RE-CLASSIFICATION OF THE TRIBE PSORALEAE USING MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERS

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Introduction

Legumes have long been associated with food or forage, due to their ability to nodulate and fix N_2 (Sprent, 2001). The positive attributes of legumes have been known for centuries, like for example in some parts of tropical America, beans from the genus *Phaseolus* have been domesticated and grown with other crops such as maize for about 6000 years (Sprent, 2001). Moreover, it was also found that nomadic peoples of Africa and Australia have long used subterranean tubers of legumes as a vital part of their diet in hot dry periods (Sprent, 2001). Owing to the general wealth of legumes, a proper understanding and classification of the different species is important for better management of these species for sustainable exploitation and protection of biodiversity (Sprent, 2001).

Legumes have been placed in the family Leguminosae (Fabaceae). This family is subdivided into three subfamilies, namely Caesalpiniodeae, Mimososideae and Papilionoideae (Sprent, 2001; & Vinuesa, 2001). The family Leguminosae is the third largest family of flowering plants, with about 650 genera and more than 18000 species (Vinuesa, 2001). Legumes are geographically widely spread ranging from tropical to temperate countries (Sprent, 2001), with diverse growth forms ranging from canopy trees, lianas and shrubs to acquatic plants and tiny annual herbs (Vinuesa, 2001). The success of legumes has largely been attributed to their ability to form symbiosis with soil bacteria namely rhizobia, resulting in formation of nodules in which reduction of atmospheric nitrogen occur (Sy *et al.*, 2001). However not all members of the Fabaceae nodulate (Van Berkum & Eardly, 1998). Nodulation is thought to have arisen independently in the three subspecies, with an estimated 97% of species in the subfamily Papilionoideae, 90% in the subfamily Mimososideae and 23% in the subfamily Caesalpiniodeae (Vinuesa, 2001). Nodulation has long been used as a tool to identify and classify species of Leguminosae but more recently other tools such as molecular data have also been used to identified and classified species of this family. The subfamily Papilionoideae has been studied the most because members of that family are largely crop species (Sprent, 2001; & Sprent & Parsons, 1999), and also contains all the known types of nodules such as determinate or desmodioid nodules (as in cowpea), indeterminate nodules (as in M*edicago*), aeschnomenoid nodules (as in *Arachis*) and lupinoid nodules (as in *Lupinus*) (Sprent,

2001). One of the most commonly known member of the subfamily Papilionoideae is the tribe Phaseoleae.

Members of the tribe Phaseoleae are easily recognised on the basis of their tropical biogeograpic origin, broad-host nodulation characteristics, route of rhizobium entry into legume, determinate nodulation phenotype and internal nodule anatomy, xylem composition and transportable solutes of fixed nitrogen, site of nitrate reductase activity and metabolic response of N_2-fed plants to nitrate supply (Dakora, 2000). As said earlier, tropical legumes such as cowpea (*Vigna unguiculata* (L.) Walp) and common bean (*Phaseolus vulgaris*. L.) possess determinate or desmodioid nodules. The morphology and internal anatomy of a spherical determinate nodule is shown in Figure 1.

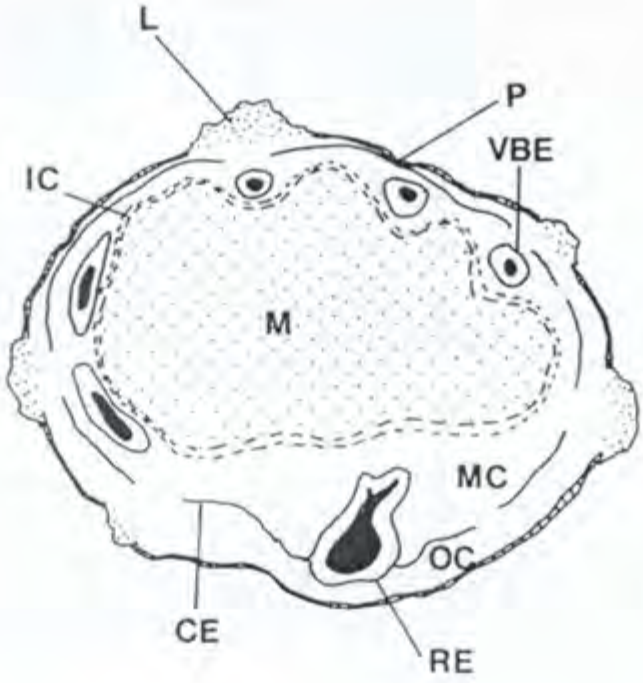


Figure 1: Schematic diagram of a transverse section of a spherical determinate legume root nodule (Dakora & Atkins, 1989). L, lenticels; P, periderm; M, medulla consisting mainly of infected cells; RE, root endodermis; VBE, vascular bundle endodermis; CE, common endodermis; OC, outer cortex; MC, middle cortex; IC, inner cortex.

According to Dakora (2000), tropical legumes differ from temperate legumes by lacking a vascular transfer cells, persistent meristem and vacuoles infected cells with calcium oxalate crystals in the outer cortex and diploid cells as opposed to tetraploids in indeterminate nodules (all of which are characteristic of temperate legumes). This distinct morphological structure (nodules) of tropical grain legumes offer an opportunity for their use as a taxonomic tool in systematic studies of the Leguminosae according to Dakora (2000). Moreover, analysis of the organic products of N_2-fixation in members of Phaseoleae has shown that they uniquely export fixed nitrogen in the form of ureides (i.e. allantoin and allantoic acid) (Dakora, 2000). Furthermore, it has also been suggested that ureides are a more efficient form of transporting nitrogen compared to amides (which are produced by temperate legumes), as they use less carbon per nitrogen, the ratio of C: N = 1 (Xian-Guo *et al*, 2000 & Dakora, 2000).

A recent study by Doyle (1998), based on *rbc*L sequencing, has provided a more recent classification of the different members of the three subfamilies of Leguminosae including the different types of nodules they produced (Fig 2).

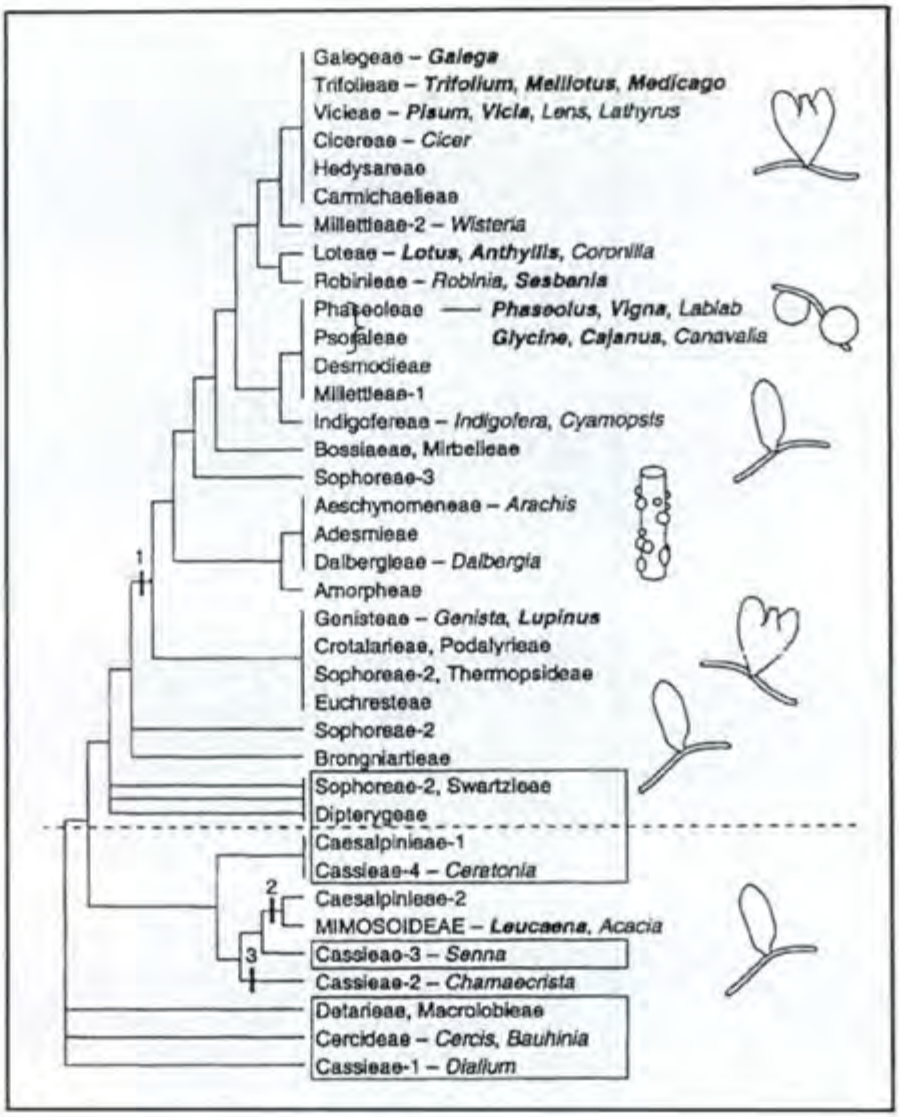


Figure 2: Phylogeny of Leguminosae, as hypothesized from rbcL sequence data (Doyle, 1998). All groups above the broken line belong to the largest subfamily Papilionoideae; below the line are Caesalpiniodeae and Mimosoideae. According to Doyle (1998), groups in boxes are not known to nodulate. The different nodule types also appear next to some of the groups in which they occur. Unbranched, elongate, indeterminate nodules are 'caeslpinioid; branched, indeterminate nodules are 'astraggaloid'; large, round, determinate nodules are 'desmodioid'; small, clustered nodules are ' aeschynomenoid' (Doyle, 1998).

According to Doyle (1998), an assessment of the major nodule types in the Leguminosae showed that the unbranched, indeterminate 'caesalpinioid' nodule type is scattered throughout the family and is notably present in Mimosoideae, Caesalpinioideae and basal Papilionoideae (Fig 2). Doyle (1998) also pointed out that the "distribution is consistent within a single origin of nodulation in the family, with the caesalpinioid nodule type evolving in the common ancestor of all nodulating legumes". Furthermore, Fig 2 also shows that within the Papilionoideae, the basic caesalpinioid nodule type has been modified to produce the determinate 'desmodioid' nodules of many Phaseoleae, such as soybean (*Glycine max*) and the common bean (*Phaseolus vulgaris*) (Doyle, 1998). Doyle (1998) also proposed that this new nodule type (determinate nodule) is responsible for biochemical innovation, like in the Phaseoleae where fixed nitrogen is transported in the form of ureides instead of the amide, which are characteristic of members of the other tribes, most of which have indeterminate nodules. Thus, nodule morphology can be a useful taxonomic tool.

From the phylogenetic tree generated by Doyle (1998), the tribes Psoraleae, Desmodieae and Millettieae are related to the tribe Phaseoleae. Consequently, it would be interesting to see whether these tribes share common morphological, anatomical and physiological features with the tribe Phaseoleae (Dakora, 2000). This is precisely the aim of the project although for the purpose of this project only Psoraleae species namely *P. pinnata*, *P. aculeata* and *P. aphylla* were examined.

The name Psoraleae was given by Linnaeus, from the Greek psoraleos, meaning mangy or warty, which apparently refer to the dots or little excrescences (glands) sprinkled over the leaves and stems (Jackson, 1982). This tribe includes shrubby plants often growing along stream banks and thin-stemmed herbs with insignificant flowers. Members of this tribe form root nodules as a result of symbiosis with soil bacteria. The aim of this study was to examine nodule morphology, anatomy and ultrastructure of *P. pinnata* as well as study the nature of fixed-N products transported in xylem of Psoraleae and determine their relatedness with the tribe Phaseoleae.

Materials and methods

Description of species

The species used for this study include *P. pinnata*, *P. aculeate*, *P. aphylla*. These species were collected from Betty's Bay – Harold Porter Botanical Garden.

P. pinnata – Erect, soft and hairy shrub which can grow up to a height of 1.5-3 m. This plant has a stout woody stem and densely leafy towards the ends of the branches (Kidd, 1983). Leaves are mostly narrow with linear leaflets. Flowers are sweetly scented (Jackson, 1982). Species used for this study were mostly collected from along a stream, which ran through the garden as well as from Silvermine near Cape Town. Nodules collected from these species had many lenticels especially those growing near waterlogged areas.

Figure 3A: Photograph of P. pinnata taken from Silvermine showing hairy shrubby plants with densely leafy towards the ends of the branches

P. aculeate – This is a densely leafy aromatic shrub, which can grow up to about 1m high. Leaves are trifoliolated and leaflets are folded and hooked at their tips (Jackson, 1980). They generally grow on hill and mountain slopes. Flowers are solitary and violet in colour and are packed close to each other and to their corresponding leaf branches (Jackson, 1980). Species for this study were collected in the Garden at Bettys Bay on the slope of the mountain; Lots of nodules were present but with fewer lenticels.

Figure 3B: Densely leafy, violet-flowered pea, P. aculeate (Jackson, 1980)

P. aphylla – An erect more or less pubescent shrub with virgate grooved branched. Leaves are distant, simple ovate-acuminate with silvery hair (Kidd, 1983). The plant consists of single flowers found on long drooping stems flowers are normally blue in colour and the petals are surrounded by a densely black calyx (Jackson, 1982). The species was collected around Betty's Bay and not in the garden. The species was found near marshy places and possess lots of nodules.

Figure 3C: P. aphylla with blue flowers on drooping stems (Jackson, 1982)

Samples collection

Leaves, stems and roots of the three species were oven dry at a temperature of 70°C for 12 days, after which the different organs were ground using a mill. Part of the ground samples together with the nodules harvested from the different species were used for the ureide assay while the other part was used to run the mass spectrometer.

Fresh nodule samples from the species *P. pinnata*, were collected from Silvermine for light and the transmission electron microscope. Two types of nodules were collected, one with lots of lenticels since the plants from which the nodules were harvested grow near a streamr and the other one with fewer lenticels, plants come from upland.

Ureide Assay

To assess whether *Psoralea* species produce ureides, efforts were made to collect xylem sap from field plants of *P. pinnata* using a mild vacuum pump. Detopping these plants failed to produce any xylem sap. Consequently, nodule extracts or hot water extracts of shoots were used for ureide assay. The assay was carried out following the procedure described by Dakora *et al* (1992). 0.2 ml of hot water tissue extract was placed into different test tubes and diluted to 2.5 ml with distilled water. Then 0.5 ml of 0.5 M sodium hydroxide was added, mixed and placed in a boiling water-bath for 10-15 minutes. After this time interval, the test tubes were removed and allowed to cool at room temperature. Following the addition of 0.5 ml of 0.65 M HCl and 0.5 ml of phenylhydrazine solution, the contents were mixed again and placed back into the water-bath for 2-4 minutes. The test tubes were then removed and immediately placed in an ice-bath for 15 minutes. Once cooled the test tubes were then removed and 2 ml of concentrated HCl was added followed by 0.5 ml of potassium ferricyanide and immediately mixed. The optical density was read on a spectrophotometer (Spectronic 20 Genesys) at 525 nm after staying for 10 minutes at room temperature. The total ureide contents of each sample were determined from a curve obtained from allantoin standards (included in the assay different allantoin concentrations, use were 10, 20, 30, 50 & 100 nmole/ml).

Microscopy

Light microscope

Fresh nodules harvested from Silvermine were fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer at pH 7.4. Free-hand sections of some nodules were done. Sections and whole nodules were stained with toluidine blue solution (1% toluidine blue and 1% borax). Some whole nodules were left unstained. These different samples were then viewed under the light microscope (Litz diaplan misroscope & Wild M400 Photomicroscope) and photographed.

Transmission Electron Microscope (TEM)

Fresh nodules harvested from *P. pinnata* (both from stream and upland) were sectioned and fixed in 2.5% gluteraldehyde in 0.1 M phosphate buffer at pH 7.4 and left overnight. Post-fixation was 1% osmium tetraoxide in phosphate buffer. After which the sections were stained in 2% aqueous uracyl acetate followed by dehydration of the tissues through a series of increasing alcohol concentrations (30%, 50%, 70%, 80%, 90%, 100% ethanol – 2 times & 100% acetone – 2 times). Following dehydration, sections were infiltrated (1:1 acetone: spurrs resin – 1:3 acetone: spurrs resin & pure resin) and embedded in epoxy resin for 24 hours in an oven at 60°C. Tissues were further sectioned to 1.0 μm thick using a Reichert Utracut-S (Leica) Utramicrotome, stained with uracyl acetate and lead citrate (Reynolds, 1963) and viewed with a TEMX 12000 EX II electron microscope

Mass Spectrometer

2 mg of the ground samples together with 2 mg of Nast and 0.5 mg of Mgel used as standard were measured into tin caps respectively. The measured samples were then run through a Finnigan Mat 252 mass spectrometer coupled to a Carlo Esba NA 1500 elemental analyses via a Couflo II open-split device for delta ^{15}N and % N analysis.

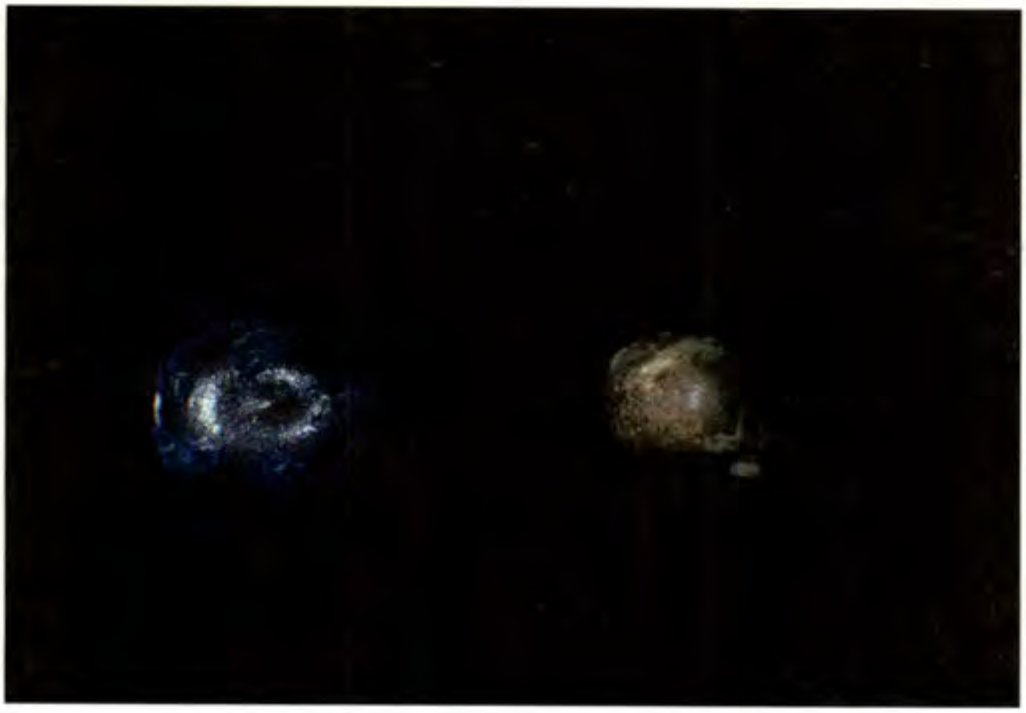
Results

Nodule morphology, anatomy and ultrastructure

Figures 4a, 4b & 4c show the nodule morphology of *P. pinnata*. These pictures show that the nodules are spherical and determinate. Lenticels can also be seen from Figure 4b & 4c. The round nodule shape is typical of the tribe Phaseoleae. Figure 4d is an example of the type of nodules formed by members of the tribe Phaseoleae. Here ~~of~~ the type of nodule formed by cowpea is shown, and clearly the nodule shape is similar for both Psoraleae and Phaseoleae tribe.

Figures 5a & 5b represents the anatomy of the nodules from *P. pinnata*. From the micrograph (Fig 5a), the medulla is made up of large infected cells, which are surrounded by the inner cortex. Moreover, the vascular bundle elements, the middle cortex and the outer cortex are also visibly seen. The nodule anatomy shown here resembles that of members of the tribe Phaseoleae such as cowpea, which is illustrated in Fig 5c. Both species have the same internal organisation. Figure 5b shows the infected and uninfected cells found in the medulla of P. pinnata nodules shot at a higher magnification (×100). Italics!

Figures 6a, 6b & 6c show the ultrastructure of the nodule. Figure 6a provides a general over-view of the infected area. Here the medulla is densely populated with bacteria. Figure 6b is a more detailed structure of the nodule. The bacteroids are clearly enclosed by a bacteroid envelope membrane and surrounded by a peribacteroid membrane. The latter separates the bacteroid from its host while allowing intensive two-way traffic of solutes and assimilates between the cytosol of the infected cell and that of the many thousands of bacteroids enclosed in the cell (Atkins, 1987). Furthermore, Figure 6b also provides an idea of the shape and size of the bacteroids. Some of the bacteroids are elongated and some are spherical in shape and from Figure 6c, it is clear that some of them contain starch granules, which function as a store of energy for the bacteroid function.



A

B

Figure 4a & 4b: Photomicrograph showing the spherical shape of the nodules of P. pinnata.

Photomicrograph A, nodules harvested from the stream possess lenticels .

Photomicrograph B, nodules harvested from upland, no lenticels present

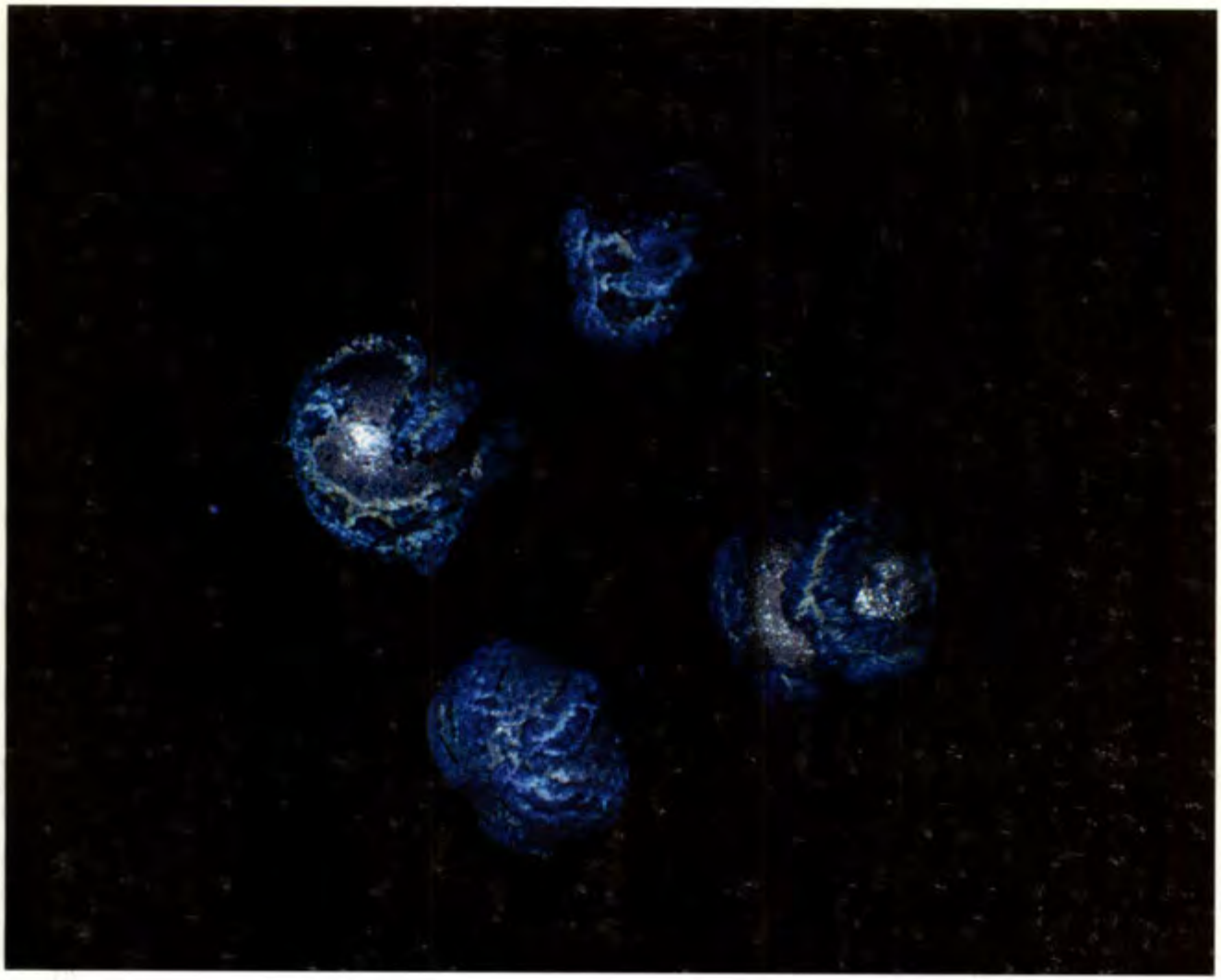


Figure 4c: Photomicrograph of nodules harvested from the stream with lots of lenticels.

Figure 4d: Micrograph of a nodule of cowpea, a typical member of Phaseoleae species with round and determinate nodule (Dakora, 1989)

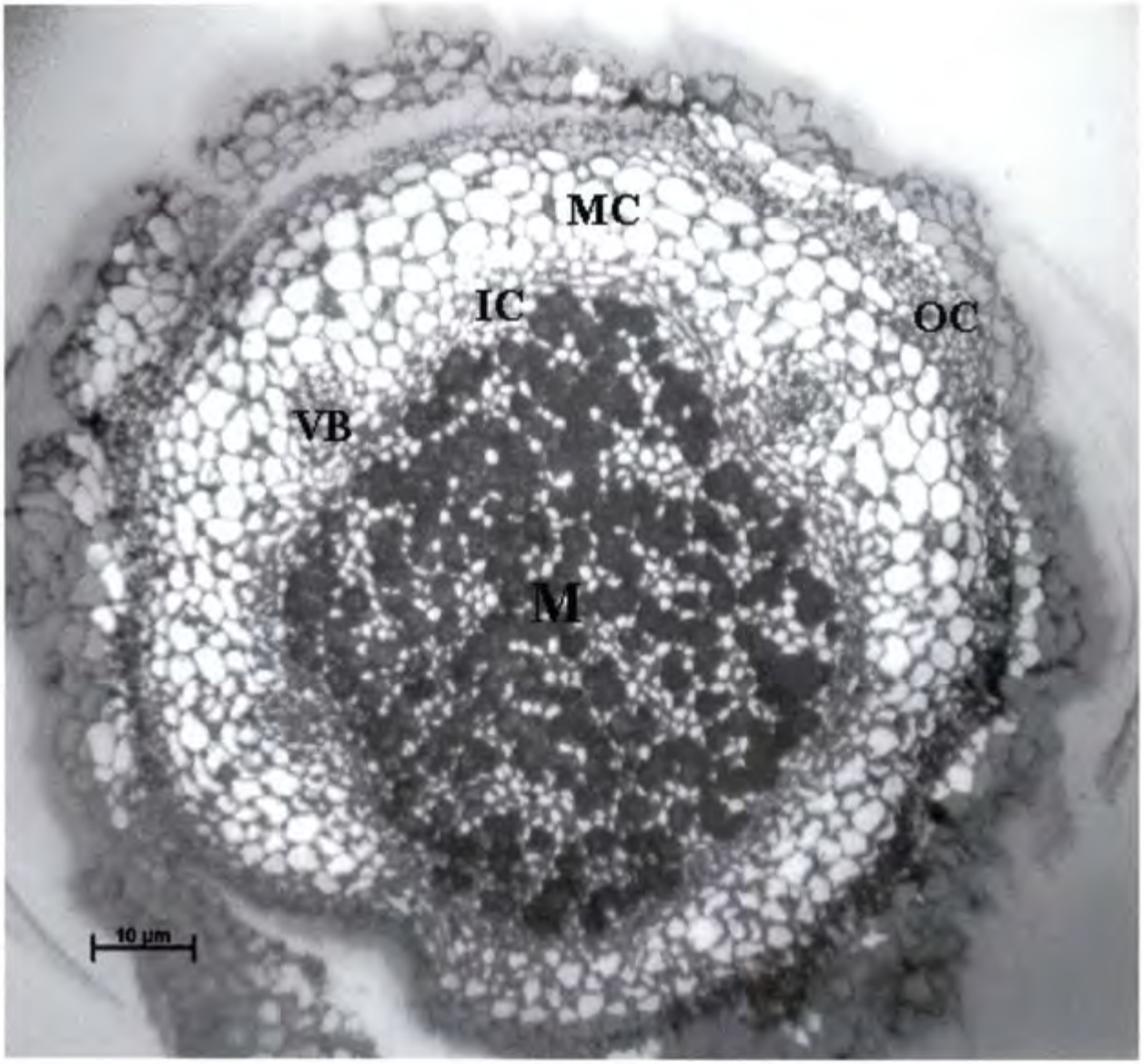


Figure 5a: Transverse section of a nodule of P. pinnata collected from Silvermine, showing the distribution of the different tissue components. M, medulla; VB, vascular bundle; IC, inner cortex; MC, middle cortex; OC, outer cortex.

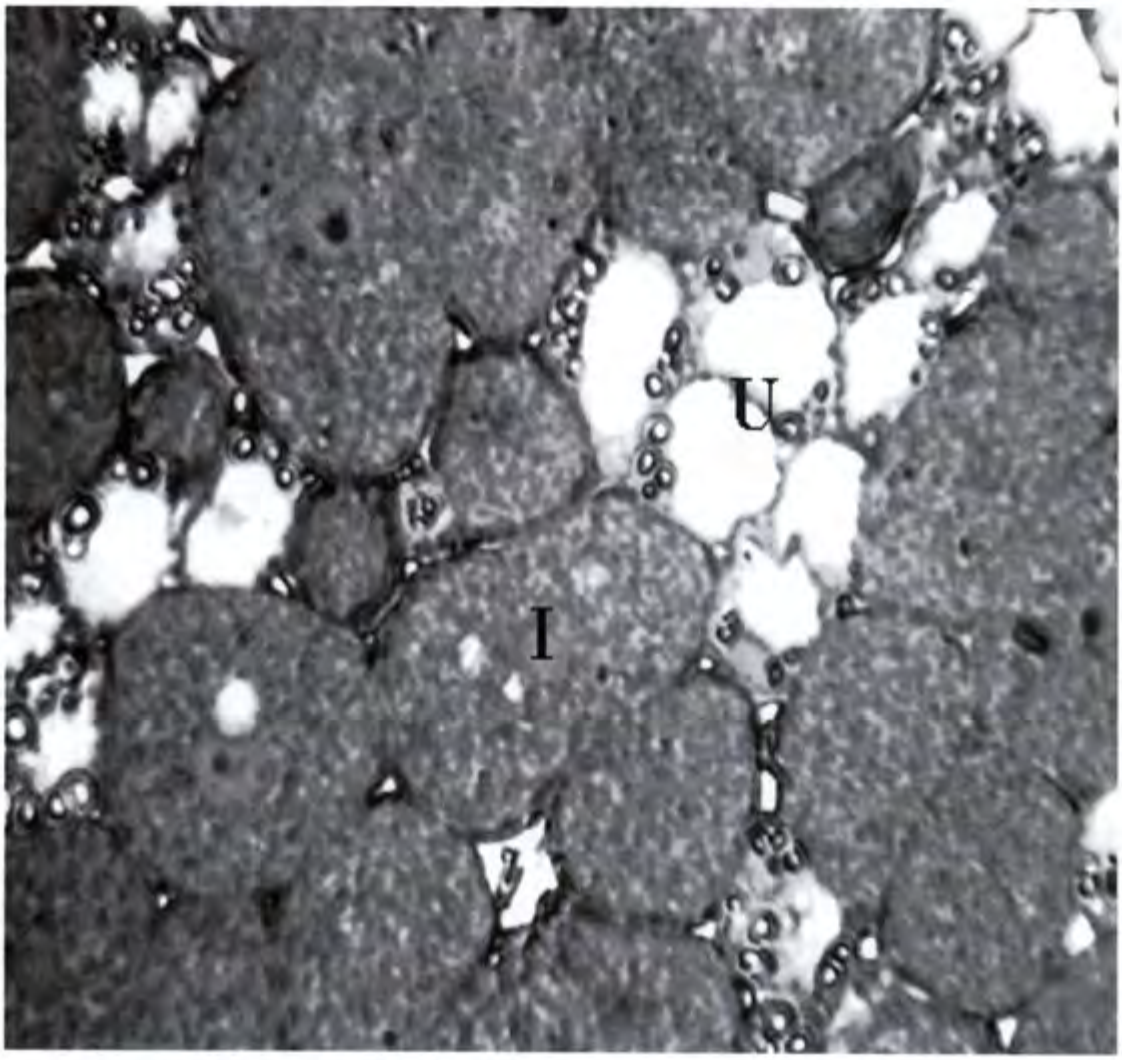


Figure 5b: Transverse section of a nodule of P. pinnata showing the medulla with the infected and uninfected cells. I, infected cells; U, uninfected cells (Magnification = × 100)

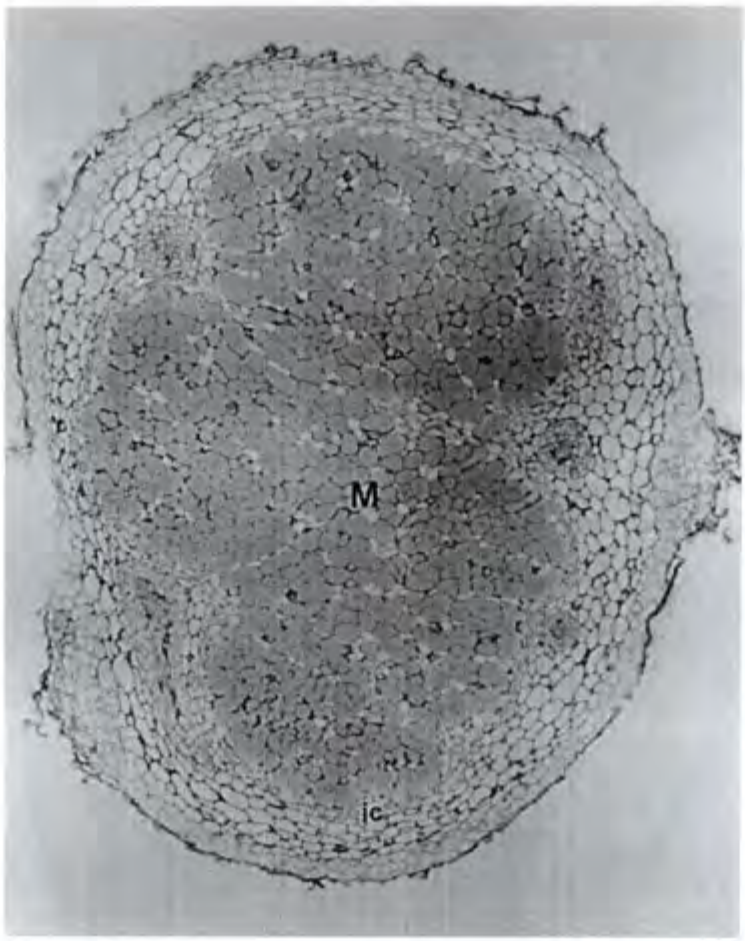
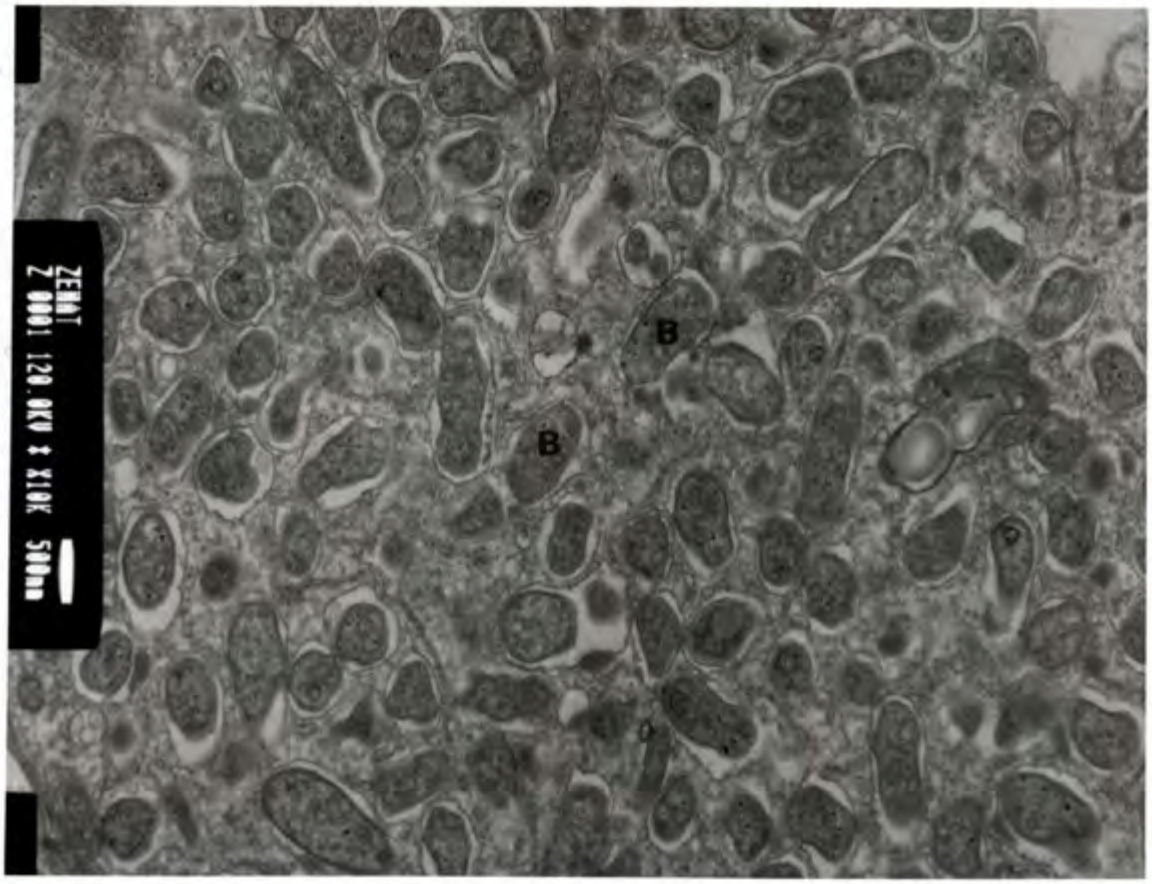
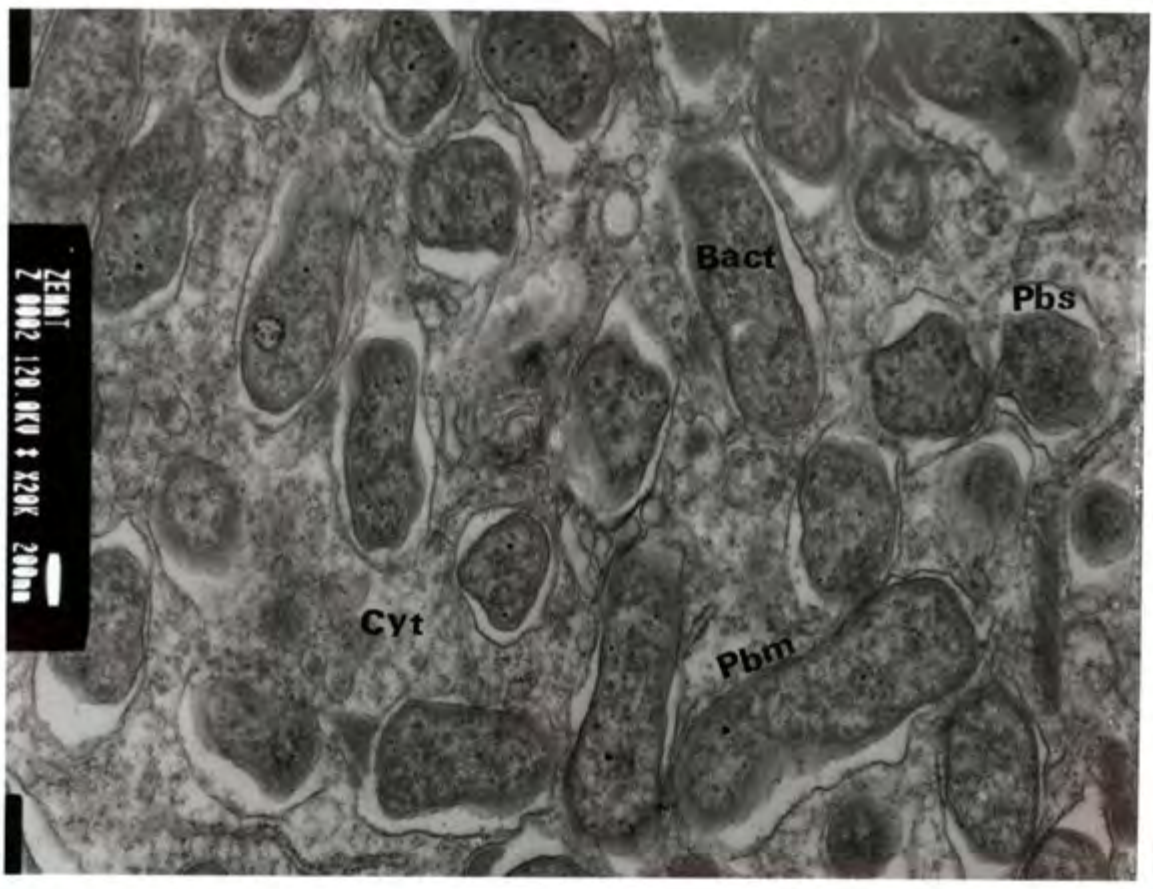


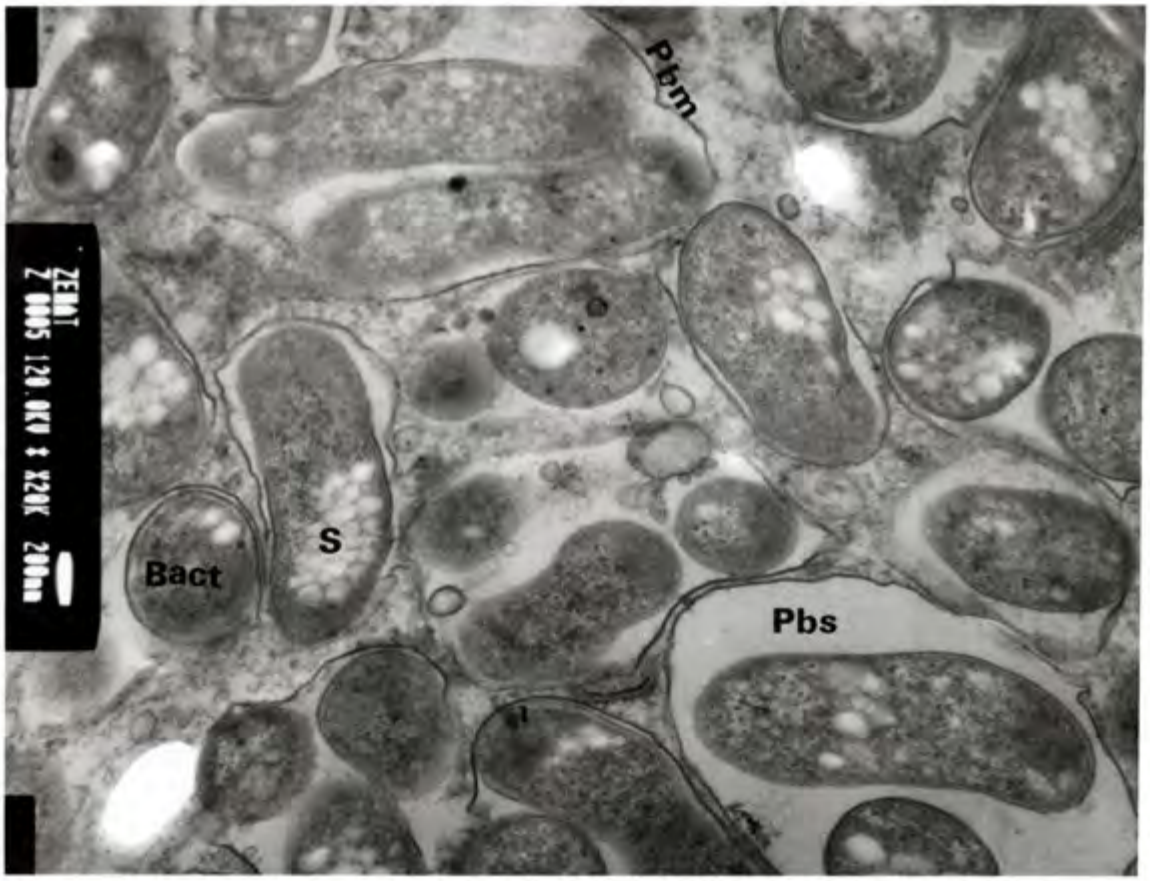
Figure 5c: Photograph of the nodule ultrastructure of cowpea (Dakora, 1989)
M – medulla, IC - inner cortex


ZEMAT
Z 0001 120.0KV 4 X10K 500nm
B
B

A


ZEMAT
Z 0002 120.0KV 4 X20K 200nm
Bact
Pbs
Cyt
Pbm

B



C

Figure 6: Electron micrograph of a nodule of P. pinnata

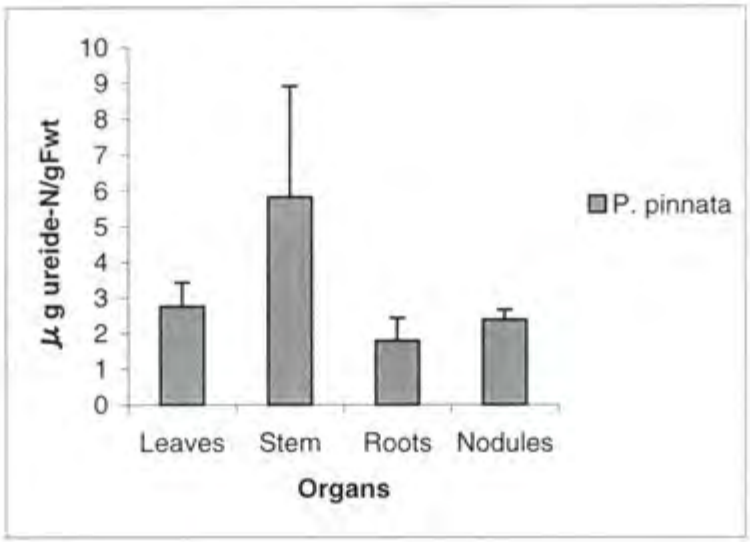
6A: An overview of the infected region, medulla densely populated with bacteria. (×500)
B, bacteria

6B & 6C: Infected area in more details (×20K)
Bact, bacteria
Cyt, cytoplasm
Pbm, peribacteriod membrane
Pbs, peribacteriod space
S, starchn granules

Ureide synthesis in nodules

The data for ureides in tissues are presented as Mean ± S. E.

A



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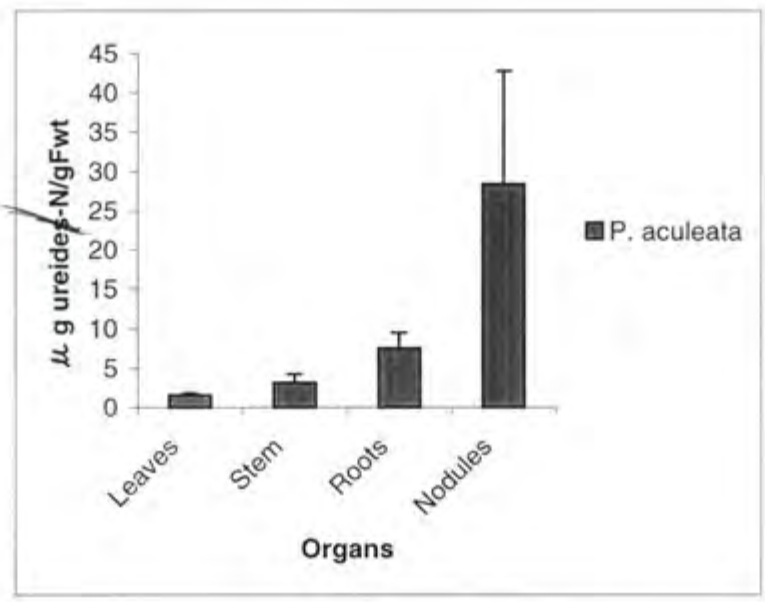

C

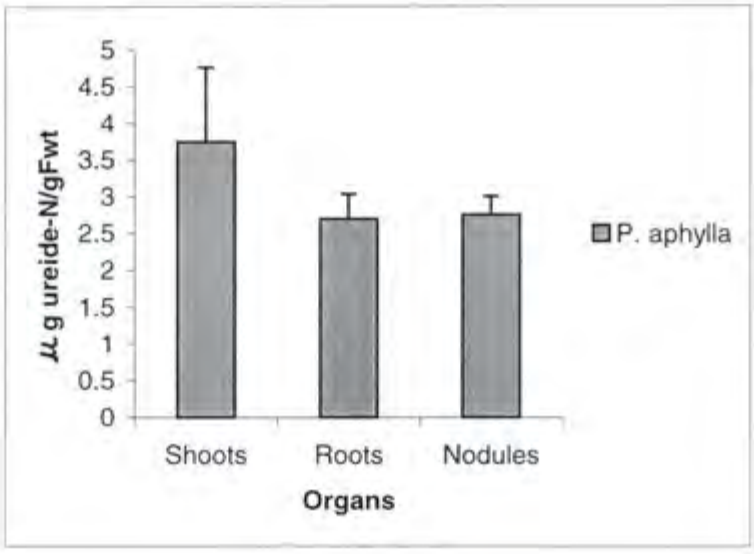

Figure 6: Concentration of ureide in the different organs; A - P. pinnata; B – P. aculeate; C – P. aphylla. Error bars are the SE of the mean.

Results obtained show that all three species used in this study produce ureide as their transportable solutes of fixed N. This is a typical characteristic of the tribe Phaseoleae. Study done by Pate *et al* (1980) on cowpea (Vigna unguiculata (L.) Walp) has shown that this species produced ureides as its transportable fixed nitrogen. Moreover the results also show that all three organs examined namely roots, stems and leaflets, ~~all had a~~ contain significant proportion of ureides although the nodulated one was much higher than the non nodulated cowpea (Pate *et al*, 1980). This is consistent with the results obtained for the study on the three *Psoralea* species mentioned above.

δ ^{15}N (‰) values

Table 1: Estimation of nitrogen fixation by the three species, *P. pinnata, P. aculeate* & *P. aphylla* using ^{15}N natural abundance. Data are Mean ± S.E.

Species	Organs	δ ^{15}N (‰)	% N
P. pinnata	Leaves	-2.106 ± 0.315	2.448 ± 0.130
	Stem	-2.400 ± 0.871	1.528 ± 0.102
	Roots	2.210 ± 3.060	2.100 ± 0.778
P. aculeata	Leaves	-1.751 ± 0.215	1.945 ± 0.060
	Stem	-1.705 ± 0.839	1.308 ± 0.033
	Roots	-0.484 ± 0.219	1.206 ± 0.043
P. aphylla	Shoots	-2.819 ± 0.167	1.639 ± 0.032
	Roots	-0.964 ± 0.308	0.826 ± 0.078

The data in Table 1 show that all three Psoraleae species have low δ ^{15}N values for their organs. These low values suggest that these species actively fix atmospheric N_2 instead of taking up soil nitrogen; these values ranged from –2.819 to 2.20.

Discussion

The tribe Psoraleae has been rearranged several times (Sprent, 2000) and the phylogenetic tree produced by Doyle (1988) indicates that this tribe is sister to the tribe Phaseoleae. However, morphological and physiological results obtained from this study clearly suggest that the tribe Psoraleae does share features related to the Phaseoleae.

Nodules morphology of *Psoralea* species is spherical in shape and determinate in growth characteristic (Fig 4). This morphological structure is typical of members found in Phaseoleae. Figure 4d gives an example of nodule morphology found in cowpea as shown by Dakora (1989). Nodule morphology of cowpea (a member of the tribe Phaseolese) is determinate and round in shape (Fig 4d, Dakora 1989), which correspond exactly to the shape of nodules found in all three *P. pinnata P. aculeata* and *P. aphylla.* Furthermore, nodules obtained from *Psoralea* species also showed the presence of lenticels (Fig 4b & 4c). It has been proposed that the function of the lenticels is to control the amount oxygen entering the cells. According to Dakora & Atkins (1989), under water stress conditions, the lenticels collapsed so as to impede the influx of gases to the infected region. This was observed in soybean and bean (*Phaseolus*) nodules and under waterlogging conditions, lenticels development is stimulated which presumably improved aeration of nodules tissues. This explains why nodules collected near the stream had more lenticels than those found further inland and further shows that they behave similarly to Phaseoleae under O_2 stress conditions. Thus, based on nodule phenotype, the evidence for relating the tribe Psoraleae to the tribe Phaseoleae is clearly apparent.

Nodule anatomy and ultrastructure (Fig 5 & 6), reveals that their internal arrangement also corresponds to that found in the Phaseoleae, cowpea (Fig 5c). The micrograph (Fig 5 & 6) shows that the nodules consist of an outer protective tissue, which presumably limit free gas exchange (Dakora & Atkins, 1989), cortical tissue and central tissue. The latter consisted of bacteroidal cells (Rhizobium-infected cells) and interstitial cells (Rhizobium-free cells). This precise location of the bacteroids is very important for the proper functioning of the enzyme nitrogenase. A more detailed structure of the nodules can be seen from Figure 6b & 6c, which shows that the bacteroids were enclosed in a membrane

shown that up to 98% of the xylem-borne N entering the shoots of nodulated cowpea plants was in the form of ureides and the highest concentration of ureides was found in the nodules. Furthermore, studies done by Reynolds *et al.* (1982) have shown that soybean, a member of the Phaseoleae, use ureides as their major transport compound. Work done by Dakora *et al* (1992) on Kersting's bean and Bambara groundnut, which belong to the tribe Phaseoleae and are tropical legumes, obtained a high concentration of ureides (> 90%) in their root xylem sap. This further supports the inclusion of the tribe Psoraleae into the Phaseoleae. Additional studies done on other members of the tribe Phaseoleae like *Phaseolus vulgaris, Phaseolus lunatus, Vigna triloba, Pueraria javanica* and *Pueraria phaseoloides,* have shown that they all have a high ureides occurrence in their xylem sap (Peoples *et al.*, 1989).

These examples further demonstrate that, since members of the tribe Psoraleae produced ureides as their major transport compound (Fig 6a, 6b & 6c), ~~clearly show that~~ they share similarities with members of the tribe Phaseoleae.

There are two nitrogen isotopes, light isotope, ^{14}N and the heavy isotope, ^{15}N. In the atmosphere, the percentage of nitrogen atoms as ^{15}N is 0.3663% and 99.6337% as ^{14}N (Unkovich, 1996). The ^{15}N isotope is considered as a reference standard for work done on estimating N_2 fixation in legumes. The $\delta\ ^{15}N$ value is considered zero since its content is constant in air (Unkovich, 1996). Consequently, if a legume is fixing atmospheric nitrogen, the $\delta\ ^{15}N$ value is expected to be low usually around zero. The low $\delta\ ^{15}N$ values obtained for plant organs of *Psoralea* species namely *P. pinnata, P. aculeata* and *P. aphylla,* clearly suggest that they are actively fixing N_2. In fact, the $\delta\ ^{15}N$ values are comparable to those of agricultural legumes such as cowpea, soybean and common bean, all members of the tribe Phaseoleae. Ecologically, these *Psoralea* species are likely to be a major source of nitrogen for associated plant species in the nutrient-poor fynbos.

Results obtained in this study clearly demonstrate that the tribe Psoraleae behaves similarly to the tribe Phaseoleae both in their nodules structure and their products of transportable N namely ureides. Moreover, the low $\delta\ ^{15}N$ values obtained further confirm the ability of this tribe to fix atmospheric nitrogen, ~~which is normally observed to~~ a function shared by members of the tribe Phaseoleae. Thus based on these results it is ~~understandable~~ suggested that the tribe Psoraleae should

envelope. Dakora (1989) observed the same anatomical structure in cowpea (Fig 5c). This further supports the fact that the tribe Psoraleae is similar to the tribe Phaseoleae.

Micrograph 6b & 6c also show two stages ~~the~~ of bacterial development in the medulla. An early stage consisting of rhizobial bacteroid enclosed in a membrane envelope and an intermediate stage where two to several bacteroids were enclosed in a membrane envelope (Tu, 1974). The intermediate stage formed what is called a symbiosome consisting of one or more bacteroids. The same type of bacteroids development was observed in cowpea (Micrograph not shown, Dakora, 1989). The similarities ~~of~~ in bacteriods development in both cowpea and the *Psoralea* species also demonstrate commonalities between the two tribes. Thus based on nodules anatomy, ultrastructure and morphology, the evidences for merging the tribe Psoraleae with that of Phaseoleae ~~are~~ is overwhelming.

Legumes are divided into tropical and temperate species. The temperate legumes are known as the amide producers while the tropical legumes are known as the ureide producers. Ureides, in the form of allantoin and allantoic acid are produced in the nodules via a purine synthesis and degradation pathway (Thomas & Schrader, 1981; Unkovich & Pate. 2000). The ureide formed is then transported to the different parts of the plant.

Studies done by Pate *et al* (1980) on eight different tropical species belonging to the tribe Phaseoleae namely *Vigna angularis, Glycine max, Vigna unguiculata, Vigna umbellate, Psophocarpus tetragonolobus, Vigna radiata, Vigna mungo, Cyamopsis tetragonoloba* and *Macrotyloma uniflorum* showed that the proportion of xylem nitrogen as ureide was much greater in nodulated plants than in non nodulated plants grown with 10 mM NO_3. Pate *et al* (1980) also obtained a proportional increased of ureide with increasing symbiotic activity in all the three organs studied namely stem, roots and leaflets, ~~study done on cowpea (*V. unguiculata*)~~. The same results were obtained for the three different Psoraleae species (*P. pinnata, P. aculeate* & *P. aphylla*) used for this study. Results obtained show that the four different organs examined namely the leaves, stems, roots and nodules, all had significant amount of ureides and the highest concentration of ureides was obtained in the nodules. The low ureide concentration obtained in the nodule of *P. pinnata* was due to inferior nodules quality. The fact that all the three species produced ureides and agree with the results of Pate *et al* (1980) further support the relatedness of the tribe Psoraleae to that of Phaseoleae. In addition work done by Atkins (1987) on xylem exudates have

be incorporated into the tribe Phaseoleae instead of being considered as a separate tribe as has been proposed by Doyle (1998).

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