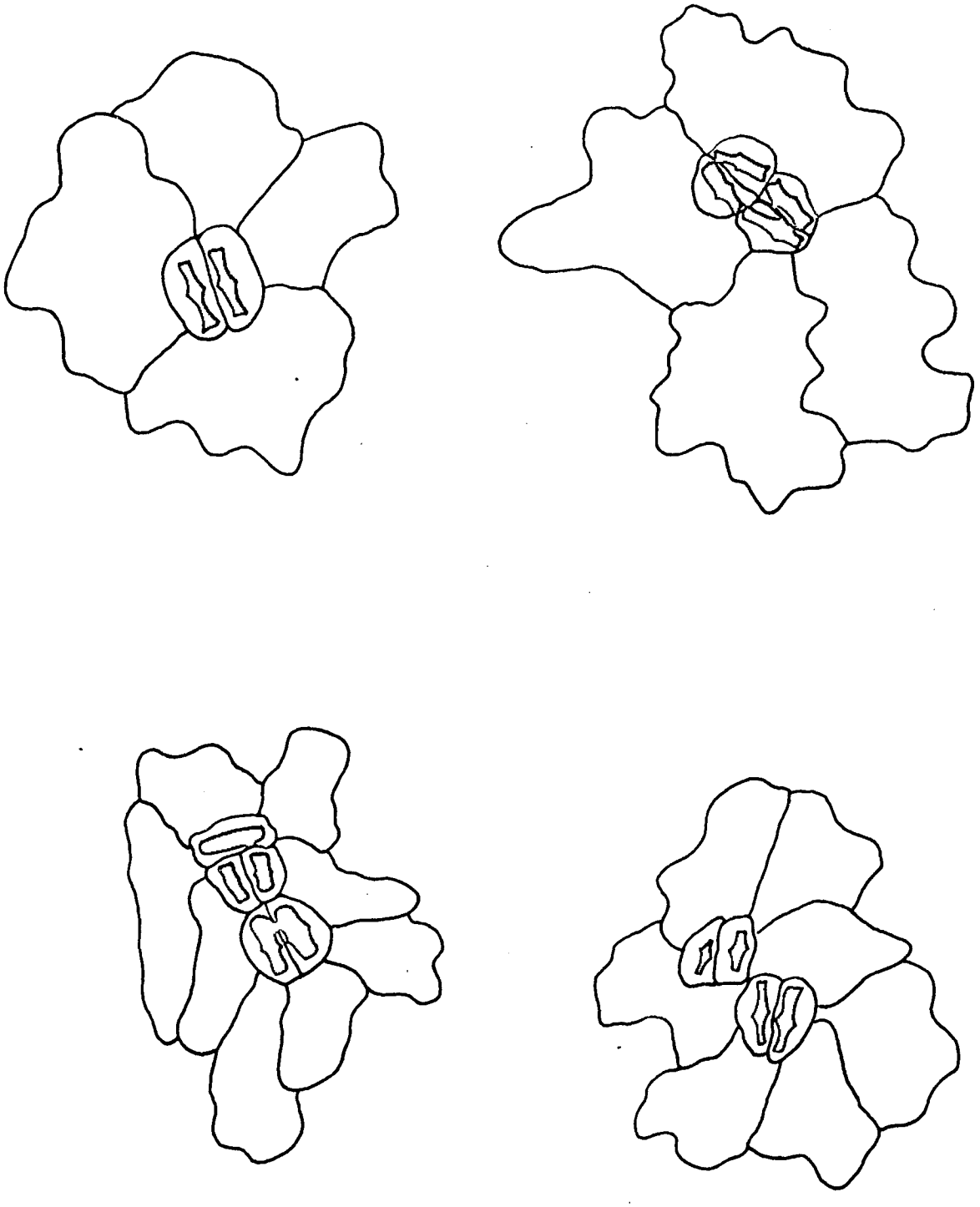


Fig 9

EPIDERMAL FEATURES IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 9

Fig. 10. Various stages leading towards the development of Idioblast crystals in Canavalia species. (x450).

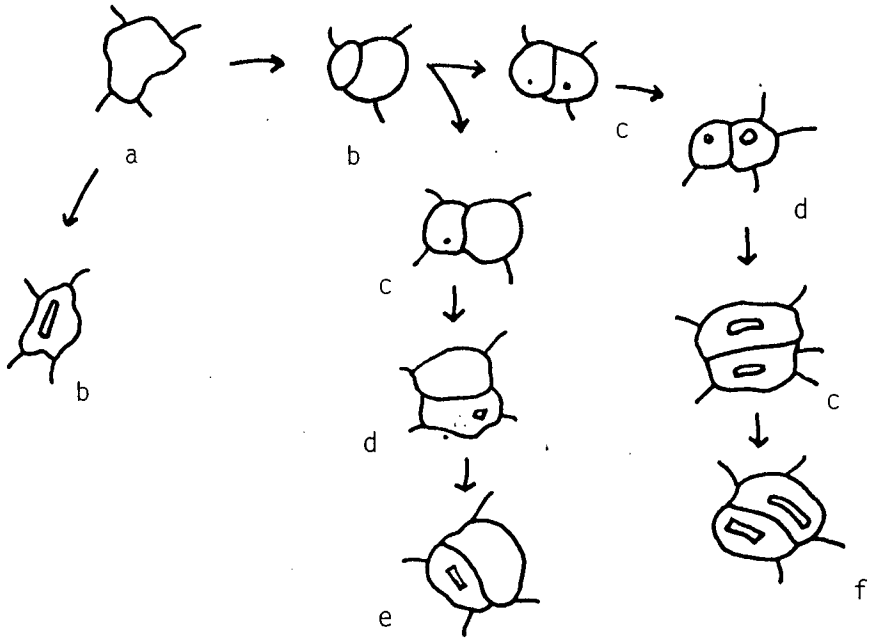


Fig. 10

Table 5 Morphology of Idioblast crystals in three Canavalia species.

species	plant part	surface	No. of crystals/ unit area	size of epidermal cell containing crystals in μm		crystal size in μm	
				length	breadth	length	breadth
C. ensiformis							
	Leaf	upper	4.6	28.9±2.5	27.5±4.9	19.2±2.9	5.0±1.1
		lower	9.3	25.2±3.4	24.9±1.7	18.9±1.7	3.8±0.9
	Anther		4.3	25.4±3.3	20.6±2.5	16.2±2.9	5.1±0.9
	Pod		3.3	26.2±4.3	22.3±2.2	17.3±1.8	5.1±1.0
C. gladiata							
	Leaf	upper	7.7	22.6±2.3	18.4±1.7	16.3±1.8	3.4±0.0
		lower	15.9	22.5±1.8	16.6±2.2	15.5±2.5	3.4±0.0
	Anther		5.2	20.2±2.1	18.0±1.6	15.4±2.6	3.6±0.0
	Pod		4.9	22.6±3.4	19.2±1.8	16.2±1.6	3.5±0.9
C. virosa							
	Leaf	upper	5.2	28.3±4.2	24.2±2.2	22.1±2.8	5.5±1.7
		lower	9.3	23.8±4.3	21.0±2.6	16.0±2.8	4.1±1.3
	Anther		4.9	27.2±4.2	23.6±2.2	17.3±2.3	5.2±1.5
	Pod		4.6	28.2±2.3	25.6±2.5	18.6±2.3	5.2±1.3

± Indicates standard deviation.

Mean of 50 readings.

Table 6 Trichome features in the leaves of three Canavalia species.

species	surface			
	Upper epidermis		Lower epidermis	
	Length in μm	breadth in μm	Length in μm	breadth in μm
C. ensiformis	256.05±87.98	15.84±2.35	270.51±35.73	16.05±1.05
C. gladiata	254.23±80.39	15.36±2.16	263.36±28.46	15.98±1.20
C. virosa	224.10±38.76	17.1 ±2.66	234.28±14.68	18.42±1.28

± Indicates standard deviation.

Mean of 50 readings.

and Martin (1977b) these sites help in the functioning of the stomata.

Idioblast with Ca-oxalate crystals are widely distributed in plant kingdom. In the present study, it is observed that the crystals in Canavalia species are of peculier nature and forms a characteristic feature of the genus Canavalia. Frank (1972) showed that the crystals in C. ensiformis are calcium oxalate monohydrate (whewellite) which was later confirmed by using infrared (IR) spectroscopy by Horner and Zindler-Frank (1982). In papilionoideae, the crystals are generally described as being solitary, either as rhomboids or styloids (Metcalf & Chalk, 1950). Earlier studies have shown that the shape and location of the crystals within a given taxon is often very specific and some investigators have used them in classification (Heintzelman & Howard, 1948).

In the present study rod shaped crystals have been located in the leaves, anthers and pods of all the three Canavalia species. Earlier studies have also revealed that these calcium oxalate crystals have been reported in many organs and tissues (Chattaway, 1956; Wattendorff & Schmid, 1973; Tilton, 1978; Stebbins et al., 1972; Guilliermond, 1978; Robertsen, 1978).

Various stages leading towards the development of Idioblast crystals, have been traced in Canavalia species. It is found that the two crystals of a pair originate as a result of the division of one mother cell. Similar observations have been made earlier (Frank, 1965).

Earlier studies have shown that the frequency of crystals is related to the calcium and oxalate content of the medium where the plants are growing (Zindler-Frank, 1975).

Very little is known about the functions of these crystals in plant. It is thought that they may be used as a protective device against foraging animals (Black, 1918). The concentration and large size of crystals on veins and pods points towards a function of mechanical support (Schneider, 1901). Stevenson (1953) reported the uptake of calcium oxalate crystals by bacteria in stipules of Rubiaceous plants possibly acts as a starting point for amino acid synthesis. Another possible explaination for their presence is that they may serve as a means of removing excess oxalic acid from the plant system, where oxalate is considered to be an end product of metabolism which is not usable to the plant (Vickery & Abrahams, 1950; Vincent & Horner, 1980).

Trichomes are also known to play important role in plants. Johnson (1975) has demonstrated the relationship between micro organisms and some epidermal features like trichomes, and the phenomenon of disease resistance in plants as a result of their interaction.

C. STARCH GRAIN STUDIES IN THREE *CANAVALIA* SPECIES

INTRODUCTION

Starch is one of the ubiquitous and abundant component of plants and has over the years assumed importance for several reasons. First and foremost, it represents the chief source of dietary carbohydrates and energy in human diet. Secondly, its hydrolysed products provides raw materials for confectionery, for brewing and for various fermentation industries. Thirdly, starch is the cheapest and most readily available colloids, useful for its thickening qualities, adhesive property and as a coating sizing agent.

Seeds are the richest source of starch and constitutes the major portion of legume carbohydrates (Sathe & Salunkhe, 1981). Starch is laid down in distinct sub-cellular bodies, the starch grains which have a characteristic appearance for each species.

Microscopic examination of starch grains under polarized light is more informative than under ordinary light because the double reflecting surface of starch grains reveal the structure of their particles under polarized light.

Legume seeds are known to contain about 50-70% of starch. The purpose of the present investigation was to isolate the starch from three *Canavalia* species and to study the starch grains with respect to morphology, dimensions, pH, gelatinization temperatures, blue value and iodine absorption spectra.

MATERIALS AND METHODS

Seeds of all the three species were collected from plants growing at the botanical garden of S.P. Chowgule College, Margao-Goa. The seeds were then air dried and decorticated. Starch was isolated from the decorticated seeds according to the method described by Schoch & Maywald (1968).

Starch grains were mounted in 5% glycerine and were observed under light and polarized microscope. Polarization equipment consisted of filter polarizer, compensators (G Red I and $\lambda/4$), attenuation and conversion filters, PK 12.5 eyepiece with graticule. Eccentricity proportion was measured by locating the position of hilum on the longitudinal (vertical) axis of the starch grain. In each case, 500 starch grains were analysed. For measuring the pH of starch, 2 grams of dried starch was mixed with 100ml of distilled water and the pH of the suspension was noted on a pH meter. Gelatinization temperatures of starch grains in all the three species were determined by the method of Mc Masters (1964). Blue value of the starches was calculated by the method described by Gilbert & Spragg (1964). Iodine absorption spectra was determined by the method of Colonna et al., (1981).

RESULTS

The study under the light microscope revealed that in all the three species, majority of the starch grains were oval in shape, while others were small round to irregular in shape. In all the three species, a distinct size variation among the starch grains was observed. The hilum appeared

Table 7. Starch grain characteristics in three Canavalia species.

Species	Length of starch grains in μm	breadth of starch grains in μm	pH of starch	gelatinization temperature	blue value
C. ensiformis	5.87-39.20	5.10-31.24	6.3-6.5	75 - 85°C	6.8
C. gladiata	6.56-58.09	5.81-37.76	6.1-6.3	70 - 80°C	6.9
C. virosa	7.26-37.76	5.81-29.04	6.0-6.2	65 - 75°C	6.6

STARCH GRAIN STUDIES IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 10

Fig. 11 Starch grains showing polarization cross in Canavalia species.

Fig. 11 a. C. ensiformis (x450)

Fig. 11 b. C. gladiata (x450)

Fig. 11 c. C. virosa (x450)

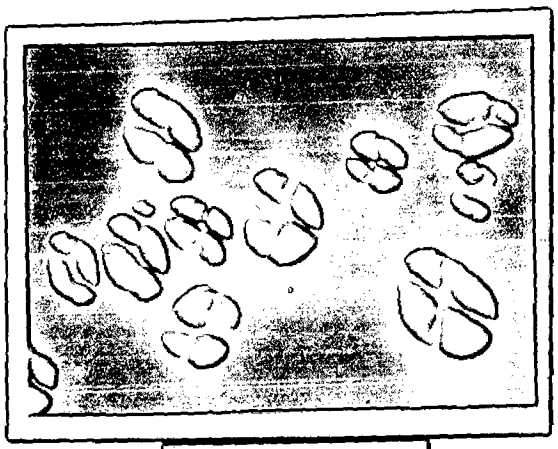


Fig. 11. (a)

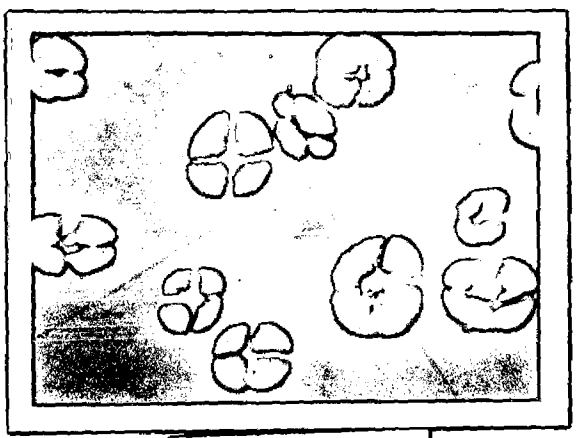


Fig. 11. (b)

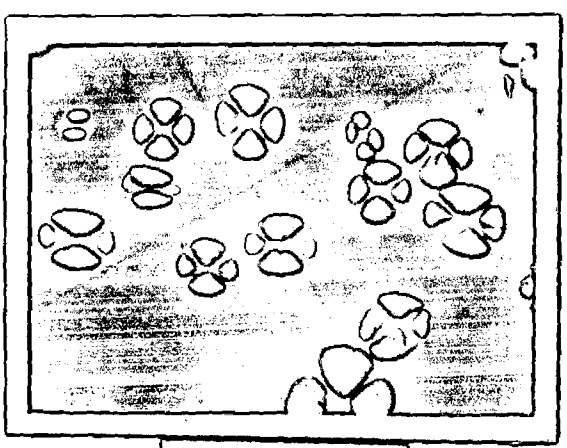


Fig. 11.(c)

Fig. 11.

as a spot on the granule and is believed to be the nucleus around which the granule has grown. Striation marks arranged eccentrically about the hilum were also clearly visible.

Under polarizing filter and compensator Gλ/4, the grains exhibited a characteristic sharp birefringence between 45^{o}-60^{o} passing through the hilum and the layers of amylose and amylopectin (Fig.11). This was specific for all the three species.

Under selenium filter (compensator G Red I) the starch grains showed a beautiful play of colours. When the attenuation filter and the compensator G Red I were at 0, one of the quadrants towards broad end and the other on the opposite side of the starch grain were of a colour between canary and butter-cup yellow and the remaining two quadrants were of the colour between turquois and ming green. The background of the preparation was faint corn flower blue. As the filter G Red I moves in the upward direction the colour starts fading in all the four quadrants resulting into a colourless starch grain at 45^{o}C and with fern green background. The colours of the quadrants reappear but in the opposite sequence as the filter (G Red I) moves to 15^{o}-0 ^{o}C. There is no significant difference in the birefringence cross and play of colours in individual starch grains of a species.

Polarization cross on eccentricity proportion was studied in the three species and was found to be 1:1.1, 1:1.09 and 1:1.15 in C. ensiformis, C. gladiata and C. virosa respectively.

Gelatinization temperature of the starches in the three species was found to spread over a range of temperature of 10^{o}C and was found to be

Table 8 Showing Iodine Absorption Spectra of starch in three Canavalia Species.

Wavelength (nm)	OPTICAL DENSITY		
	C. ensiformis	C. gladiata	C. virosa
520	0.23	0.26	0.27
530	0.24	0.27	0.28
540	0.25	0.28	0.29
550	0.26	0.31	0.31
560	0.27	0.32	0.32
570	0.28	0.33	0.34
580	0.29	0.35	0.35
585	0.30	0.36	0.39
590	0.34	0.38	0.38
595	0.32	0.36	0.35
600	0.31	0.33	0.33
610	0.28	0.32	0.32
620	0.26	0.31	0.31
630	0.24	0.30	0.30
640	0.23	0.29	0.28
650	0.21	0.28	0.27
660	0.20	0.27	0.26
670	0.19	0.26	0.25
680	0.17	0.25	0.24
690	0.16	0.24	0.23
700	0.15	0.23	0.22

STARCH GRAIN STUDIES IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 11

Fig. 12 Iodine absorption spectra of starches in three Canavalia species.

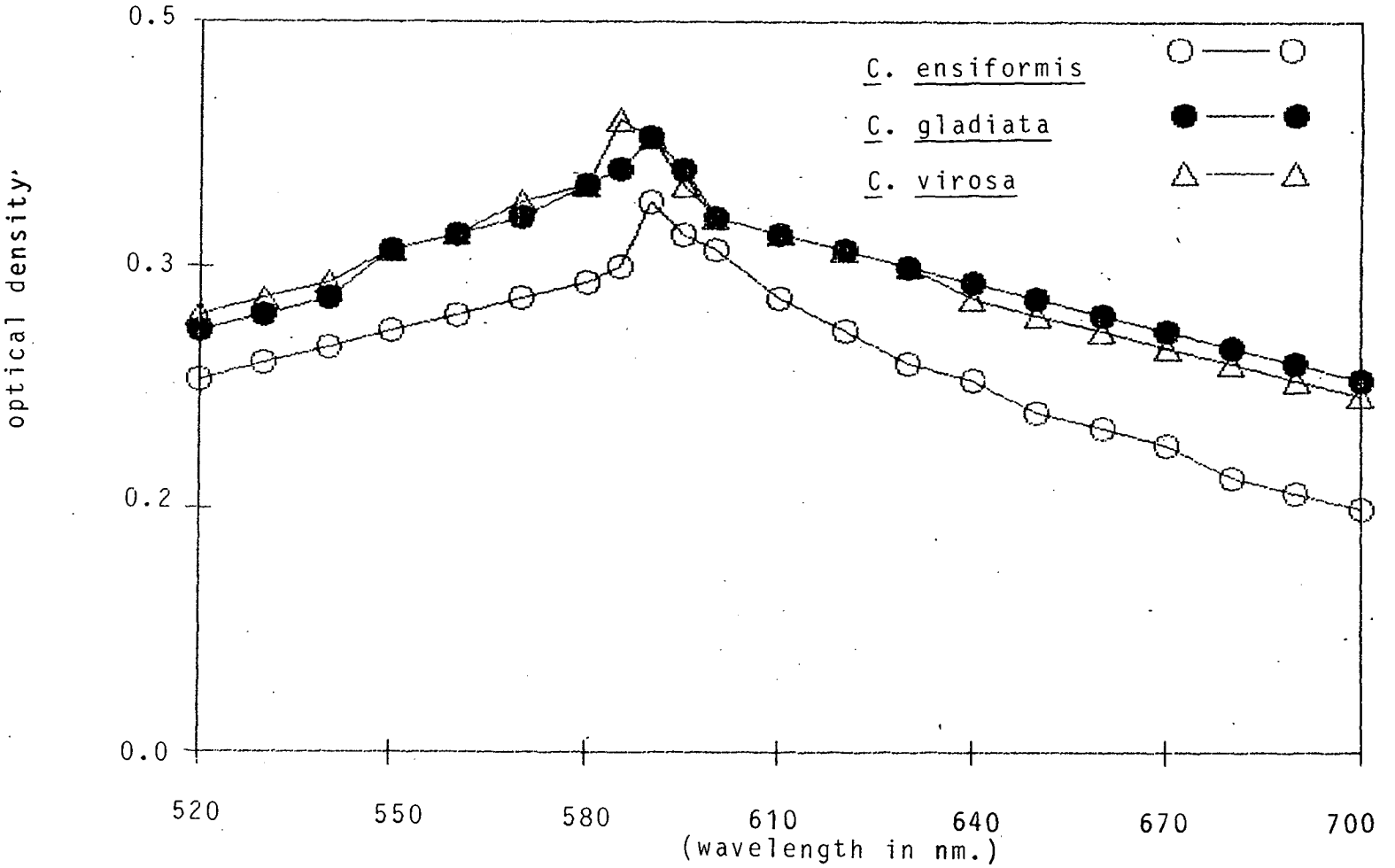

0.5
0.3
0.2
0.0
optical density
C. ensiformis
C. gladiata
C. virosa
520
550
580
610
640
670
700
(wavelength in nm.)

Fig. 12

70°-80° C in C. ensiformis, 65°-75° C in C. gladiata and 75°-85° C in C. virosa. The pH valus of all the starch samples were found to be acidic. viz. 6.3-6.5 in C. ensiformis, 6.1-6.3 in C. gladiata and 6.0-6.2 in C. virosa.

Blue values of the starches in the three species have been calculated and were found to be 6.8,6.9 and 6.6 for C. ensiformis, C. gladiata and C. virosa respectively (Table 7).

The wavelength of maximum absorption (λmax) of the Iodine-starch complex for both C. ensiformis and C. gladiata was 590 nm, while for C. virosa it was 585 nm (Table 8; Fig.12).

DISCUSSION

The use of organic molecules as a tool in plant taxonomy is well established. This is inspite of some scepticism from a few traditionally oriented botanists. Their caution is partly a reaction to others who have made exaggerated claims for this method, especially when small molecules are used for differentiation.

It is well known that the size and shape of the starch grains varies from species to species and it is a characteristic feature of a particular species. In the present study, it was observed that the shape of the starch grains was quite varient, ranging from small round to large oval to irregular. Similar observations with regard to the shape of starch grains in different legume starches have been reported earlier (Li & Varriano-Marston, 1979; Sathe & Salunkhe, 1981).

In the present study, it was observed that under polarizing filter and compensator $\lambda/4$, the granules exhibited sharp birefringence. This property of birefringence suggests that the granule is actually a sphaerocrystal (Badenhuizen, 1965). The characteristic birefringence of the ungelatinized starch granule under polarized light is sufficient indication that the molecules have been deposited in some sort of orderly arrangement. Under such circumstances, the associative forces of adjacent molecules would be mutually satisfied, hydration tendencies would be at a minimum, and consequently the granule would be insoluble in cold water. If a water suspension of starch is slowly heated, the granules lose their birefringence before swelling becomes pronounced. This could be ascribed to partial dehydration of starch molecules, thereby disrupting their orderly arrangement (Schoch, 1942).

Values of pH of all the starches indicated acidic nature. Gelatinization temperature of the three species was found to spread over a range of temperature of $10\,^{o}C$, which is in confirmity with that of Mc Masters (1964). The granules lose their birefringence cross beyond this temperature range. The present results obtained confirm the earlier findings of Schoch and Maywald (1968) who stated that smaller the size of starch granules, higher the gelatinization temperature.

Further studies with regard to starch grain compositions (amylose and amylopectin) may help to clear some more points and which will help for considering the chemotaxonomy of this genus.

D. PALYNOLOGY AND POLLEN PHYSIOLOGY OF THREE *CANAVALIA* SPECIES

INTRODUCTION

Palynology, a science based on pollen grains and spores, has major application in plant taxonomy. These spores and pollen offer a wide range of precisely definable morphological features and in many a taxon morphology of spores or pollen can characterise the taxon and serve to identify it without reference to other morphological data.

Pollen offers an elegant experimental material and there is need to study palynology with reference to problems of male fertility in economic plants. Based on palynology alone, definite trends of evolution have been recognised within plant groups. During pollen formation, considerable qualitative and quantitative reshuffling in genes and chromosomes take place and how much of variability, thus generated, is actually utilized in nature in plant evolution, and in field in plant breeding, depends on the extent of selection that operates during maturation of gametes, fertilization and embryo formation. These studies are of direct relevance to plant breeders.

Pollen physiology has attracted the attention of plant breeders and horticulturists ever since the discovery of the pollen tube by Amici (1924). Horticulturists and plant breeders often fail to get fertile seeds in spite of all the care taken during artifical pollination. Unless sterility is the main cause, failure of seed setting may be due to slow growth of the pollen tube or its early degeneration in the style.

Perusal of literature indicates that no work has been done on the palynological aspects on any of the Canavalia species. Hence the present investigation was conducted to study the pollen morphology, pollen physiology and their relationships in three Canavalia species viz., C. ensiformis, C.gladiata and C. virosa.

MATERIALS AND METHODS

Pollen grains from fresh flowers of C. ensiformis, C. gladiata and C. virosa were used for each micro-preparation. For the study of pollen morphology, pollen grains from 10 flowers were taken. Methods of acetolysis (Erdtman, 1952; Nair, 1962) were employed for the detail study of pollen grains. Fresh and unstained pollen grains were also examined under the laboval microscope (Carl Zeiss). All measurements were made by using stage micrometer.

Two methods of viability testing were employed: TTC staining and in vitro germination. These tests were done at the same time for all pollen lots.

TTC was prepared as a 0.5% solution (w/v) with 0.15 M, pH 7.0, tris buffer. The solution was stored in brown glass and used on the following day. A modified procedure (Cook & Stanley, 1960) was used to stain the pollen with TTC : 50mg of pollen from each lot was placed in a test tube, 1 ml of TTC solution was added, and the mixture was incubated for 1h at 30 °C in a water bath. The pollen was then examined microscopically using blue filtered transmitted light. Three test tubes per pollen lot were incubated, four slides per test tube were prepared, and 100 pollen grains from each of the 12 samples were examined for staining. Heavily stained dark red pollen with uniform

distributed colour in the grains was considered viable. For the study of pollen germination, the following method was used: A 0.6% solution of Difco Bacto-agar was prepared in distilled water with (1) no additive (2) 5% sucrose (3) 10% sucrose (4) 15% sucrose (5) 20% sucrose (6) 25% sucrose and (7) 30% sucrose. The agar solution was autoclaved at 15 lbs pressure for 15 minutes. Approximately 10ml. of autoclaved agar solution was poured into 5-cm sterilized petri dishes to a uniform refrigerator untill use on the following day. Approximately 5mg of pollen was sprinkled uniformly over the agar medium using a camel's hair brush. The petri dishes were kept at 28°C - 30°C. A pollen sample was removed from the petri dish and mounted on a clean slide stained with an aqueous solution of safranin and examined under the microscope. Three petri dishes per pollen lot were maintained. Four slides were prepared from each petri dish by removing loops of pollen from four places on the agar medium. One hundred pollen grains were counted for percent germination in each of the 12 samples. Germination counts were made after 48 hours because by that time the pollen tubes had more or less attained their maximum lengths and practically no further growth occured after this period.

RESULTS

The following are the features of pollen grains as observed under the microscope:

(a) C. ensiformis

Pollen grains are yellow when matured. They are 3-zonoporate, ambtri-angular, pollen mass size 40 µm - 59 µm, pores are equilateral, Circular. Diameter of the pores is 7 µm - 8 µm. Exine is 2 µm - 3 µm thick; surface

of the pollen grains is faintly faveolate; ectoexine is as thick as endoexine.

(b) C. gladiata

Pollen grains are yellow when matured. They are syncolporate and ambtriangular; pollen mass size 43 μm - 58 μm; endocolpium is circular. Diameter of endocolpium is 7 μm - 8 μm. Exine thickness is 3 μm - 4 μm. Ectoexine is thinner than endoexine.

(c) C. virosa

Pollen grains are yellow when matured. They are syncolporate, rarely paracolporate, ambtriangular; pollen mass size 42 μm -55 μm; endocolpium is circular. Diameter of endocolpium is 7 μm - 8 μm. Ectoexine is thicker than endoexine. Surface of the pollen is faveolate.

Pollen staining for all the species with TTC solution started after approximately 30 minutes and maximum staining was reached after approximately 1h. The colouration appeared as yellow, light red, or dark red. The percentage of dark red stained pollen (counted as viable) in all the three species ranged from 42.08 to 54.83% (Table 9.). The light red stained pollen ranged from 17.42 to 23.84%. The rest of the pollen was unstained (Yellow). In some grains, the red colour was not uniformly distributed over the grain; instead it was concentrated in globules.

Table 9. Pollen viability indicated by TTC staining in three Canavalia species

Plant species	Dark red stained pollen grains %	Light red stained pollen grains %	Total viability %(mean±SD)
C. ensiformis	54.83±7.21	23.84±3.21	78.67±10.42
C. gladiata	47.67±7.51	18.49±2.42	66.16±9 .93
C. virosa	42.08±7.17	17.42±2.21	59.50±9 .38

Table 10. In vitro germination of pollen grains in three Canavalia species.

Plant species	% sucrose concentration						
	0%	5%	10%	15%	20%	25%	30%
C. ensiformis	56.64 ±8.46	59.82 ±9.16	62.12 ±9.82	69.14 ±9.96	63.82 ±8.64	62.18 ±8.32	59.14 ±8.19
C. gladiata	50.27 ±8.65	53.82 ±8.64	60.26 ±9.96	58.29 ±9.18	57.26 ±9.36	55.26 ±8.16	52.89 ±8.28
C. virosa	42.23 ±8.72	49.26 ±8.86	54.28 ±9.27	52.80 ±8.26	50.29 ±8.16	49.83 ±8.42	47.27 ±8.19

Germination of pollen grains in agar begins within 1h. Maximum germination was recorded after 24hrs. Results showed that there were differences in pollen germination for different sucrose concentration. Again, it was observed that the percentage of pollen germination in all the three species was comparatively higher in sucrose solutions as compared to the pollens germinating in agar alone. There was germination in all the concentrations (ranging from 5% to 30%) of sucrose used. In C. ensiformis, higher germination percentage was observed in agar supplemented with 15% sucrose, while in C. gladiata and C. virosa highest germination percentage was observed in agar supplemented with 10% sucrose (Table 10).

DISCUSSION

From the present study, it is seen that the pollen grains of C. gladiata and C. virosa differ from the pollen grains of C. ensiformis in the character of colpus and exine. Thus further study of the complex of characters of the species would provide a very suitable taxonomic tool for resolving the biosynthesis of the genus Canavalia.

TTC has been widely used as a rapid and simple test of the germination capacity of seeds. TTC solution is colourless and is reduced by oxidative and dehydrogenase enzymes to a dark red formazan complex in living cells (Smith, 1951). The capacity to germinate is assumed to be correlated with the activity of these enzymes. However, contradictory results have been obtained when TTC was used as an assay of pollen viability. It has been found satisfactory for

maize (Vieitez, 1952), pine (Cook & Stanley, 1960), and plum (Norton, 1966) pollen, but it tended to overestimate the viability of apple, grape, peach, and pear pollen (Oberle & Watson, 1953) as compared to vitro germination tests.

In vitro germination of pollen grains showed that optimum sucrose concentration for germination of pollen grains is 15% in C. ensiformis and 10% in C. gladiata and C. virosa. This can be correlated to the different pollen sizes. The above results are in confirmity with that of Rao and Ong (1972). According to them there is every indication that the larger germinating units possess high internal osmotic concentration than do the smaller entities.

PART II

NODULATION STUDIES IN *CANAVALIA ENSIFORMIS.*

A. EXTERNAL AND INTERNAL MORPHOLOGY OF ROOT NODULES.

B. SCREENING AN EFFECTIVE NATIVE RHIZOBIA.

A. EXTERNAL AND INTERNAL MORPHOLOGY OF ROOT NODULES IN *CANAVALIA ENSIFORMIS*

INTRODUCTION

Nodules are complex, coordinated organs with many functions and attributes. They are in fact "sheltered and controlled environments within which the root nodule bacteria provide a source of nitrogenous compounds which are avidly picked up by the host plant and translocated rapidly to growing plant tissues (Bergersen, 1981).

Nodules arise in the cortex of legume root following infection by rhizobia, originating as a group of dividing cortical cells which will give rise to the nodule meristem. The spatial dispositions adopted by the dividing cells of the meristem cause the nodules of various legumes to differ in shape and gross anatomy as well as there are associated differences in the fine structure.

Sprent (1979) has classified nodules in two broad catagories viz., determinate and indeterminate nodules based on the nature of meristems, type of vascular system and the mode of infection. Recently, Corby (1980) has recognized five basic shapes for nodules which he found to have taxonomic and evolutionary significance, especially among the tribes of the papilionoideae.

Perusal of literature reveal that studies on morphological and histological aspects of nodules in *Canavalia* species are almost negligible, and have been already reviewed under "Previous work". Hence the present investigation was carried out to study the external and internal morphological features of root nodules in *Canavalia ensiformis*.

MATERIALS AND METHODS

Plants growing at the S.P. Chowgule College garden were used for the study. Plants of various stages with intact root system were carefully uprooted and washed in running water taking care not to damage the nodules. Various morphological features of the nodules, such as position on the root system, shape, colour, surface etc. were critically studied.

For histological studies, the method described by Vincent (1970) was found suitable. The nodules were fixed for 12 hrs., washed, dehydrated, embedded in 58 ^{o}C m.p. paraffin and cut in serial sections 10-12 μm thick. Heidenhains haematoxylin with counter stain (Picric acid, aq. satd.) gave satisfactory staining. A large number of rough hand-cut sections of the nodules were also studied under light microscope.

RESULTS

In Canavalia ensiformis the first nodule appears on the secondary root on the seventh day of plant growth, when the first true leaves unfold. Nodulation was found to occur on the secondary, tertiary and quarternary roots. However, nodulation does not occur on the primary root (Fig. 13).

The nodule when young is spherical but at maturity it may retain the same spherical shape or may attain either elongate, bi-lobed or fan-like shape. Both effective and ineffective nodules were observed. The effective nodules were either typically elongate, fan shaped, or were spherical in appearance and organization, where as ineffective nodules were minute, spherical, and when cut lacked pink colour indicative of the presence of leg-

haemoglobin. All the nodules were light brown in colour with rough surface. The nodule size varied from 1.0mm to 21.0mm.

The mature nodule differentiates into an inner bacteriod and an outer certical region (Fig. 14). The inner bacteriod region is composed of two types of cells, viz., the larger parenchymatous cells whose cytoplasm is filled with the symbiotic forms of rhizobia, the bacteriods; and the smaller parenchymatous cells which are devoid of any symbiotic forms. The cytoplasm of infected cells in ineffective nodules showed uniform staining resulting into a "clumped" appearance.

The nodule cortex is heterogenous with a discontinous sclerenchymatous layer being sandwiched by parenchyma, thus differentiating into an outer and an inner cortex. The outer cortex of the nodule is very spongy, with large intercellular spaces. The outermost layer of the outer cortex is composed of dead cells, but beneath lie many layers of intact cells. The cells of the inner cortex are tightly packed.

The vascular traces lie in the inner cortical region. The number of vascular traces that surround the central bacterial zone in cross section varies from 9 to 15. The vascular traces that pass around the central tissue join at the base of the nodule and enter the vascular system of the roots. Each vascular trace is surrounded by its own endodermis, within which lie 2-3 layered pericycle. The vascular bundles are conjoint, amphicribral (Hadrocentric) in nature, the xylem cells of which have spirally thickened walls.

NODULATION STUDIES IN *CANAVALIA ENSIFORMIS*

EXPLANATION OF PLATE - 12

Fig. 13. Root System showing nodules in *C. ensiformis*.

Fig. 14. T.S. of root nodule of *C. ensiformis*. (x100)

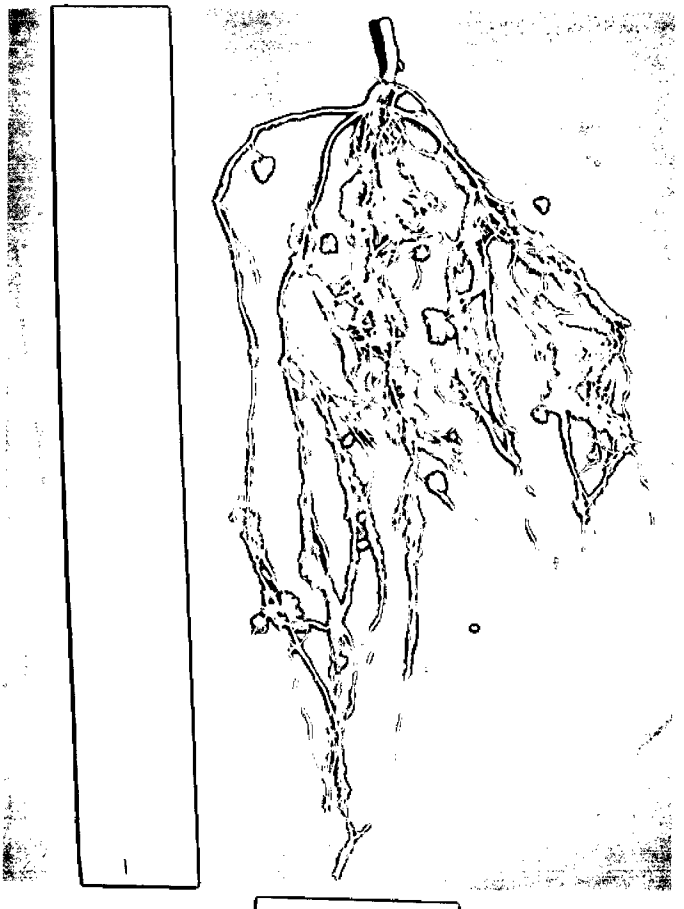


Fig. 13.

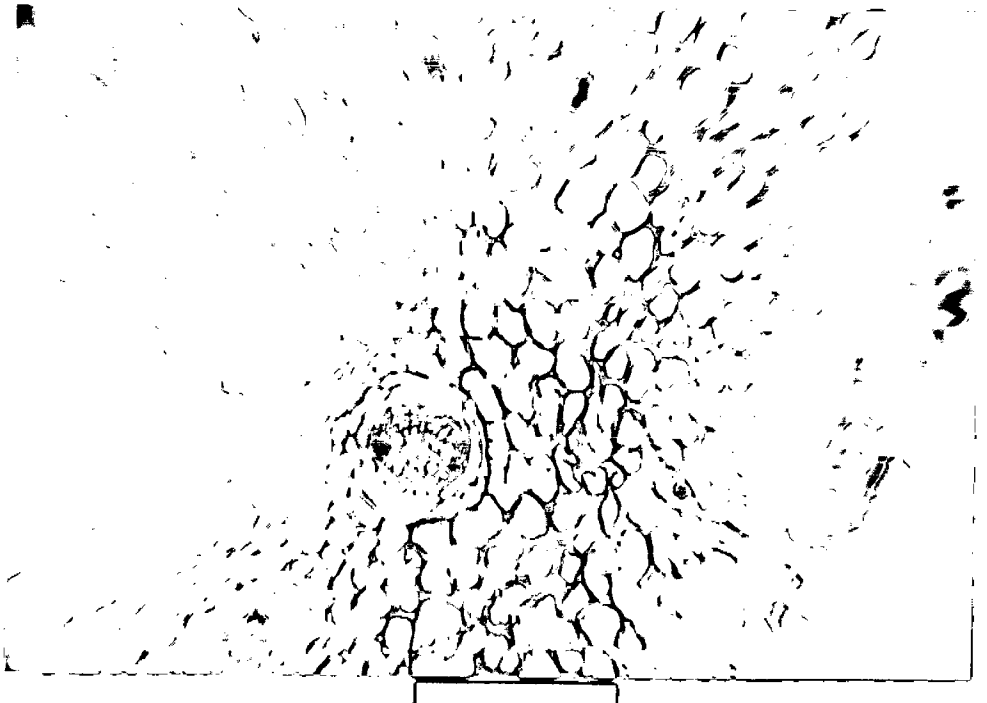


Fig. 14.

At maturity, the degeneration of the nodule progresses, from the base upwards, ultimately resulting in a empty shell whose wall is composed of the cortex traversed by the vascular bundles.

DISCUSSION

From the present study, it is clear that the appearance of the first nodule synchronises with the unfolding of the true leaves. Similar observations have been made in other legumes by earlier workers (Narayana & Gothwal, 1964). However, Bieberdorf (1938) failed to observe such as type of correlation.

A wide range of variation in both shape and size of nodules was observed. Both effective and ineffective nodules were found.

The effective nodules are well developed, possess pink colour due to the presence of a pigment 'leghaemoglobin' (akin to the haemoglobin of human blood) and the bacteriod tissue is well organized with plenty of bacteriods (Subba Rao, 1988).

The bacteriod containing cells are the most prominent cells of the nodule and they comprise nearly 70% of the total cells of the central tissue in C. ensiformis. Narayana and Gothwal (1964) reported that nearly 49% of the total cells of the central tissue were infected by Rhizobium in C. gladiata. The volume of this central tissue and its duration in an intact state are the major determinants of the extent of nitrogen fixation (Chen & Thornton, 1940).

The cytoplasm of the infected cells in effective nodules stained uniformly, resulting into a "clumped" appearance. Similar observations have been recorded earlier (Virginia et al., 1984).

The outer cortex of the nodule was soft and spongy in nature. This is because of the loosely packed parenchymatous cells which have large intercellular spaces. According to Bergersen and Goodchild (1973) many of the intercellular spaces in the outer cortex are gas-filled with very little cytoplasm.

At maturity, the nodule senescence starts from the inner bacteriod region which is a characteristic feature of determinate nodules. Similar observations have been made earlier in determinate nodules (Bergersen, 1981), who stated that senescence seems to begin with the infected cells, loosing ultrastructural features; changes in host nucleus and decay of bacteriods follow. Lastly, bacteria enter the damaged cells from infection-thread-remnants and there is usually spread of bacteria between the cell walls leading to intracellular masses of bacteria. He further argues that senesced tissue may serve as a reservoir of infection from which new nodule growth arises in nodules which over-winter or are perennial.

B. SCREENING AN EFFECTIVE NATIVE RHIZOBIA FOR *CANAVALIA ENSIFORMIS* (L.)DC.

INTRODUCTION

The beneficial effects of legumes on agricultural productivity were known to the ancients. The oldest agricultural records available indicate that leguminous crops have been cultivated for centuries and that they were valued for food and soil enrichment long before their ability to work symbiotically with bacteria was understood (Fred *et al.*, 1932).

In the tropics, leguminous plants are prevalent and constitute one of the largest groups or flora (Lim & Burton, 1982). Norris (1956) suggested that legumes originated in the tropics; in fact tropical trees comprise a large part of the family which is said to be basically a tropical arborescent family (Tutin, 1958). In India, the leguminosae are considered to be the second most dominant family in the order of abundance (Puri, 1960).

The nodule is the focal point of reaction between the *Rhizobium* and its host plant. When a susceptible leguminous plant and a compatible strain of rhizobia are brought together under conditions favourable for growth and infection, a nodule will form (Lim & Burton, 1982). Although nodule formation is regarded as a general characteristic of legumes, not all legumes have been reported as nodulating. The subfamily papilionoideae contains a high proportion of nodulating genera. Sixty percent of all genera (comprising of

2592 species) have been examined for nodulation which amounts to 21% of the species in the subfamily out of which 97% of the species examined can nodulate (De Faria, 1989).

The leguminous plant with nodules on its roots induced by an effective strain of Rhizobium, is considered to be in symbiosis with the bacteria. The symbionts are considered to be mutually beneficial, but their relationship may shift with age. Allen and Allen (1954) suggested that in the early stages of nodule development, the bacteria are the dominant factor. As the nodule matures structurally, there is a period of balance when each symbiont is supplying and being supplied by the other. This is a period of mutual benefit; nitrogen is being supplied to the plant by the bacteria, and carbohydrates are being supplied to the bacteria for growth and nitrogen fixation. When the plant matures and begins fruiting, it becomes the dominant controlling factor and eventually initiates senescence in the nodule.

The strain of a single species of Rhizobium differ markedly in their biochemical characteristics and their ability to fix atmospheric nitrogen (Muthusamy et al., 1973). Careful isolation and inoculation of these effective strains of Rhizobium are known to improve the yield and quality of legume crop.

Canavalia ensiformis (L.)DC. is a robust legume, which produces nutritious pods in marginal soils where other pulses fail. It also provides

plentiful green manure and forage. The available literature clearly shows that no work has been carried out to screen an effective rhizobia for C. ensiformis or any of the Canavalia species (Allen & Allen, 1981) although reciprocal host-infection tests have been carried out to confirm that the Rhizobium infecting Canavalia species belong to the Cowpea group (Wilson, 1939). Hence the present investigation was undertaken to search for an effective native strain of Rhizobium for this plant and to study the effect of inoculation with isolated native strains on different characteristics of C. ensiformis.

MATERIALS AND METHODS

A) Collection of legume sample:

Thirty five to forty days old plants of C. ensiformis with intact root system were carefully uprooted and washed gently under running water to remove the soil without disturbing the nodules. Observations on the degree of nodulation, location, morphology and size of the nodules were recorded.

B) Isolation of root nodule bacteria:

Healthy, pink, unbroken nodules of C. ensiformis were selected for the isolation of root nodule bacteria (Vincent, 1970; Date, 1976).

The nodules were removed from the root leaving a small portion of the root attached to each of them for ease of handling. They were then washed thoroughly in running tap water to remove gross surface contamination and then immersed in 0.1% (w/v) acidified solution of mercuric chloride

for 4-5 minutes, after which they were transfered to 70% ethanol and agitated in the solution for 3-5 minutes. Finally, they were washed thoroughly in sterile distilled water to remove all traces of disinfectant. The nodules were then crushed in a small aliquot of sterile water in a sterile test tube with the help of a sterile glass rod. The milky exudate thus obtained, was inoculated in two plates of Yeast extract mannitol agar incorporated with Congo red dye (CRYEMA).

The plates were then incubated at 28^{o}C for 7 days and the typical isolated colony was subcultured on CRYEMA plates. The well isolated colony obtained from the sub-culture was transferred to yeast extract mannital agar (YEMA) slants containing 0.1% Calcium carbonate for maintainance and further studies. The slants were kept at 4^{o}C in the refrigerator with regular transfers after every three months. The cultures were coded according to the botanical name of the host plant from which they were isolated.

C) Identification of root nodule bacteria:

In order to confirm that the isolates belongto genus Rhizobium and not Agrobacterium or other non-rhizobial contaminants, the following tests were performed .

a) Colony characteristics:

Colony characteristics of the rhizobial cultures on YEMA media (with Congo red) were noted after 7-10 days incubation at 28^{o}C (Vincent 1970).

b) Staining:

Gram staining of the rhizobial cultures were determined by the staining method described by Vincent (1970).

c) Biochemical properties:

i) Growth and reaction in Litmus milk:

Tubes of sterile litmus milk inoculated with rhizobial cultures were incubated at 28°C for 4 weeks and examined for acid production and formation of serum zone (Vincent, 1970).

ii) Ketolactose test:

The production of ketolactose by the isolates was tested on a modified medium of Bernaerts and De Ley (Gaur et al ., 1973).

iii) Nile Blue reduction test:

The ability of the isolates to reduce Nile Blue was tested by Skinner's method (Skinner. 1977).

iv) Hydrogen sulphide production:

The ability of the rhizobial cultures to produce hydrogen sulphide was checked in nutrient broth tubes provided with lead acetate paper. After 14 days incubation at 28°C blackening of the strip was taken as proof of H_2S production.

v) Growth in Hofer's alkaline medium :

The ability of the isolates to grow in Hofer's medium (Hofer, 1935) with pH 11.0 was tested.

vi) Urease activity :

Inoculated urea agar slopes were incubated for one week before examination (Christensen, 1946).

vii) Nitrate reduction :

Yeast extract mannitol broth containing 0.1% K-nitrate was inoculated with rhizobial cultures and reduction of nitrate to nitrite, if any, was examined after 7 day incubation at 28°C by adding 1 ml of N-Naphthyl-ethylene-diamine-dihydrochloride and 1 ml of sulfanilic acid (Anon, 1957).

viii) Methyl Red test :

Tubes containing glucose phosphate broth were inoculated and after incubation for 7 days at 28°C, 5 drops of methyl red indicator were added. A red colour denoting pH 4.5 or less was considered as positive reaction (Conn et al ., 1957).

ix) Production of Indole ;

tubes of trypton water were inoculated with the rhizobial cultures. After incubation at 28°C for 7 days, 0.5 ml of Kovac's indole reagent was

added and tubes were shaken gently, allowed to stand for 10 minutes, and observed for the development of any deep red colour (Conn et al 1957).

x) Utilization of Citrate :

Plates containing Kosers medium were inoculated, incubated for 14 days and then examined for growth (Koser, 1923).

xi) Catalase activity :

A loopful of the growth of rhizobial culture from YEMA slant were stirred in 10% (w/v) H_2O_2 and observed for any evolution of gas (Graham & Parker, 1964).

xii) Oxidase activity :

Oxidase activity in the isolates was tested according to the method of Kovaks (1956).

xiii) Tolerance of Sodium chloride:

Plates of YEMA medium in which the concentration of NaCl had been increased to 2% and 3% w/v NaCl were inoculated, incubated for seven days, then examined for growth (Graham & Parker, 1964).

d) pH tolerance :

The ability of the rhizobial cultures to grow at various pH values was tested (Graham & Parker, 1964).

e) Utilization of Carbohydrates:

All the isolates were tested for their ability to grow when provided with various carbohydrates as the sole source of carbon.The carbohydrates tested were maltose, L-rhamnose, D-glucose, sucrose, mannitol, galactose, D-fructose, L-arabinose, D-xylose and starch (Graham & Parker, 1964).

D) plant infection tests :

Plant infection tests were performed to confirm the isolates as rhizobia by using Vigna sinensis savi. (cowpea) as a test plant which is an authentic host of the cowpea cross-inoculation group. Isolates showing the ability to nodulate the cowpea plants, were considered as Rhizobium cultures (Vincent, 1970).

E) Screening

a) Selection of strains:

Twenty-three isolates obtained from the root nodules, all of which were identified to be rhizobia based on the morphological and biochemical characteristics and confirmed by plant inoculation test.
from these isolates, four morphologically and biochemically different strains namely 1,10, 17 and 22 were selected and designated as CE-1, CE-2, CE-3 and CE-4 respectively, which were later used to screen out for the most effective native strain for C. ensiformis. Viable cell counts in diluted inoculum suspensions were routinely determined by plating.

b) Maintainance of selected Rhizobium strains:

The selected Rhizobium cultures were maintained on Yeast extract mannitol agar (YEMA) slants containing 0.1% Calcium carbonate. Inocula were grown in Yeast extract-mannitol broth (YEMB) having the same composition of YEMA excluding agar (Vincent, 1970).

c) Inoculation

For pot experiments, powdered clayey loam soil (pH 5.8) was used. The soil was sterilized for 1h at 15 lbs to eliminate native rhizobia and other contaminants. Pots (26cm diameter) were filled with 8Kg of sterilized soil.

Seeds of C. ensiformis were surface sterilized with acidified 0.1% $HgCl_2$ for 4-5 minutes and washed several times with sterile distilled water. The surface sterilized seeds were kept aseptically on the plain (water) agar plates at 29-30^{0}C for germination. The sprouted seeds were carefully transfered to pots (one seed/pot) filled with sterilized soil. One week old seedlings were inoculated with 2ml of inoculant of test strains. The plants were watered daily to field capacity with distilled water and received Crone's solution on the third day of every week.

Six plants of each treatment were selected at random on the 60th day of growth. Observation on the degree of nodulation, location, morphology

and size of the nodules were recorded. Various morphological characteristics were recorded. Plant and nodule dry weight were recorded separately after drying to constant weight. Plants were harvested at maturity and yield was recorded.

RESULTS

During the course of Rhizobium study, twenty-three isolates were obtained from the nodules of C. ensiformis plant. When these were grown on yeast extract mannitol agar containing Congo red, all the strains gave slimy colonies, but did not absorb the dye from the media. Microscopic observations showed that the cells of all the isolates were motile, gram-negative, tiny rod-shaped structures containing polyhydroxybutarate granules. It was observed that isolates 1 to 21 were fast growers on yeast extract mannitol agar, while isolates 22 and 23 were slow growers (Table 11).

Litmus milk was changed to acidic and serum zone was formed in all the isolates except in isolates 22 and 23, which gave negative results. All the isolates were negative to ketolactose test, Nile Blue reduction test and Hydrogen-sulphide test. None of the isolates could grow in Hofer's alkaline medium. Isolates 1 to 16 could produce urease, while the remaining isolates were unable to do so. All the isolates except isolates 22 and 23 could reduce nitrate. All the isolates were positive to methyl red but were indole negative. Citrate utilization test was found to be

Table 11. Morphological characteristics of the isolates obtained from the root nodules of *C. ensiformis*.

Isolate No.	Growth on Congo-red YEMA plates	Colony characteristics on Conge red YEMA plates	Colony surface	Gram staining	Polyhydroxy butarate granules
1.	+	Large, Circular, Watery white & gummy.	convex	-	+
2.	+	"	"	-	+
3.	+	"	"	-	+
4.	+	"	"	-	+
5.	+	"	"	-	+
6.	+	"	"	-	+
7.	+	"	"	-	+
8.	+	"	"	-	+
9.	+	"	"	-	+
10.	+	Medium, irregular, watery white & gummy	"	-	+
11.	+	"	"	-	+
12.	+	"	"	-	+
13.	+	"	"	-	+
14.	+	"	"	-	+
15.	+	"	"	-	+
16.	+	"	"	-	+
17.	+	Medium, Circular, Watery white & gummy.	"	-	+
18.	+	"	"	-	+
19.	+	"	"	-	+
20.	+	"	"	-	+
21.	+	"	"	-	+
22.	+	Medium, Circular, Creamy white & gummy.	"	-	+
23.	+	"	"	-	+

negative for all the isolates. Isolates 1 to 9 and 16 to 21 could produce catalase, while others gave negative results. Isolates 1 to 21 gave positive results with oxidase test while remaining isolates gave negative results. It was observed that none of the isolates could grow in a medium containing either 2% or 3% of sodium chloride (Table 12).

All the isolates showed maximum growth at pH 5.5-8.0. Except for isolates 22 and 23, all the others showed growth at pH 4.5, 5.0 and 8.5. Isolates 1 to 16 showed growth at pH 4.0, where as isolates 1 to 9 showed growth at pH 9.0, while the remaining isolates did not show any growth. It was observed that pH 3.5, 9.5 and 10.0 were lethal and none of the isolates could grow (Table 13).

It was observed that all the isolates utilized Glucose, Sucrose, Fructose, Arabinose, Xylose and Mannitol, while none of the isolates could utilize starch. The slow growing isolates did not utilize Maltose and Rhamnose, but utilized Galactose. Isolates 16 to 21 could not utilize Rhamnose, while isolates 1 to 21 could not utilize Galactose (Table 14).

All the isolates could form nodules on the test plant Vigna sinensis, an authentic host of the cowpea cross-inoculation group.

Thus on the basis of morphological and biochemical similarities, all the twenty-three isolates could be grouped into four different groups. Of the four groups, three were found to be fast growers, while only one was found to be a slow grower. One representative from each group was later selected to screen out the best and effective native rhizobia for C. ensiformis plant.

Table 12. Biochemical properties of the isolates from the root nodules of *C. ensiformis*.

Biochemical test	Isolates																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
1. Serum zone, litmus milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
2. Acid reaction, litmus milk	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
3. Ketolactose test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4. Nile Blue test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5. H_2S test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6. Hofer's alkaline media	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7. Urease Production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-
8. Nitrate reduction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
9. Methyl Red test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10. Indole production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11. Citrate utilization	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12. Catalase test	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	+	+	+	+	-	-
13. Oxidase test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
14. Tolerance to 2% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15. Tolerance to 3% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 13 Tolerance of pH by the isolates obtained from the root nodules of *C. ensiformis*.

pH	Isolates																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
3.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
4.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
5.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
5.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
8.5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
9.0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-
9.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 14 Utilization of carbohydrates by the isolates obtained from the nodules of *C. ensiformis*.

Carbohydrate	Isolates																						
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
Rhamnose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Viable cell counts per ml. of the selected Rhizobium strains are depicted in Table 15. It is seen that maximum cell counts were observed in CE-1 (9.32×10^8/ml) strain, while minimum cell counts were recorded in CE-4 (1.85×10^8/ml) strain.

Nodule morphology in the plants inoculated with selected native rhizobia was studied and the results are depicted in Table.16. No nodulation was observed in control plants. This can be attributed to the sterilized conditions. Maximum nodulation was found to occur in plants inoculated with rhizobial strain CE-1, where the nodules were distinctly large. Their shape varied from globose to highly irregular showing extensive branching. The nodules when sectioned, showed bright pink colouration indicating the presence of leghaemoglobin. Majority of the nodules formed in plants inoculated with rhizobial strain CE-2 were globose to flattened, while the plants inoculated with rhizobial strain CE-3 showed globose to cylindrical nodules. In both the cases, the nodules were found to be effective. The plants inoculated with rhizobial strain CE-4 showed minute, ineffective globose nodules that lacked pink colouration.

A significant increase in the plant height and leaf length was observed in plants inoculated with rhizobial strain CE-1 and CE-2 over uninoculated control. Maximum number of nodes were observed in plants inoculated with CE-1 strain, thus resulting in higher number of leaves (Fig.15). It was observed that inoculation with CE-1 strain increased the root length considerably over all the other treatments and uninoculated control (Fig.16).

Table 15. Showing viable counts per ml of selected Rhizobium strains isolated from root nodules of C. ensiformis.

Rhizobium strains	Dilution	Counts/ml
CE-1	10^{-8}	9.32×10^{8}
CE-2	10^{-8}	7.99×10^{8}
CE-3	10^{-8}	4.24×10^{8}
CE-4	10^{-8}	1.85×10^{8}

Table 16. Nodule morphology of C. ensiformis plants inoculated with selected native Rhizobium strains.

Rhizobium strains	Degree of nodulation	Position on root system	Shape	size in mm.	Colour		Surface
					outside	Inside	
Control	-	-	-	-	-	-	-
CE-1	+++	Lateral	Globose to highly irregular showing extensive branching	5.0-15.0	Brown	Pink	Rough
CE-2	++	Lateral	Globose to flattened	4.5-7.2	Brown	Pink	Rough
CE-3	++	Lateral	Globose to flattened	3.0-6.5	Brown	Pink	Rough
CE-4	+	Lateral	Globose	2.0-5.0	Light brown	Pink	Rough

Legend :

+ = sparse nodulation = <25 nodules/plant.

++ = moderate nodulation = 25-50 nodules/plant

+++ = abundant nodulation = 50-100 nodules/plant.

++++ = very abundant nodulation = >100 nodules/plant.

NODULATION STUDIES IN *CANAVALIA ENSIFORMIS*

EXPLANATION OF PLATE - 13

Fig. 15 Effect of selected native rhizobial strains on plant growth in *C. ensiformis*.

Fig. 16 Effect of selected native rhizobial strains on root growth in *C. ensiformis*.

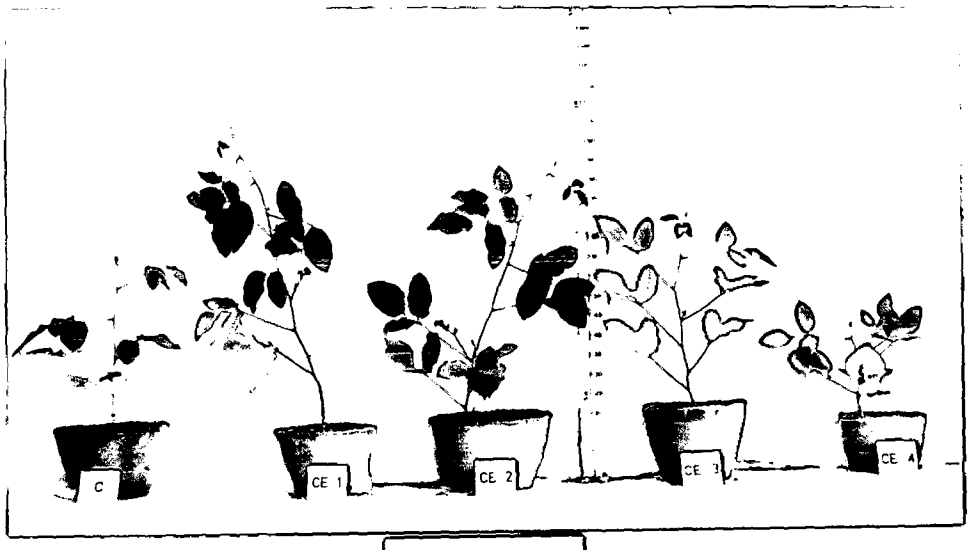

C
CE 1
CE 2
CE 3
CE 4

Fig. 15.

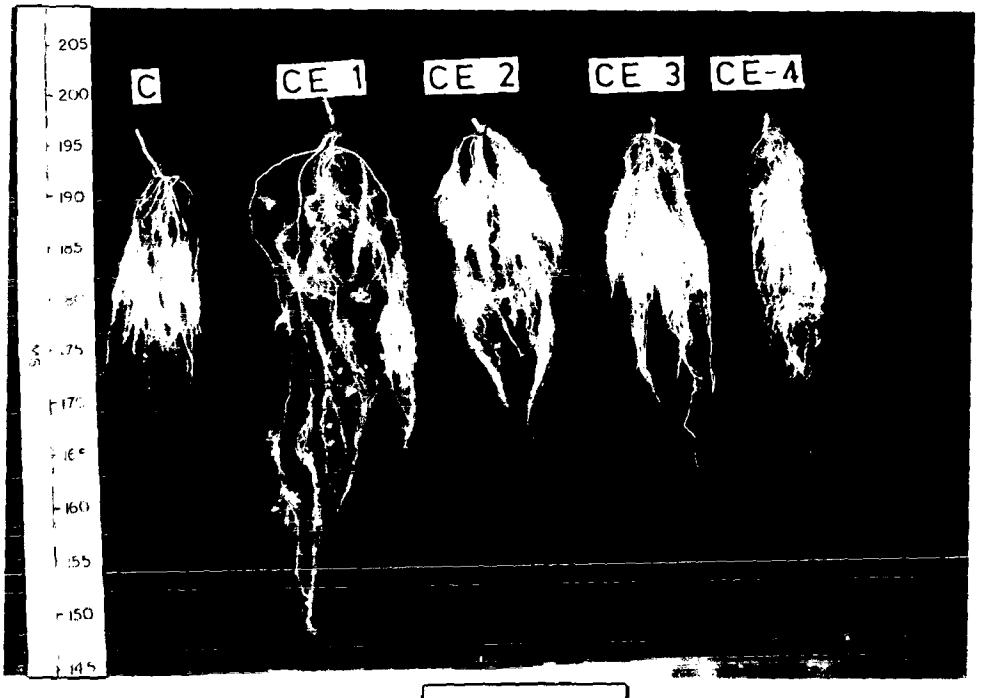

205
200
195
190
185
175
160
155
150
145
C
CE 1
CE 2
CE 3
CE-4

Fig. 16.

Table 17. Vegetative and reproductive characteristics of C. ensiformis plants inoculated with selected native Rhizobium strains.

Characters	Rhizobium strains				
	Control	CE-1	CE-2	CE-3	CE-4
Plant height cm	42.5±3.87	67.3±5.00	62.1±4.65	59.1±4.55	30.5±2.65
No. of leaves/plant	9.0±0.82	11.0±1.14	10.0±0.82	9.0±0.00	8.0±0.00
No. of nodes/plant	7.0±0.71	10.0±0.93	9.0±1.09	8.0±0.84	7.0±0.71
Leaf length cm (3rd node)	10.6±0.23	15.9±0.32	14.9±0.27	8.6±0.16	7.4±0.16
Leaf breadth cm (3rd node)	6.5±0.11	8.2±0.14	7.8±0.13	6.1±0.09	5.9±0.07
Petiole length cm (3rd node)	16.0±0.29	14.2±0.28	9.8±0.18	9.1±0.19	7.3±0.16
Root length cm	34.2±3.16	42.2±2.82	36.8±1.63	35.9±1.41	32.3±1.83
No. of nodules/plant	-	69.8±18.8	39.6±2.16	33.2±1.45	9.2±0.63
Shoot dry wt./plant g	2.60±0.03	3.58±0.03	3.32±0.05	3.28±0.04	2.54±0.01
Root dry wt./plant g	0.67±0.02	1.27±0.04	1.05±0.03	0.92±0.03	0.58±0.02
Nodule dry wt./plant g	-	0.30±0.02	0.12±0.03	0.09±0.02	0.02±0.00
No. pods/plant	3.0±0.71	6.4±1.48	4.0±0.81	2.0±0.69	2.0±0.65
Length of pod cm	10.5±0.21	13.6±0.31	10.6±0.28	9.1±0.23	8.4±0.19
No. of seeds/pod	4.0±0.71	6.0±0.86	5.0±0.79	5.0±0.79	3.0±0.58

± Indicates standard deviation.

Shoot, root and nodule dry weights were significantly higher in plants inoculated with CE-1 strain over plants inoculated with any of the strains or uninoculated control.

As regards to the yield characteristics, a significant increase in the yield was observed in plants inoculated with CE-1 strain. Although a significant increase in the pod length was observed, no significant increase was seen in the number of seeds per pod in plants inoculated with CE-1 strain (Table 17).

DISCUSSION

All the twenty-three isolates obtained from the root nodules of C. ensiformis were identified to be rhizobia. All the isolates showed growth on yeast extract mannitol agar supplemented with congo red. None of the isolates were stained with congo red, thus clearly differentiating them from Agrobacteria. The characteristic Gram negative staining was observed in all the isolates. All the isolates contained polyhydroxybutarate granules, which were stained with Sudan black B and were found to be soluble in Chloroform. All the isolates showed gummy (extracellular polysaccharides) nature. Trinick (1982) reported that the water soluble gum (exopolysaccharide) produced in abundance seems to have no morphological role. However, Subba Rao (1988) presumes that these extracellular polysaccharides (slime) might help in binding soil particles together. Of the twenty-three isolates, isolates 1 to 21 were found to be fast growers, while isolates 22 and 23 were found to be slow growers.

Biochemical characteristics of the isolates are depicted in Table 12. Litmus milk was changed to acidic and serum zone was formed in all the isolates, except for isolates 22 and 23. Lang(1961) reported that slow growing soyabean and cowpea strains do not form serum zone. Acid was produce in litmus milk by fast growing strains, where as the slow growing strains gave an alkaline reaction. The slow growing non-acid producing rhizobia have been considered to be the ancestral forms of rhizobia since they are associated with primitive tropical legumes growing in alkaline enviroment (Vincent, 1970).

None of the isolates could form 3-Ketolactose on the modified medium of Barnaerts and De Ley. This result is in agreement with the earlier observations that Rhizobium is negative to ketolactose test (Barnaerts & De Ley, 1963; Gaur et al., 1973; Skinner, 1977). All the isolates failed to reduce Nile Blue dye. This confirms the earlier report of Skinner, (1977) who stated that responses of rhizobia to these tests were not related to nodulating ability or effectiveness in fixing nitrogen. None of the isolates were able to produce hydrogen sulphide even though Cruickshank et al., (1975) have reported that hydrogen sulphide is produced at least in small amounts from sulphur-containing amino acids by a large number of bacteria. However, according to Holt (1979) Rhizobium produces a little or no hydrogen sulphide on bismuth sulphite agar.

None of the isolates could grow on Hofer's alkaline medium. This can be attributed to the high pH (11.0) level of the medium. Production of strong ureolytic enzymes was observed in the isolates 1 to 16, while the rem-

-aining isolates showed negative results. All the fast growing isolates could reduce nitrate, where as the slow growing isolates failed to reduce nitrate. This finding contrasts with the earlier finding of Neal and Walker (1938). All the isolates were positive to methyl red, thus showing that there is production of sufficient acid during the fermentation of glucose. None of the isolates were able to decompose the amino acid tryptophane to indole. None of the isolates showed growth in the citrate medium suggesting that they do not utilize citrate. Isolates 1 to 9 and 16 to 21 were catalase positive where as the fast growing isolates gave positive results with oxidase test. It is observed that none of the isolates could grow in the medium containing either 2% or 3% of sodium chloride. These results are in agreement with those observed by Graham and Parker (1964). Yadav and Vyas (1971) reported that some pea strains were sensitive to 0.3% sodium chloride but others were killed by 0.2% salt concentration.

Tolerance of various levels of pH by the isolates obtained from the root nodules of C. ensiformis plant are depicted in Table 13. From the results, it is seen that all the isolates showed maximum growth at pH 5.5-8.0, where as pH 3.5, 9.5 and 10.0 were found to be lethal and none of the isolates showed any growth. Prucha (1951) reported that large amounts of alkali inhibit growth of legumes bacteria and interfere with their ability to infect the root. Brayan (1923) found that alfalfa nodule bacteria were killed at pH 4.5-4.7 and pH 3.5-3.9 respectively. Wilson(1926) concluded that the legume bacteria disappeared in proportion to increase in soil acidity. Mulder et al., (1960) found that low pH makes soil conditions inadequate for the growth of rhizobia. Graham and Parker (1964) reported that none of the Rhizobium species is able to tolerate pH 10.0.

Utilization of carbohydrates by the isolates obtained from the root nodules of C. ensiformis plants are depicted in Table 14. Rhizobium strains are known to utilize a wide range of carbohydrates, but did not utilized starch. Singh et al., (1967) and Singh and Sharma (1971) have indicated the variations in the utilization of various carbohydrates by rhizobia. Graham & Parker (1964) reported that strains of the fast growing rhizobia generally utilize a wider range of carbon compounds than the slower growing rhizobia.

All the twenty-three isolates could form nodules on Vigna sinensis, an authentic host of cowpea cross-inoculation group. According to Kleez owska et al., (1969) biochemical characters are not diagnostic in case of Rhizobium but the ability to form root nodules is the most important characteristic, distinguishing Rhizobium from other contaminant thus exhibiting a characteristic host specificity.

Inoculation with Rhizobium strain CE-1 increased the nodule number and size as compared to Rhizobium strain CE-4, which produce minute, ineffective nodules that lacked pink colour. Inoculation with Rhizobium strain CE-1 showed significant increase in the total plant biomass and yield over all other strains and uninoculated control. Similar variations among the different strains of the same species to benefit the host have been reported by many workers in different leguminous crops (Vaishya & Gajendragadkar, 1982; Vaishya et al., 1982; Chahal & Joshi, 1978; Pawar et al., 1977).

Thus from the present study, it can be concluded that all the strains were not equally efficient. Strain CE-1 was most efficient as it showed maximum number of nodules, plant biomass and total yield.

PART III

AGRONOMIC STUDIES IN *CANAVALIA* SPECIES

A. SOIL ANALYSIS.

B. VA MYCORRHIZAL STUDIES IN *C. ENSIFORMIS* AND *C. GLADIATA*.

C. GROWTH RESPONSE OF *C. GLADIATA* PLANT IN MINE REJECT AMENDED WITH DIFFERENT LEVELS OF FARM YARD MANURE (FYM).

A. SOIL ANALYSIS

INTRODUCTION

Soil is the outer covering of the earth which consists of loosely arranged layers of materials composed of inorganic and organic constituents of different stages of organization. It is the natural medium in which plants live,multiply and die and thus provide a perennial source of organic matter which could be recycled for plant nutrition. It provides the physical support needed for the anchorage of the root system and also serves as the reservoir of air, water and nutrients which are so essential for plant growth.

A detailed study of the soil type, is a basic step towards successful cultivation. This study helps in knowing the organic and inorganic status of the soils, which when found deficient, can be amended with the necessary plant nutrients.

The present investigation was undertaken to study the physical and chemical properties of the soils, which generally favours the growth of Canavalia species.

MATERIALS AND METHODS

Soil samples from four localities (Margao, Colva, Macasana and Pale) of Goa were collected. Of these four localities, Canavalia plants were found growing in the first three, while from Pale the mine rejects were analysed since a study on the feasibility of this legume to grow on these mine rejects was undertaken.

Thoroughly mixed composite mine reject samples were collected from Pale mines. All the samples were then air dried and used for physical and chemical analysis.

Table 18. Physical analysis of the soil samples collected from different localities.

Characters	Locality			
	Margao	Colva	Macasana	Pale (mine reject)
Soil texture :				
Silt %	17.2	20.2	21.6	19.3
Clay %	18.6	13.4	20.2	33.9
Coarse sand %	10.3	3.9	5.8	2.1
Fine sand %	52.6	57.5	50.2	42.2
pH	5.8	5.8	5.9	6.1
Water holding capacity %	41.5	38.9	44.6	62.9
Electrical conductivity (mhos.)	0.09	0.10	0.11	0.09

To determine the proportion of silt, clay, coarse and fine sand, the soils were sieved with the help of standard mesh size sieves. All the soil samples were analysed for their pH, water holding capacity and Electrical conductivity as described by Piper (1957). Organic carbon in the samples was estimated according to the method of Walkely and Black (1934). Available Phosphorus in the samples was estimated by Bray I method. Available Potassium, CaO and MgO in the soils was calculated by using the neutral Ammonium acetate method. Inorganic metal analysis were carried out by using Atomic absorption spectrophotometry following acid digestion.

RESULTS

All the results of the physical and chemical analysis of the four soil samples have been depicted in Tables 18 & 19 respectively.

The present study reveals that the soils from all the four localities were sandy in nature with fine sand constituting more than half of the weight of the entire soil sample, except for mine reject. The Macasana soils contained higher amounts of silt (21.6%) as compared to the other soil samples, while the mining reject showed higher amount of clay (33.9%). Margao soils contained higher amount of coarse sand (10.3%).

All the soils were found to be acidic in nature whose pH varied from 5.8 to 6.1.

Water holding capacity was found to be maximum (62.9%) in mine reject, followed by Macasana soils (44.6%), Margao soils (41.5%) and Colva soils (38.9%).

Electrical conductivity was maximum in Macasana soils (o.11 mhos.), closely followed by Colva soils (o.10 mhos.), while the Margao soils and

Table 19. Chemical analysis of the soil samples collected from different localities.

Characters	Locality			
	Margao	Colva	Macasana	Pale (mine reject)
Organic carbon %	2.1	2.4	2.8	1.2
Available P lbs/acre	4.0	2.0	4.0	6.0
CaO %	0.11	0.06	0.08	0.13
MgO %	0.02	0.02	0.01	0.07
Available K lbs/acre	120	90	60	90
Inorganic elements: (mg/g)				
Copper (Cu)	0.06±0.00	0.03±0.00	0.08±0.00	0.05±0.00
Iron (Fe)	34.24±0.32	29.92±0.16	46.72±0.64	153.28±0.96
Manganese (Mn)	1.01±0.01	0.22±0.00	0.52±0.00	4.96±0.01
Cobalt (Co)	0.07±0.00	0.01±0.01	0.06±0.00	0.11±0.00
Nickel (Ni)	0.12±0.00	0.04±0.00	0.09±0.01	0.14±0.00
Zinc (Zn)	0.7 ±0.00	0.4 ±0.00	0.6 ±0.00	0.8 ±0.00
Magnesium (Mg)	1.97±0.01	0.75±0.00	1.91±0.00	1.0 ±0.00
Calcium (Ca)	0.7 ±0.00	0.2 ±0.00	2.2 ±0.00	2.1 ±0.00
Cadmium (Cd)	Trace	Trace	0.003±0.001	0.007±0.001

the mine reject showed same (0.09 mhos.) electrical conductivity.

Organic carbon was found to be more in Macasana soils (2.8%), followed by Colva soils (2.4%), Margao soils (2.1%) and mine reject (1.2%).

Mine rejects showed more amount (6.0 lbs/acre) of available P, while Colva soils showed very less (2.0 lbs/acre) amount of available P.

Margao soils were found to be rich in available K (120 lbs/acre), followed by Colva soils (90 lbs/acre), mine reject (90 lbs/acre) and Macasana soils (60 lbs/acre).

Mine rejects showed more amount of CaO and MgO when compared with other soil samples.

As regards to inorganic micro elements, mine rejects contained higher amounts of Fe, Mn, Co, Ni, Zn and Cd; Macasana soils contained more amount of Cu and Ca, while Margao soils were richer in Mg content.

DISCUSSION

In all a total of four soil samples were analysed from four different localities.

From the results, it is clear that all the four samples showed variations in soil texture, although they contained high proportion of fine sand. Texture is an important soil characteristic because it will, in part, determine water intake rates (absorption), water storage in the soil, the ease of tilling the soil, the amount of aeration (vital for root growth), and will influence soil fertility. In fact, the physical properties of a soil depend on its texture.

The pH of all the soil samples ranged from 5.8 to 6.1. This shows that Canavalia plants favour nor too acidic soils. It is well known that legumes only do well in slightly acidic to moderately basic soils because of a high calcium demand or inability to tolerate soluble aluminium. Strongly acidic soils (pH 4-5) is usually known to have high and toxic concentrations of soluble aluminium and manganese (Brenes & Pearson, 1973).

Mine reject showed maximum water holding capacity. This is mainly because the mine reject contains a high proportion of clay in it, which makes the water difficult to drain.

The conductance of the soil solution gives a clear idea of the total soluble salts of the soil. The Macasana soils showed higher electrical conductivity compared to other samples, indicating a higher amount of total soluble salts.

Results indicate that the mine reject contain less amount of organic matter as compared to other soil samples. This is mainly due to lack of vegetation and exposure of lower horizon of soil to the surface. According to Donahue et al., (1987) most cultivated soils contain only 1-5 percent organic matter which is mostly situated in the top 25cm of the soil. The nature and the amount of organic matter greatly influences a soils physical properties and strongly affect its chemical and biological properties (Pandeya et al., 1968). Soil organic matter is the principle storehouse for large amounts of the nutrient anions. Decomposition of organic matter by microorganisms make these nutrient ions available for absorption by plants.

Results indicate that the reject soils showed higher amount of Fe, Mn, Co, Ni, Zn and Cd when compared with other soil samples, while Macasana soils contained more amount of Ca and Cu; and Margao soils were rich in Mg content. Although minute quantities of the micronutrients are required, they are vital to plant growth.

B. VA-MYCORRHIZAL STUDIES IN TWO *CANAVALIA* SPECIES

INTRODUCTION

Vesicular-arbuscular mycorrhizal (VAM) fungi belong to Endogonaceae, a family in mucorales of Phycomycetae. These are the most prevalent type of mycorrhizal fungi which are virtually obiquitous, found in almost every soil type and are present in association with major plant groups such as angiosperms, gymnosperms, pteridophytes and bryophytes. The association is a finely balanced, complex system. The fungus is believed to be obligatory dependent on the plant. The chief benefits of the VAM association to the fungal endophyte are an abundant supply of carbohydrates from the host plant and a niche where it is protected from the microbial antagonisms of the soil (Hayman, 1983).

It is well estiblished that VAM fungi favours plant growth. The improvement of plant growth is due to increased uptake of phosphorus and other mineral nutrients especially in soils of low fertility (Baylis, 1967). Phosphorus deficiency is probably the major limitation to the growth of legumes in many soils, particularly those in tropical areas with especially high capacities of fixing phosphorus (Abbott *et al.*,1979). Bowen (1978) pointed out that most tropical soils are low in available phosphate for even grasses to respond markedly to mycorrhizal inoculation as the phosphate applied to the soil is fixed as Fe and Al phosphates. In mycorrhizal infected plants, the fungi carry a loose hyphal network extending into the

surrounding soil and thus providing an extensive surface area for absorption of nutrients by plant root (Gerdemann, 1975).

Perusal of the literature revealed that no work has been done on the response of any of the Canavalia species to VAM fungi. Hence, the present investigation was undertaken to study the effect of VAM-Rhizobium interaction on growth, yield and nutrient uptake in two Canavalia species viz., C. ensiformis and C. gladiata.

MATERIALS AND METHODS

Clayey loam soil (pH 5.8) of low phosphorus status was used. The soil was sterilized for 1h at 15 lbs to eliminate naturally occurring endophytes and other contaminants. Pots (26cm diameter) were filled with 8Kg of sterilized soil. Canavalia ensiformis plants were inoculated with two isolates of VAM fungi, namely, Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. and Trappe and Gigaspora margarita Becker and Hall, while Canavalia gladiata plants were inoculated with three isolates of VAM fungi, namely Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. and Trappe, Gigaspora margarita Becker and Hall, and Glomus albidum Walker and Rhodes. The purity of the isolates was checked. The inoculum was placed 5 cm below the soil surface to produce mycorrhizal plants. At planting, to ensure nodulation seedlings of C. ensiformis and C. gladiata were inoculated with 2ml (9.8×10^8 cells/ml and 8.2×10^8 cells/ml respectively) of effective Rhizobium strains.

Surface sterilized seeds of Jackbean and Swordbean were soaked overnight

in distilled water and were allowed to germinate on moist filter papers in petridishes at 30°C. After two days healthy and uniform seedlings were transferred to pots (one seed/pot) containing sterilized soil. In case of C. ensiformis, the experiment was performed in Oct'87 and consisted of six treatments with six replications. The treatments include : 1.Uninoculated control 2. Inoculated with Rhizobium 3. Inoculated with Glomus fasciculatum 4. Inoculated with Gigaspora margarita 5. Inoculated with Glomus fasciculatum and Rhizobium and 6. Inoculated with Gigaspora margarita and Rhizobium. In case of C. gladiata, the experiment was conducted in Oct'88 and consisted of eight treatments and six replications. The treatments include : 1.Uninoculated control. 2. Inoculated with Rhizobium 3. Inoculated with Glomus fasciculatum 4. Inoculated with Gigaspora margarita 5.Inoculated with Glomus albidum 6. Inoculated with G. fasciculatum and Rhizobium 7. Inoculated with G. margarita and Rhizobium and 8. Inoculated with G. albidum and Rhizobium.

At weekly intervals, the plants received a culture solution (100ml/pot) which was lacking nitrogen and phosphorus ($MgSO_4$ 1.5mM; k_2SO_4 2.0mM; $CaCl_2$ 4.0mM). All pots were watered daily to field capacity with distilled water. In both the experiments, the mycorrhizal colonization in the root was estimated on the 45th day after clearing the roots with 10% KOH and staining with Trypan blue (Phillips and Hayman, 1970), using the formula

$$\frac{\text{Number of VAM position segments}}{\text{Total number of segments scored}} \times 100 = \% \text{ Colonization.}$$

Six plants of each treatment were selected at random and harvested on the 60th day of growth. Mycorrhizal spore numbers in the root-zone soil were estimated by wet-sieving and decantation method (Gerdemann & Nicolson, 1963). The degree of nodulation was estimated by counting the nodules under a low power stereomicroscope. Plant dry weight was recorded after drying to constant weight. Yield characteristics were noted at maturity. Phosphous content was estimated by the Vanadomo -lybdate method (Jackson, 1973) and Zn, Cu, Mn and Fe by atomic absorption spectrophotometry following acid digestion.

Table 20. Percentage mycorrhizal infection of the roots of C. ensiformis and spores in soil as influenced by VA mycorrhizal fungi and Rhizobium.

Treatment	Infection %	Spores/100ml soil
Glomus fasciculatum	61.8	228.6
Glomus fasciculatum + Rhizobium	74.9	284.3
Gigaspora margarita	60.3	128.3
Gigaspora margarita + Rhizobium	65.9	196.4

RESULTS

The present study reveals that in both the species viz., C. ensif-ormis

VA-MYCORRHIZAL STUDIES IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 14

Fig. 17. Vesicles in G. fasciculatum.

Fig. 17a. Vesicles (x100).

Fig. 17b. Vesicles (x450).

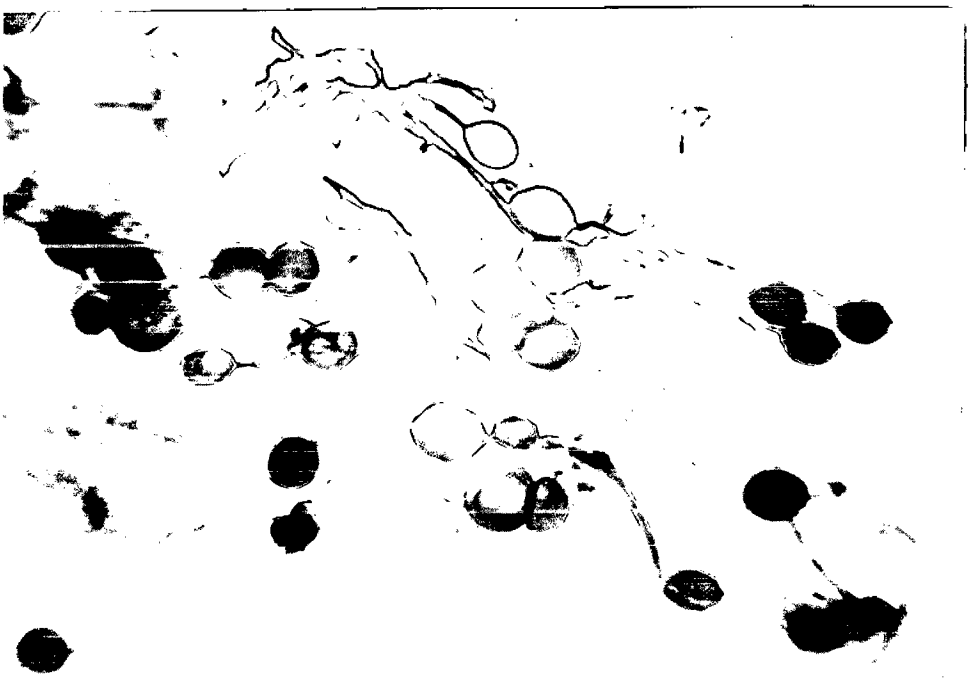


Fig. 17. (a)

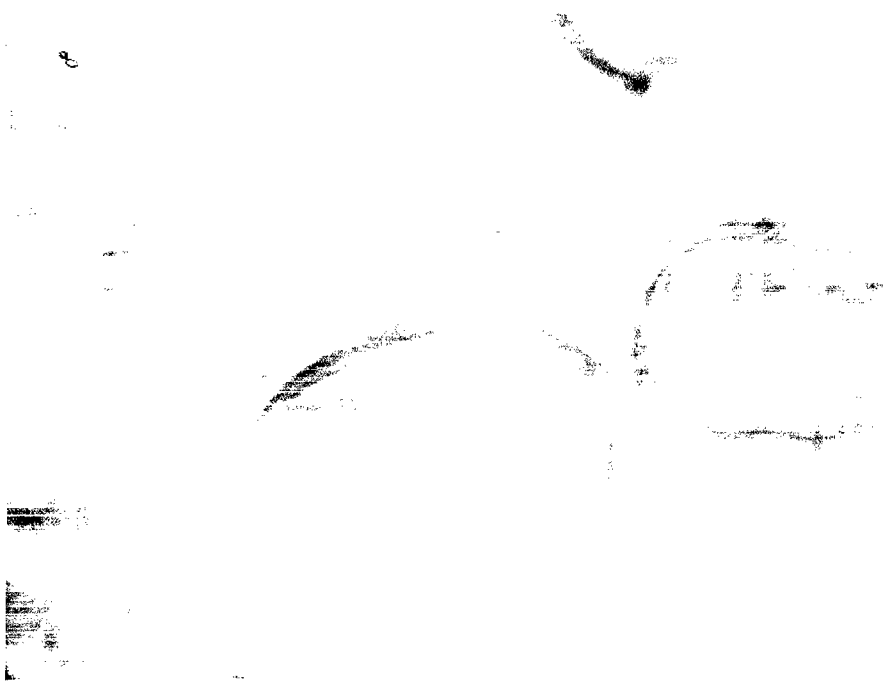


Fig. 17. (b)

Fig. 17.

Table 21. Percentage mycorrhizal infection of the roots of C. gladiata and spores in soil as influenced by VA mycorrhizal fungi and Rhizobium.

Treatment	Infection %	Spores/100 ml soil
Glomus fasciculatum	74.8	248.6
Glomus fasciculatum + Rhizobium	86.2	318.2
Gigaspora margarita	63.5	208.2
Gigaspora margarita + Rhizobium	66.8	237.6
Glomus albidum	69.8	212.8
Glomus albidum + Rhizobium	73.5	246.8

VA-MYCORRHIZAL STUDIES IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 15

Fig. 18 Arbuscles and spores of G. fasciculatum (x100).

Fig. 19 A broken sporocarp of G. fasciculatum (x100).

Fig. 18.

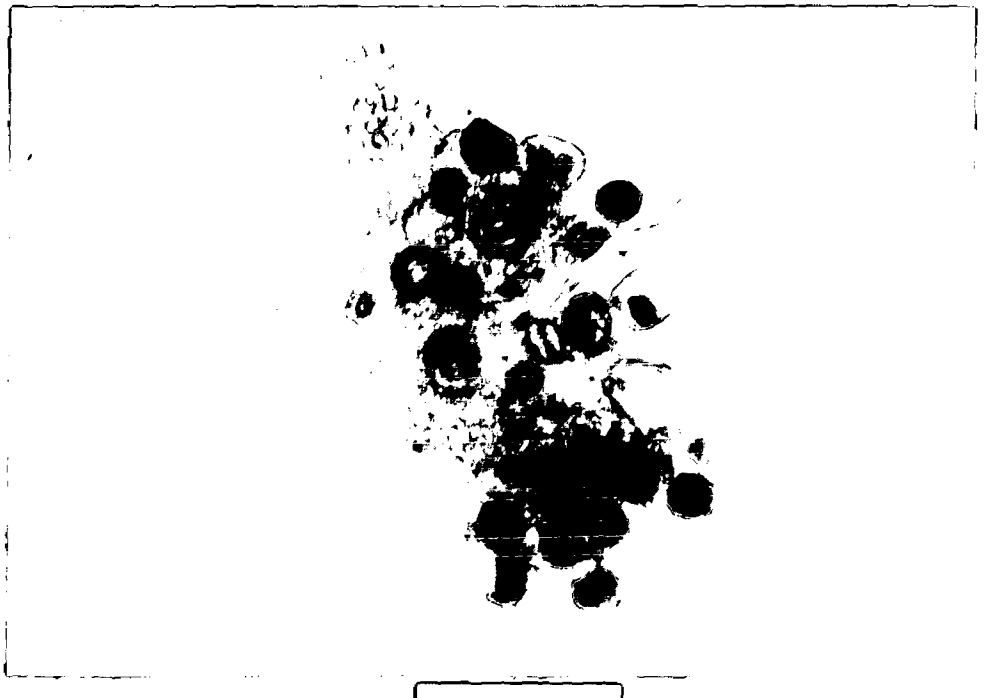


Fig. 19.

VA-MYCORRHIZAL STUDIES IN *CANAVALIA* SPECIES

EXPLANATION OF PLATE - 16

Fig. 20. Spores of *C. fasciculatum*.

Fig. 20a. A young spore (x450).

Fig. 20b. A matured spore (x450).

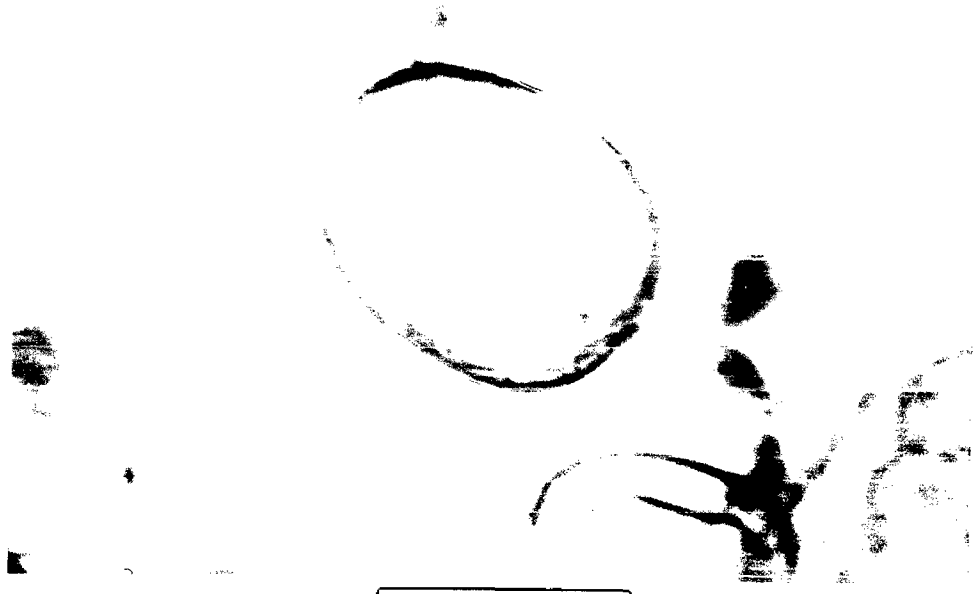


Fig. 20.(a)

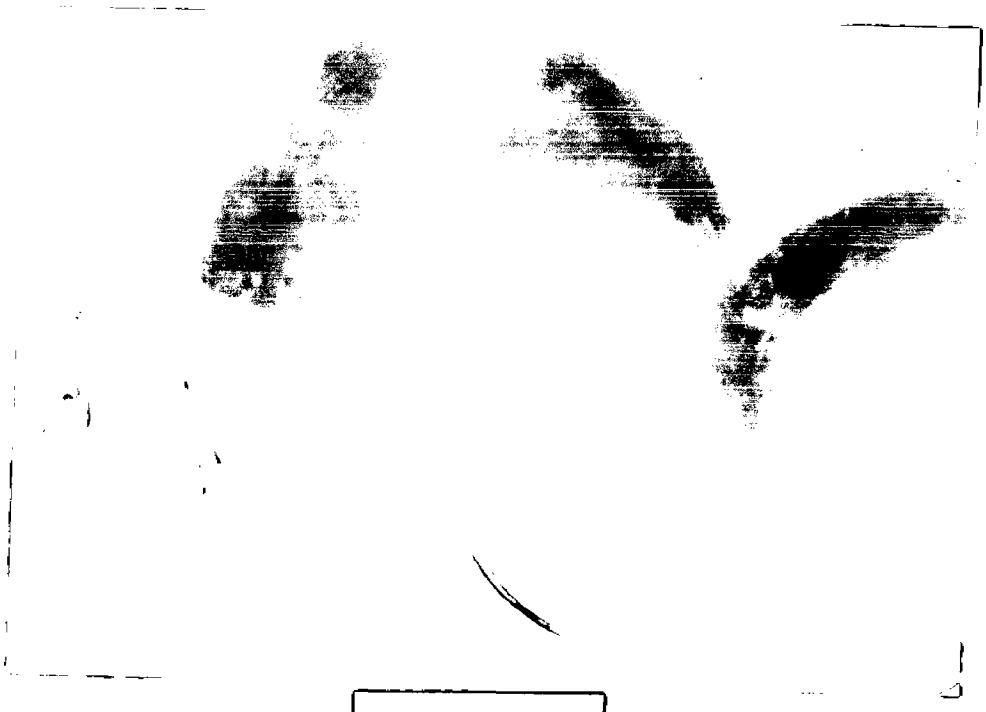


Fig. 20.(b)

Fig. 20.

Table 22. Effect of VAM- *Rhizobium* interaction on nodule morphology of *C. ensiformis* plant.

Treatment	Degree of nodulation	Position on root system	Shape	Colour		Surface
				Outside	Inside	
Control	-	-	-	-	-	-
Rhizobium	+++	Lateral	Globose	Brown	Pink	Rough
G. fasciculatum	-	-	-	-	-	-
G. margarita	-	-	-	-	-	-
G. fasciculatum + *Rhizobium*	++++	Lateral	Globose	Brown	Pink	Rough
G. margarita + *Rhizobium*	+++	Lateral	Globose	Brown	Pink	Rough

Legend:

+	=	Sparse nodulation	= < 25 nodules/plant.
++	=	Moderate nodulation	=25-50 nodules/plant.
+++	=	Abundant nodulation	= 50-100 nodules/plant.
++++	=	Very abundant nodulation	= >100 nodules/plant.

Table 23. Effect of VAM-*Rhizobium* interaction on nodule morphology of *C. gladiata* plant.

Treatment	Degree of nodulation	Position on root system	Shape	Colour		Surface
				Outside	Inside	
Control	-	-	-	-	-	-
Rhizobium	+++	Lateral	Globose	Brown	Pink	Rough
Glomus fasciculatum	-	-	-	-	-	-
G. albidum	-	-	-	-	-	-
G. margarita	-	-	-	-	-	-
G. fasciculatum + *Rhizobium*	+++	Lateral	Globose	Brown	Pink	Rough
G. albidum + *Rhizobium*	+++	Lateral	Globose	Brown	Pink	Rough
G. margarita + *Rhizobium*	+++	Lateral	Globose	Brown	Pink	Rough

Legend:

\+ = Sparse nodulation = < 25 nodules./plant.

++ = Moderate nodulation = 25-50 nodules/plant.

+++ = Abundant nodulation = 50-100 nodules/plant.

++++ = Very abundant nodulation = > 100 nodules/plant.

and C. gladiata, dual inoculated plants with VAM and Rhizobium recorded highest percentage of mycorrhizal infection than the plants inoculated with any other treatments and uninoculated control (Fig.17a, b & Fig.18). Similarly, spore numbers in the root zone soil of both the species inoculated with VAM and Rhizobium were higher than the plants inoculated with any other treatment (Table 20 & 21). The structure of sporocarp and the spores of G. fasciculatum has been depicted in Fig. 19, 20a & 20b. There was no infection in plants inoculated with Rhizobium alone or uninoculated control. This can be attributed to the sterilized soil conditions.

There was no nodulation at all in the uninoculated control plants and also in plants inoculated only with VA mycorrhizal fungi. However, plants inoculated with Rhizobium nodulated well. Effect of VAM-Rhizobium interaction on nodule morphology of both the species have been depicted in Table 22 & 23.

Effect of VAM-Rhizobium interaction on the morphological and yield characteristics of both the species have been depicted in Table 24 & 25.Results indicate that dual inoculated plants showed better growth (Fig.21a & b). It was interesting to note that there was considerable decrease in the root length of plants inoculated with VAM alone when compared to the uninoculated plants. The total plant dry weight in dual inoculated plants was more than that of uninoculated control. A significant increase in yield was observed in dual inoculated plants.

Table 24. Effect of VAM-*Rhizobium* interaction on the morphological and yield characteristics of *C. ensiformis* plant.

Character	Treatment					
	Control	R	GM	GF	GM + R	GF + R
Plant height (cm)	42.5 ±3.87	67.3 ±5.00	63.4 ±4.34	75.3 ±5.54	113.0 ±9.43	137.0 ±15.79
No. of leaves/ plant	9.0 ±0.82	11.0 ±1.14	8.0 ±0.69	12.0 ±0.96	13.0 ±1.29	13.0 ±1.29
Leaf length (cm) (3rd node)	10.6 ±0.23	15.9 ±0.32	13.8 ±0.58	15.4 ±0.31	15.1 ±0.58	17.1 ±0.58
Leaf breadth (cm) (3rd node)	8.2 ±0.14	7.8 ±0.13	8.6 ±0.16	10.5 ±0.18	8.9 ±0.20	9.2 ±0.14
Root length (cm)	48.5 ±3.62	48.8 ±4.54	35.6 ±3.53	34.9 ±3.16	42.3 ±3.22	38.9 ±3.62
No. of nodules/ plant	-	69.8 ±18.8	-	-	76.0 ±22.4	112.0 ±32.6
Total plant dry wt. (g)	3.27 ±0.05	±4.85 ±0.07	4.37 ±0.07	5.20 ±0.08	6.41 ±0.10	6.89 ±0.12
Nodule dry wt./ plant (g)	-	0.03 ±0.02	-	-	0.29 ±0.02	0.49 ±0.04
No. of pods/plant	3.0 ±0.71	6.4 ±0.41	5.0 ±0.98	9.0 ±1.98	7.0 ±1.28	11.0 ±2.41
Length of pod (cm)	10.5 ±0.91	13.6 ±0.31	12.5 ±1.58	14.3 ±1.86	13.4 ±1.41	15.3 ±1.55
No of seeds/pod	4.0 ±0.71	6.0 ±0.78	6.0 ±0.93	7.0 ±1.08	6.0 ±0.98	8.0 ±1.20

± Indicates standard deviation.

GM = *Gigaspora margarita*; GF = *Glomus fasciculatum* ; R = *Rhizobium*.

Table 25. Effect of VAM-Rhizobium interaction on the morphological and yield characteristics of C. gladiata plant.

Characters	Treatments							
	Control	R	GM	GA	GF	GM+R	GA+R	GF+R
Plant height (cm)	51.12 ±6.81	77.93 ±11.23	78.28 ±20.16	85.47 ±11.64	98.02 ±7.53	133.65 ±18.68	152.58 ±12.37	177.90 ±18.62
No. of leaves/plant	9.67 ±0.52	11.33 ±1.03	12.33 ±1.03	12.83 ±1.17	13.17 ±1.47	15.0 ±0.63	15.33 ±1.37	18.0 ±1.47
Leaf length (cm) (3rd node)	8.32 ±0.70	10.33 ±0.68	10.93 ±0.93	10.97 ±0.65	11.05 ±0.87	11.52 ±0.49	11.53 ±0.42	12.05 ±0.77
Leaf breadth (cm) (3rd node)	4.92 ±0.54	5.8 ±1.06	6.42 ±0.63	6.93 ±0.31	7.08 ±0.60	7.20 ±0.38	7.34 ±0.4	7.52 ±0.26
Root length (cm)	50.97 ±1.96	54.38 ±4.04	41.75 ±3.26	42.03 ±3.16	39.32 ±3.55	48.67 ±3.36	48.12 ±2.58	48.27 ±2.85
No. of Nodule/plant	-	56.0 ±4.98	-	-	-	68.0 ±4.90	71.5 ±8.41	89.33 ±12.16
Plant dry wt. (g)	7.05 ±0.17	9.34 ±0.80	10.03 ±0.12	10.32 ±0.58	12.64 ±0.94	13.19 ±0.58	14.71 ±0.79	16.52 ±0.72
Nodule dry wt (g)	-	1.12 ±0.10	-	-	-	1.25 ±0.07	1.37 ±0.10	1.96 ±0.28
No. of pods/plant	3.4 ±1.14	5.4 ±1.14	6.6 ±1.98	6.6 ±1.03	7.2 ±1.47	9.4 ±1.21	9.6 ±1.28	12.0 ±1.12
Average pod length (cm)	13.6 ±1.21	21.9 ±1.09	24.6 ±0.97	25.1 ±0.58	26.1 ±1.21	26.3 ±1.49	26.9 ±0.79	31.2 ±1.14
Average no. of seeds/pod	5.3 ±1.41	9.0 ±1.58	10.2 ±1.02	11.1 ±1.12	12.9 ±0.98	13.3 ±1.17	13.8 ±0.97	14.0 ±0.98

± Indicates standard deviation .

GM = Gigaspora margarita ; GF = Glomus fasciculatum ; GA = Glomus albidum ; R = Rhizobium.

VA-MYCORRHIZAL STUDIES IN *CANAVALIA* SPECIES

EXPLANATION OF PLATE - 17

Fig. 21a & b. Effect of VAM-*Rhizobium* interaction on plant growth in *C. ensiformis*. (C=control; R =*Rhizobium*; GF = *Glomus fasciculatum* ; GM=*Gigaspora margarita*).

Fig. 21. (a)

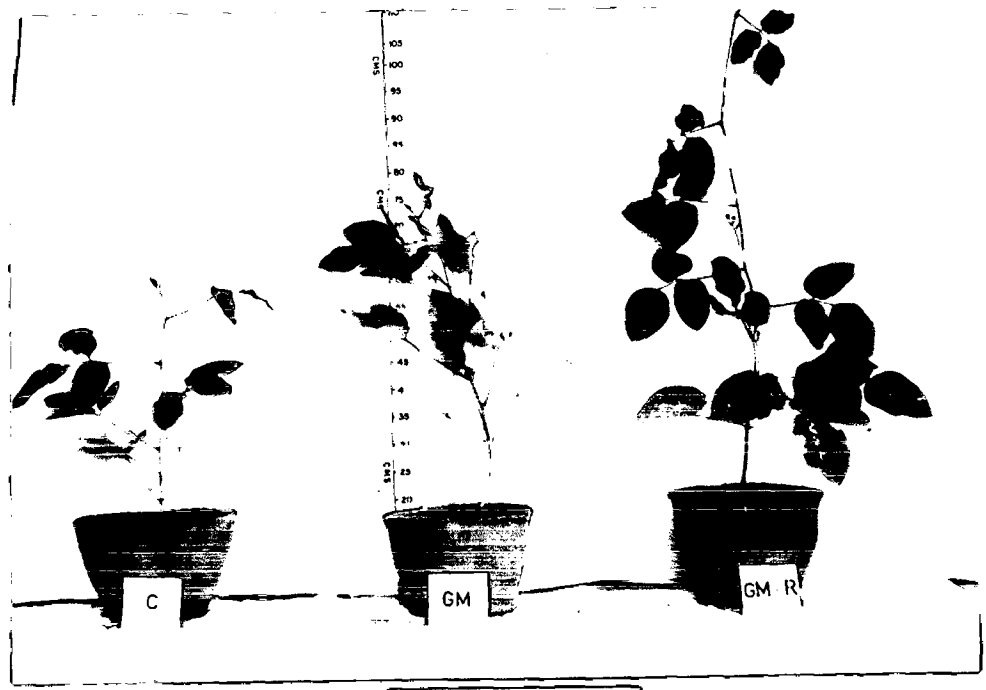


Fig. 21. (b)

Table 26. Effect of VAM-*Rhizobium* interaction on nutritient uptake in *C. ensiformis* plant.

Treatment	Element (µg/plant dry wt.)				
	P	Cu	Zn	Mn	Fe
Control	5.04 ±0.59	41.20 ±9.97	20.4 ±3.42	132.11 ±44.21	446.09 ±228.44
Rhizobium	7.86 ±1.07	66.93 ±15.13	21.3 ±3.51	196.91 ±69.24	693.16 ±345.42
Gigaspora margarita	12.11 ±1.40	73.85 ±14.68	46.2 ±4.12	355.28 ±139.28	710.74 ±344.14
Glomus fasciculatum	14.72 ±1.92	90.48 ±17.63	54.3 ±4.60	428.48 ±169.30	870.06 ±415.58
Gigaspora margarita + *Rhizobium*	17.95 ±2.18	123.07 ±13.47	79.6 ±5.12	593.57 ±229.24	1124.70 ±571.26
Glamus fasciculatum + *Rhizobium*	19.77 ±2.83	126.76 ±27.42	84.3 ±5.19	653.17 ±265.82	1234.41 ±630.16

± Indicates standard deviation.

Mean of six readings.

Table 27. Effect of VAM-*Rhizobium* interaction on nutrient uptake of *C. gladiata* plant.

Treatment	Elements (µg/plant dry wt.)				
	P	Cu	Zn	Mn	Fe
Control	11.60 ±1.22	98.79 ±10.24	30.62 ±2.46	304.55 ±49.38	877.24 ±202.32
Rhizobium	15.89 ±1.33	123.67 ±18.26	32.26 ±3.22	387.50 ±71.23	1051.86 ±296.82
Gigaspora margarita	28.02 ±1.82	182.06 ±19.29	74.14 ±3.19	855.79 ±112.19	1352.47 ±312.41
Glomus fasciculatum	36.24 ±1.89	217.46 ±21.12	99.18 ±3.96	1108.82 ±178.36	1520.35 ±329.46
Glomus albidum	32.69 ±1.84	196.54 ±19.46	90.78 ±3.24	940.92 ±129.46	1502.38 ±322.16
Gigaspora margarita + *Rhizobium*	37.99 ±2.16	243.35 ±23.42	132.11 ±4.92	1278.71 ±222.69	2241.27 ±489.90
Glomus fasciculatum + *Rhizobium*	52.32 ±2.27	318.39 ±29.16	163.76 ±6.29	1626.27 ±289.72	2888.87 ±596.42
Glomus albidum + *Rhizobium*	42.88 ±2.10	275.49 ±28.36	146.88 ±5.36	1447.09 ±246.86	1640.66 ±575.68

± Indicates standard deviation.

Mean of six readings.

Phosphorus uptake by both C. ensiformis and C. gladiata was enhanced significantly due to dual inoculation. Apart from phosphorus uptake, mycorrhizal association also resulted in increased uptake of micronutrients i.e. Zinc, Manganese, Copper and Iron (Table 26 & 27). However, the higher amount of nutrients recorded in mycorrhizal plants and dual inoculated plants can be attributed to increased plant dry weight of these plants.

DISCUSSION

Although legumes are, in general, nodular and mycorrhizal in nature (Mosse, 1977) inoculation with selected efficient strains of rhizobia and VAM fungi improved nodulation, nitrogen fixation, growth and phosphorus nutrition in some forage and grain legumes (Bagyaraj et al., 1979; Redente & Reeves, 1981).

The results indicate that in both the species, the plants infected with G. fasciculatum and Rhizobium recorded highest percentage of mycorrhizal infection and also more amount of spore number in the root zone soil. The ability to produce rapid and extensive infection is one of the factor contributing to the effectiveness of a Vesicular-arbuscular mycorrhizal (VAM) fungus at increasing nutrient uptake (Abbott & Robson, 1981 b). According to Azcon-G de Aguilar et al., (1980) Rhizobium may enhance establishment of VA mycorrhizae in legumes by polysaccharide production leading to increased synthesis of polygalacturonase at the infection site.

Better growth and nutrient uptake in dual inoculated plants over plants

inoculated with either organism or uninoculated control suggest their possible synergistic effect in the two legumes used in the present study. This confirms the findings of Smith & Daft (1977); Bagyaraj et al., (1979) Azcon-Aguilar et al., (1982) that VA mycorrhiza can have important effects on nodulation and nitrogen fixation in legumes.

Infection with the mycorrhizal fungi alone cause a decrease in the root length. According to Hayman and Mosse (1972), the absorbing area of mycorrhizal roots is the same or smaller than that of non-mycorrhizal roots. Hence the increase in phosphorus uptake by mycorrhizal plants may either be due to external hyphae decreasing the distance of diffusion of phosphorus to root surface (Abbott & Robson, 1982) or to a greater efficiency of mycorrhizal roots in absorbing phosphorus from low concentrations in the soil solution (Mosse et al., 1973).

Soil application of VA mycorrhizal fungi alone increased the phosphorus concentration in the plant, although dual inoculation with VAM and Rhizobium registered the highest status (Table 26 & 27). Similar results have been earlier reported in other legumes (Manjunath et al., 1984; Subba Rao et.al., 1986). Asai (1944) demonstrated that several legumes grow poorly and failed to nodulate in autoclaved soil unless they are mycorrhizal. This was probably due to phosphorus deficiency since an adequate phosphorus supply is important for satisfactory nodulation and nitrogen fixation (De meterio et.al., 1972). The increase in phosphorus concentration is mainly due to the VAM fungus

although additive effect is seen in dual inoculated plants. The mycorrhizal fungi scavange the phosphorus in the soil by extending their hypae beyond the root hair and phosphorus depletion zone (Sanders & Tinker, 1971). White and Brown (1979) suggest that active arbuscules are the probable sites of breakdown of polyphosphate granules. Cox et al ., (1975) proposed that phosphorus might be translocated to the root, from mycelium in the soil, by the transport of polyphosphate-containing vacuoles by cytoplasmic streaming.

Dual inoculated plants showed an increased uptake of Zn, Cu, Mn, and Fe as compared to plants inoculated seperately with either VAM or Rhizobium or uninoculated control (Table 26 & 27). Evidences for increased uptake of Zn, Cu, Mn and Fe have been reported earlier (Krishna & Bagyaraj, 1984). Uptake of minor elements important in nodulation and nitrogen fixation, such as Copper (Greenwood, 1958) and Zinc (Kapur et al., 1975) has been reported earlier.

Dual inoculated plants inoculated with VAM and Rhizobium increased the yield over plants inoculated seperately either with VAM or Rhizobium or uninoculated control (Table 24 & 25). Such synergistic effect on the yield of crops due to interaction between VAM and Rhizobium have been reported earlier (Subba Rao et al ., 1986).

While the principle effect of mycorrhiza on nodulation is undoubtably phosphorus-mediated, it is also known to aid other processes involved in nodulation and nitrogen fixation such as supply of photosynthate, trace elements

or plant hormones (Munns & Mosses, 1980; Bagyaraj, 1983).

Whatever the mechanism of action, the present study suggests that dual inoculation with selected mycorrhiza and Rhizobium will help the growth of Jackbean and Swordbean in P-deficient soils.

C. GROWTH RESPONSE OF *CANAVALIA GLADIATA* PLANT IN MINE REJECTS AMENDED WITH DIFFERENT LEVELS OF FARM YARD MANURE (FYM).

INTRODUCTION

Mine spoils and tailings are not true soils but are derived mostly from crushed bedrock and/or glacial deposits. They are very low in organic matter and normally have low levels of available nitrogen (N), phosphorus (P) and sometimes potassium (K) (Jeffrey *et al.*, 1975; Grandt, 1978).

Since mine wastes are generally low in organic content, the addition of organic amendments is an integral part of many reclamation programmes. These organic supplements are known to help the initial plant establishment by changing the moisture regime, moderating surface temperatures, decreasing erosion, improving fertility status by supplying nutrients, increasing cation exchange capacity or detoxifying toxic metals. Materials such as peat moss, animal manure, straw, cellulose fibres, sawdust and municipal refuse have been applied as surface mulches or incorporated the top layer of the waste material (Brown *et al.*, 1976; Moore & Zimmermann, 1977).

Leguminous plants are commonly used as part of many mine waste revegetation programmes (Johnson et al., 1977; Moore & Zimmermann, 1977). Perusal of the literature reveals that no such work has been carried out with regards to any of the *Canavalia* species. Hence, the present study was conducted

to study the response of the legume (C. gladiata) to the mine rejects amended with various levels of Farm Yard Manure (FYM).

MATERIALS AND METHODS

A composite mine reject was collected from the iron ore mines of Chowgule and Co. Ltd., Pale. Different compositions of mixtures of mine rejects along with either Farm yard manure (FYM) or garden soil were prepared as given below.

1. 100% Garden soil.
2. 50% Reject + 50% Garden soil.
3. 50% Reject + 50% FYM.
4. 60% Reject + 40% FYM.
5. 70% Reject + 30% FYM.
6. 80% Reject + 20% FYM.
7. 90% Reject + 10% FYM.
8. 100% Reject.

The mixtures were then filled separately in pots and labelled accordingly. Seeds soaked in water overnight were planted in these pots (one seed/pot). The plants were watered daily to field capacity. Vegetative characteristics of the plants were recorded on the 60th day of growth. Yield characteristics were recorded at maturity.

Soil samples were collected from the iron ore waste dumps and agricultural soils. They were analysed for total bacterial population by dilution plate technique, using nutrient agar.

RESULTS

It is seen that the total bacterial population per gram in the mine reject is very low (6.89×10^4) when compared to that of cultivated soils (60.80×10^6) (Table 28).

Table 28 Total bacterial population per gram in mine rejects and agricultural soils.

Samples	Total bacterial population/g of dry soil.
Mine reject	6.89×10^4
Agricultural soil	60.80×10^6

All the results of growth responses of C. gladiata plants in mine rejects amended with different levels of FYM are depicted in Table 29.

Plants growing in different composition of iron ore mine reject supplemented with FYM showed various morphological and growth responses. It was observed that height of plants growing in 100% reject was much less (30.60 cms) compared to plants growing in either garden soil (47.32 cms) or in any of the other treatments. Maximum growth was observed in pots amended with 30% FYM (173.78 cms). Significant increase in growth was observed in all the treatments as compared to the control.

There was depression in the nodule number (36.4) and nodule dry weight (0.07 g) in plants grown in 100% reject as compared to plants grown in Garden soil (Table 29). The nodules were smaller and lacked pink colour, while the plants growing in pots containing 30% FYM showed markable increase in nodule number (266) and nodule dry weight (2.20 g). The nodules were larger in size and were rich in leghaemoglobin which indicate their effectiveness.

Maximum plant dry weight (25.15 g) was recorded in plants growing in pots containing 30% FYM. However, plants growing in pots containing 100% reject showed poor dry weight (5.94 g).

Maximum pod yield (11.3) was recorded in plants grown in pots containing 30% FYM. The pods were larger (30.4 cm) and bore more seeds (13.4) over

Table 29. Response of C. gladiata plants growing on the mine rejects amended with various levels of Farm Yard Manure (FYM).

Characters	Treatment							
	100% R	100% GS	50% R + 50% GS	50% R + 50% GS	60% R + 40% FYM	70% R + 30% FYM	80% R + 20% FYM	90% R + 10% FYM
Plant height (cm)	30.60 ±2.85	43.32 ±5.40	81.74 ±10.79	139.08 ±10.54	164.83 ±13.25	173.78 ±13.91	102.10 ±7.91	58.74 ±13.98
No. of leaves/plant	5.80 ±1.64	10.20 ±2.28	11.60 ±1.52	13.20 ±1.30	17.20 ±1.30	22.20 ±1.86	15.80 ±1.79	12.4 ±1.82
Leaf length (cm) (3rd node)	6.84 ±1.19	8.70 ±1.96	9.30 ±0.71	12.18 ±1.40	12.10 ±1.10	13.42 ±1.63	12.20 ±1.08	10.02 ±0.71
Leaf breadth (cm) (3rd node)	4.70 ±0.76	6.18 ±0.57	6.36 ±0.36	8.60 ±1.17	8.78 ±0.39	9.98 ±1.01	8.86 ±0.97	7.24 ±0.65
No. of branches/plant	0.00 ±0.00	0.45 ±0.20	1.60 ±1.52	2.80 ±0.45	2.80 ±0.84	4.20 ±1.30	1.40 ±1.34	1.64 ±1.20
Root length (cm)	38.09 ±2.82	46.67 ±3.28	50.23 ±2.92	52.38 ±1.92	54.26 ±2.86	56.28 ±4.82	53.22 ±1.92	49.36 ±1.28
No. of nodules/plant	36.40 ±5.22	54.0 ±5.61	124.0 17.07	145.6 ±6.19	163.6 ±8.65	266.0 ±21.02	191.2 ±10.06	133.4 ±8.08
Plant dry wt. (g)	5.94 ±0.35	8.61 ±0.49	11.52 ±0.60	19.45 ±1.80	21.15 ±1.03	25.15 ±1.21	23.19 ±1.28	8.64 ±0.87
Nodule dry wt. (g)	0.07 ±0.01	0.25 ±0.06	0.55 0.02	0.92 ±0.02	1.37 ±0.25	2.20 ±0.14	1.13 ±0.09	0.66 ±0.06
No. of pods/plant	2.3 ±0.93	4.4 ±1.14	5.4 ±1.14	7.0 ±1.21	8.3 ±1.21	11.3 ±2.86	8.8 1.14	5.9 ±1.09
Average pod length (cm)	12.3 ±0.84	18.9 ±1.09	20.5 ±0.75	22.6 1.58	23.8 ±2.36	30.4 ±2.92	24.3 ±1.14	23.6 ±1.17
Average no. of seeds/pod	4.0 ±0.84	6.0 ±1.58	9.0 ±1.58	10.2 ±1.21	10.6 ±1.86	13.4 ±1.28	10.7 ±1.98	10.8 ±1.20

± Indicates Standard deviation. FYM = Farm Yard Manure.

all other treatments. Plants grown in 100% reject gave poor pod yield (2.3). The pods were smaller in size (12.3 cm) and contained less seeds (4.0), where as the plants grown in garden soil did not give very significant pod yield (4.4). The pods were slightly larger (18.9 cm) and contained more seeds (6.0) as compared to plants grown in 100% reject.

DISCUSSION

Reclamation of mine wastes have been attempted using physical, chemical and Vegetative methods with varying degrees of success. Vegetative stabilization is normally the most desirable technique. Leguminous plants are usually favoured for initial stabilization efforts since they provide high protein grain and herbage as well as improves soil fertility. With suitable amendments and adequate site preparation, they provide a quick and effective ground cover.

Since C.gladiata is a woody and drought resistant legume, its use as a cover crop on the mine rejects was tried. The present study showed that this legume exhibited a greater tolerance to grow on mine rejects although it showed poor growth as compared to other treatments.

It has been found that the soils on the mines are acidic in nature with pH ranging from 5.2 to 6.7. The soil taken for the present investigation had pH 5.2. Numerous studies have shown that leguminous plants gave poor growth under acid conditions (Iswaran et al., 1970; Palazzo & Duell, 1974). Planting trials on mine waste have also shown similar results with very poor legume survival and growth below pH 5.0 (Nielson & Peterson, 1972; Miles et al., 1973).

Analysis of the unvegetated mine wastes showed less number of micro-organisms than the cultivated soils. Similar observations have been made earlier (Jurgensen, 1978). Less microbial population in the mine waste may be due to the inherent physical and chemical properties of the iron ore mine waste. Climatic condition of the site may be the additional contributory factor. There is always a direct corelation between the organic matter of the soil and microbial population. Mine wastes were poor in organic matter, and hence microbial population was less. Although, the plants growing on 100% mine rejects showed fair amount of nodulation, it does not mean that the bacteria are effective nitrogen fixers. Nodules may be present, but depending on the Rhizobium strain and environmental conditions, nitrogen fixation may not be occuring. This is because of the fact that the nodules in plants grown in 100% reject were much smaller and lacked pink colour. In general, the production of nodules appear to be inhibited in an acid condition (Lie, 1971). The effectiveness of Rhizobium strains also apparently decrease drastically as acidity increases (Holding & Lowe, 1971).

Although C. gladiata plants survived in the iron ore mine wastes, their growth was poor when compared to the control. This is possibly due to lack of non availability of essential elements that are required for growth. Jackson (1967) suggested that lack of available Ca or Mo in acid waste could be a possible factor in legume survival. Increased solubilities of heavy metals under highly acidic conditions have been attributed in the poor survival of leguminous plants on some wastes (Jeffrey et al., 1977; Johnson et al., 1975). Nutrient status of the mine waste can be improved by the addition of Farm Yard Manure. It contains high amount of organic matter and also known to be a carrier of some

minor elements. Bonner (1946) observed that Farm Yard Manure maintains an adequate supply of available iron to the plant. Farm Yard Manure has the ability to form stable chelates in which metal ions are held between atoms of complex organic molecules, thereby eliminating toxicity until the organic matter decomposes through natural process.

The proportion of 30% FYM and 70% reject gave encouraging results both in terms of vegetative and reproductive growth. From this study we can conclude that initial stabilization of iron ore mine waste is possible provided an adequate amount of fertilizers such as FYM is used.

PATH IV

PATHOLOGY

A STUDIES OF SEED MYCOFLORA OF C. ENSIFORMIS AND C. GLADIATA.

B. FRUIT ROT DISEASE OF C. ENSIFORMIS.

A. STUDIES ON SEED MYCOFLORA OF TWO *CANAVALIA* SPECIES.

INTRODUCTION

A wide variety of microorganisms are known to be present epiphytically and endophytically in stored seeds. It is believed that these microorganisms have a tremendous influence on the grade and keeping quality of the seeds under stored conditions (Christensen & Kaufmann, 1969). Fungi haboured in dormant condition are reported earlier in stored pulses. (Karmakar *et al.*, 1980).

Canavalia ensiformis (Jackbean) and *C. gladiata* (Swordbean) are two robust, high yielding, closely related species which produce nutritious pods and high-protein seeds. It was observed that the seeds of both the species (*C. ensiformis*, in particular) were infected by fungi during germination, which affects the further development, thus reducing germination percentage.

The present investigation was carried out to assess the mycoflora associated with the seeds of two *Canavalia* species under storage conditions.

MATERIALS AND METHODS

The external and internal mycoflora of seeds of the two *Canavalia* species was studied by using the method given by Narsimhan and Rangaswami (1969).

One year old seeds of each species were divided into two batches. From the first batch, 5g of seeds were put in 50ml of sterilized distilled water. The flask was then kept on a shaker for 10-15 minutes. Dilutions were made from 10^{-1}

to 10^{-6}. From the second batch, 5g of seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution, followed by ten serial transfers in sterile distilled water. The seeds were then crushed to a paste in an alcohol sterilized pestle and mortar and suspended in 50ml of sterilized distilled water. The mixture was kept on a shaker for 10-15 minutes. Dilutions were made from 10^{-1} to 10^{-6}.

Each dilution (0.5 ml) was plated on Rose Bengal (Sigma) Agar by surface plating technique. Plates were then kept in an incubator at $28^{o}\pm 1^{o}C$. Observations were recorded after 4-5 days. The various colonies that appeared after this period were purified on Czapek's agar plates by single hyphal tip method. Colony characters of the fungal isolates on Czapek's agar were noted. Morphological characters of the different colonies were observed under the microscope.

RESULTS

In all a total of nine distinct colonies of fungi were recorded in both the species undertaken for the study. All these nine fungal specimens were identified at the Commonwealth Mycological Institute (CMI), Kew, England.

The study revealed that fungi were present both externally as well as internally (Table 30). It was observed that the fungal count was higher in internal flora than that of external flora (Table 31). Aspergillus was found to be the dominant genus in the seeds of both the species. Table 31 and 32 depicts the distribution of mycoflora (expressed per gram of seeds) and morphological grouping (expressed as 1% of individual groups) of the isolates in the stored seeds respectively. A reduction in the viability percentage was observed in the seeds of both the species. No discoloration of the seeds was observed.

Table 30 Distribution of external and internal mycoflora from the seeds of two *Canavalia* species.

Location	PLANT SPECIES					
	C. ensiformis			*C. gladiata*		
	Sp. no.	Herb. IMI no.	Identification	Sp. no.	Herb. IMI no.	Identification
EXTERNAL	06	330506	*Aspergillus flavus* Link	09	330509	*Aspergillus japonicus* Saito
	09	330509	*Aspergillus japonicus* Saito	02	330502	*Rhizopus rhizopodiformis* (Cohn) Zopf
	08	330508	*Dacylaria dimorphospora* Veenbaas-Rijks	06	330506	*Aspergillus flavus* Link
INTERNAL	04	330504	*Aspergillus aculeatus* Iizuka	05	330505	*Pithomyces sacchari* (Speg.) M.B. Ellis
	03	330503	*Fusarium solani* (Mart.) Sacc.	07	330507	*Aspergillus versicolor* (Vuill.) Tiraboschi
	01	330501	*Fusarium moniliforme* Sheldon			

Table 31. Distribution of mycoflora in the seeds of two Canavalia species (Population expressed per gram of seeds).

Plant species	Viability	Externally-borne	Internally-borne
C. ensiformis	54%	2.1×10^3	3.0×10^3
C. gladiata	68%	1.6×10^3	3.2×10^3

Table 32 Morphological grouping of the isolates in stored seeds of two Canavalia Species (Expressed as % of the individual groups).

Plant species	Fungus	Externally-borne	Internally-borne
C. ensiformis			
	Aspergillus flavus	53.3	-
	Aspergillus japonicus	36.2	-
	Aspergillus aculeatus	-	72.2
	Fusarium moniliforme	-	12.6
	Fusarium solani	-	15.2
	Dacylaria dimorphospora	10.5	-
C. gladiata			
	Aspergillus flavus	52.8	-
	Aspergillus japonicus	27.6	-
	Aspergillus versicolor	-	92.4
	Pithomyces sacchari	-	7.6
	Rhizopus rhizopodiformis	19.6	-

DISCUSSION

The observations revealed that nine fungal species were associated with the seeds of two Canavalia species. The results indicated that fungi were recorded both externally and internally in both the Canavalia species. Both the species harboured higher population of internally-borne fungi than the externally-borne ones, which is similar to the earlier results for a varieties of ragi and rice (Srinivasa et al., 1972).

Aspergillus is found to be the major fungal genus associated both externally and internally with the seeds of the two Canavalia species. Two species of Fusarium viz., F. moniliforme and F. solani were found among the internal mycoflora of seeds in C. ensiformis. Karmakar et al., (1980) have reported that F. moniliforme was one of the prevalent fungi associated with soybean seed.

Aspergillus flavus and A. japonicus were found to occur externally in the seeds of both the species. Ram Babu (1977) reported the occurrence of A. flavus among the fungal isolates from the external surface of deteriorating seeds of Cannabis sativa L. The other fungal species, namely Rhizopus, Pithomyces and Dacylaria were found in small number. The incidence of different genera present in varying concentration is in agreement with the earlier reports of various pulses (Khare et al., 1971; Karmakar et al., 1980). No discolouration in the seed colour was observed, although several organisms associated with discolourations of soybean seeds have been reported (Kilpatrick, 1952; Sherwin & Kreitlow, 1952).

B FRUIT ROT DISEASE OF CANAVALIA ENSIFORMIS

INTRODUCTION

Canavalia ensiformis, commonly known as Jackbean is usually cultivated in the kitchen gardens in Goa by marginal farmers. Although this plant is used as green manure and a fodder crop, the main use lies with the immature nutritious pods which are eaten as vegetables.

Although C. ensiformis plant is known to be resistant against pathogenic attacks, quite a number of diseases have been reported earlier (Sam Raj & Jose, 1969; Laximinarayana & Reddy, 1976; Singh & Shrivastava, 1979).

The present investigation reports a new disease, which predominantly occurs during the monsoon season (June to September) and brings about the decay of pods, thus reducing the yield considerably.

MATERIALS AND METHODS

Infected pods were carefully detached from the plants growing in the botanical garden of S.P.C. College, Margao. The diseased tissues were cultured on Czapek-Dox agar medium and the fungus was brought into pure culture by single hyphal tip method on the same medium. Both macro- and microscopic characteristics of the fungus were noted. The pure, non contaminated culture tubes were sealed and labelled. Later, they were carefully packed and sent to International Mycological Institute (IMI) Kew, England, for identification.

RESULTS

The causal organism was identified and confirmed as Fusarium solani (Mart.) Sacc. The culture has been deposited at CMI, Kew, England, under the accession no. IMI 330503.

The disease was marked by decay of a small portion of the pod which is confined to the skin of the fruit in the early phase of infection, which later on enters in the deeper zone and spreads to the entire fruit, thus bringing about fruit rot. Externally there is extensive development of cottony white septate mycelium in the early stages, which later on blackens as the disease advances (Fig. 22).

Conidia are borne sparsely on septate mycelium. Two types of conidia, are found. Macroconidia are large, formed in aerial mycelium from loosely branched conidiophores and are 1 to 4 septate. They are thin walled and pointed at both ends. Their size varies from 9 µm - 11 µm in one septate, 16 µm - 18 µm in two septate, 27 µm-29µm in three septate and 34 µm - 36 µm in four septate. Microconidia are borne on simple phialides arising alternately on the hyphae or form short sparsely branched conidiophores. Microconidia are abundantly variable in size and shape. They are oval, ellipsoidal, cylindrical, straight to curved ranging from 4 µm - 6 µm. Chlamydospores develop from hyphal or conidial cells. They are thick walled, globose, smooth, single or in short chains, terminal as well as intercalary in position and ranging from 6 µm - 8 µm in size (Fig. 23).

Pathogenicity test was performed and found positive.

PATHOLOGY

EXPLANATION OF PLATE - 18

Fig. 23 Fruit rot of C. ensiformis.

Fig. 24 Mycelium and conidia of the causal organism, Fusarium solani (Mart.) Sacc. (x450).

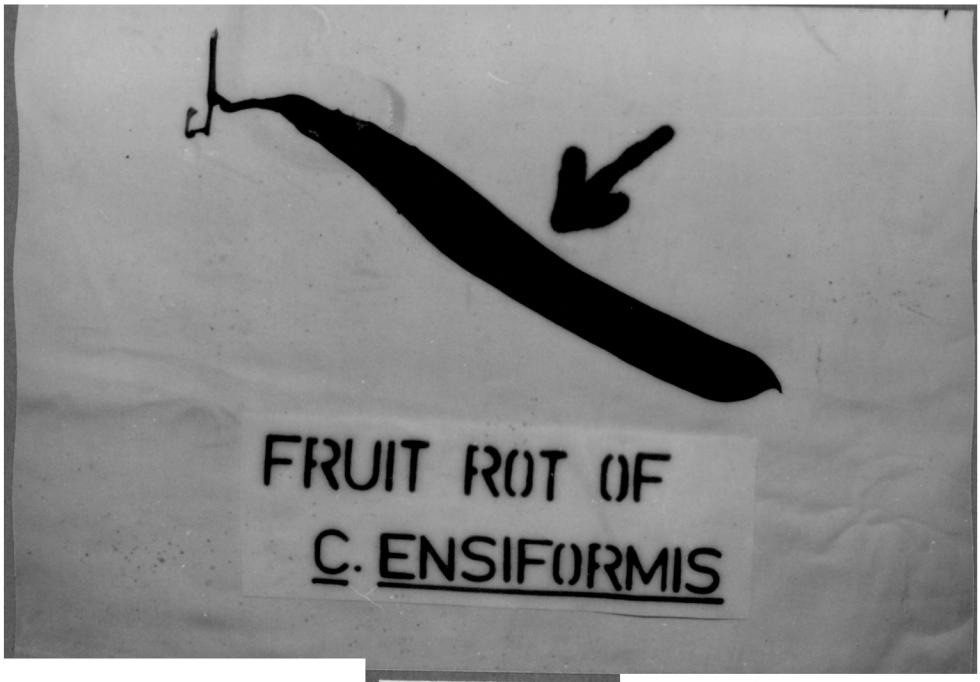


Fig. 23.

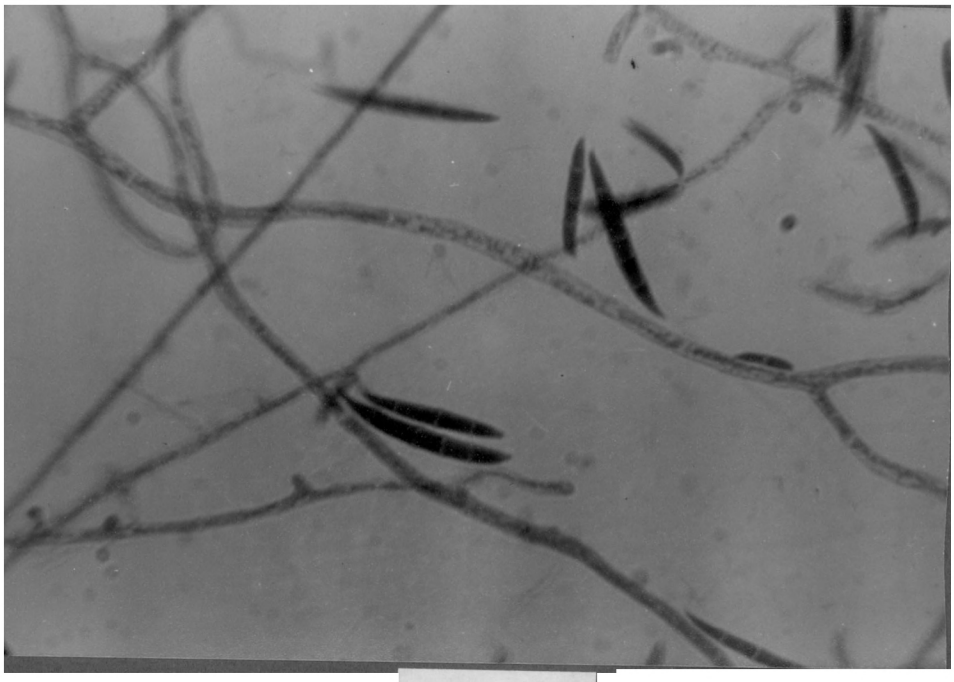
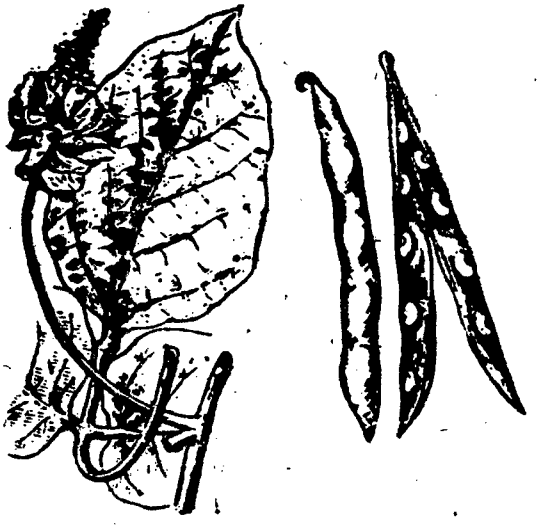


Fig. 24.

DISCUSSION

The fungus was claimed as pathogen only after satisfying the Koch's postulates. Perusal of literature revealed that this disease has not been reported earlier on Jackbean. However, there are two earlier reports of fruit rot caused by different pathogens in C. ensiformis. Laximinarayana and Reddy (1976) first reported fruit rot disease in C. ensiformis caused by Coleophoma empetri (Rostr.) Patrak. Later, a fruit rot disease caused by Trichothecium roseum (Pers.). in C. ensiformis was reported (Singh & Shrivastava, 1979).



PART V

TOXICOLOGY AND CHEMICAL ANALYSIS

A. DETECTION OF CANAVANINE IN *CANAVALIA* SPECIES

B. DISTRIBUTION OF CANAVANINE IN *CANAVALIA* SPECIES.

C. LECTIN ACTIVITY IN THE SEEDS OF *CANAVALIA* SPECIES.

D. CHEMICAL ANALYSIS.

STUDIES ON CANAVANINE IN *CANAVALIA* SPECIES.

INTRODUCTION

Canavanine (α- amino-ʃ- guanidinoxybutyric acid) was originally discovered in three closely related species of *Canavalia* viz., *C. ensiformis* (Kitagawa & Tomiyama, 1929); *C. lineata* (Kitagawa, 1937), and *C. obtusifolia* (Damodaran & Narayanan, 1939). It is known to occur in the free state only in the family Leguminosae, subfamily papilionideae, where it has been reported in 60% of the approximately 540 species examined and 35% of the 150 genera (Turner & Harborne, 1967).

The weight of evidence indicates that canavanine is an important metabolite in those seeds in which it occurs. It is sometimes present in high concentration in seeds, and circumstantial evidence points strongly to its role in the transport as well as storage of nitrogen (Tschiersch, 1959).

It has been suggested that canavanine is a natural substrate of the enzyme arginase, which is known to occur in those legumes containing free canavanine (Bell, 1958). L-canavanine, which is a toxic structural analog of L-arginine, is known to exhibit potent insecticidal properties (Rosenthal *el al.*, 1977). Canavanine is known to stimulate the growth of young rats (Kitagawa, 1937). Besides, it is extensively used as an important research biochemical.

Studies of canavanine biosynthesis have been studied in *C. ensiformis* by various workers Naylor (1966); Rosenthal (1970) & (1971); Downum *et al.*, (1983).

Practically no work has been reported in C. gladiata and C. virosa.The present investigation was carried out with an objective to estimate canavanine in C. ensiformis, C. gladiata and C. virosa. Among these species, c. ensiformis and C. gladiata are cultivated, where as C. virosa is found growing in wild state and is unsuitable for human consumption, hence it can possibly be exploited for extraction of canavanine.

The apparent restriction of canavanine to the leguminosae (Fearon & Bell, 1955) suggested that its biosynthesis might be directly or indirectly associated with the presence of nitrogen-fixing bacteria in the root nodules of these plants. Hence canavanine has been tested for in both the cells and growth media on which it was allowed to grow.

Distribution of canavanine with regards to its amount, location and fluctuation at various stages of development have been studied in detail in all the three Canavalia species.

A. DETECTION AND ESTIMATION OF CANAVANINE IN CANAVALIA SPECIES.

Materials and methods

Seeds from matured pods of three Canavalia species were collected from plants growing at the S.P.Chowgule College garden.

a) Qualitative analysis of seeds :-

Finely ground seed powder (0.1g) was stirred with 1ml of 0.1N HCl at at room temperature and kept for 18 hours. The suspension was then neutralized with 1ml of 0.1N NaOH and the supernatant was used for paper chromatography.

For chromatography, the solvents used were phenol-water(4:1,w/v),butanol-pyridine-acetic acid-water (4:1:1:2, by vol.) and methyl ethyl ketone-acetone-water-formic acid (80:4:12:2, by vol.). Chromatograms were run on Whatman no.1 paper for 24 hours in the first two solvents and for 5 hours in the third. The chromatograms run in the second solvent were sprayed with phosphate buffer and redried in air to remove the last traces of pyridine (Bell, 1960). All chromatograms were sprayed with Pentacyanoammoniferrate (PCAF) reagent (Bell, 1958).

b) Quantitative determination of canavanine :

Seeds were finely ground and dried at 110°C for 18-20 hours.

The powder (0.1g) was stirred with 2ml of 0.1N HCl and kept for 24 hours. The mixture was neutralized with 2ml of 0.1N NaOH, stirred with 50mg of Kieselguhr and filtered.

To 1ml of the clear filtrate, 0.5ml of phosphate buffer was added and the final volume was made to 12ml with the same buffer. When the colour had fully developed (40 min.) the optical density was measured in an spectronic-20 colorimeter and the concentration of canavanine was read from a standard curve as described by Fearon and Bell (1955).

c) Qualitative analysis of bacterial extracts and media:

Rhizobia were isolated from the root nodules and were grown in a medium containing :-

Sucrose	-----0.5%
Yeast extract(bakers yeast)	-----0.5%
K_2HPo_4	-----0.5%
$MgSo_4.7H_2O$	-----0.1%
$FeCl_3$	-----0.001%
$Na_2MoO_4.2H_2O$	-----0.0001%

The pH was adjusted to 7.0 with 0.1N NaOH. The colonies were allowed to establish themselves for 21 days. Later, the bacteria were centrifuged and the culture media was decanted. The sediment (0.5ml) containing bacterial cells was ground with fine sand and stirred with 2ml of 0.1N HCl. After 1 hour the supernatant liquid and the decanted culture medium were examined by paper chromatography.

Table 33. Concentration of canavanine in the seeds of three Canavalia species.

Plant species	Total canavanine (% of dry wt.)
C. ensiformis	3.47
C. gladiata	3.06
C.virosa	2.86

Each value represents the average of six separate determinations.

RESULTS

Qualitative analysis by paper chromatography revealed that canavanine reacted with Pentacyanoammonioferrate (PCAF) reagent at pH 7 to give magenta coloured spots in the dry seeds of all the three species, which matched with the spots obtained by using authentic canavanine.

Canavanine was found present in the cotyledon and embryo of the dry seed, while it was absent in the seed coat.

Quantitative analysis of seeds in all the three species gave a magenta reaction with PCAF at pH 7, thus confirming the presence of canavanine in all the three species. Concentration of canavanine in the dry seeds of three Canavalia species have been depicted in Table 33. No canavanine was detected in either the bacterial cell extracts or from the culture medium on which they were allowed to grow.

DISCUSSION

Canavanine has been detected and estimated in C.ensiformis, C. gladiata and C.virosa. From the results, it is seen that C. ensiformis contains maximum canavanine content (3.47%) as compared to C. gladiata (3.06%) and C. virosa (2.86%). According to Kitagawa (1937), canavanine constitutes about 2.5% of the dry substance of the jackbean. Damodaran and Narayanan (1939) detected canavanine in the seeds of Canavalia obtusifolia and found that the canavanine

yield amounts to above 20g per Kg of the fat-free seed meal. Fearon and Bell (1955) reported 4.7% and 3.3% of canavanine on dry weight basis in the seeds of Colutea arborescens and Canavalia ensiformis, respectively, while no canavanine was detected in the soybean seeds. Although the presence of canavanine has been detected in C. gladiata (Birdsong et al.1960),no quantitative analysis was reported. The occurrence of this rare amino acid in high concentrations in the seeds of Canavalia species possibly suggests its function as a nitrogen-storage product. Bell (1971) contends that while this unique amino acid functions primarily as storage metabolite, it may also provide an adaptive advantage to the plants by rendering the plants less susceptible to attack by various animals and lower plants. Indeed the capacity of canavanine to inhibit bacterial growth (Volcani & Snell, 1948); yeast and algal development (Walker, 1955); tissue culture of human (Miedena & Kruse, 1966) and other mammalian cell lines (Kruse et al., 1962) lends credence to this concept.

Results obtained by paper chromatography indicate that canavanine is present in the cotyledons and embryo of the dry seed and no canavanine was detected in the seed coat in any of the three species studied. Both these findings confirm the earlier report of Naylor (1966).

In conclusion, the present study revealed the presence of canavanine in all the three species studied. Among these, C. virosa is of particular interest as it is widly found and is unsuitable for human consumption. Since

it contains appreciably high amount of canavanine in the seed, it can be commercially exploited for extracting canavanine.

B. DISTRIBUTION OF CANAVANINE IN THE DEVELOPING PLANTS OF CANAVALIA SPECIES.

Materials and methods

Seeds of three Canavalia species viz., C. ensiformis, C gladiata and C. virosa were collected from the botanical garden of S.P.Chowgule College. Seeds of uniform size were selected and soaked separately in distilled water for 24 hours at 28°C. The fully hydrated seeds were planted in plastic bags containing thoroughly washed vermiculite. Each bag was watered with 100ml of distilled water everyday and received Hoagland's solution on the 7th day of growth. Six plants were harvested randomly at the required intervals, washed with distilled water and were subdivided into four samples; namely cotyledon, root, leaf and stem.

a) Quantitative determination of canavanine:

Preparation of plant extract and further assay for canavanine was performed according to the method described by Rosenthal (1970).

RESULTS

Various samples of three Canavalia species; viz., C. ensiformis, C. gladiata and C. virosa were assayed for canavanine, the results of which are depicted in Tables 34, 35 and 36 respectively. In each case, the canavanine content of various samples was plotted as a function of time (Fig.24,25, & 26).

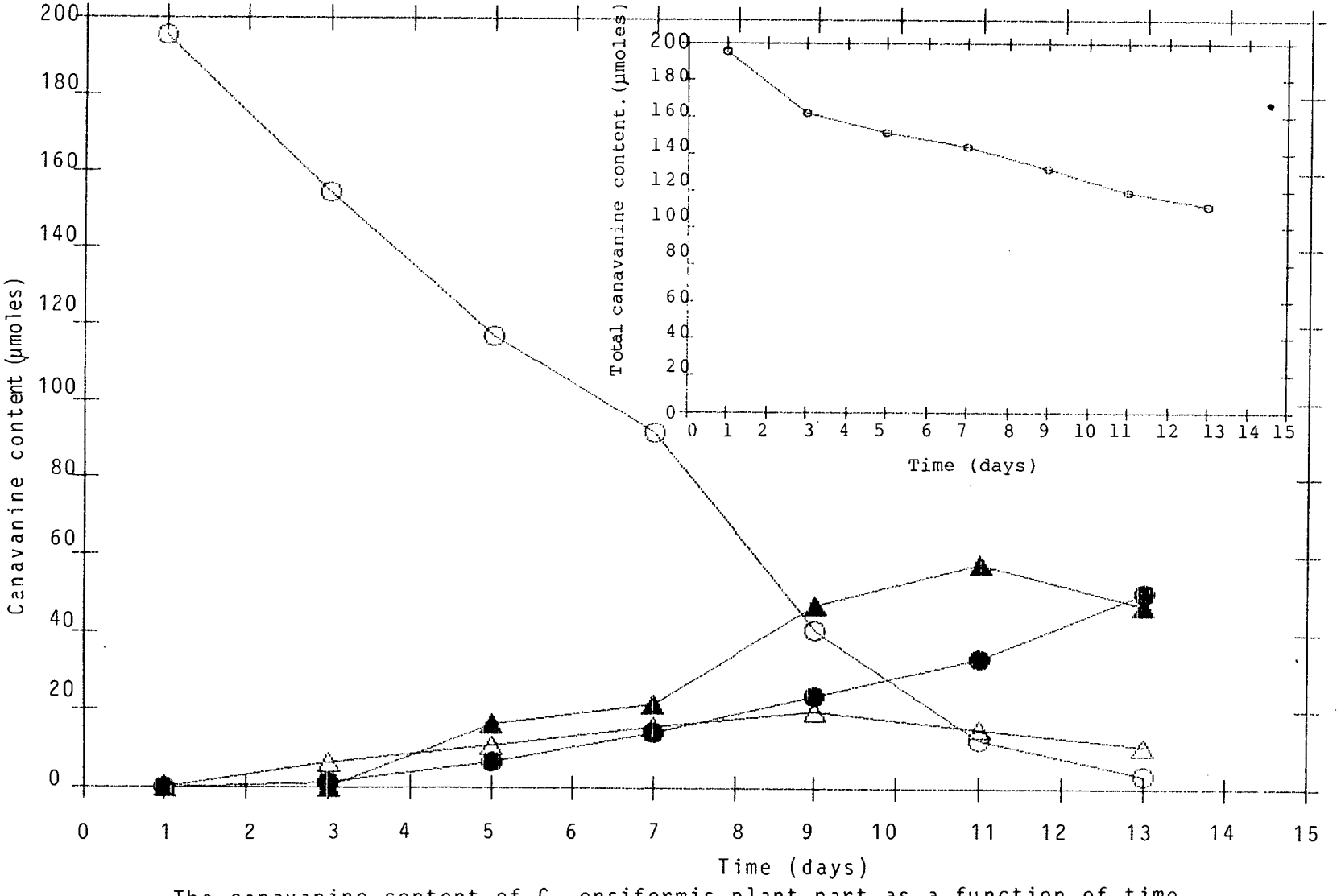
Table 34 Assays of *Canavalia ensiformis* plant.

Time (Days)	Plant Part	Canavanine content (μnmoles/plant)	Total canavanine content (μnmoles/plant)
1	Cotyledon	195.23	195.23
3	Cotyledon	154.37	161.75
3	Leaf	1.14	
3	Root	6.24	
5	Cotyledon	116.91	150.96
5	Leaf	6.81	
5	Root	10.78	
5	Stem	16.46	
7	Cotyledon	91.94	143.59
7	Leaf	14.19	
7	Root	15.89	
7	Stem	21.57	
9	Cotyledon	40.86	131.67
9	Leaf	23.84	
9	Root	19.86	
9	Stem	47.11	
11	Cotyledon	12.49	119.19
11	Leaf	33.49	
11	Root	15.32	
11	Stem	57.89	
13	Cotyledon	3.41	111.81
13	Leaf	50.51	
13	Root	10.78	
13	Stem	47.11	

STUDIES ON CANAVANINE IN CANAVALIA SPECIES

EXPLANATION OF PLATE - 20

Fig. 24. Distribution of canavanine in the developing plant of C. ensiformis.



The canavanine content of C. ensiformis plant part as a function of time

○——○ cotyledons ; ●——● Leaf ; △——△ root ; ▲- - -▲ Stem.

Insert: The canavanine content of the entire plant as a function of time.

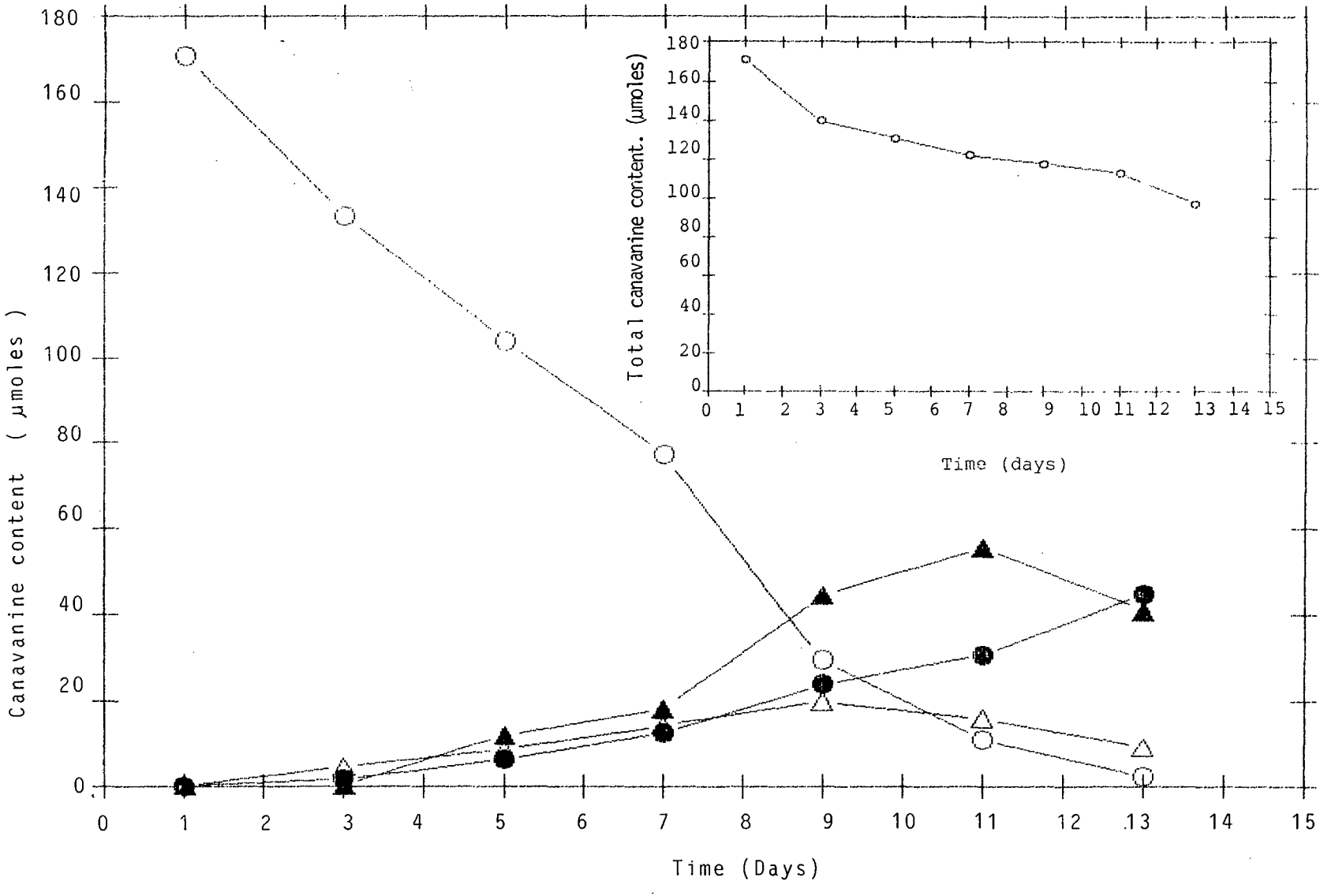
Table 35 Assays of Canavalia gladiata plant.

Time (Days)	Plant part	canavanine content (μmoles/plant)	Total canavanine content (μmoles/plant)
1	Cotyledon	170.83	170.83
3	Cotyledon	133.37	139.61
3	Leaf	1.70	
3	Root	4.54	
5	Cotyledon	103.86	130.53
5	Leaf	6.24	
5	Root	8.51	
5	Stem	11.92	
7	Cotyledon	77.19	122.03
7	Leaf	12.49	
7	Root	14.19	
7	Stem	18.16	
9	Cotyledon	29.51	177.64
9	Leaf	23.84	
9	Root	19.86	
9	Stem	44.43	
11	Cotyledon	10.78	112.80
11	Leaf	30.65	
11	Root	15.89	
11	Stem	55.48	
13	Cotyledon	2.27	97.05
13	Leaf	44.84	
13	Root	9.08	
13	Stem	40.86	

STUDIES ON CANAVANINE IN *CANAVALIA* SPECIES

EXPLANATION OF PLATE - 21

Fig. 25. Distribution of canavanine in the developing plant of *C. gladiata.*



The canavanine content of C. gladiata plant part as a function of time.

○ —— ○ Cotyledons ; ▲ —— ▲ Stem ; ● —— ● Leaf ; △ —— △ Root .

Insert: The canavanine content of the entire plant as a function of time.

Fig. 25

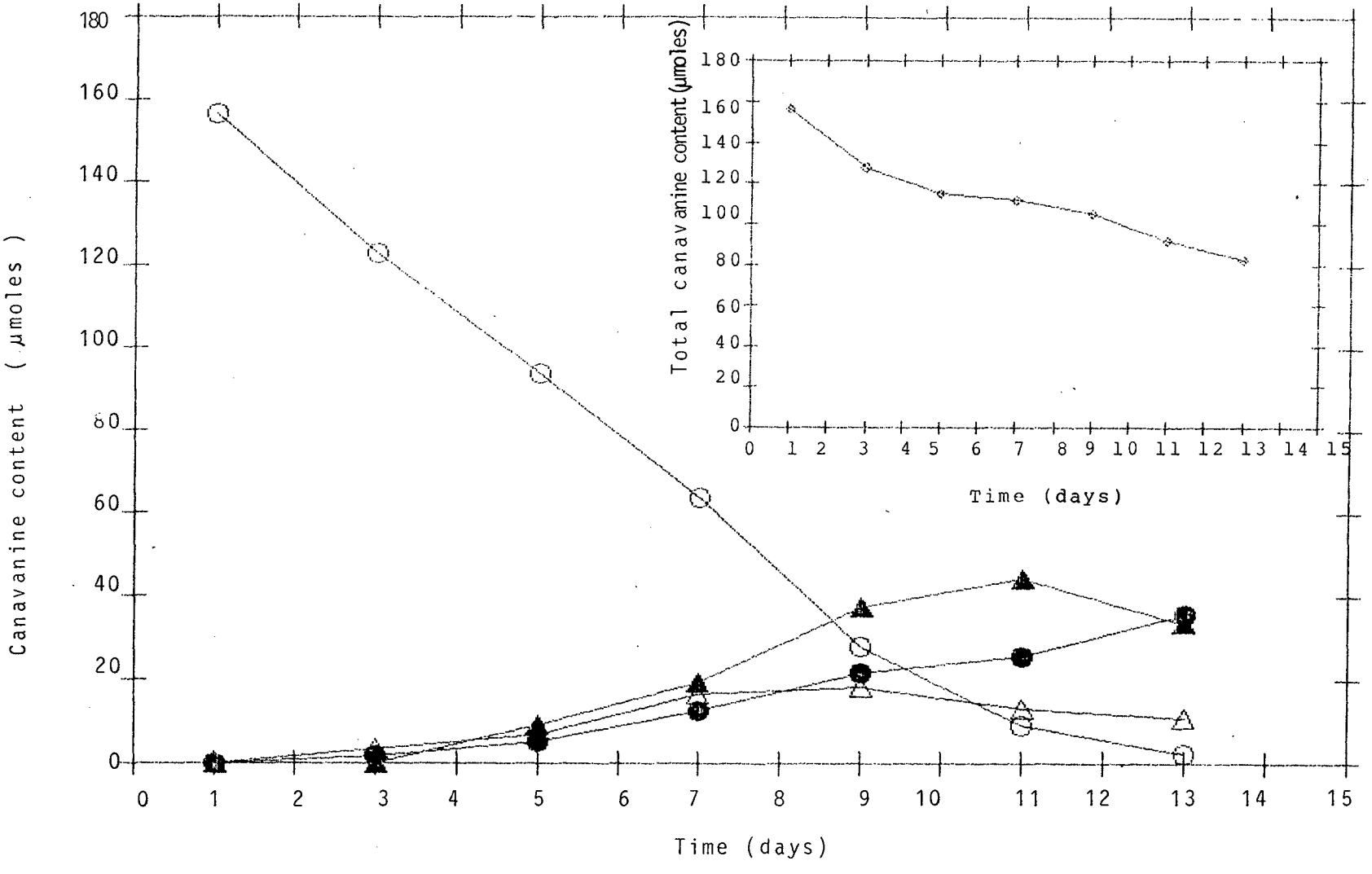
Table 36 Assays of Canavalia virosa plant.

Time (Days)	Plant part	canavanine content (µmoles/plant)	Total canavanine content (µmoles/plant)
1	Cotyledon	154.64	156.64
3	Cotyledon	122.59	127.70
3	Leaf	1.70	
3	Root	3.41	
5	Cotyledon	93.64	114.64
5	Leaf	5.11	
5	Root	6.81	
5	Stem	9.08	
7	Cotyledon	63.56	111.11
7	Leaf	12.49	
7	Root	16.46	
7	Stem	19.30	
9	Cotyledon	27.81	105.00
9	Leaf	21.57	
9	Root	18.16	
9	Stem	37.46	
11	Cotyledon	9.08	91.94
11	Leaf	25.54	
11	Root	13.05	
11	Stem	44.27	
13	Cotyledon	2.27	82.30
13	Leaf	35.76	
13	Root	10.78	
13	Stem	33.49	

STUDIES ON CANAVANINE IN *CANAVALIA* SPECIES

EXPLANATION OF PLATE - 22

Fig. 26 Distribution of canavanine in the developing plant of *C. virosa.*



The canavanine content of C. virosa plant part as a function of time.

○——○ cotyledons ; ●——● leaf ; △——△ root ; ▲——▲ Stem ;

Insert: The canavanine content of the entire plant as a function of time.

Canavanine content with reference to different plant parts showed similar fluctuations in all the three species studied. A sharp decrease in the canavanine level was recorded in the cotyledons. Shrivelling of the cotyledons occurred between the 13th and 14th day, while abscission was observed between the 15th and 18th day. The level of canavanine in the roots showed gradual increase during the first 9 days of plant growth, after which a gradual decrease was observed; while canavanine level in the stem showed a constant increase untill the 11th day after which a decrease was observed. Moreover, a gradual increase in the canavanine level in the leaves was observed throughout the experimental period.

DISCUSSION

Canavanine distribution in the developing plants in three Canavalia species have been studied in detail. The ontogenetic data in all the three species (Table 34, 35,36 & Fig. 24,25,26) indicate that the development in these plants is linked to canavanine depletion in the cotyledons and its utilization by the growing regions of the plant. Bell (1958) showed that the concentration of canavanine in the seeds of Medicago sativa fall rapidly during germination. Rosenthal (1972) presented clear evidence that degeneration of canavanine to canaline and urea is the principle step in canavanine utilization. According to Bell (1958) canavanine stored in the leguminous seeds provide a readily available supply of nitrogen for the developing embryo. This suggests the existence of an enzyme system associated with its utilization. Kitagawa (1937) first reported the presence of canavanase (obtained from liver), an enzyme which hydrolysis canavanine to urea and canaline. Damodaran and Narayanan (1940) reported its existence in the seeds of Canavalia ensiformis which brought about the hydrolysis

of canavanine to urea and canaline. They showed that this enzyme was identical with Jackbean arginase, though the optimum pH of hydrolysis was 9.4 for arginine and 7.5 for canavanine. However, recently Downum et al., (1983) have provided clear experimental evidence to prove that arginase and canavanase is one and the same enzyme whcih mediates the hydrolysis of both L-arginine and L-canavanine in C. ensiformis.

Studies on jackbean ontogeny by Sehgal and Naylor (1966) revealed a peak urease activity in the cotyledons within the first week of growth. Jackbean urease would complete the utilization of nitrogen stored in the canavanine molecule by converting urea to Co_2 and ammonia. Perhaps the pronounced urease activity in the jackbean seed is partly due to the role of this enzyme in canavanine utilization.

Thus from the present study, it can be concluded that during germination, canavanine in the cotyledons is translocated to the developing leaves, which perhaps explains the increase in the leaf canavanine level. The overall decrease in the level of canavanine during germination indicates its degradation and utilization in the developing plants of all the three Canavalia species.

C. LECTIN STUDIES IN THREE *CANAVALIA* SPECIES

INTRODUCTION

Lectins or haemagglutinins are sugar-binding proteins that agglutinate cells or precipitate glycoconjugates and are found both in plants and animals. Among plants they are abundant in seeds of legumes where they have great importance because of their toxic properties. About 54% of higher plant families tested contain lectin-positive species (Tom & Western, 1971).

The wide spread distribution of lectins throughout the plant kingdom and their abundance in many plants suggest that these molecules are of physiological importance to the plants. Indeed, the diversity in lectin structure and distribution found among different plant families, as well as recent evidence suggesting the presence of different lectins in different tissues of the same plant, indicate that during evolution different lectins may have become adapted for unique function in different tissues or species. Although at present the role(s) of plant lectins is still unknown, a variety of hypothesis on lectin function have been proposed (Lis & Sharon, 1981; Etzler, 1985).

Since Stillmark (1889) who was the first to communicate the agglutinating capacity of a *Ricinus communis* extract, several researchers in different branches of science have studies these glycoproteins. However, no much work has been done on lectins found in *Canavalia* species as evident from the literature which is already reviewed under "Previous work". The present study deals with the agglutination activity of seed extracts of three *Canavalia* species viz., *C. ensiformis*, *C. gladiata* and *C. virosa* on different human blood groups, namely A, B, O and Oh Oh (Bombay group).

MATERIALS AND METHODS

The seeds of three Canavalia species viz., C. ensiformis, C. gladiata and C. virosa were collected from the botanical gardens of S.P. Chowgule College, Margao.

The seeds were powdered and passed through a 60mm mesh sieve. Five grams powder was taken in a stoppered bottle. To this 50ml of 0.9% sodium chloride solution (saline) was added and the suspension was vigorously shaken. It was allowed to stand overnight at 6^{o}C and then filtered. The clear extract obtained was used for testing haemagglutinating activity.

Four percent suspensions of red blood cells from the whole blood of four different blood groups, namely A, B, O and Oh (Bombay group) were freshly prepared by the method described by Liener & Hill (1964). The haematocrit value was determined on a portion of whole blood, using a haematocrit tube. The whole blood was centrifuged and the cells repeatedly washed with saline till the supernatent was clear. From the haematocrit value and the quantity of whole blood initially used, a 4% cell suspension was prepared in saline.

Agglutination reactions were performed in a small test tubes by mixing constant amounts of saline with a dilution series of the cell extract. The highest dilution of antiserum which gave a detectable agglutination is taken as the end point (the 'titre') of that reaction.

RESULTS AND DISCUSSION

Extracts of all the three Canavalia species studied gave positive reaction with all panel of human red cells including Oh phenotype. Titre of seed extracts of all the three species with A, B, O and Oh phenotypes are given in Table 37.

Table 37. Agglutination activity of cell extracts of three Canavalia species on different human blood groups.

Blood group	PLANT SPECIES		
	C. ensiformis	C. gladiata	C. virosa
A	128	16	32
B	64	16	32
O	64	32	08
Oh (Bombay group)	256	64	16

Lectins have capacity of agglutinating specifically red blood cells of different blood groups (Jaffé & Brucher, 1972). This agglutinating capacity is due to their ability of combining specifically with certain cell membrane polysaccharide (Kornfeld & Kornfeld, 1969). Thus plants which contain hemagglutinins are important since these haemagglutinins can be used as blood-typing reagents. This is because of the fact that sera of human and animal origin are much expensive and require more complicated preparations.

From the present study, it is seen that saline extracts of C. gladiata may be used as a cheap and easily prepared anti-A and anti-B blood grouping reagent while saline extracts of C. virosa may be used as a cheap and easily prepared anti-O and anti-Oh (Bombay group) blood grouping reagent.

D. CHEMICAL ANALYSIS IN THREE CANAVALIA SPECIES

INTRODUCTION

Phytochemical characteristics have been used for solving taxonomic problems for years. Chemical analysis of inter-specific variations based on botanical characterization is an essential prerequisite for the breeding programme of the species. Hegnauer (1965) suggest that both organic and inorganic constituents be considered for chemotaxonomic studies. Of late, with the advances in biochemical research, botanists have opined that chemical constituents of plants should serve as one of the basis for taxonomic classification.

Certain biochemical compounds may be a characteristic feature of a family or a genera. According to Erdtman and Mitre (1956) such compounds may be formed by certain metabolic processes in plants which are retained when the group in question undergoes further evolution and differentiation. Bate-Smith (1959) discussed its aspects and considered that chemical processes in plants are probably the essential events distinguishing certain groups of plants from others.

Canavalia ensiformis and C. gladiata are two robust, high yielding, closely related species producing nutritious pods and high-protein seeds. They are capable of providing food in marginal areas where other pulses fail and they can provide a plentiful green manure and forage.Although they are already widely eaten by marginal farmers, their full potential is limited by growth inhibiting proteins that must be carefully detoxified before the seeds are edible.Reduction and/or elimination of these factors would make these legumes more acceptable

as a source of inexpensive nutritious proteins and maximize their utilization for human food. However, it has been found that all the three Canavalia species viz., C. ensiformis, C. gladiata and C. virosa are rich sources of lectins which are used as research biochemicals.

Perusal of the literature revealed that no work on the systematic chemical analysis of Canavalia species has been done earlier. Hence, the present investigation was undertaken to differentiate these species on the basis of chemical contents and to see for any possible taxonomic correlation between the species.

MATERIALS AND METHODS

Plants growing in the botanical gardens of S.P. Chowgule college, Margao, were used for the experiment. TAN and Proline content were estimated from the leaf material. Titratable Acid Number (TAN) was determined by titrating an aliquot of fresh leaves with 0.1 N alkali (NaOH) from a microburette using Phenolpthalein as an indicator (Thomas & Beevers, 1949). Proline was extracted from fresh leaves and estimated according to the method described by Bates et al., (1973). Total soluble sugars were estimated by the method of Somogyi (1952). Total nitrogen was estimated following the method of Hawk et al., (1954). From the total nitrogen values, the protein contents were calculated by multiplying it with an usual conversion factor 6.25. Starch was estimated by using the method of Mc Cready et al., (1950). Fat, Fibre and Ash content in the seeds was calculated by using the standard AOAC methods (1970). Inorganic metal analysis in

the seed material were carried out on Atomic absorption spectrophotometry following acid digestion.

RESULTS

All the results obtained from the above mentioned different estimations are based on mean values of three determinations and are depicted in Table 38.

TAN was found to be maximum in C. ensiformis (23.6ml), followed by C. virosa (17.8ml) and C. gladiata (17.4ml).

Proline content was maximum in C. virosa (0.004%), followed by C. gladiata (0.002%) and C. ensiformis (0.001%).

Fat content was found to be more in C. ensiformis (2.9%) while it was 1.6% in C. virosa and 0.8% in C. gladiata.

Seeds of C. gladiata (33.92%) were found to be rich in protein contents, closely followed by C. ensiformis (32.23%), while it was much less in the seeds of C. virosa (17.38%).

Total soluble sugars were found to be maximum in C. gladiata (5.8%), followed by C. ensiformis (4.2%) and C. virosa (2.2%).

Starch content was found to be maximum in the seeds of C. virosa (47.98%), followed by C. gladiata (42.82%) and C. ensiformis

Table 38. Chemical analysis of three Canavalia species.

Component	Plant species		
	C. ensiformis	C. gladiata	C. virosa
TAN*	23.6	17.4	17.8
Proline	0.001	0.002	0.004
Fat+%	2.9	0.8	1.6
Protein+(N x 6.25)	32.23	33.92	17.38
Nitrogen+%	5.16	5.43	2.78
Total Soluble sugars° %	4.20	5.80	2.20
Starch + %	34.94	42.82	47.98
Fibre+ %	9.4	13.3	8.6
Ash+ %	3.2	4.0	2.1
Mineral composition+ (mg/100g d.m)			
Phosphorus (P)	338	363	302
Calcium (Ca)	247	447	117
Magnesium (Mg)	144	153	133
Zinc (Zn)	43	43	33
Copper (Cu)	10	20	10
Nickel (Ni)	4	1	1

° Expressed as glucose.

* Leaf material used for analysis.

+ Seed material used for analysis.

TAN is expressed in ml of 0.1 NaOH required to neutralize the acids present in 100g fresh tissue. All other values are expressed in g/100g dry weight except for Proline which is expressed in g/100g fresh weight.

(34.94%).

Fibre content was found to be more in the seeds of C. gladiata (13.3%) while it was 9.2% in C. ensiformis and 8.6% in C. virosa.

Ash content was more in the seeds of C. gladiata (4.0%) while it was 3.2% in C. ensiformis and 2.1% in C. virosa.

Seeds of C. gladiata were rich in Phosphorus (363mg/100g), Calcium (447mg/100g) and Magnesium (153mg/100g) as compared to the other two species. The Copper content in the seeds of C. gladiata (20mg/100g) was twice than that of the other two species.

DESCUSSION

In the present study all the three Canavalia species showed a considerable variation in their chemical contents. Maximum quantity (23.6ml) of 0.1N NaOH was required to neutralize the acids in C. ensiformis, while minimum (17.4ml) in C. gladiata. The amount of TAN value is a characteristic of a species and it changes from species to species. TAN values also depend on diurnal fluctuations of C_3 and C_4 acids.

Maximum proline content was observed in C. virosa, while the other two species showed less amount of proline content. C. virosa is found to be more stress tolerant as compared to the other two species. Substantial quantities of the amino acid proline is known to accumulate under stress conditions (Aspinall & Paleg, 1981). The biochemical mechanism leading

to the induction of proline accumulation and its physiological significance have not been elucidated although it has been suggested that proline may be involved in osmoregulation (Carcellar & Franschina, 1986).

It is observed that the seeds of C. ensiformis and C. gladiata are rich in protein content, while the seeds of C. virosa showed maximum starch content.

Fibre and ash content was more in the seeds of C. gladiata while seeds of C. ensiformis were richer in fat content.

Mineral contents were found to be more or less same in the seeds of the three Canavalia species. It was observed that the seeds of all the three species were a rich source of Phosphorus, Calcium and Magnesium.

Thus, from the present study, it is seen that the chemical constituents differ clearly in quantity and this can be used as one of the criteria for taxonomic differentiation of the three species.

LITERATURE CITED

Abbott, L.K., A.D. Robson & C.A. Parker 1979. Double symbiosis in Legumes. Proceedings of a symposium on Soil Microbiology and Plant Nutrition Kuala Lumpur.

Abbott, L.K. & A.D. Robson 1981. Infectivity and effectiveness of versicular -arbuscular mycorrhizal fungi: Effect of inoculum type. Aust. J. Agri. Res., 32:631-639.

Abbott, L.K. & A.D. Robson 1982. The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. Aust. J. Agri. Res., 33:389-408.

Ahmad, K.J. 1979. Epidermal characters in Acanthaceae. In: Progress in Plant Research. Vol. I Ed. Khoshoo, T.N. & P.K. K. Nair. Today & Tomorrow's Printers & Publishers. Karolbagh, New Delhi.

Allen, O.N. & E.K. Allen 1954. Abnormal and Pathological plant growth. Brookshaven symposia in Biology, 6; 209-234.

Allen, O.N. & E.K. Allen 1981. The Leguminosae:A source book of characteristics, uses, and nodulation. 1st edn. The University of Wisconsin press, Madison.

*Amici, G. B. 1824. Ann. Sci. Nat. Bot., 2:41.

Anonymous 1957. Manual of Microbiological methods. Society of American Bacteriologists, Mc Graw Hill Book Co. INC., New York.

AOAC 1970. "Methods of Analysis". 11th edn. Association of Offical Analytical Chemists, Washington, DC.

* Asai, T. 1944. Über die Mykorrhizenbildung der leguminosen Pflanzen. Japanese Journal of Botany, 13: 463

Aspinall, D. & L.G. Paleg 1981. Proline accumulation. Physiological aspects. In: The Physiology and Biochemistry of Drought Resistance in plants. Ed. Paleg, L. G. & Aspinall, D. Academic Press, New York - Sydney - London.

Azcón-G DE Aguilár, C., J.M. Barea & J. Olivares 1980. Effect of Rhizobium polysaccharides on VA mycorrhiza formation. 2nd International symposium of Microbial Ecology, University of Warwick, Coventry, U.K. Abst. No. 18.7

Azcón-Aguilar, C., J.M. Barea, R. Azcon & J. Olivares 1982. Effectiveness of Rhizobium and VA mycorrhiza in the introduction of Hedysarum coronarium in a new habitat. Agric. Environ., 7:199-206.

Babcock, E. B. & D.R. Cameron 1934. Chromosomes and Phylogeny in Crepis II. The relationships of 108 species. Univ. Calif. Publ. Agr. Sci., 6:287-324.

Badenhuizen, N.P. 1965. Starch, Chemistry and Technology Ed. Whistler, R.L. & E.F. Paochall Vol I.

Bagyaraj, D.J., A. Manjunath & R.B. Patil 1979. Interaction between a vesicular-arbuscular mycorrhiza and Rhizobium and their effects on soybean in the field. New Phytol., 82:141-145.

Bagyaraj, D.J. 1983. Biological Interactions with VA mycorrhizal fungi. In: VA mycorrhiza. Ed. Powell, C.L. & D.J. Bagyaraj. CRS Press, Boca Raton. USA.

Barnaerts, M.J. & J. De Ley. 1963. A biochemical test for crown gall bacteri a. Nature, 197:406-407.

Bate-Smith, E.C. 1959. A Chemical to Plant Taxonomy. Proc. Linn. Soc., 169:198-211.

Bates, L.S., R.P. Walden & I.D. Teare 1973. Rapid determination of free proline for water stress studies. Plant & Soil, 39:205-207.

Battaglia, E. 1955. Chromosome morphology and terminology. Caryologia, 8:179-187.

Baylis, G.T.S. 1967. Experiments on the ecological significance of Phycomycetous mycorrhizas. New Phytol., 66:231-243.

Bell, E.A. 1958. Canavanine and related compounds in Leguminosae. Biochem. J., 70:617-619.

Bell, E.A. 1960. Canavanine in the Leguminosae. Biochem. J., 75:618-620.

Bergersen, F.J. 1982. Root Nodules of Legumes:Structure and Functions. Research studies Press John Wiley & Sons Ltd. New York.

Berry, E.W. 1916. The lower Eocene floras of southeastern North America. U.S. Geol. Surv. Prof. Paper,91:1-353.

Berry, E.W. 1925. The tertiary flora of the island of Trinidad, B.W.I. Johns Hopkins University Studies Geol., 6:71-161.

Berry, E.W. 1930. Revision of the lower Eocene Wilcox flora of the southeastern states. U.S. Geol. Surv. Prof. Paper, 156:1-144.

Bhandari, N.N., S.L. Tandon & S. Jain. 1969. Some observations on the cytology and cytomixis in Canavalia DC. Cytologia, 34:22-29.

Bieberdorf, F.W. 1938. The cytology and histology of the root nodules of some Leguminosae. J. Amer. Soc. Agron., 30:375-389.

Birdsong, B.A., R. Alston & B.L. Turner 1960. Distribution of canavanine in the family Leguminosae as related to phyletic groupings. Can. J. Bot.,38(4):499-505.

Black , O.F. 1918. Calcium oxalate in the Dasheen. Am. J. Bot., 5:447-451.

*Bonner, J. 1946. Bot. Gaz., 108:267.

Bown, G. D. 1978. Mycorrhizal roles in tropical plants and ecosystems. In: Tropical Mycorrhiza Research. Ed. Micola, P. Oxford University Press. London.

* Brayan, O.C. 1923. Soil Sci., 15:37.

Brenes, E. & R.W. Pearson. 1973. Root responses of three Gramineae species to soil acidity in an oxisol and an ultisol. Soil Sci., 116:295-302.

Brown, R.W., R.S. Johnston, B.Z. Richardson & E.E. Farmer. 1976. Rehabilitation of alpine disturbances: Beartooth Plateau, Montana. In: High-altitude revegatation workshop no.2.Ed. Zuck, R.H. & L.F. Brown. Colo. st. Univ. Ft. Collins.

* Carcellar, M. & A. Fraschina 1986. Bot. Gaz., 147:98.

Chahal, V.P.S. & P.K. Joshi 1978. Effect of inoculation with different strains of Rhizobium on gram (Cicer arietinum L.) Ind. J. Microbiol., 18(2): 101-102.

Chattaway, M.M. 1956. Crystals in Woody tissues Part II. Tropical Woods, 104:100-124.

Chatterjee, D 1948. Indian species of Canavalia DC. J. Ind. Bot. Soc., 28:63-95.

Christensen, W.B. 1946. Urea decomposition as a means of differentiating Proteus ans paracolon-organisms from each other and from Salmonella and Shigella types. J. Bacteriol., 52:461-466.

Christensen, C.M. & H.H. Kaufmann 1969. Grain storage - The role of fungi in quality loss. Univ. Minnesota Press, Minneapolis, Minn.

Colonna, P., A. Buleon & C. Mercier 1981. Pisum Sativum and vicia faba carbohydrates.Structural studies of starches. J. Food Sci., 46:88-93.

Cook, S.A. & R.G. Stanley 1960. Tetrazolium chloride as an indicator of pine pollen germinability. Silvae Genet., 9:134-136.

Con, H.J. M.W. Jennison & O.B. Weeks 1957. Routine tests for the identification of bacteria. In: Manual of Microbiological Methods. Soc. of Am. Bacteriologists, Mc Graw-Hill Book Co., INC., New York.

* Corby, H.D. L. 1980. Rhizobial Root Nodules in the classification of the Leguminosae. Ph.D. thesis, University of Rhodesia.

* Covas, G. 1949. Studios cariológicos en Antófitas III. Darwiniana, 9 (1): 158-162.

Cox, G., F.E. Sanders, P.B. Tinker & J.A. Wild 1975. Ultrastructural evidence relating to host-endophyte transfer in a vesicular- arbuscular mycorrhiza. In: Endomycorrhizas. Ed. Sanders, F.E., B. Mosse & P.B. Tinker. Academic Press, London.

Cruickshank, R.,J.P. Duguid., B.P. Marmion & R.H.A. Swain 1975. Medical Microbiology. 12th edn. Vol II Edinburgh: Churchill Livingstone.

Daisuke, Y. & T. Minamikawa 1986. In vivo studies of Protein synthesis in developing seeds of Canavalia gladiata DC. Plant & Cell Physiol., 27(6): 1033-1041.

Darlington, C.D. & A.P. Wylie 1955. Chromosome Atlas of flowering plants. George Allen & Unwin Ltd., London.

Date, R.A. 1976. In: Symbiotic Nitrogen Fixation in plants. Ed. Nutman, P.S. Cambridge University Press. London-New York.

Dayanand, A., N.S. Padshetty & K. Ramaswamy 1986. Screening an effective native strain of Rhizobium for green gram (Vigna radiata). Proc. 7th Nat. Symp. Life Sci., Gulbarga.

De Faria,S.M., G.P. Lewis, J.I. Sprent & J.M. Sutherland 1989. Occurrence of nodulation in the Leguminosae. New Phytol., 111:607-619.

* Delaunay, L. 1926. Phylogenetische chromosomenverkurzung. Zeitschr.Zellf. u. milr. Anat. 4:338-364.

De Meterio, J. L., R. Ellis JR. & G.M. Paulsen 1972. Nodulation and nitrogen fixation by two soybean varieties as affected by phosphorus and Zinc nutrition. Agronomy Journal, 64:566.

Donahue, R.L., R.W. Miller & J.C. Shickluna 1987. Soils: An introduction to soils and plant growth. 5th edn. Prentice-Hall of India Pvt. Ltd. New Delhi.

Downum, K.R., G.A. Rosenthal & W.S. Cohen 1983. Arginine and L-canvanine metabolism in Jackbean, Canavalia ensiformis (L.) DC. and Soybean,

Glycine max (L.) Plant Physiol., 73:965-968.

Erdtman, G. 1952. Pollen morphology and Plant taxonomy Angiosperms. Almqvist and Wiksell, Stockholm.

Erdtman, G. & Vishnu Mittre. 1956. On terminology in pollen and spore morphology. Paleobotanist, 5:109-111.

Etzler, M.E. 1985. Distribution and function of plant lectins. In: The chemistry and biology of lectins. Ed. Liener, I.E., N. Sharon & I.J.. Goldstein. Academic Press, New York.

Fearon, W.R. & E.A. Bell 1955. Canavanine : Detection and Occurrence in Colutea arborescens. Biochem. J., 59:221-224.

Fors, A.L. 1955. Use of Canavalia ensiformis as a soil improver in cane fields. Mon. XXVIII Conf. Ar. A. Soc. Tec. Azuc. Caba. pp. 67-72.

* Frank, E. 1965. Ein neues Verfahren zur Beobachtung der morphologischen differenzierungsschritte in der lebenden Epidermis, und sein Ergebnis bei der Analyse einer Musterbildung. Ber. dtsch. bot. Ges., 78:119-122.

Frank, E. 1972. The formation of crystal idioblast in Canavalia ensiformis DC. at different levels of calcium supply. Z. Pflanzenphysiol., 67:350-358.

Fred, E.B., L.L. Baldwin & E. Mc Coy 1932. University of Wisconsin studies

in science. No. 5. Root Nodule bacteria and leguminous plants. University of Wisconsin Press, Madison.

Garcia, E.C. 1977. Studies on the in vitro growth of cotyledon explants from Canavalia ensiformis . Plant Physiol. (Suppl.), 59(6):3.

Gaur, Y.D., A.N. Sen & N.S. Subba Rao 1973. Usefulness and limitation of Ketolactose test to distinguish Agrobacteria from rhizobia. Curr. Sci., 42:545-546.

Gerdemann, J.W. & T.H. Nicolson 1963. SPores of mycorrhizal Endogone species extracted by wet sieving and decanting .Trans. Br. Mycol. Soc., 46:235-244.

Gerdemann, J.W. 1975. VA mycorrhizae. In: Development and function of roots Ed. Torrey, J.G. & D.T. Clarkson. Academic Press, London.

Ghosh, B.N., B. Dasgupta & P.K. Sircar !985. Lectin Concanavalin A distribution at different stages in the tissues of Canavalia gladiata. Curr. Sci., 54(2):80-82.

Gilbert, G.A. & S.P. Spragg 1964. Methods in Carbohydrate chemistry. vol. 4. Ed. Whistler, R.L. Academic Press, New York and London.

Goswami, L.C. 1979. Karyological studies of thirty two varieties of Black gram (Phaseolus mungo L.). Cytologia, 44:549-556.

Graham, P.H. & C.A. Parker 1964. Diagnostic features in the characterization of root nodule bacteria of legumes. Plant & Soil, 20:383-396.

Grandt, A. F. 1978. Mined-land reclamation in the interior coal province. J. Soil Water Cons., 33:62-68.

Greenwood, E.A.N. 1958. The interaction of copper and phosphorus in legume nutrition. In: Nutrition of the legumes. Ed. Hallsworth, E.G. Butterworths:London.

Guppy, H.B. 1906. Observations of a naturalist in the Pacific, II. Plant Dispersal. Macmillan London.

Guppy, H.B. 1917. Plants, seeds, and current in the west Indies and Azores. Williams & Norgate, London.

Hawk, B.P., B.L. Oser & W.H. Summerson 1954. Practical Physiological Chemistry. 13th edn. Blakiston, New York.

Hayman, D.S. 1983. The physiology of vesicular-arbuscular endomycorrhizal symbiosis. Can. J. Bot., 61:944-963.

Hayman, D.S. & B. Moses 1972. Plant growth responses to vesicular arbuscular mycorrhiza. III. Increased uptake of labile P from soil. New Phytol., 71:41-47.

* Hegnauer, R. 1965. Chemotaxonomic der pflanzen. Vol. 4 Birkhauser-Verlag, Basle.

Heintzelman, C.E., JR. & R.A. Howard 1948. The comparative morphology of the leacinaceae. V. The pubescence and crystals. Am. J. Bot., 35:45-52.

Herman, E.M. & L.M. Shannon 1884. Immunocytochemical localization of concanavalin A in developing Jack-bean cotyledons. Planta, 161:97-104.

Hofer, A.W. 1935. Methods fordistinguishing between legume bacteria and their common contaminants. J. Amer. Soc. Agron.,27:228-230.

Holding,A.J. & J.F. Lowe 1971. Some effects of acidity and heavy metals on the Rhizobium-leguminous plant association. Plant & Soil, spec. vol. pp. 153-166.

Holt, J.G. 1979. The shorter Bergey's Manual of Determinative Bacteriology. 8th edn. The Williams & Wilkins Company/Baltimore.

Horner, H.T. & E. Zindler-Frank 1982. Histochemical, spectroscopic, and x-ray diffraction identifications of the two hydration forms of calcium oxalate crystals in three legume and Begonia. Can. J. Bot., 60:1021-1027.

Huprikar, S.V. & Kamala Sohonie 1961. Haemagglutinins in Indian Pulses: Part I-Detection of Haemagglutinins & effect of autoclaving

& germination on haemagglutinating activity. J. Sci. Industr. Res., 20:82-85.

Hutchinson, J. 1964. The genera of flowering plants. Clarendon Press, Oxford.

Huziwara, Y. 1962. Karyotype analysis in some genera of compositae. VIII. Further studies on the chromosome of Aster. Amer. J. Bot., 49:116-119.

Iswaran, V., K.S.B. Sharma & M. Comhaire 1970. Soil fertility, legumes and Rhizobium efficiency. AGRI Digest, 19:3-19.

Jackson, M.L. 1973. Soil chemical analysis. Prentice Hall of India (Pvt) Ltd. New Delhi.

Jackson, W.A. 1967. Physiological effects of soil acidity. In: Acidity and Liming. Agron. Monogr. No.12. Ed. Pearson, R.W. & F. Adams. Amer. Soc. Agron., Madison.

Jeffrey, D.W., M. Maybury & D. Levinge 1975. Ecological approach to mining waste vegetation. In: Minerals and the enviroment Ed. Jones, M.J. Institution of Mining & Metallurgy, London.

Johnson, H.B. 1975. Plant Pubescence: An ecological perspective, Bot. Rev., 41(3):233-258.

Johnson, M.S., T. Mc Neilly & P.D. Putwain 1977. Revegetation of metalliferous mine spoil contaminated by lead and zinc. Environ. Pollut., 12:261-277.

Joseph, L.S. & J.C. Bouwkamp 1978. Karyomorphology of several species of Phaseolus and Vigna. Cytologia, 43:595-600.

Jurgensen, M.F. 1978. Microorganisms and the reclamation of mine wastes. In:Forest soils and land use. Ed. Youngberg, C.T. Proc. of the fifth north American Forest soils conference. Aug. 6-7 at Colorado State University.

Kapur, O.C., M.S. Gangwar & K.V.B. Tilak. 1975. Influence of zinc on symbiotic nitrogen fixation by soybean (Glycine max Linn.) in silt loam soil. Indian J. Agric. Res., 9:51-56.

Karmakar, S., S. Subuddhi & B. Bandyopadhyay 1980. Studies on seed mycoflora of soybean. Ind. J. Microbiol., 20(3):236-238.

Kawakami, J. 1930. Chromosome numbers in Leguminosae. Bot. Mag. Tokyo, 44:319-325.

Kedarnath, S. 1950. A note on the chromosome numbers of some plants. Indian J. Genetics & Plant Breeding, 10(2):96

Kilpatrick, R.A. 1952. Fungi associated with soybean seeds within the pods at Stoneville, Mississippi. Phytopathology, 42:285.

Kitagawa, M. 1937. A diamino acid, canavanine, and a monoamino acid, canaline. J. Biochem. (Japan) 25:23-41.

Kitagawa, M. & T. Tomiyama 1929. A new amino-compound in the Jack bean and a corresponding new ferment. Biochem. J.,75:618-620.

Khare, M.N., R.P. Mishra, S.M. Kumar & J.N. Chand 1971. Seed mycoflora of Khesari (Lathyrus sativum) its pathology and control. Indian Phytopathology, 25:69-75.

Kleezkowska, J., P.S. Nutman, F.A. Skinner & J.M. Vincent 1968. The identification and classification of Rhizobium. In: Identification Methods for Microbiologists. Ed. Gibbs, B.M. & D.A. Shapton. Academic Press, New York and London.

Koser, S.A. 1923. Utilization of the salts of organic acids by the Colon-aerogenes group. J. Bacteriol., 8:493.

Kovaks, N. 1956. Identification of Pseudomonas pyocyanea by the oxidase reaction. Nature (London), 178:703.

Krishna, K.R. & D.J. Bagyaraj 1984. Growth and nutrient uptake of peanut inoculated with mycorrhizal fungus Glomus fasciculatum compared with non-inoculated ones. Plant & Soil, 77:405-408.

Kruse, P.F.,JR., H.A. Carter & P.B. White 1962. Some effects of canavanine

and canaline on arginine and ornithine metabolism in mammalian tumor cell cultures. Sci. Proc. Amer. Assn. Cancer Res., 3:336.

Lai, C.C. & E. Varriano-Marston. 1979. Studies on the characteristics of black bean starch. J. Food Sci. 44:528.

Lande, R.T. 1961. Nodule bacteria associated with the indigenous leguminosae of south-western Australia. J. Gen. Microbiol., 26:351-359.

Laximinarayana, P. & S.M. Reddy. 1976. A new disease of sword bean. Curr. Sci. 45(9):530-531.

Lentz, D.L. 1986. Ethnobotany of the Jicaque of Honduras. Economic Botany, 40(2):210-219.

Levan, A., K. Fredga & A.A. Sandberg 1964. Nomenclature for centromeric positions on chromosomes. Hereditas, 52:201-220.

Lewitsky, G.A. 1931. The morphology of chromosomes. Bull. Appl. Bot. Genet. & Pl. Breed., 27:103-173.

Lie, T.A. 1971. Symbiotic nitrogen fixation under stress conditions. Plant & Soil, spec. vol. pp. 117-128.

Liener, I.E. 1964. Seed Hemagglutinins. Economic Botany, 12:27-33.

Lim, G. & J.C. Burton 1982. Rhizobium. In:Nitrogen fixation. Ed. Broughton, W.J. Vol. II Clarendon Press. Oxford.

Lis, H. & N. Sharon 1981. Lectins in higher plants. In:The biochemistry of plants. Ed. Marcus, A. Academic Press. New York.

* Macallum, A.B. 1905. J. Physiol., London. 32:95-128.

Manjunath, A., D.J. Bagyaraj & H.S. Gopala Gowda 1984. Dual inoculation with VA mycorrhiza and Rhizobium is beneficial to Leucaena. Plant & Soil, 78:445-448.

Mc Cready, R.M., J. Guggolz, V. Silviera & R.S. Owen 1950. Determination of starch and amylose in vegetables. Analyst. Chem., 22:1156.

Mc Masters, M.M. 1964. Methods in Carbohydrate chemistry Vol 4, Ed, Whistler, R.L. Academic Press, New York and London.

*Menzel, P. 1920. Uber pflanzenreste aus Basalttuffen des Kamerungebietes. Beitrage zur geologischen Erforschung der deutschen Schutzgebiete 18.

Metcalfe, C.R& L. Chalk. 1950. Anatomy of the Dicotyledons. Clarendon Press, Oxford Vol 1 & 2.

Miedena, E. & P.F. Kruse 1966. Effect of canavanine of proliferation and metabolism of human cells in vivo. Proc. Soc. Exp. Biol. Meol., 121:1220-1222.

* Miège, J. 1960. Nombres chromosomiques de plantes d'Afrique occidentale. Rev. cytol. et. Biol. Vég. 21(4):373-384.

Miles, V.C., R.W. Ruble & R.L. Bond. 1973. Performance of plants in relation to spoil classification in Pennsylvania. In: Ecology and Reclamation of Devastated lands. Vol. 2 Ed. Hutnik, R.J. & G. Davis. Gorgen and Breach, New York.

Molina, M.R. & Bressani 1974. Protein-starch extraction and nutritive value of the Jack bean and Jack bean protein isolate. In: Nutritional aspects of common beans and other legume seeds as animal and human foods. Ed. Jaffe, W.G.

Moore, T.R. & R.C. Zimmermann 1977. Establishment of vegetation on serpentine asbestos mine wastes, southeastern Quebec, Canada. J. Appl. Ecol., 14:589-599.

Mosse, B. 1977. The role of mycorrhiza in legume nutrition on marginal soil.In:Exploiting legume-Rhizobium symbiosis in tropical agriculture. Ed. Vincent, J.M., A.S. Whitney & J.Bose. University of Hawaii. Miscellaneous Publication no. 145.

Mosse, B., D.S. Hayman & D.J. Arnold 1973. Plant growth responses to vesicular-arbuscular mycorrhiza. V. Phosphate uptake by three plant species from P-deficient soils labelled with ^{32}P. New Phytol., 72: 809-815.

*Mulder, E.G. & W.L. Veen 1960. Plant & Soil, 13:91.

Munns, D.N. & B. Mosse 1980. Mineral nutrition of legume crops. In: Advances in legume science. Ed. Summerfield, R.J. & A.H. Bunting. HMSO London.

Nair, P.K.K. 1962. Pollen grains of cultivated plants - III. Great millet and maize. Indian J. Agric. Sci., 32:196-200.

Narayana, H.S. & B.D. Gothwal 1964. A contribution to the study of root nodules in some legumes. Proc. Ind. Acad. Sci., B, Vol. LIX:351-359.

Narsimhan, K.S. & G. Rangaswami 1969. Microflora of Sorghum grain. Mys. J. Agric. Sci., 3:215-226.

Naylor, A.W. 1966. Synthesis and degradation of canavanine, an analog of Arginine in Canavalia ensiformis (L.)DC. Science, 154:425.

Neal, O.R. & R.H. Walker 1938. Physiological studies on Rhizobium. J. Bacteriol., 30:178-187.

Nielson, R.F. & H.B. Peterson 1972. Treatment of mine tailings to promote vegetative stabilization. Utah State Agric. Exp. Sta. Bull. 485.

Norris, D.O. 1956. Legumes and the Rhizobium symbiosis. Empire J. Exp. Agric., 24:247-270.

Norton, J.D. 1966. Testing of plum pollen viability with tetrazolium salts. Proc. Soc. Hortic. Sci., 89:132-134.

Oberle, G.D. & R. Watson. 1953. The use of 2,3, 5- triphenyl tetrazolium chloride in viability tests of fruit pollen. Proc. Soc. Hortic. Sci.,61:299-303.

Palazzo, A. J. & R.W. Duell. 1974. Responses of grasses and legumes to soil pH. Agron. J., 66:678-682.

Pandeya, S.C., G.S. Puri & J.S. Singh 1968. Research methods in Plant ecology. Asia Publishing House.

Pawer, N.B., A.M. Shirsat & J.N. Ghulgule 1977. Effect of seed inoculation with Rhizobium on grain yield and other characters of cowpea (Vigna unguiculata). Tropical grain legume bulletin, 7:3-5.

Phillips, J.M. & D.S Hayman. 1970. Improved procedures for clearing and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55:158- 160.

Piper, C.S. 1957. Soil and Plant analysis. Hans Publishers, Bombay.

*Poucques, M.L. de 1945. Etude caryologique sur quelques Légumineuses exotiques. Rev. Cytol. et Cytophysiol. Vég., 8,1.4:117-128.

*Prucha, M.J. 1915. (Cornell) Agri. Col. Mem (cited in Fred et al., 1932).

Puri, G.S. 1960. Indian forest ecology. Vol. I, Primlani, India.

Purseglove, J.W. 1968. Tropical crops:Dicotyledons I. John Wiley and Sons, New York.

Ram Babu, A.N. Roy, Y.K. Gupta & M.N. Gupta. 1977. Fungi associated with deteriorating seeds of Cannabis sativa L. Curr. Sci., 46(20): 719-720.

Ramachandran, C., K.V. Peter & P.K. Gopalakrishnan 1980. Drumstick (Moringa oleifera): A multipurpose Indian vegetable. Economic Bot.,34 (3):276-283.

*Rao, A.N. & E.T. Ong. 1976. Grana, 12(2):113-120.

Redente, E.F. & F.B. Reeves. 1981. Interactions between vesicular-arbuscular mycorrhiza and Rhizobium and their effect on sweet vetch growth. Soil Sci., 132:410-415.

Riley, H.P. 1960. Chromosomes of some plants from the Kruger National Park. Jour. S. African Bot., 26(1):37-44.

Rôbertson, B.L. 1978. Raphide- sacs as epidermal appendages in Jubaeopsis caffra Becc. (Palmae). Ann. Bot., 42:489-490.

Rosenthal, G.A. 1970. Investigations of canavanine biochemistry in the Jackbean Plant, Canavalia ensiformis (L.) DC. Plant Physiol., 46:273-276.

Rosenthal, G.A. 1971. An Ontogenetic study of canavanine formation in the fruit of Jackbean, Canavalia ensiformis (L.) DC. Plant Physiol., 47:209-211.

Rosenthal, G.A. 1972. Investigations of canavanine biochemistry in the Jack bean Plant, Canavalia ensiformis (L.) DC. Plant Physiol., 50:328-331.

Rosenthal, G.A., D.H. Janzen & D.L. Dahlman 1977. Degradation and Detoxification of canavanine by a specialized seed predator. Science, 196:658-660.

Saldanha, C.J. 1984. Flora of Karnataka. Vol I Oxford IBH Publishing Co., New Delhi.

Sam Raj, J. & P.C. Jose. 1969. Collar root of sword beans (Canavalia ensiformis D.C.) Sci & Cult., 35(11):623-624.

Sammour, R.H., J.A. Gatehouse, J. Gilroy & D. Boulter 1984. The homology of the major storage protein of Jack bean (Canavalia ensiformis) to pea vicilin and its separation from α-mannosidase.

Sanders, F.E. & P.B. Tinker 1971. Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature, 233:278-279.

Sarbhoy, R.K. 1978. Cytogenetical studies in genus Phaseolus Linn. I and II. Somatic and meiotic studies in fifteen species of Phaseolus

(Part I). Cytologia, 43:161-170.

Sarbhoy, R.K. 1978. Cytogenetical studies in genus Phaseolus Linn. I and II. Somatic and meiotic studies in fifteen species of Phaseolus (Part 2). Cytologia, 43:171-180.

Sarbhoy, R.K. 1980. Karyological studies in the genus Phaseolus Linn. Cytologia, 45:363-373.

Sathe, S.K. & D.K. Salunkhe 1981. Solubilization and electrophoretic characterization of the Great Northern bean (Phaseolus vulgaris L.) proteins J. Food Sci. 46(1):82.

Sauer, J. 1964. Revision of Canavalia. Brittonia, 16:106-181.

Schertz. K.F., W.C. Boyd, W. Jurgelsky JR. & E. Cabanillas 1960. Seed extracts with Agglutinating activity for human blood. Economic Bot., 14:232-240.

Schneider, A. 1901. The probable function of Calcium oxalate crystals in plants. Bot. Gaz., 32:142-144.

Schoch, T.J. & E.C Maywald 1968. Preparation and properties of various legume starches.Cereal Chem. 45:564.

Schoch, T.J. 1941. Physical Aspects of starch behavior Cereal Chem., 18(2):121-128.

Sehgal, P.P. & A.W. Naylor 1966. Ontogenetic study of urease in Jack bean, Canavalia ensiformis (L.) DC. Bot. Gaz., 127:27-34.

Sharma, P.C. & P.K. Gupta 1982. Karyotypes in some Pulse crops. The nucleus, 25(3):181-185.

Sherwin, H.S. & K.W. Kreitlow 1952. Discolouration of soybean seeds by the Frogeye fungus Cercospora sojina. Phytopathology, 42:568-572.

*Shibata, K. 1962. Estudio Citólogicos de plantas Columbianas silvestres y. cultivadas. (Cytological studies on some wild and cultivated plants of Columbia). J. Agric. Sci. Tokyo Nogyo Daigaku, 8:49-62.

Simmonds, N.W. 1954. Chromosome behaviour in some tropical plants. Heredity, 8:139-150.

*Singh, A. & P.B. Sharma 1971. Ind. J. Microbiol., 11:33.

Singh, P.L. & A.K. Shrivastava 1979. Fruit rot disease of Canavalia ensiformis DC. A new record. Curr. Sci., 48(4):168-169.

*Singh, R., N. Singh & G.S. Sidhu 1967. Ind. J. Microbiol., 7:143.

Sinha, S.S.N. & P. Kumar 1971. Cytological studies in some varieties of Cajanus cajan (Linn.). J. Cytol. Genet., 6:18-24.

Skinner, F.A. 1977. An evaluation of the Nile Blue test for differentiating rhizobia from Agrobacteria. J. Appl. Bacteriol., 43:91-98.

Smith, F.E. 1951. Tetrazolium salt. Science,113:751-754.

Smith, S.E. & M.J. Daft 1977. Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal Medicago sativa. Aust. J. Plant Physiol., 4:403-413.

Smith, S.C., S. Johnson, J. Andrews & A.Mc Pherson. 1982. Biochemical characterization of canavalin, the major storage protein of Jack bean. Plant Physiol., 70:1199-1209.

Somogyi, M. 1952. Estimation of sugars. J. Biol. Chem., 185:19-23.

Sprent, J.I. 1979. The biology of nitrogenfixing organisms. Mc Graw-Hill, London.

Srinivasa, H.P., G. Oblisami & G. Rangaswami 1972. Microflora of finger millet and rice seeds. Mys. J. Agric. Sci., 6:271-284.

Stace, C.A. 1965. Cuticular studies as an aid to plant taxonomy. Bull. Br. Nat. Hist., 4(1):1.78.

Stebbins, G.L. JR. 1950. Variation and evolution in plants. Columbia University Press, New York.

Stebbins, R.L., D.H. Dewey & V.E. Shull. 1972. Calcium crystals in apple stem, petiole, and fruit tissue. Hort. Sci., 7: 492-493.

Stevens, R.A. & E.S. Martin. 1977a Ion-adsorbent substomatal structures in Tradescantia pallidus. Nature, 268:364-365.

Stevens, R.A. & E.S. Martin 1977b The morphogenesis of substomatal structures in Polypodium vulgare. Can. J. Bot., 55:2873-2878.

Stevenson, G.B. 1953. Bacterial symbiosis in some New Zealand plants. Ann. Bot., 17:343-345.

*Stillmark, H. 1889. Uber ricin. Arch. Pharmakol. Inst. Dorpat., 3:59.

Subba Rao, N.S., K.V.B.R. Tilak & C.S. Singh. 1986. Dual inoculation with Rhizobium sp. and Glomus fasciculatum enhances nodulation, yield and nitrogen fixation in chickpea (Cicer arietinum Linn.) Plant & Soil, 95:351-359.

Subba Rao, N.S. 1988. Biofertilizers in Agriculture. Oxford and IBH Publishing Co. Pvt, Ltd. New Delhi.

Sumner, J.B. 1919. The Globulins of the Jack bean, Canavalia ensiformis. J. Biol. Chem., 37:137-142.

Sumner, J.B. & S.F. Howell 1936. The identification of the hemagglutinins of the Jack bean with concanavalin A. J. Bacteriol., 32:227-237.

Thomas, M. & H. Beevers 1949. New Phytol., 48:421-447.

*Tilton, V.R. 1978. A developmental and histochemical study of the female reproductive system in Ornithogalum caudatum Ait. using light and electron microscopy. Ph.D. Dissertation. Iowa State University, Ames.

Tjio, J.H. & A. Levan 1950. The use of Oxyquinoline in chromosome analysis. Ann. Estae. Exp. Aula. Dei., 2:21-64.

Toms, G. C. & A. Western 1971. In:Chemotaxonomy of the Leguminosae. Ed. Harborne, J.B., D. Boulter & B.L. Turner. Academic Press, London and New York.

Trinick, M.L. 1982. Nitrogen fixation. In:Rhizobium Ed. Broughton, W.J. Clarendon Press.

*Tschiersch, B. 1959. Uber canavanine. Flora, 147:405-416.

Turner, B.L. & J.B. Harborne 1967. Distribution of canavanine in the plant kingdom. Phytochemistry, 6:863-866.

Tutin, T.G. 1958. In: Nutrition of the legumes. Ed. Hallsworth, F.G. Butterworths. London.

Vickery, H.B. & M.D. Abrahams 1950. The metabolism of the organic acids of tobacco leaves. III. J. Biol. Chem., 186:411-416.

*Vieitez, E. 1952. El uso del cloruro 2,3,5-triphenyl tetrazolium para determinar la vitalidad del polen. Ann. Edafol. Fisiol. Veg., 11:297-308.

Vincent, J.M. 1970. A manual for the practical study of root-nodule bacteria. Blackwell Scientific Publications, Oxford and Edinburgh.

Vincent, R.F. & H.T. Horner JR. 1980 Calcium oxalate crystals in plants. The Bot. Rev. 46(4):361-427.

Virginia, R.A., L.M. Baird, J.S. LA Favre, W.M. Jarell, B.A. Bryan & G. Shearer 1984. Nitrogen fixation efficiency, natural ^{15}N abundance, and morphology of mesquite (Prosopis glandulosa) root nodules. Plant & Soil, 79:273-284.

Volcani, B.E. & E.E. Snell 1948. The effect of arginine and related compounds on the growth of bacteria. J. Biol. Chem., 147:893-902.

Ustimenko-Bakumovsky, G.V. 1983. Plant growing in the tropics and subtropics. 1st edn. Mir Publishers, Moscow.

Walker, J.B. 1955. Canavanine and homoarginine as antimetabolites for arginine and lysine in yeast and algae. J. Biol. Chem., 212:207-215.

Walkley, A. & I.A. Black 1934. An examination of the Detjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci., 37:29-38.

* Wattendorff, J. & H. Schmid 1973. Prufung auf perjodat-reaktive Feinstrukturen in den suberinisierten Kristallzel-Wanden der Rinde von Larix and Picea. Z. Pflanzenphysiol., 68:422-431.

Wellendorf, M. 1966. The microscopic structure of some papiliona - ceous starches II. Bot. Tidsskr., 62(1):50-56.

White, J.A. & M.F. Brown. 1979. Utrastructure and X-ray analysis of phosphorus granules in a vesicular-arbuscular mycorrhizal fungus. Can. J. Bot, 57: 2812 - 2818.

Wilson, J.K. 1939. A relationship between pollination and nodulation of the leguminosae. J. Am. Soc. Agron., 31:159-170.

*Wilson, V.K. 1926. J. Amer. Soc. Agron., 18:280.

*Yadav, N.K. & S.R. Vyas 1971. Ind. J. Microbiol., 11(2):97.

Vaishya, U.K. & G.R. Gajendragadkar 1982. Effect of rhizobial inoculation on nodulation and yield of different genotypes of urid (Vigna mungo (L.) Wilzek) Ind. J. Microbiol., 22(2):132-134.

Yamauchi, D. &T. Minamikawa 1987. Synthesis of canavalin and concan-

avalin A in maturing Canavalia gladiata seeds. Plant Cell. Physiol., 28(3):421-430.

Zindler-Frank, E. 1975. On the formation of the pattern of crystal idioblasts in Canavalia ensiformis DC. VII Calcium and oxalate content of the leaves in dependence of calcium nutrition. Z. Pflanzenphysiol., 77:80-85.

*Literature not Cited.

SYNOPSIS

S Y N O P S I S

The genus Canavalia DC. belongs to the subfamily papilionoideae of the family Leguminosae and includes 51 species (Sauer, 1964) widely distributed throughout the tropics of the Old World and the New World; Islands of the West Indies and South Pacific (Allen & Allen, 1981). The generic name Canavalia is the latinized version of Kanavala, the vernacular Malabar Indian name for a plant species, presumably Canavalia maritima (Aubl.) Thou. (Sauer, 1964).

Two species extensively cultivated in India are C. ensiformis (L.) DC., Jackbean, and C. gladiata (Jacq.)DC, Swordbean. Both these species are closely related, robust, high yielding and produce nutritious pods, containing protein-rich seeds. They are capable of providing food in marginal areas where other pulses fail. Young seeds and pods of both these species are cooked and eaten as vegetables, but once they mature and become hard, they are not delectable. This is mainly due to the presence of growth inhibiting compounds. These are proteins Canavalin, Concanavalin A and B, the enzyme Urease, and the amino acid Canavanine. However, the dry, fully mature seeds are also edible, provided they are extensively boiled with one or two changes of boiling water and peeling of the seed coat. They can also be detoxified by fermentation to "tempeh", as is done with soybeans in Asia. The other species C. virosa is found growing wild in India and is non-edible.

All these species are grown as green manure and as cover and rotation crop because of their rapid and heavy growth, semi-drought-resistance, deep-

rooted habit, and shade tolerance. They serve as excellent soil-binders and soil-improvement plants. Nutritionally the foliage appears acceptable, but cattle find it coarse and unpalatable.

All the species are an important source of Lectins,Urease and Gibberellins. Recently, Con A has been used by biochemists to isolate blood group substances (immunoglobulins and glycoprotein), thus become an important tool in medical analysis. There is now speculation that Con A is a plant antibody (lectin) that protects these beans against diseases caused by mircoorganism infections. The Urease extracted from Canavalia species is used in analytical laboratories. It converts urea into carbon dioxide, water and ammonia, and is used as a reagent for determining urea concentrations.

The genus Canavalia also has a great pharmacological importance.Canavalia ensiformis has a cooling, denulcent, antibilious and cordial action, while C. virosa has a narcotic action. Decoction of leaves is taken for nervous disorders in Honduras. Throughout Polynesia, the orchid-colour maunaloa flowers of Canavalia species are strung into ornamental garland leis.

In Goa, all the three Canavalia species viz, C. ensiformis, C. gladiata and C. virosa are found growing luxuriently.

A search through available literature, however, indicates that very little work has been done on these plants in the field of Taxonomy (Sauer, 1964); Cytology (Bhandari et al., 1969); nodule morphogenesis (Narayana & Gothwal, 1964); Pathology (Singh & Shrivastava, 1979); Toxicology (Downum et al., 1983; Ghosh et al., 1985); and Chemical analysis (Molina & Bressani, 1974).

The present work is divided into five parts:

PART I - deals with Mitosis and Karyotype analysis; Epidermal features; Starch grain morphology; Palynology and pollen physiology in Canavalia species.

PART II - deals with nodulation studies in C. ensiformis.

PART III - deals with agronomic studies in Canavalia species.

PART IV - deals with mycoflora and diseases of Canavalia species.

PART V - deals with toxicological and chemical analysis of Canavalia species.

PART I A. MITOSIS AND KARYOTYPE ANALYSIS

Somatic counts of three Canavalia species viz.,C. ensiformis, C. gladiata and C. virosa have been found to be 2n = 22. One pair of SAT-chromosome has been found in C. gladiata. Mitosis was found to be normal in all the three species. The total chromatin length has been calculated in all the three species.

PART I B. EPIDERMAL FEATURES

The leaves of all the three Canavalia species are amphistomatic, the stomata being predominantly paracytic associated with anisocytic type of stomata. Stomatal frequency and stomatal size varied in the three species. No ion-adsorbent substomatal structures were found.

The crystalliferous cells containing two crystals separated by a median wall (rarely three) usually occurs singly among the epidermal cells. The frequency of crystalliferous cells was more in the leaves, followed by anthers and pods.

Non glandular type of trichomes with pointed tips were found on the leaf surfaces of all the three Canavalia species.

PART I C. STARCH GRAINS -

Starch grain studies were carried out under ordinary and polarized light microscope. Morphology of starch grains in all the three Canavalia species has been studied. Besides this, other important characteristics of starch grains studied include dimensions, pH, gelatinization temperature, blue value and iodine absorption spectra.

PART I D. PALYNOLOGY AND POLLEN PHYSIOLOGY

Pollen morphology in all the three species has been studied in detail. In C. ensiformis, the pollen grains were 3-zonoporate, while in C. gladiata and C. virosa they were syncolporate.

Pollen viability has been calculated in all the three Canavalia species. Maximum pollen viability was observed in C. ensiformis (78.67%), while it was 66.16% in C. gladiata and 59.5% in C. virosa.

In vitro germination percentage of pollen grains by using different concentrations of sucrose have been studied in all the three Canavalia species. In C. ensiformis,maximum germination percentage (69.14%) was recorded in 15% sucrose solution, while in C. gladiata (60.26%) and C. virosa (54.28%) was recorded in 10% sucrose solution.

PART II NODULATION STUDIES -

Various morphological features of the nodules in C. ensiformis such as position on the root system, shape, colour, surface etc.were critically studied. A large number of microtome sections (10-12 μm) from histological preparations, as well as handcut sections were studied under light microscope.

It has been found that nodules in C. ensiformis were of determinate type. Nodulation did not occur on primary root. Both effective and ineffective nodules were observed.

The mature nodule was differentiated into an inner bacteriod and an outer cortical region. Nearly 70% of the cells of central tissue were infected with bacteriods.

In all a total of 23 isolates were isolated from the root nodules of C. ensiformis. Various morphological and biochemical tests were performed by using an authentic host to confirm the rhizobial cultures. Four morphologically and biochemically diff-

erent strains viz. CE-1, CE-2, CE-3 and CE-4 of rhizobia were selected and these were then used to screen out for the most effective strain for C. ensiformis.

The study revealed that all the four strains were not equally efficient. The rhizobial strain CE-1 was most efficient as it showed maximum number of nodules, plant biomass and total yield.

PART III A SOIL ANALYSIS

Soil samples were collected from different localities of Goa where Canavalia plants were found growing. Physical and chemical analysis of the same were carried out.

PART III B. VESICULAR-ARBUSCULAR (VA) MYCORRHIZAL STUDIES

Vesicular-arbuscular mycorrhizal studies have been carried out in two Canavalia species viz., C. ensiformis and C. gladiata. For C. ensiformis, two mycorrhizal isolates viz., Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. and Trappe, and Gigaspora margarita Backer and Hall were tried, while for C. gladiata three mycorrhizal isolates viz., Glomus fasciculatum (Thaxter sensu Gerd.) Gerd. and Trappe; Gigaspora margarita Becker and Hall, and Glomus albidum Walker and Rhodes were tried. The effect of VAM-Rhizobium interaction has also been studied.

The study revealed that in both the species viz., C. ensiformis and C. gladiata, dual inoculated plants with VAM and

Rhizobium recorded highest percentage of mycorrhizal infection and higher number of spores in the root zone soil.Dual inoculated plants showed better growth and yield. Mycorrhizal infection resulted in increased uptake of phosphorus and other micronutrients i.e. Zinc, Manganese, Copper and Iron. Overall, the study showed that dual inoculation with selected VA-mycorrhiza and Rhizobium will help the growth of C. ensiformis and C. gladiata plants in phosphate deficient soils.

PART III C. GROWTH RESPONSE OF C. GLADIATA PLANT IN MINE REJECTS -

Mine reject and cultivated soils were analysed for total bacterial population. The results showed that the total bacterial population in the mine reject is very low (6.89×10^{4}/g), while it was much higher in cultivated soils (60.80×10^{6}/g).

Plants grown in 100% reject showed poor growth and nodulation. Addition of various levels of Farm Yard Manure (FYM) to the reject brought about the much desired changes in the plant growth. Among the different levels of FYM used, it was observed that 30% FYM gave best results in terms of vegetative and reproductive growth. Since C. gladiata plants survived in the mine rejects, it can possibly be used in mine waste revegetation programmes.

PART IV A. SEED MYCOFLORA

Nine distinct isolates of fungi were recorded in C. ensiformis and C. gladiata. All these nine fungal specimens were identified at the Commonwealth Mycological Institute (CMI) Kew, England.

Fungal count was higher in internal flora than on the external flora. The genus Aspergillus was found to be dominant in the seeds of both the species.

PART IV B. FRUIT ROT DISEASE OF C. ENSIFORMIS

A new disease, which predominantly occures during the monsoon season (June to September) and brings about the decay of pods, thus reducing the yield considerably has been reported for the first time. The casual organism was identified and confirmed at the International Mycological Institute (CMI) Kew, England and was found to be Fusarium solani (Mart.) Sacc. Pathogenicity test was performed and was found positive.

PART V. TOXICOLOGY AND CHEMICAL ANALYSIS

Canavanine has been detected in three Canavalia species viz., C. ensiformis, C. gladiata and C. virosa. Distribution of canavanine with regards to its amount, location and fluctuations at various stages of development have been studied in detail in all the three species. The study revealed that during germination canavanine in the cotyledons is translocated to the developing leaves, which perhaps explains the increase in the leaf canavanine level. The overall decrease in the level of canavanine during germination indicates its degradation and utilization in the developing plant.

All the three Canavalia species were analysed for TAN, Proline, Fat, Protein, Total soluble sugars, Starch, Fibre, Ash and Inorganic metals.

REFERENCES

Allen, O.N. & E.K. Allen 1981. The Leguminosae : A source book of characteristics, uses, and nodulation. 1st edn. The University of Wisconsin Press, Madison.

Bhandari, N. N., S. L. Tandon & S. Jain 1969. Some observations on the cytology and cytomixis in Canavalia DC. Cytologia, 34:22-29.

Downum, K. R., G. A. Rosenthal & W.S. Cohen 1983. Arginine and L-canavanine metabolism in Jackbean, Canavalia ensiformis (L.) DC. and soybean, Glycine max (L.). Plant Physiol., 73:965-968.

Ghosh, B. N., B. Dasgupta & P. K. Sircar 1985. Lectin Concanavalin A distribution at different stages in the tissues of Canavalia gladiata. Curr. Sci., 54:80-82.

Molina, M. R. & R. Bressani 1974. Protein-starch extraction and nutritive value of the Jackbean protein isolate. In : Nutritional aspects of common beans and other legume seeds as animals and human foods. Ed. Jaffe, W. G.

Narayana, H. S. & B. D. Gothwal 1964. A contribution to the study of root nodules in some legumes. Proc. Ind. Acad. Sci., B, Vol. LIX : 351-359.

Sauer, J. 1964. Revision of Canavalia. Brittonia, 16:106-181.

Singh, P. K. & A. K. Shrivastava 1979. Fruit rot disease of Canavalia ensiformis DC. - A new record. Curr. Sci., 48(4) :168-169.