Chapter-III

Results & Discussion

1. Habitat analysis

a) Soil Analysis

Salt flats associated with estuarine mangrove under strong influence of seasonal rainfall in the tropics constitute an interface between terrestrial halophytic and non-halophytic vegetation. The two *Derris* species selected for the present investigations also reflected this situation. It was noticed that plants of *D. trifoliata* grew among the various mangroves right in the core of estuarine vegetation (Fig.3a). On the other hand the plants of *D. scandens* were at the border of this vegetation. The climber *D. trifoliata* was found to be associated with several mangrove such as *Rhizophora apiculata; Avicennia officinalis; Avicennia marina; Sonneratia alba; Acanthus ilicifolius; Clerodendrum inerme; Excoecaria agallocha* and *Salvadora persica.* On the other hand, the plant species like *Pongamia glabra* served as support for growth of *D. scandens* (Fig.3b). In the study site selected for the present investigation these plants were about 120 meter apart from each other. The soil analysis of these two soils is recorded in Table 3. It is clear from the table that the soil supporting growth of *D. trifoliata* has got higher salt content. Both with respect to sodium and chloride than the one collected from rootzone of *D. scandens*. Joshi (1982)has reported significant levels of Na^+ and Cl^- in the estuarine soil inhabited by halophytes. The potassium

Fig. 3a *Derris trifoliata* habit

Fig. 3b *Derris scandens* habit

a

b

Table 3 - Inorganic elements in soils collected from root zone of <u>Derris</u> species

Root zone - soil sample	pH	Na^+	Cl^-	K^+	Ca^{+2}	Mg^{+2}	Fe^{+3}	Cu^{+2}	Co^{+2}	Mn^{+2}	Zn^{+2}
D. trifoliata	6.7	0.31	0.177	0.165	0.185	0.0014	0.00015	0.000	0.000	0.00015	0.0011
D. scandens	7.5	0.08	0.106	0.035	0.183	0.0031	0.0001	0.000	0.000	0.00135	0.00075

Values are expressed as g 100^{-1}g dry weight status of the soil in the rootzone of D.trifoliata is quite high as compared to the other soil samples. The calcium levels in the two soil samples are more or less similar. The soil supporting the growth of D.scandens is having relatively higher levels of magnesium and manganese and lower levels of the micronutrients, iron and zinc. There is also a difference in the pH values of the soil supporting the two Derris species. The pH in case of D. trifoliata is slightly acidic, while that D. scandens is slightly alkaline. It is quite obvious that this difference in soil pH can also exert differential influence on the mineral uptake in the two Derris species.

In estuarine vegetation proximity to the coast and seasonal distribution of rainfall result in pronounced seasonal variation in soil salinity and availability of superficial fresh water. Such seasonal variation in the water in the Ratnagiri mangrove creek has been clearly demonstrated in the experiments of Jamale (1975), Ghevade (1983) and Kadam and Bhosale (1985). Moreover Kadam and Bhosale (1985) recorded that the physical and chemical parameters of estuarine soils in Bhatye estuary also show great variations with respect of different months of the year. Thus it is obvious that the habitat where the Derris species are growing is a continuously changing medium and this in turn can influence various metabolic and growth process in these two species. It is further clear that such influence would be more effective in case of D. trifoliata growing with various mangrove members rather than in D.scandens.

2. Plant analysis

a) Leaf anatomy

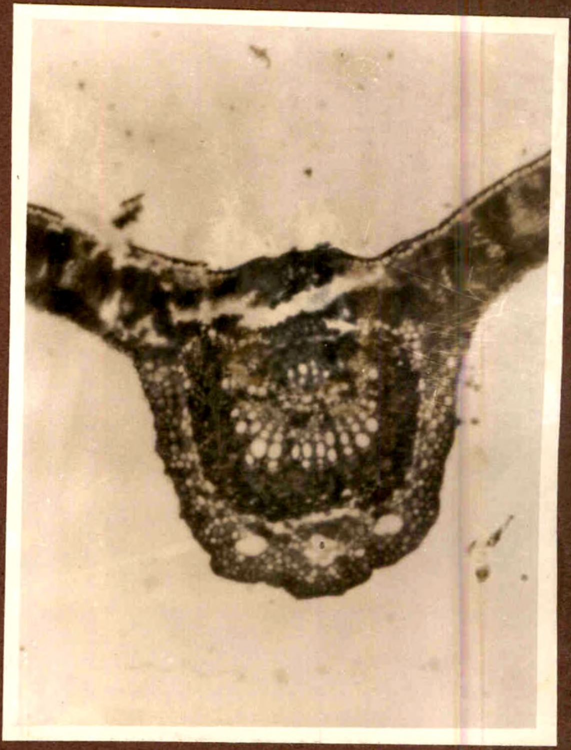
The anatomical features as revealed in transverse sections of the leaves of Derris scandens and Derris trifoliata are depicted in Fig.4. Both the leaves are typically bifacial or dorsiventral as palisade tissue occurs on side of leaflet and the spongy tissue on the other. The two species differ with respect to the nature of the epidermis on the two leaf surfaces; thus in D. trifoliata both surfaces have epidermis with cuticular layers on the surface. On the other hand in D. scandens the abaxial epidermal cells are papillose with poorly developed cuticle. According to Metcalfe and Chalk (1979) cuticular thickness appears to show a variable response to environmental conditions. There is a general belief that plants from arid regions frequently have strongly cutinized leaves. Several researchers have demonstrated that the plants with thick cuticle have reduced rate of cuticular transpiration. It has been pointed out Uphof (1941) that the epidermis of xerosucculents and coastal halophytes is characterized also by a thick cuticle and a cover of waxy layers. Sidhu (1974) made a critical study of epidermis of 24 mangrove species from the East and West coast of India. He observed that the cuticle in all species except the succulent herbs,

Fig.4 Leaf anatomy of Derris species

a) Transection of D.trifoliata leaf passing through midrib (3.2 X 6.3)

b) Transection of D. scandens leaf passing through midrib (3.2 X 6.3)

a



b

was thick and tenaciously attached to the epidermis. In dorsi-ventral leaves, cuticle on the lower leaf surface was invariably thicker than that on the upper surface. Wax coatings were present on both surfaces of leaves of most of the tree species.

Our observations indicate that the cuticular development is more pronounced in the leaves of D. trifoliata and it can be regarded as an adaptive feature similar to other mangrove species. It is evident from the figure that in both species the upper epidermal cells are larger than the lower epidermal cells. Similar observations have been recorded by Sidhu (1974) in other mangrove species. In both the species the mesophyll is differentiated into palisade and spongy parenchyma. The layers of palisade tissue vary in the two species. In D. trifoliata the palisade is double layered whereas in D. scandens the palisade is multiseriate. Below the palisade lies the spongy parenchyma. The mesophyll tissue in D. trifoliata is less compact as compared to that of D. scandens due to presence of large intercellular spaces in the spongy parenchyma zone.

Certain observations suggest a correlation between the development of an extensive intercellular system and specialization with regard to photosynthesis. A high ratio of internal to external surface may be accompanied by a high concentration of chlorophyll (Turrell, 1939). Leaves with prominent intercellular spaces may produce a larger gain in total dry matter per unit area of leaf per day than the more compactly built leaves (Pickett, 1937). However the ratio of internal to external surface is strongly and positively correlated with the rate of transpiration (Turrell, 1944) so that the structure favourable for photosynthesis induces at the same time a high loss of water. The compactly arranged, cutinized, and cuticularized epidermis and the presence of fine film of fatty material on the walls of the mesophyll cells exposed to the intercellular spaces apparently reduce but do not completely control the high transpiration which is a concomitant of the structural specialization for photosynthesis (Wylie, 1947). Thus it is apparent that the lacunose nature of mesophyll in D. trifoliata may be an adaptive feature as the plant is growing in swampy habitat.

The centre of the ventral part of the midrib in both the species is occupied by fibres above which is a zone of collenchymatus cells. In this region three well defined sacs have been noticed in case of D. scandens whereas only one sac is seen in case of D. trifoliata. Presence of such sacs in leaves and stem of Derris has been mentioned by Metcalfe and Chalk (1957). Our observations indicate that there may be difference in the number of sacs among various Derris species In the midrib of the leaf the vascular tissue in the ventral half occurs in the continuous arched band with phloem or the outside and xylem within. The internal phloem strands are also evident in both the leaves in the central region. The vascular tissue is surrounded by well defined sheath of sclerenchymatus cells and a layer of tannin rich cells in D. scandens. Such sheath in case of D. trifoliata is rather irregular. The absence of typical Kranz anatomy in the Derris species clearly rules out the possibility of operation of C_4 pathway as has been reported for some mangroves from our laboratory. The presence of large number of tannin rich cells in the leaf tissue was clearly noticiable in case of D. scandens. While only few such cells were seen in D. trifoliata. These observations are consistent with the observations of total polyphenol contents (Fig.26), in the leaves of two species.

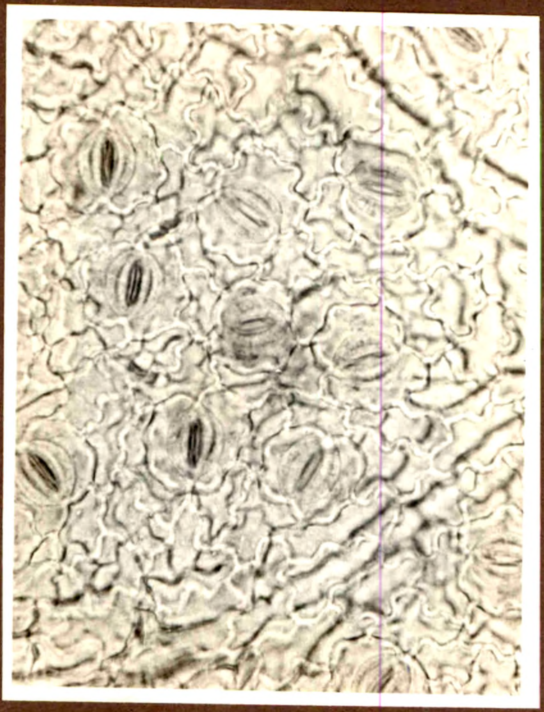
In both the species the stomata were confined to the lower surface. Sidhu (1974) made stomatal study in leaves of 24 mangrove species. He observed that the stomata in mangrove species were restricted to the lower epidermis except in amphistomatal leaves of Salvadora persica and Sonneratia apetala. The stomata restricted to one surface rather than occurring both surfaces can improve water relations of the plants but at the same time can pose limitations on photosynthetic CO_2 input. The stomata in Derris species were found to be belonging to paracytic type (Fig.5).

Metcalfe and Chalk (1979) recorded variation in the

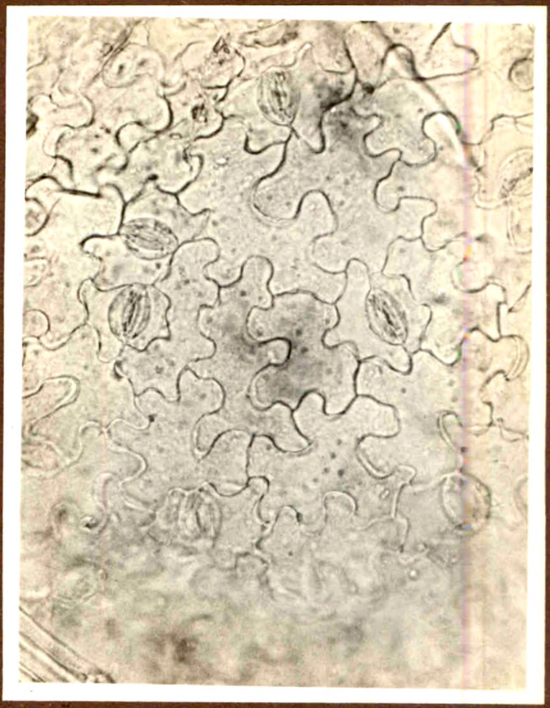
Fig.5 Stomata of Derris species

a) Lower epidermis of D.trifoliata leaf showing paracytic stomata(40 X 3.2 X 10)

b) Lower epidermis of D.scandens leaf showing paracytic stomata (40 X 3.2 X 10)



a



b

appearance of the guard cells and their outer stomatal ledges (rims) in mangrove species. They recorded that there is a single outer stomatal rim in *Conocarpus*, *Laguncularia* and *Lumnitzera* whereas the rims of *Bruguiera*, *Ceriops*, *Kandelia* and *Rhizophora* are conspicuously two lipped such single outer stomatal rim is quite prominent in mangrove associate *D. trifoliata* in contrast to *D. scandens*.

The study of stomatal Index showed that *D. trifoliata* leaves had stomatal Index of 33.74 while that of *D. scandens* was 24.15. Salisbury (1927) likewise emphasized that the frequency of stomata is high when the size of the epidermal cell is low and that the frequency is low when the epidermal cells are large. After further research Rowson (1946) made an extensive study of stomatal Index values in different species of *Cassia* and came to the following conclusions concerning the stomatal Index i) It does not vary significantly at different positions upon the leaf surface, ii) it is independent of leaf size and plant habitat, iii) it is the same for different varieties within a species, iv) co-generic species may be differentiated by means of stomatal Index; and v) the stomatal Index value is more uniform upon the lower than the upper surface, except in isobilateral leaves. However, Van Staveren and Baas (1973) found that stomatal Index of *Apodytes dimidiata* E. Meyer ex Arn. (Icacinaceae) was too variable (7-21) to be of any use as a taxonomic character.

Sidhu (1974) determined stomatal Index in 24 mangrove species. He reported that the stomatal Index varied from 1.5 (*Ceriops candolleana*) to 22.00 (*Acanthus ilicifolius* and *Sesuvium portulacastrum*) and majority of mangrove species had the stomatal Index about 2 to 12. Our observations indicate that the stomatal Index of *D. trifoliata*, a mangrove associate is quite higher than the other mangrove species and *D. scandens*.

b) Nodulation studies

Legumes are bestowed with a capacity to fix atmospheric nitrogen through the symbiotic association with *Rhizobium* in their root nodules. Although nodulation in leguminosae is more or less a common feature, some legume species do show absence of nodules under natural conditions (Sprent *et al.*, 1989). During the present ecophysiological investigations the author noticed occurrence of profuse nodulation on roots of both Derris species (Fig.6). The nodulation was present both below the soil and at ground level and it was particularly predominant during rainy season. There are about 80 species belonging to genus *Derris* and so far the occurrence of nodules in *D.elliptica* (Roxb.) Benth, *D. microphylla* (Miq.) Val., *D. phillipinensis* Merr. and *D. scandens* (Roxb.) Benth has been reported (Allen and Allen, 1981). It is interesting to note here that the present observations can be regarded as the first

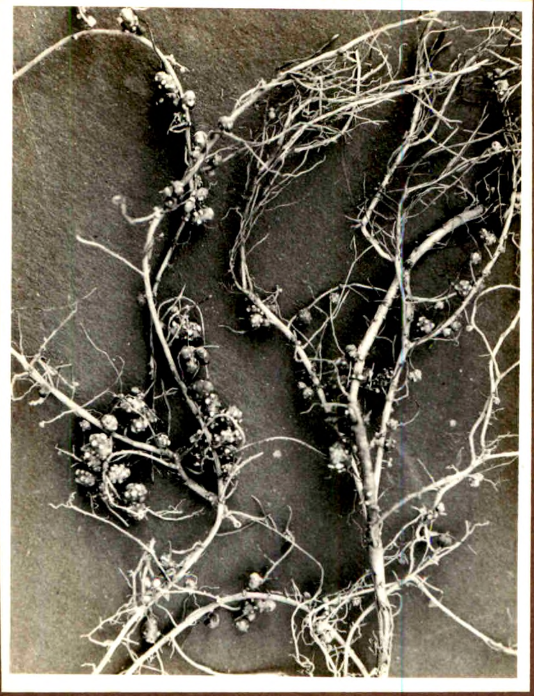
Fig.6 Root nodules and nodule anatomy of Derris species

a) Nodules on roots of D. trifoliata

b) Nodules on roots of D.scandens

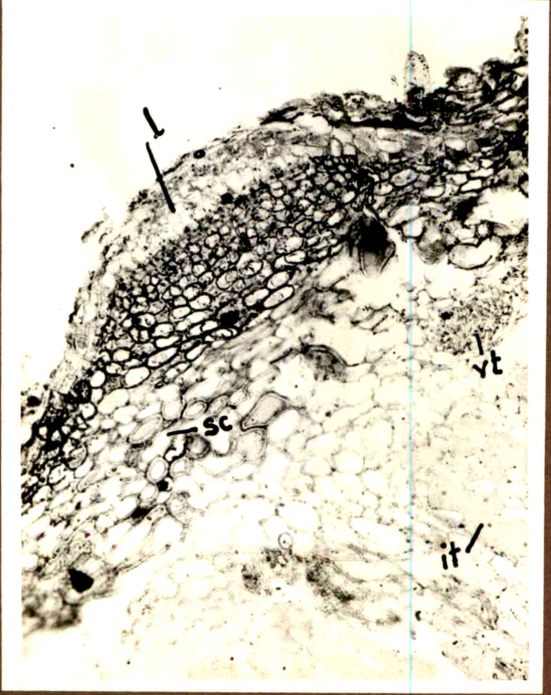
c) Transection of nodule of D. trifoliata showing lenticel (l), Vascular tissue(Vt), Sclerides (Sc) and infected tissue (it) (12.5 X 3.2)

d) Transection of nodule of D.scandens showing lenticel (l), Vascular tissue (vt) sclerides (sc) and infected tissue (it) (12.5 X 3.2)

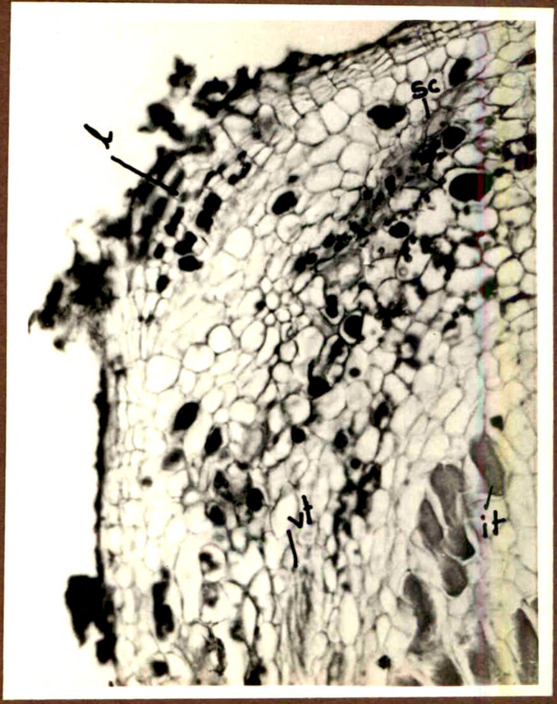


a

b



c



d

report on occurrence of nodules in Derris trifoliata Lour. (Sprent personal communication, 1990).

The nodules of both Derris species were found to be spherical in shape, short lived and of determinate nature. The surface of nodules appeared little rough due to the presence of lenticels. The fact that these nodules were produced on Derris roots due to infection by Rhizobium was confirmed with the help of crystal violet staining. In order to have more insight into nodule structure, anatomical studies were performed. These studies revealed that the nodules possess prominent lenticels, besides cortex and central infected tissue (Fig. 6).

The cortex showed presence of vascular tissue and irregular layer of sclerides, such extensive scleride production has been recorded in the nodules of several woody legumes including Derris guilleminianus (Sprent et al., 1989). Our observations indicate that the sclerides occur in nodules of even the climbing legumes like D. trifoliata. The presence of lenticels in nodules of Derris species is quite interesting.

A formation of enlarged lenticels on cowpea nodules under waterlogged conditions has been reported by Minchin and Summerfield (1976). The presence of lenticels on the root nodules of D. trifoliata can be certainly regarded as an adaptive feature for aeration in coastal swampy habitat and this is in tune with the occurrence of pneumatophores in some other mangrove species.

Since nodulation process directly takes place in the soil medium, it is quite natural that the soil constraints exert marked influence on this process. Zahran and Sprent (1986) carried out a detailed study of the effects of sodium chloride salinity on the infection of Vicia faba root hairs by Rhizobia. They noticed that NaCl treatment reduced the number of root hairs and their lateral expansion. It was further noticed that the number of nodules was approximately halved by NaCl salinity. Sprent and Zahran (1988) reported following effects of 100 mol/m^3 NaCl salinity on nodule development; 1) mitoses in the nodule apical meristem were reduced in number, 2) infected cells showed accelerated senescence and 3) bacteroids did not differentiate completely into the pleomorphic forms typical of this symbiosis. They further reported that salinity adversely affected nitrogen fixing activity as estimated by the acetylene reduction assay.

It is evident foregoing account that the process of nodulation and nitrogen fixation in glycophytic legumes is quite sensitive to salinization. Absolutely no information is available about nodulation in halophytic species. We will see in the next part that nodules of both Derris species accumulate appreciable levels of various inorganic constituents and still they are able to maintain adequate total nitrogen content in different plant parts. Similarly the extent of nodulation on both species under saline conditions is quite appreciable. These finding lead the author to suggest the probable utility of <u>D. trifoliata</u> in the mangrove reforestation programmes in view of the nitrogen dynamics of the coastal ecosystems.

C) Analysis of inorganic constituents

i) Sodium :

The sodium contents in different plant parts of *Derris trifoliata* and *D. scandens* are recorded in Fig.7. It is evident from the figure that in both the species, the young leaves contain the lowest levels of sodium. The nodules also contain appreciable amount of sodium. in both the species. Among the essential plant parts of *D. trifoliata*, the highest sodium level are recorded in the root tissue followed by petiole. In case of *D. scandens*. Petioles contain the maximum amount of sodium which is followed by the root tissue.

Harmer and coworkers (1953) were able to recognise four different categories of plants depending upon their sodium requirement a) plant requiring sodium in the absence of potassium (lucerne, barley, oat, tomato); b) plants requiring low sodium under potassium deficiency conditions (maize, red clover, lettuce, onions, potatoes); c) plants displaying moderate requirements for sodium when adequately supplied with potassium (some members of cruciferae, wheat, peas); d) plants showing a strong dependence on sodium when adequately supplied with potassium (beet, turnips, and particularly sugarbeet).

Although there are controversies regarding the necessity of sodium for plant metabolism, it has been shown in several experiments that sodium plays an important role in halophytes. This was clearly evident in halophyte *Atriplex vesicaria*

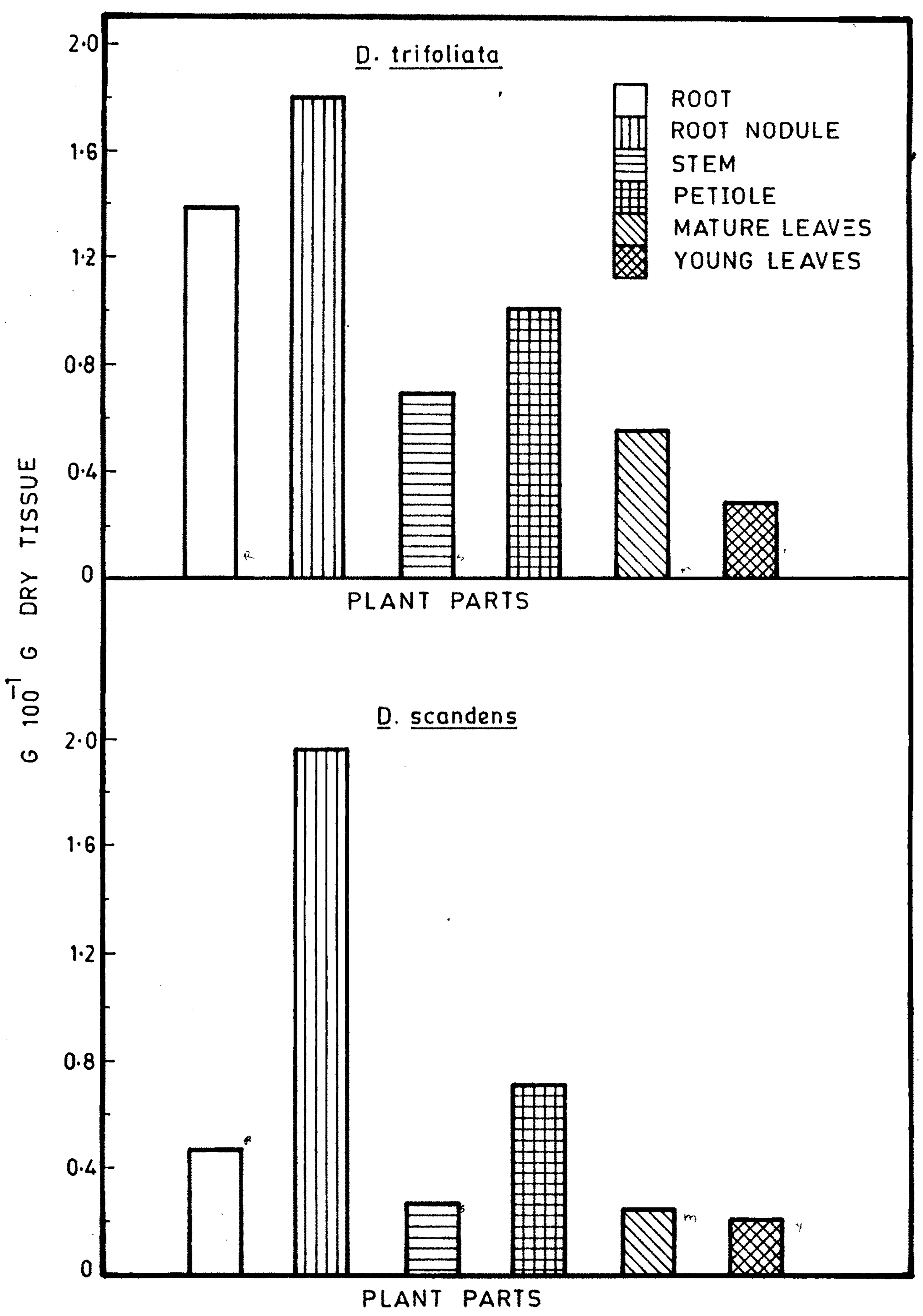


FIG 7 – SODIUM CONTENTS IN DIFFERENT PARTS OF <u>DERRIS</u> SPECIES whose growth ceased and pronounced deficiency symptoms were visible in the absence of sodium (Brownell and Wood, 1957). The deficiency symptoms disappeared only after addition of sodium. Walter (1968) observed increase in biomass of Salicornia due to increase in salinity. In case of mangrove Rhizophora mangle, Pannier (1959) observed that growth of seedlings in diluted seawater was 3-5 times higher than in rain water or undiluted seawater. The exact reasons for growth stimulation due to salinity in halophytes are not known. Jennings (1968a) assumed that sodium excesses cross the plasmolemma without any need for energy expenditure and that the overall process of sodium transport into vacuole results in ATP production. It was suggested that such ATP is later used for additional growth, thus explaining growth stimulation caused by NaCl in halophytes. The role of sodium in osmotic adjustment in halophytes is also very well documented (Flowers, 1985)and this may also contribute for growth stimulation as the plants may not depend on the production of organic solute for the process of osmoregulation and in one way check the energy expenditure.

According to Marschner (1971) sodium can at least partially replace potassium in plants and the extent of replacement of potassium by sodium depends decidedly on the plant species. Potassium is replaceable to a large extent by sodium in the vacuole. Sodium can also partially replace potassium in activating several enzyme systems. Marschner further argues that in certain plant species like sugarbeet, sodium shows a high mobility in the phloem and hence it should be able to replace potassium in its function in long distance transport processes. Rains (1972) has indicated that sodium plays an important role in maintaining favourable water balance. Besides halophytes, sodium is also required in at least small amounts by plants having C_4 pathway (Brownell and Crossland, 1972). Johnsten et al. (1984) observed low chlorophyll a to b ratio in sodium deficient C_4 plants.

There are several attempts to measure the sodium levels in various halophytic species, especially from the leaf tissue. Albert (1975) recorded that sodium levels in the dicot halophytes were remarkably higher than those in monocot halophytes. Walsh (1974) tried to summarise the data (obtained from Golley, Sidhu and Hollister) regarding the elemental composition of mangrove leaves. This summary reveals that the sodium content in the mangrove leaves varies from 0.41 (Conocarpus erectus) to 5 (Avicennia marina) per cent dry weight. The seagrasses studied by Birch (1975) ranged in a sodium content from 2.6% to 6.3%, while certain species of Atriplex a terrestrial halophyte contained 4.5 % to 7.1% sodium (Beadle et al. 1957). Spain and Holt (1980) studied the elemental status of the foliage of seven mangrove species from Northern Queensland. Their study showed that sodium content varies from 7.73 % (Ceriops tagal) to 3.11 % (Avicennia marina). The sodium status in saline plants has been analysed in our laboratory also. According to Joshi et al. (1975) the sodium content in saline plants ranges from 1.5 to 9.9% · The range of values for sodium reported by Kotmire and Bhosale (1979) in mangroves is from 0.4 to 4.6 %. Sidhu (cf Walsh, 1974) has recorded that the leaves of D. trifoliata contain 1.23 % sodium, whereas the work of Kotmire and Bhosale has shown that the sodium content in leaves of D. trifoliata is·4% Our observations indicate that there is interspecific variation with respect to sodium levels in the leaf tissue in Derris species and the sodium levels in D. trifoliata are higher than those recorded for D. scandens. It is further interesting to note that the leaf sodium concentration increases with age and in the senescent leaves sodium levels as high as 1.64 (D. trifoliata) and 0.4 (D. scandens) were recorded, These observations are in agreement with the suggestion of Karmarkar (1982) that the senescent leaves of halophyte show a marked tendency of salt accumulation. Albert (1975) suggested that shedding of salt saturated old leaves is a strategy of salt regulation in some halophytes. It was observed in the present investigation that in month of February a marked leaf senescence and leaf shedding occurred in D. trifoliata. Thus it is apparent that this strategy can at least be partly operating in D. trifoliata also.

Although the leaf analysis of various halophytes has been done by several investigators, the whole plant analysis has been attempted by only few workers and hence this creates great difficulties in analysis of salt regulation at a plant level. In Atriplex hastata, Black (1956) observed that the site of sodium accumulation was in buds and very young leaves. In Suaeda monoica sodium tended to accumulate in young leaves and buds, while its content in roots and stem was low (Waisel, 1972). Hill (1908) observed that the content of sodium in various organs of Suaeda and Salicornia increased from the root upwards. Walsh (1974) reported that the stem and roots of some mangrove species (Avicennia nitida, Conocarpus erectus, and Rhizophora mangle) contained relatively very low sodium levels as compared to leaves. Our observations with Derris species indicate exactly opposite pattern. Thus in D. trifoliata the site of sodium accumulation appears to be the root tissue and the leaf tissue is protected from the heavy accumulation of sodium. The excellent work of Lauchli and Wieneke (1979) has demonstrated that the salinity tolerance in leguminous crops like soybean depends on the efficiency with which the root system can limit access of sodium and chloride to the above ground plant parts. Our observations indicate for the first time that in leguminous halophytes also this strategy is in operation especially with respect to sodium distribution. The high levels of sodium in petiole are also interesting since this adaptive feature may protect the leaf tissue from toxic effects of sodium. Similar tendency has been reported to be operating in salt tolerant leguminous species. Sesbania grandiflora (Chavan and Karadge, 1986). The nodules of both the species of Derris are found to be rich in sodium and this probably indicates that sodium may not be interfering with nitrogen fixation process in these species.

ii) Chlorides :

The chloride contents in different parts of the two Derris species are recorded in Fig.8. It is clear from the figure that the distribution pattern of chloride differs in these two species. In case of D. trifoliata, the chloride contents are considerably high in petiole and mature leaves in contrast to the root and stem tissue. On the other hand, in case of D. scandens, the roots accumulate considerable amount of chloride. On the whole, the chloride contents in D. trifoliata are higher than those of D. scandens.

Although chloride was detected in the plant tissues several years ago the exact participation of chlorides in the plant metabolism has been not yet confirmed. However, it is realized that this element is essential for growth and development in small quantities. Resenblum and Waisel (1969) recorded stimulation of growth of Atriplex halimus by chloride. Halket (1911) noticed essentiality of chloride for flowering in Salicornia olivieri. Critchley (1982, 1983) demonstrated the role of chloride in photosynthetic oxygen evolution in

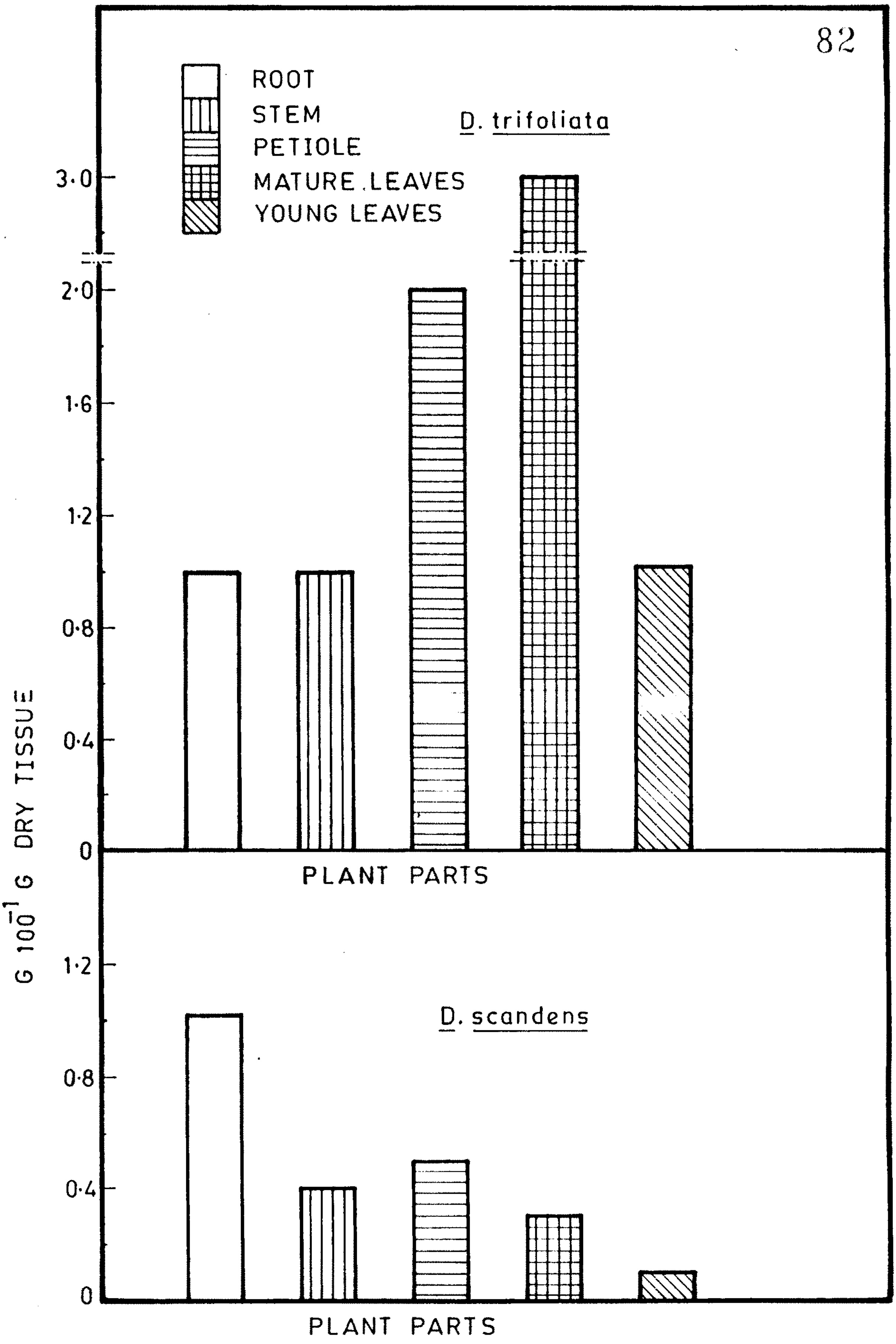


FIG. 8 – CHLORIDE CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

higher plants. He showed that photosystem II activity in some mangrove chloroplast had a high chloride demand. Flowers (1985) has high-lighted the role of chloride in osmotic adjustment in dicot halophytes.

According to Waisel (1972) sodium and chloride are taken up by roots of halophytes in non-equivalent quantities and they also differ in the patterns of their distribution within plant organs. It is quite understandable that the halophytes are rich in chlorides. Waisel (1972) observed 6.8 % chloride in Suaeda monoica. Sen et al. (1982) observed that the chloride contents in the desert halophytes of Rajasthan varied from 2.42 to 36.16 %. Breckle (1976) observed 6.5 % chlorides in Atriplex confertifolia. Joshi and Bhosale (1982) observed that the chloride content varies from 2.5 to 11.73 % in various mangrove species. Spain and Holt (1980) observed that the chloride contents in the leaves of mangrove species from Queensland was in the range of 3.25 to 6.07 % and in all the cases the chloride content were always higher than the corresponding sodium content. Kotmire and Bhosale (1979) observed that the leaves of D. heterophylla plants growing in Deogad and Mumbra creeks had 1.86% and 2 % chlorides respectively. It is evident from our observations that the chloride content in Derris leaves increased with leaf age and may reach a value as high as 3%.

There are very few attempts to determine the chloride levels in different parts of halophytes. Hill (1908) observed that the content of sodium and chloride in various organs of *Suaeda* and *Salicornia* plants increased from the root upward. Waisel (1972) observed that the stem tissue of *Suaeda monoica* contained less chlorides than the leaves. Jamale (1975) observed that distribution of chloride in various parts of *Rhizophora mucronata* was in the order mature leaves > stem > roots. Spain and Holt (1980) observed that the branch wood of the seven mangrove species - *Aegiceras corniculatum* (L.) Blanco, *Avicennia marina* (Forsk.) Viern., *Ceriops tagal* (Perr.) C.B.Rob., *Excoecaria agallocha* L., *Osbornia octodonta* F. Muell., *Rhizophora stylosa* Griff. and *Xylocarpus australasicus* Ridley.

In the present investigation it is evident that the order of chloride content in different parts of *D. trifoliata* is mature leaves > petiole > young leaves > stem and roots. On the other hand in case of *Derris scandens* the order is as follows : root > Petiole > Stem > mature leaves > young leaves. The relatively high levels of chloride in *D. trifoliata* in comparison to *D. scandens* clearly throw light on the halophytic nature of the earlier species. It is further clear from our observations that in case of *D. trifoliata*, except the root tissue in all other plant parts the chloride levels are higher than the corresponding sodium levels. Thus even though this halophytic species is able to regulate the sodium excess in the shoot tissue, it is unable to prevent the chloride accumulation in the aerial tissue. Further studies are essential to localise the chloride in different compartments of the cells of Derris leaves.

iii) Potassium :

The potassium contents in different plant parts of the two Derris species are recorded in Fig.9. It is clear from the figure that the potassium contents in different plant parts of D. trifoliata are higher than those recorded in D. scandens. The nodules of both the species contain appreciable amount of potassium. The young leaves show relatively higher levels of potassium than mature leaves in both the species. The amount of potassium is rather low in the root tissue.

Potassium plays several key roles in plant metabolism and hence this element is highly indispensable element for plant growth and development. It can move both through xylem and phloem and hence can be readily redistributed in the plant. Potassium plays an important role in water relation because of its osmotically active nature (Mengel, 1977). The involvement of potassium in turgor regulation of higher plants is also well documented. The stomatal movements depend on potassium status in the guard cell and potassium plays a role in stomatal opening and closure (Fischer and Hsiao,1968). Besides these processes potassium plays a direct role in plant metabolism. Suelter (1970) has listed some 58 enzymes which

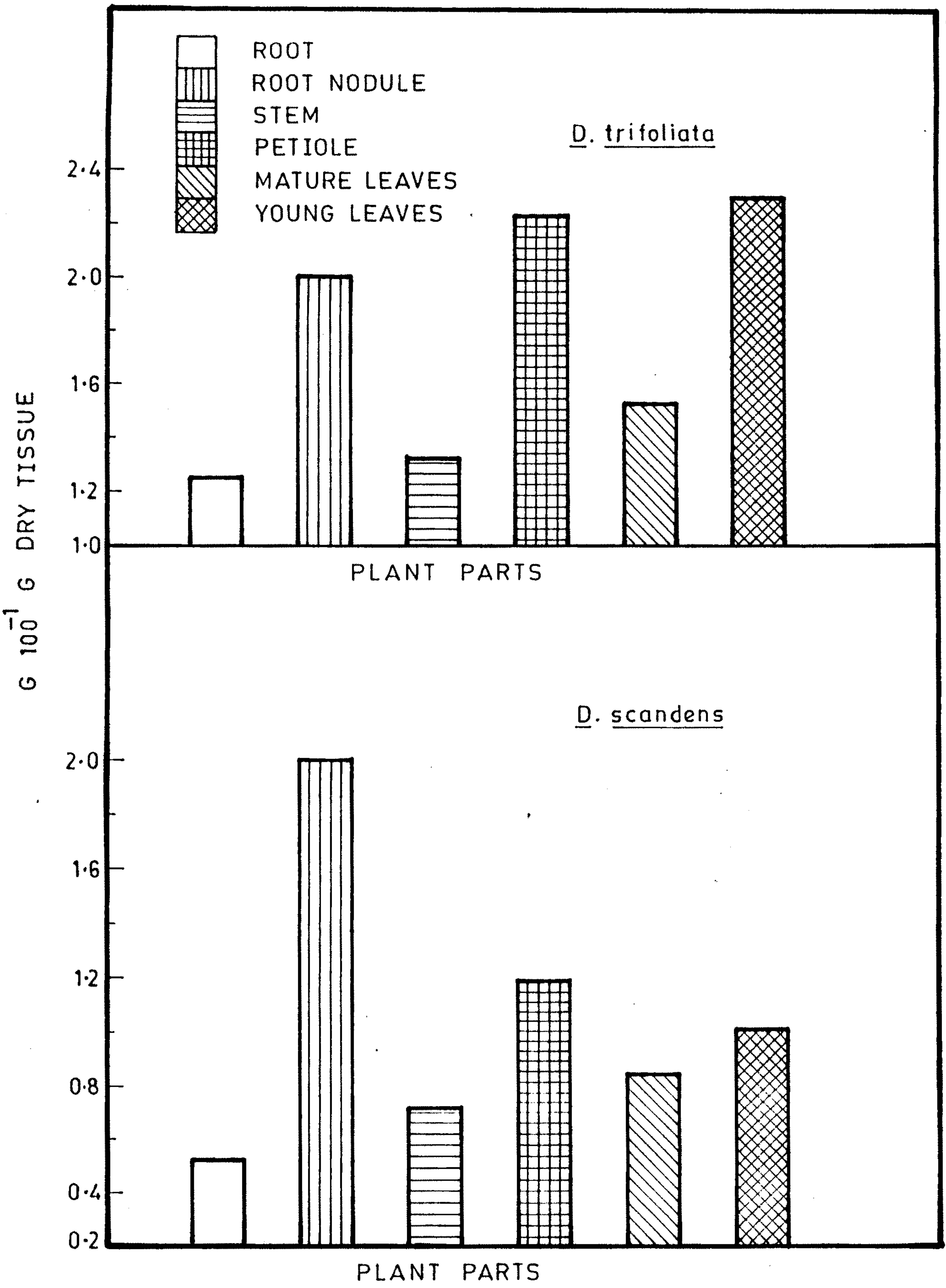


FIG. 9 – POTASSIUM CONTENTS IN DIFFERENT PARTS OF <u>DERRIS</u> SPECIES.

need potassium for maximum activity. Research in the last two decades has clearly revealed that potassium is important in plant metabolism particularly through its action on, processes of photosynthesis and respiration.

Potassium merits special attention in the halophyte physiology. Larsen (1967) has demonstrated the important role of potassium in salt tolerance of Halobacterium. Role of potassium in developing salt tolerance in mangroves and succulents has been documented in the work from our laboratory (Karmarkar and Joshi, 1968; Joshi et al., 1972, 1975). The role of K^+ has been further confirmed by Rozema (1975, 1976) who states that potassium provides a relatively important contribution to the total osmotic potential, which together with the independence of the K^+ concentration to the increasing Na^+ content as salinity increases could form the basis of salt tolerance. Gorham (1980) reported that a preferential potassium uptake is also a feature of monocotyledons halophytes. A preferential potassium uptake under saline conditions was also demonstrated in Heritiera littoralis and Hibiscus tiliaceus by Popp et al. (1985). There are few attempts to measure the the potassium levels in coastal halophytes. Walsh (1974) who summarized the observations of Sidhu, Golley and Holliester reported that the potassium content in the leaves of various mangroves varies from 0.31 % (Sonneratia apetala) to 2.9% (Avicennia officinalis). Potassium concentrations of the foliage of the seagrasses studied by Birch (1975) are in the range from 0.6 to 2.4 %. Mishra (1967) recorded nearly 3% K^+ content in Clerodendron inerme. Chirputkar (1969) reported upto 1.33% K^+ in the mangroves, Bruguiera parviflora and Ceriops candollena. The K^+ content reported for the mangroves from West coast of India ranges from 0.24 to 1.56% (Joshi et al., 1975). Spain and Holt (1980) observed that the potassium level in the foliage of mangroves from Queensland ranged from 0.48 (Aegiceras corniculatum) to 1.79 %(Xylocarpus australasicus). Ghevade (1983) recorded that the potassium content in seagrass Halophila beccarii was 1.58%. Sidhu (cf Walsh, 1974) observed that the potassium content in Derris uliginosa leaves was 2.58 %. Kotmire and Bhosale (1979) observed that the potassium levels in D. heterophylla leaves were of the order 0.68 to 0.8%. The values recorded in the present investigation for young leaves of D. trifoliata are nearer to those observed by Sidhu. The relatively high levels of potassium in young leaves of both the species, clearly indicate its participation in the active metabolic machinery of young leaves. The potassium contents in mature, leaves are lower than those of young leaves. Similar observations were recorded by Popp et al. (1985) in case of Ceriops tagal. It was further noticed that there is a great decline in the potassium levels of senescent leaves of both species because in senescent leaf tissue of D. trifoliata 0.42 %

and in D. scandens 0.1% potassium contents were recorded. These observations clearly revealed that similar to other terrestrial plant species potassium is retranslocated during leaf senescence in halophytic plants like Derris.

Although potassium status has been studied in several coastal halophytes these studies are mainly concerned with leaf tissue. Walsh (1974) has recorded that the roots of the halophyte. Laguncularia racemosa contain as high as 8.1 % potassium. Spain and Holt (1980) reported that the potassium levels in branchwood of mangroves were relatively low as compared to those of foliage. In the present investigation also it is noticed that the potassium levels in the stem tissue are lower than those recorded for young and mature leaves. The high levels of potassium in the roots of D. trifoliata are quite interesting and they suggest the involvement of this element in salt regulation at root level. The nodules of both the species are also rich in potassium. It is also evident from our studies that in most of the plant parts of both Derris species the K/Na ratio is greater than one and this clearly indicates a preferential uptake of potassium under saline conditions. Thus similar to other halophytes. Potassium nutrition is of great significance in salt tolerance process of Derris species.

iv)Calcium :

The distribution of calcium in different plant parts of two *Derris* species is depicted in Fig.10. It is evident from the figure that there is not much variation in calcium levels in different parts of *D. trifoliata* and the calcium content varies from 0.32 to 2.49 %. On the other hand, in *D. scandens* the foliar parts like petiole and leaves appear richer in calcium as compared to stem and root system. It is interesting to note that in both the cases the highest calcium content is recorded in petiolar tissue.

Although calcium is one of the major inorganic nutrients found in plant tissue, there are great controversies regarding the exact calcium requirements and the definite role of calcium in plant metabolism. Calcium has a relatively large ionic radius (0.99 nm) compared with other divalent ions and has a coordination number of six or higher (often seven or eight) to form octohedral complexes. Calcium has ability to bind with proteins and other macromolecules with low geometrical demand. Ligands of Ca^{2+} complexes often consist of high amounts of carboxylates, little water, and usually with at least one protein chain carboxyl-O group. Ligand exchanges with Ca^{2+} are relatively rapid and the binding strength of Ca^{2+} is not dependent on the number of carboxylate ligands. According to Clarkson and Hanson (1980) a major role of calcium appear to be its binding with proteins, nucleic acids

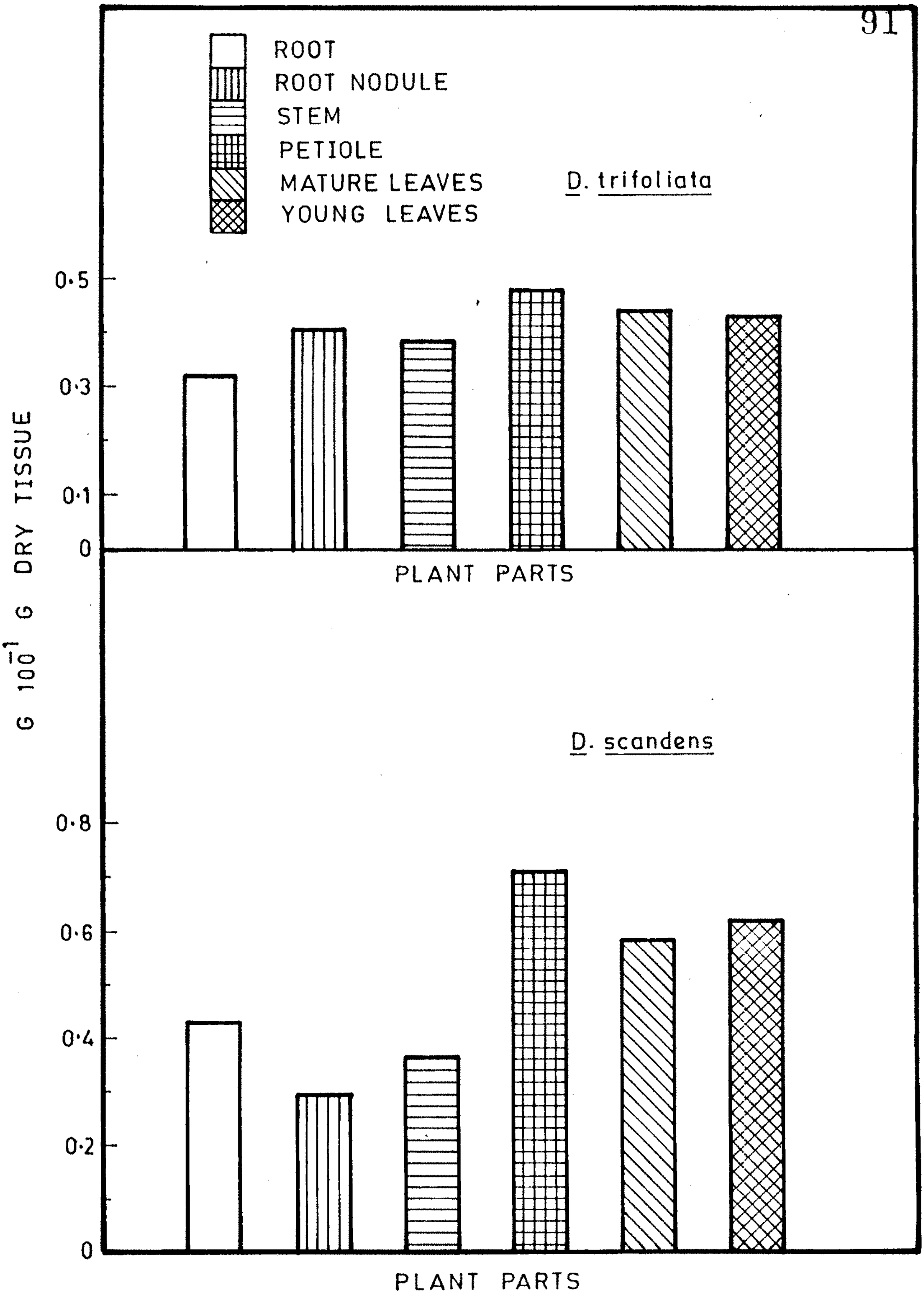


FIG. 10 – CALCIUM CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

and lipids to affect cell adhesion, membrane and chromatin organization and enzyme conformation. Calcium plays an important role of maintaining membrane stability. In cell wall also it is an essential component of middle lamella in the form of calcium pectate. Calcium influences the activities of several enzymes such as α-amylase, esterase, pectinesterase, lipoxygenase, nucleases, proteinkinase and adenosine triphosphatase (Clark, 1984). It has been argued that calcium plays an important role in salt tolerance mechanism by preventing membrane damage by sodium (Epstein 1972, Lessani and Marschner 1978).

According to Waisel (1972) the calcium content in various halophytic plants is low and usually does not exceed 0.5% of their dry weight. He further noticed that in halophytes like *Atriplex, Suaeda monoica* and *Aleuropus litoralis* the calcium content is affected by the presence of NaCl in the medium. He further pointed out that in certain hydrohalophytes, uptake of calcium is extremely slow and precipitates of calcium carbonate are found on leaf surface of plants which grow in alkaline water. Although this is the case with the above halophytes, it has been observed in case of several mangrove species that the calcium content varies from 0.65 to 2.48% (Walsh, 1974). Imbaba (1972) noticed that the calcium content in *Rhizophora mucronata* and *Bruguiera gymnorhiza* was 1 and 1.57% respectively. Spain and Holt (1980) noticed that the calcium levels in the mangrove species of Queensland varied from 0.27 (*Aegiceras corniculatum*) to 2% (*Xylocarpus australasicus*). Joshi *et al.* (1975) observed that the leaves of mangroves from Ratnagiri coast contain 0.42 to 1.25% calcium. Sidhu (cf Walsh, 1974) noticed that the leaves of *D. uliginosa* contain 1.76 % calcium while Kotmire and Bhosale (1979) found that the leaves of *D. heterophylla* growing in Deogad and Mumbra creeks contained 1.06 and 2.8% calcium respectively. The values of calcium recorded in present investigation are rather low. There is limited information about the distribution of calcium; in different parts of saline plants. On the whole, plant level and calcium generally moves passively in the transpiration stream (Waisel, 1972). Walsh (1974) recorded that in case of *Conocarpus erectus* roots contain very high levels of calcium (2.12 %) as compared to stem and leaf tissue. On the other hand, in *Avicennia nitida* the calcium level in the stem was higher than that in the roots. Spain and Holt (1980) observed that the branchwood contained more calcium than the foliage in case of *Aegiceras corniculatum*, *Avicennia marina*, *Ceriops tagal*, *Excoecaria agallocha* and *Rhizophora stylosa* while opposite situation was noticed in case of *Osbornia octodonta* and *Xylocarpus australasicus*. Ghevade (1983) observed that the leaves of seagrass *Halophila beccarii* had slightly higher calcium contents than those of the rhizome. It is evident from our observations that the leaf tissue is richer in calcium than either root or stem tissue of both *Derris* species. It is generally observed that calcium shows a tendency to accumulate with increasing leaf age in halophytes (Amonkar, 1977, Kotmire, 1983, Popp *et al.*, 1985). At the same time Jamale (1975) observed a marked decline in calcium content of old leaves of *Excoecaria agallocha*. It is apparent from our observations that a general halophytic trend is shown by *D. trifoliata* leaves to a small extent while in *D. scandens* leaves, the young leaves are richer in calcium than the mature leaves. In either case calcium does not seem to play a major role in ion regulation in *Derris* species under saline conditions as the calcium levels are relatively low in all the plant parts.

V) *Magnesium* :

Fig.11, records the magnesium contents in different parts of *D. trifoliata* and *D. scandens.* It is evident from the figure that nodules of both the species contain very large amounts of this divalent cation. The petioler tissue also has appreciable levels of magnesium. It is clear from the figure that the stem tissue of both the species contains lower amount of magnesium in comparison to either leaf or root tissue.

Besides its involvement in the structure of chlorophyll molecule, magnesium is essential for several physiological processes. Magnesium serves as a cofactor in almost all

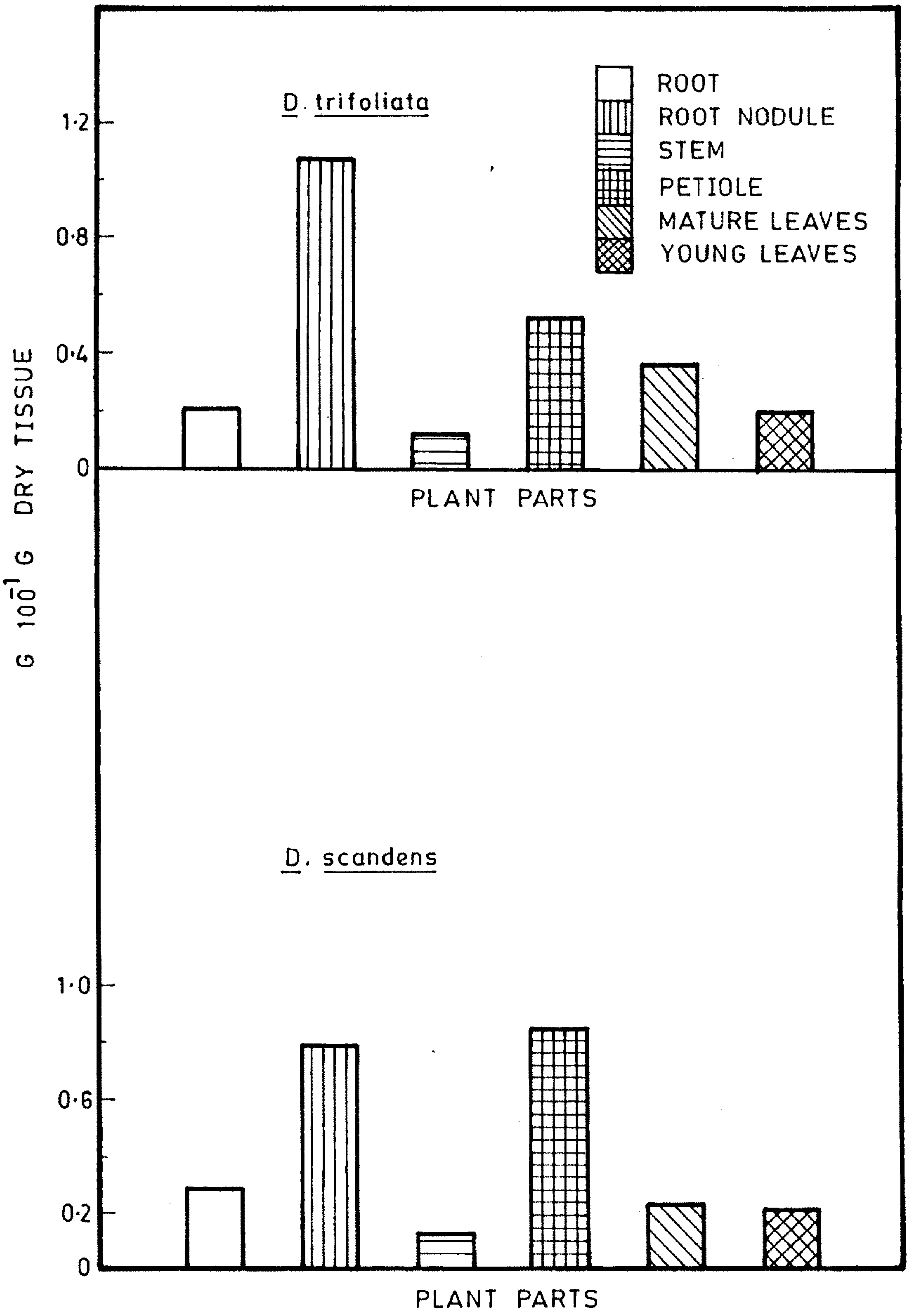


FIG. 11 – MAGNESIUM CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

enzymes activating phosphorylation processes. It creates a bridge between the pyrophosphate structure of ATP or ADP and the enzyme molecule. According to Balke and Hodges (1975) the activation of ATPase by Mg^{2+} is brought about by this bridging function. Enzymes like phosphokinases, dehydrogenases and enolases are activated by magnesium. In these enzymes, however, the magnesium action is not specific and manganese is often a more efficient activator. A key reaction of magnesium is the activation of ribulose biphosphate carboxylase. Bandurski (1955) reported that PEP case in spinach requires magnesium (1×10^{-3}M) for its optimum activity. Magnesium regulates integrity and stabilizes nucleic acids and membranes. The lack of Mg^{2+} probably causes the dissociation of ribosomes into their subunits and destroys the ribosomal configuration that is necessary for protein synthesis (Watson, 1965). The transfer of amino acyls from amino acyl transfer ribonucleic acid (+ RNA) to a polypeptide chain appears to be activted by Mg^{2+} (Clark, 1984). Magnesium is also essential for enzymes of sucrose biosynthesis. Thus this divalent cation plays an importatnt role in protein metabolism as well as carbohydrate metabolism.

There are great variations in the magnesium content in various halophytes. Walsh (1974) has recorded that the magnesium level in the leaf tissue of mangroves varies from 0.23% (Avicennia nitida) to 1.56% (Lumnitzera racemosa). The levels of magnesium recorded by Spain and Holt (1980) in leaves of various species of mangroves from Queensland are in the range of 0.39 to 0.94%. On the other hand, Popp (1984) observed appreciable levels of magnesium in members of family Rhizophoraceae. Sidhu (cf Walsh, 1974) has observed that the magnesium content in the leaves of *Derris* ~~*uliginosa*~~ trifoliata were 1.24% while Kotmire and Bhosale (1979) observed that the magnesium contents in *D. heterophylla* leaves at two localities were 0.31 and 0.47 %. In the present investigation the values of magnesium in the leaves of two categories in two species vary from 0.20% to 0.36% and the leaves of *D. trifoliata* contain more magnesium than those of *D. scandens.*

Very little information is available regarding the distribution of magnesium in different parts of halophytes. Walsh (1974) recorded that there was not much variation in the magnesium levels in different parts of *Avicennia nitida* while the stem tissue of *Lumnitzera racemosa* contained less magnesium as compared to leaf tissue. Spain and Holt (1980) noticed that the magnesium levels in the branchwood of different mangrove species were considerably lower than those in the foliage of corresponding species. Our observations with *Derris* also indicate a similar trend, as the stem tissue contains very low levels of magnesium as compared to root and leaf tissue.

Some attempts have been made to clarify the role of magnesium in salt tolerance. In case of a mangrove *Agielitis* annulata, Atkinson et al. (1967) suggested that the maintenance of salt tolerance was due to increase in magnesium content in the leaves. Konigshofer (1983) noticed that Plantago species tended to exclude sodium from leaf tissue by enhancing magnesium uptake for charge balance under saline conditions. Bernstein (1975) is of the opinion that magnesium maintains the ionic balance in the leaves and the tolerance capacity of a particular species depends on its ability to absorb adequate levels of Ca^{2+} and Mg^{2+} from the soil under saline conditions. Epstein (1972) has indicated that the adequate level of magnesium for optimum growth is 0.2%. Our observations indicate that both the Derris species have efficient mechanism of magnesium uptake under saline conditions as in almost all plant parts the magnesium levels are higher than this optimum level. The high levels of magnesium in nodule tissue are particularly interesting and they indicate active participation of magnesium in the nodule metabolism.

vi) Phosphorus :

The phosphorus contents in different plant part of D. trifoliata and D. scandens are recorded in Fig.12. It is evident from the figure that the phosphorus levels are quite significant in the young leaves of D. trifoliata. The phosphorus contents are also quite high in nodules of both the species. In other parts of D. trifoliata, there is not much variation in phosphorus status. The stem and petiole tissue of D.scandens

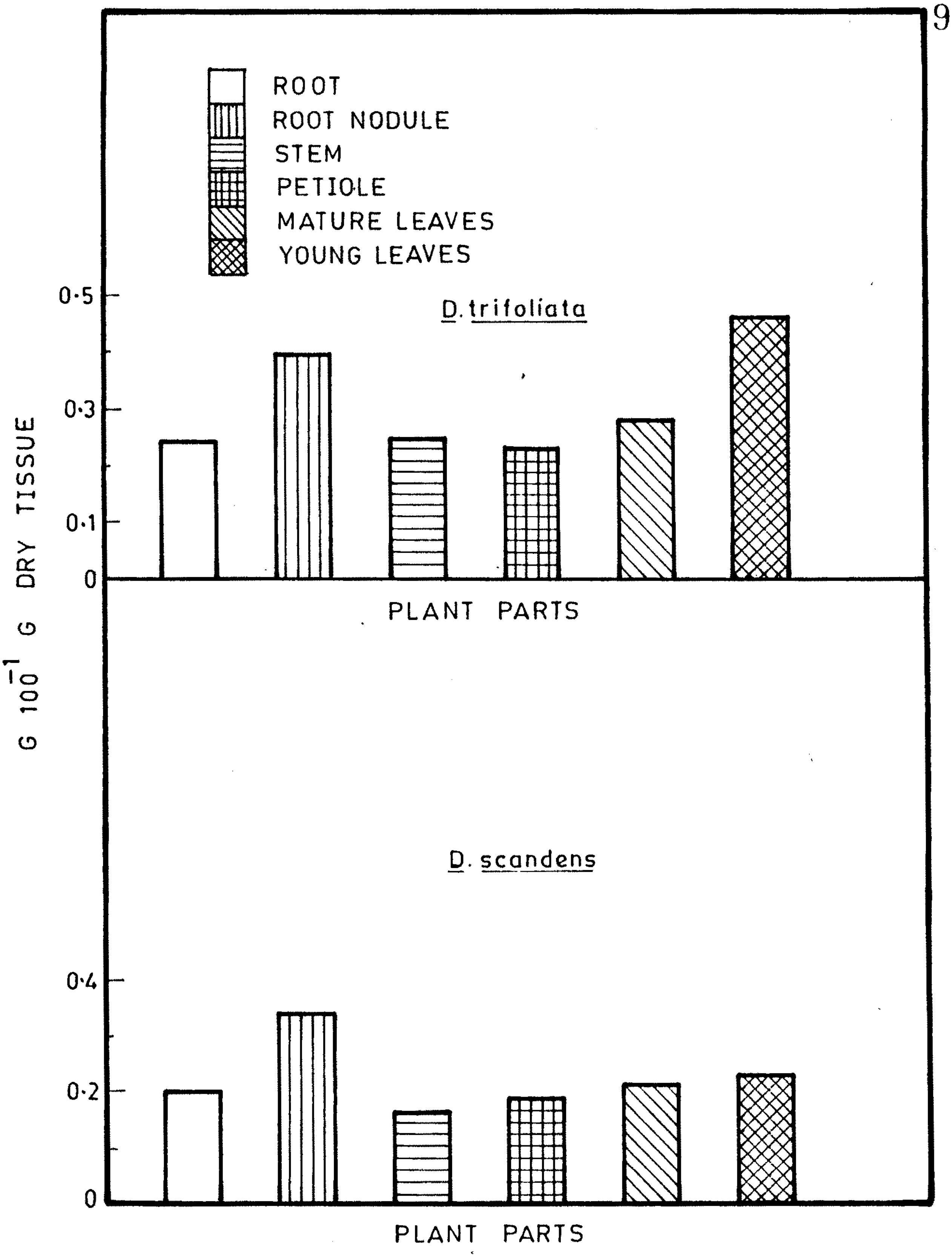


FIG. 12 – PHOSPHORUS CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

contain relatively low levels of phosphorus.

Phosphorus is regarded as one of the key components of cellular chemistry. This element is a constituent of important cellular metabolites including DNA. The sugar phosphates play a major role during the process of glycolysis. The phospholipids are essential constituents of membrane structure. The role of phosphorus in energy transfer is very well established. This element in combination with other organic molecule like ADP and NAD is energy currency of plant metabolic processes.

It has been suspected by Waisel (1972), that halophytes may have an insufficient supply of phosphorus. According to Van den Honert (1937) uptake of phosphorus by plants in far higher from slightly acid solutions than from alkaline once because under such conditions, phosphorus is available as the monovalent $H_2PO_4^-$ ion and not as di or trivalent once. There are some attempts to measure phosphorus status in the leaves of various halophytes. The data presented by Walsh (1974) indicates that phosphorus content in leaves of mangroves varies from 0.14 (Rhizophora mangle) to 0.9% (R. brevistyla). Spain and Holt (1980) observed that the phosphorus content in the mangrove leaves varied from 0.09% (Ceriops tagal) to 0.21 % (Excoecaria agallocha). Sidhu (Cf Walsh, 1974) has recorded 0.26% phosphorus in D. uliginosa leaves. The observations of Kotmire and Bhosale (1979) indicated that the leaves of

D. heterophylla plants growing in Mumbra creek had 0.05 % phosphorus while those in Deogad creek had 0.11% phosphorus. The present observations indicate relatively higher phosphorus levels and a rather luxuriant consumption of phosphorus in plants growing Ratnagiri locality. Not much literature is available regarding phosphorus levels in different plant parts of halophytes. Spain and Holt (1980) noticed that the phosphorus levels in the branchwood of various mangrove species are relatively very low as compared to those in foliage. Ghevade (1983) noticed that the leaves of seagrass Halophila beccarii contained four times more phosphorus than rhizome. In the present investigation also high levels of phosphorus are noticeable in the leaf tissue as compared to the other plant parts. This is quite understandable in the light of the fact that leaf is a major metabolic centre. In view of Wilson et al. (1970) resistance to secondary salt induced stress in Glycine falcata is due to its ability to maintain high P^{5+} content in presence of salt in the medium. Rozema (1976) however, noticed that phosphate makes a very small contribution to osmotic adjustment in Juncus species. The work of Waisel and Eshl (1970) revealed that in the leaf cells of Suaeda monoica all of the phosphorus was concentrated in the cytoplasm and its content inside the vacuoles was negligible. According to Awad et al. (1990) the requirement for more phosphorus in salinized plants could be related to its role in energy fixation and carbohydrate partitioning and transport. At the same time phosphorus is also involved in the formation of cell membrane lipids, which play a vital role in ionic regulation (Bieleski and Ferguson, 1983). In the light of above hypothesis the high phosphorus status of the *Derris* leaves can be definitely regarded as an adaptive feature. The high level of phosphorus in root nodules is also noteworthy in this respect.

vii) Iron :

Iron contents in different parts of the two *Derris* species are presented in fig. 13. It is clear from the figure that the root nodules of both species have highest iron contents and they are followed by the root tissue. The stem tissue of *D. trifoliata* also contain appreciable amount of iron. The iron content in the young leaves is very low as compared to other plant parts.

Iron is one of the key mineral elements of plant metabolism. The prosthetic groups of the cytochromes, haemoglobins in root nodules and enzymes such as catalase are iron porphyrins. Iron porphyrins when attached to specific proteins make enormous enhancement of enzyme activities and at the same time hemes are also stabilized and protected. The importance of iron enzymes in respiration has been well documented. Most plant and animal cells contain the cytochromes which consist of a iron porphyrin enzymes. Iron porphyrins are found in all

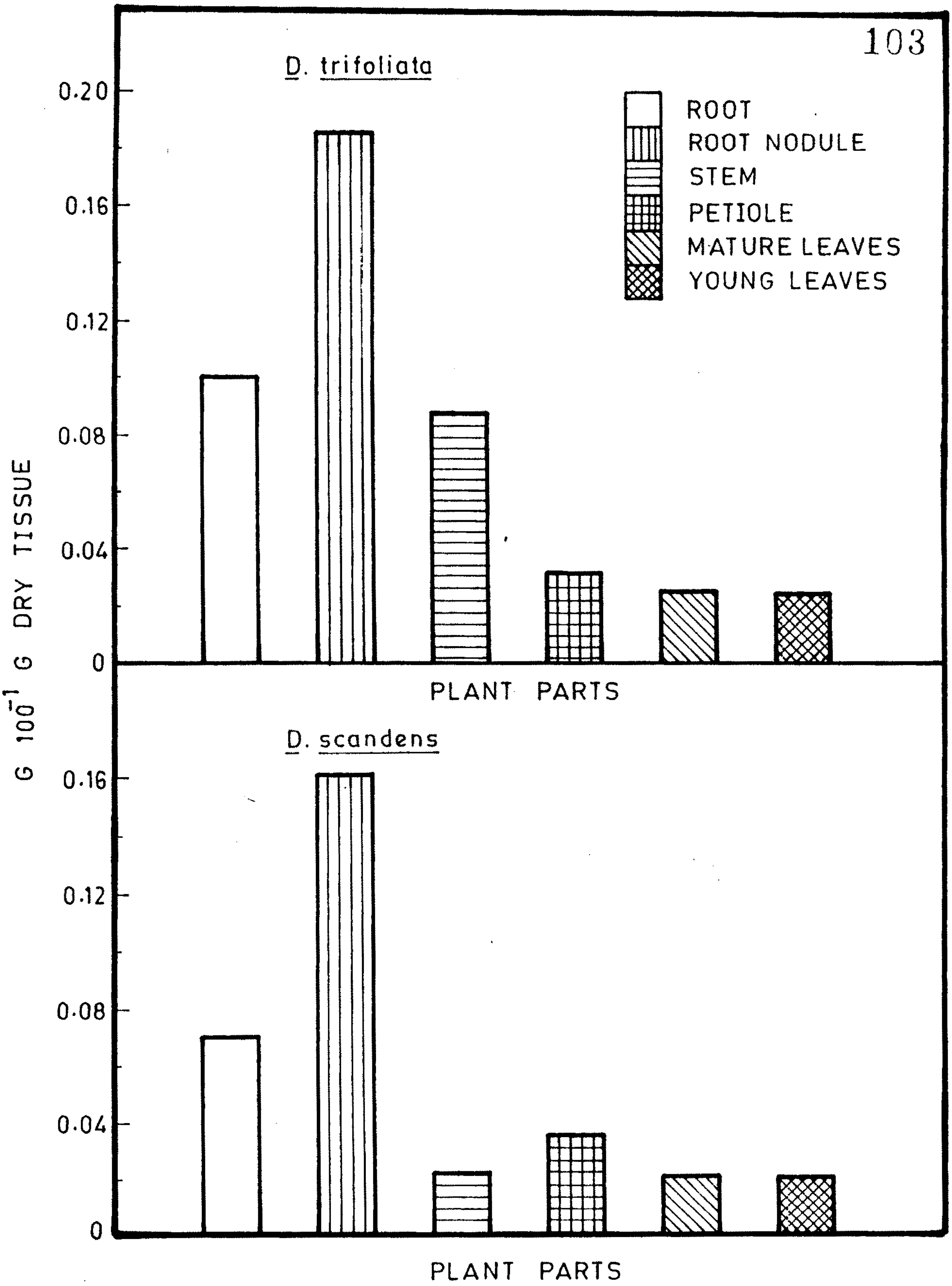


FIG. 13 – IRON CONTENTS IN DIFFERENT PARTS OF
DERRIS SPECIES.

aerobic forms of life and are involved in the respiratory chain and photosynthetic electron transport.

The formation of the chlorophylls and haemoglobin two of the most important pigments known in plants and higher animals is dependent upon an adequate supply of iron. Deficiency of iron within the cells of the leaf causes chlorosis or failure of chlorophyll synthesis which results in a yellowish or whitish colour in leaf. There are evidences for the involvement of ferrous ion in the condensation of succinic acid and glycine to form α-aminoleavalinic acid (ALA) which then condenses to form pyrrole groups. Afterwards prctoporphyrin IX is formed and magnesium is then incorporated into the molecule to form chlorophyll. It has been noticed that only about 0.1% of the iron in a plant leaf is involved in the heme pigments (Dekock et al., 1960). The remaining iron appears to be stored as a ferric phosphoprotein termed phytoferritin due to its similarity to animal ferritin (Muir, 1960). Another form of iron occurs in the chloroplast and is termed as ferrodoxin containing 10 atoms of iron per molecule of which 8 are probably attached to sulphahydryl groups in the ferrous form.

Ferredoxin is a nonhaem-iron protein which participates in oxidoreduction process by transferring electron (Mengel and Kirkby, 1982). Terry (1980) earlier indicated that iron stress causes a failure in the formation of photosynthetic units. The possible role of iron in protein metabolism has been suspected from the findings of a number of authors who have observed that in iron deficiency the protein fraction decreases simultaneously with an increase in the level of soluble organic nitrogen compounds (Perur *et al.*, 1961). The short term experiments with the alga *Euglena gracilis* indicate that iron is directly implicated in nucleic acid metabolism (Price *et al.*, 1972). According to Mengel and Kirkby (1982), the iron content of green plant tissue is low as compared to macronutrient and generally in the order of about 100 ppm in the dry matter. Waisel (1972) observed that the salt marsh species *Spartina alternifolia* has got high iron requirement and if grown in media with a low iron content the plants become chlorotic. Golley and Hollister (Cf Walsh, 1974) observed that the iron content of mangrove leaves varied from 0.008% (*Rhizophora brevistyla*) to 0.0305 %(*Conocarpus erectus*). Kotmire and Bhosale (1979) studied the iron levels in leaves of various mangrove species growing in Deogad and Mumbra creeks. They found that the iron content varied from 0.012 to 2.80%. They further recorded that the iron levels in *D. heterophylla* leaves were in the range 0.026 to 0.052 %. The iron levels recorded in the present investigation in the leaves of two *Derris* species range from 250 ppm of dry matter to 262 ppm of the dry matter and this indicate that the iron nutrition in this legume is quite adequate under saline conditions.

There are few attempts to study the distribution of iron in halophytes. Golley and Hollister (Cf Walsh, 1974) observed that the roots of Conocarpus erectus and Laguncularia racemosa contained significant levels of the iron and these levels were far above those recorded for the leaf tissue. It was further noticed that the stem tissue of Rhizophora brevistyla and Avicennia nitida was also rich in iron. As high as 0.1% iron in the mangrove tissue was recorded by these two workers. The present investigation also indicate that the roots of both Derris species contain considerably higher amount of iron as compared to leaves. This may be due to immobile nature of iron (Brown, 1956). It is interesting to note that nodules of both the species have very high levels of iron which indicates an active participation of iron in the nitrogen fixation process.

viii) Manganese :

The levels of trace element manganese in different parts of the Derris species are shown in Fig.14. It is clear from the figure that the manganese level in the plant parts like young leaves, mature leaves, petiole and nodules are relatively very high in D. scandens as compared to D. trifoliata. However, the pattern of manganese distribution appears more or less similar in both the species except for the fact that in D. trifoliata maximum manganese content is seen in root tissue and root nodules, while in D. scandens mature leaves contain highest amount of manganese.

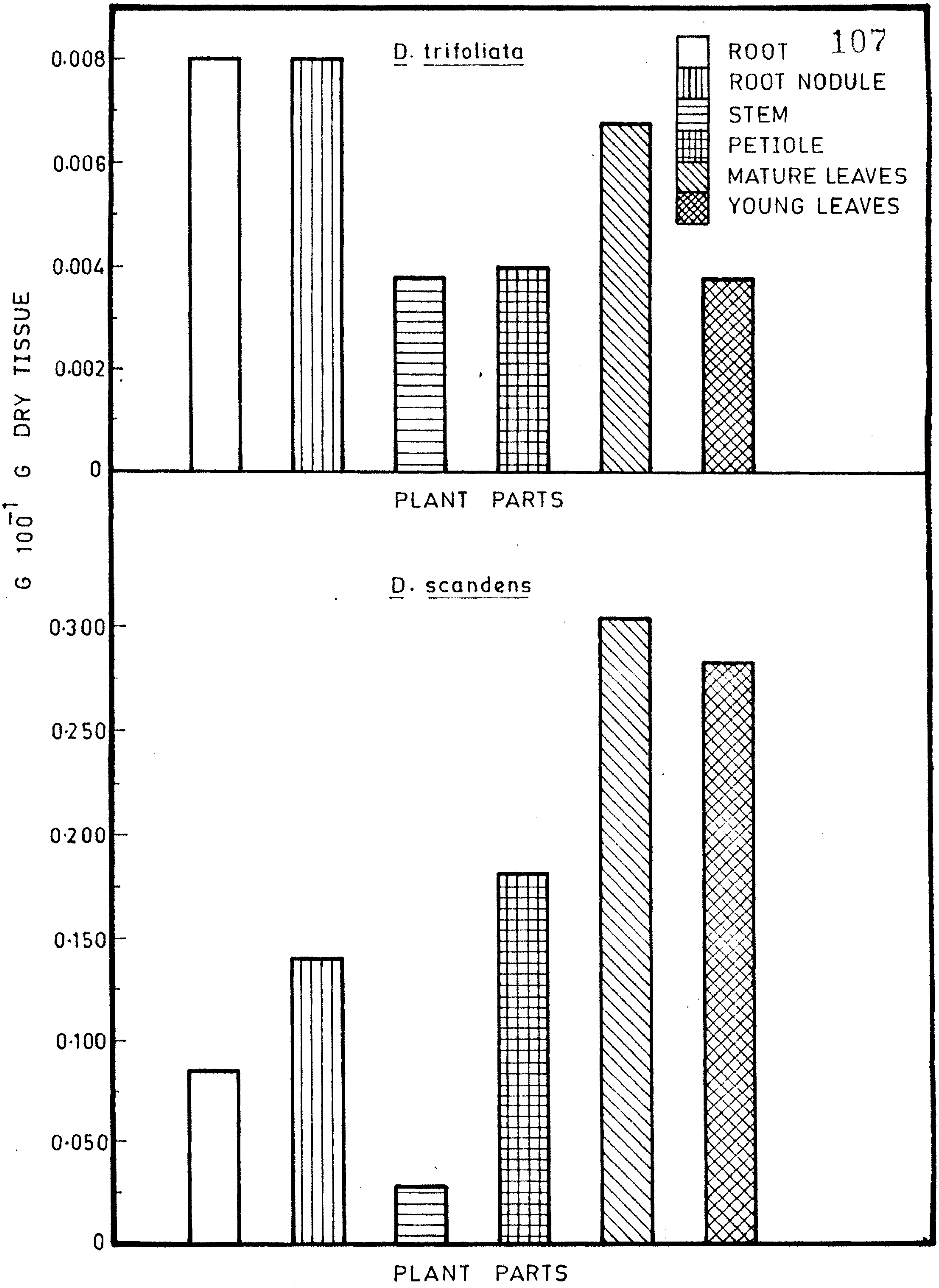


FIG. 14 – MANGANESE CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

Manganese although a micronutrient, plays several vital roles in plant metabolism. Basiouny and Briggs (1976) indicated that the photosystem II contains manganese protein which catalyses the early stages of oxygen evolution. Manganese is also essential for the activities of enzymes of TCA cycle such as PEP carboxylase, NADP-malic enzyme and NAD-malic enzyme. It is now very well realized that manganese can replace magnesium in many of the phosphorylating and group transfer reactions. Teichler Zallen (1969) have demonstrated the requirement of manganese for maintenance of chloroplast structure.

According to Stout (1961) the level of manganese adequate for the growth of multicellular plant is 50 ppm. There are very few observations regarding manganese status of halophytes. Morton (1965) observed that the leaves of Rhizophora mangle contain 0.003 % manganese. Golley and Hollister (Cf Walsh, 1974) observed that the manganese content in mangrove leaves varies from 0.005% (Conocarpus erectus) to 0.0387 % (Rhizophora brevistyla). Spain and Holt (1980) studied the manganese levels in the foliage of different mangrove species growing in Northern Queensland. Their observations indicate that the manganese levels ranged from 0.0016 % (Osbornia octodonta) to 0.0318 % (Rhizophora stylosa). From our laboratory Joshi and Bhosale (1982) reported that the manganese content in the leaves of mangroves varied from

0.00258 % to 0.0158%. In case of D. heterophylla Kotmire and Bhosale (1979) recorded that the manganese content in the leaves varied from 0.014 to 0.02%. These observations concur with the observations made with D. scandens in the present investigation. However the values recorded in both young and mature leaves of D. trifoliata are quite low.

Except for the report of Spain and Holt (1980) particularly no information is available regarding the distribution of manganese in different parts of halophytes. These workers recorded that except Rhizophora stylosa the branchwood of all other mangrove species contained very low levels of manganese compared to corresponding leaf tissue. In the present investigation such situation appears to prevail in case of D.scandens while in D. trifoliata the stem tissue contains appreciable amount of manganese. In case of D. trifoliata, the roots and root nodules contain higher levels of manganese as compared to the aerial parts. A negative influence of salinity on manganese uptake and its accumulation in the leaf tissue was evident in the experiments of Soufi and Wallace (1982) with Atriplex hymenelytra. Our observations indicate that similar situation may be there in case of D. trifoliata.

ix) Zinc :

In Fig.15 the contents of zinc in different parts of D. trifoliata and D. scandens plants growing under natural

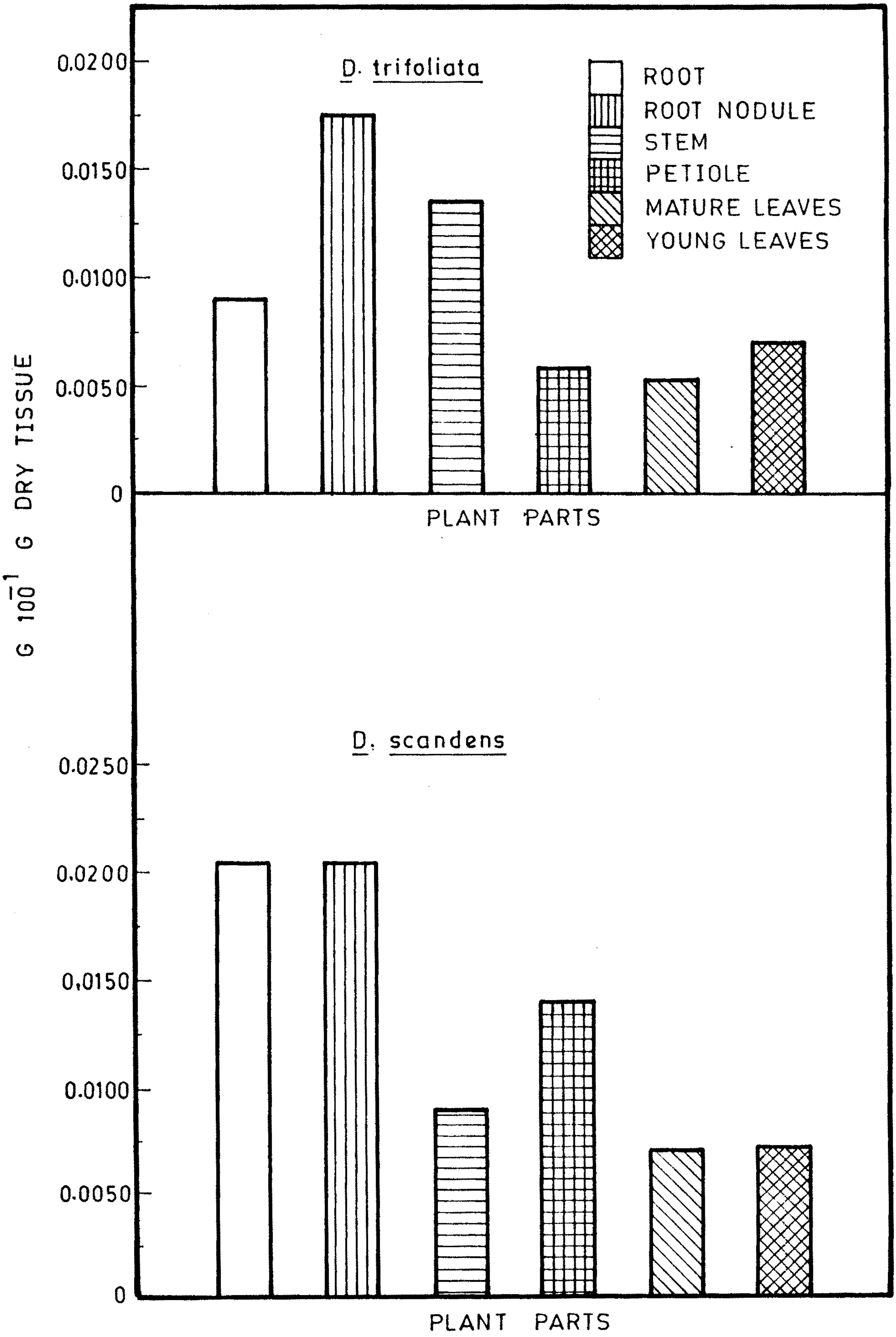


FIG. 15 - ZINC CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

conditions are depicted. It is clear from the figure that the pattern of zinc accumulation in the two species differ. In case of D. trifoliata the zinc levels are considerably high in the root and stem tissue. On the other hand, in D. scandens young leaves and root tissue are rich in zinc. Nodules of both the species have very high levels of zinc.

In last few year considerable attention has been paid to Zinc nutrition of the agricultural crops. The association of zinc with various enzymes,has been established in recent years. The enzyme specifically activated by zinc was first shown to be carbonic anhydrase. This enzyme catalyses the reaction

$$H_2O + CO_2 \rightleftharpoons H^+ + HCO_3$$

Other enzymes which need zinc include a number of dehydrogenases in particular glutamic acid dehydrogenase, lactic acid dehydrogenase, alcohol dehydrogenase as well as proteinases and peptidase (Vallee and Wacker, 1970). Zinc is also a component of an important protective enzyme superoxide dismutase. The work of Indian workers has clearly shown the involvement of zinc in nitrogen metabolism of crop plants (Naik and Asana, 1961, Ghildiyal et al., 1986, Reddy and Rao, 1979). Zinc requirement of plant is relatively very small. But some times zinc may reach toxic concentration in the soil and can cause number of metabolic disorders (Fitter and Hill 1987). It has been recorded that zinc resistance is a multiple phenomenon partly involving exclusion in the root cell walls and partly localization in the cell vacuoles, where the ion is complexed with malate. Mathys (1977) has suggested that zinc is stored in vacuoles complexed with oxalate and transferred through the cytoplasm bound to malate. Mengel and Kirkby (1982) have suggested that up to 0.01% (100 ppm) zinc contents are present in plant tissues. Boehle and Lindsay (1969) indicated that 0.002 to 0.007% zinc levels can be regarded as sufficient for soybean. There are few attempts to record zinc levels in halophytes. Golley and Hollister (Cf Walsh 1974) observed that the zinc contents in mangrove leaves varied from 0.001% (*Rhizophora brevistyla*) to 0.017% (*Lumnitzera racemosa*). Spain and Holt (1980) recorded relatively low levels of zinc in the leaves of seven mangroves from Northern Queensland. They found that the zinc content varied from 0.0005% (*Rhizophora stylosa*) to 0.0047% (*Excoecaria agallocha*). The observations from our laboratory indicated that the zinc levels in leaves of mangrove species varied from 0.0008% to 0.0029% (Joshi and Bhosale, 1982). The present observations indicate that the leaves of *D. trifoliata* contain high zinc levels as compared to the values recorded by Joshi and Bhosale and Spain and Holt but still they are in the satisfactory range recorded by Boehle and Lindsay (1969). It is evident from the present investigation that young leaves of *D. scandens* contain excess of zinc which may prove to be toxic.

There are some attempts to find out distribution of zinc in different parts of halophytes. Hollister (Cf Walsh, 1974) observed not much difference between zinc levels in different parts of Conocarpus erectus. On the other hand, in Laguncularia racemosa, he observed marked accumulation of zinc in the root tissue. In case of Lumnitzera racemosa the leaves had seven times more zinc contents than the stem tissue. Relatively high levels of zinc in foliage as compared to branch wood tissue were also recorded by Spain and Holt (1980) for mangrove species of North Queensland. In the present investigation, however, the stem tissues of D. trifoliata show higher levels of zinc as compared to the leaf tissue. In D. scandens roots and root nodules of D. trifoliata, we can find marked accumulation of Zinc. Tyler (1976) observed accumulation of zinc in roots of Anemone nemorosa as compared to shoot and he found that the root to shoot ratio of zinc content was 3.6. Such tendency is shown by D. trifoliata plants also. It is very clear from the present investigation that D. scandens is more efficient in zinc uptake than D. trifoliata as in all plant parts of this species, the zinc levels are higher.

X) Copper :

The levels of copper in different plant parts of the two Derris species are shown in fig.16. The roots of D. trifoliata contain high levels of copper in comparison to the stem and leaf tissue. On the other hand, in young leaves of D.scandens

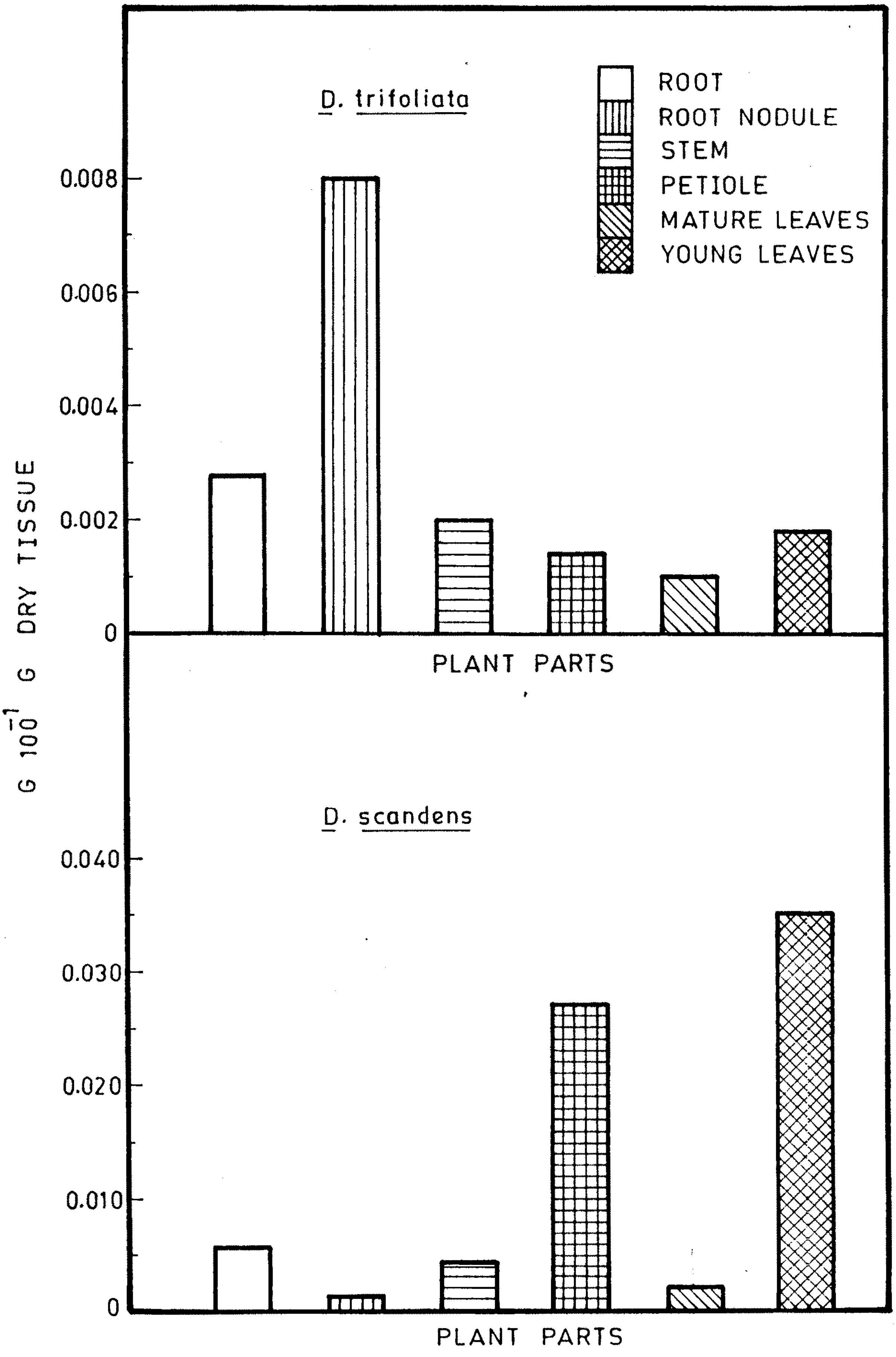


FIG. 16 – COPPER CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

maximum copper contents are recorded. The petiolar tissue of this species also contain high levels of copper. Appreciable amount of copper has been recorded in root nodules of D. trifoliata.

The oxidative enzymes such as cytochrome oxidase, ascorbic acid oxidase, polyphenol oxidase and laccase contain copper as an essential component. Copper is also a component of enzyme superoxide dismutase. According to Bishop (1966) the chloroplast protein, plastocyanin which plays an important role in photosynthetic electron transport has copper as an essential component. There are some reports which indicate that copper participates in protein, carbohydrate and fatty acid metabolism. In some cases copper toxicity is also noticed and copper resistance involve the complexing of copper with polyamino polycarboxylic acids so as to maintain the toxic ion in an inactive form in the metabolic compartment. The copper content in most plant is generally between 2 to 20 ppm in dry plant material (Tiffin, 1972).

There are few attempts to measure copper status of halophytes. Spain and Holt (1980) observed that the copper content in mangrove foliage varied from 0.0007% (Osbornia octodonta) to 0.002% (Avicennia marina). Joshi and Bhosale (1982) observed that the copper content in mangrove leaves varied from 0.00041% to 0.0009%. The values recorded for the leaves of D. trifoliata are quite above these values.

The copper contents in other plant organs of halophytes are recorded by very few workers. Hollister (Cf Walsh, 1974) observed that the roots of *Conocarpus erectus* contained 3 times more copper as compared to stem tissue, while in *Avicennia nitida* there was not much difference in copper levels of both the tissues. The work of Spain and Holt (1980) with seven mangrove species from Northern Queensland indicated that the branchwood contained lower copper level than the foliage.
In the present investigation with *D. trifoliata* it can be seen that the root tissue contains more copper as compared to stem and leaf tissue. Such tendency of copper accumulation in roots has been noticed by Tyler (1976) in *Anemone nemorosa*. Observations of Hallsworth *et al* (1960) indicated that there is a particular requirement of copper in symbiotic nitrogen fixation. The nodulation of *Trifolium subterraneum* was appreciably depressed at low levels of copper nitrition. Although, as yet no mechanisms for this has been identified. Hallsworth *et al.* (1960) also suggested that copper may be involved in leghaemoglobin synthesis. The high levels of copper recorded in nodules of *D. trifoliata* clearly indicate efficient copper uptake mechanism of this species under saline conditions in order to facilitate satisfactory nitrogen fixation.

xi) Cobalt :

In Fig.17 the contents of cobalt in different parts of *D. trifoliata* and *D. scandens* plants growing under natural

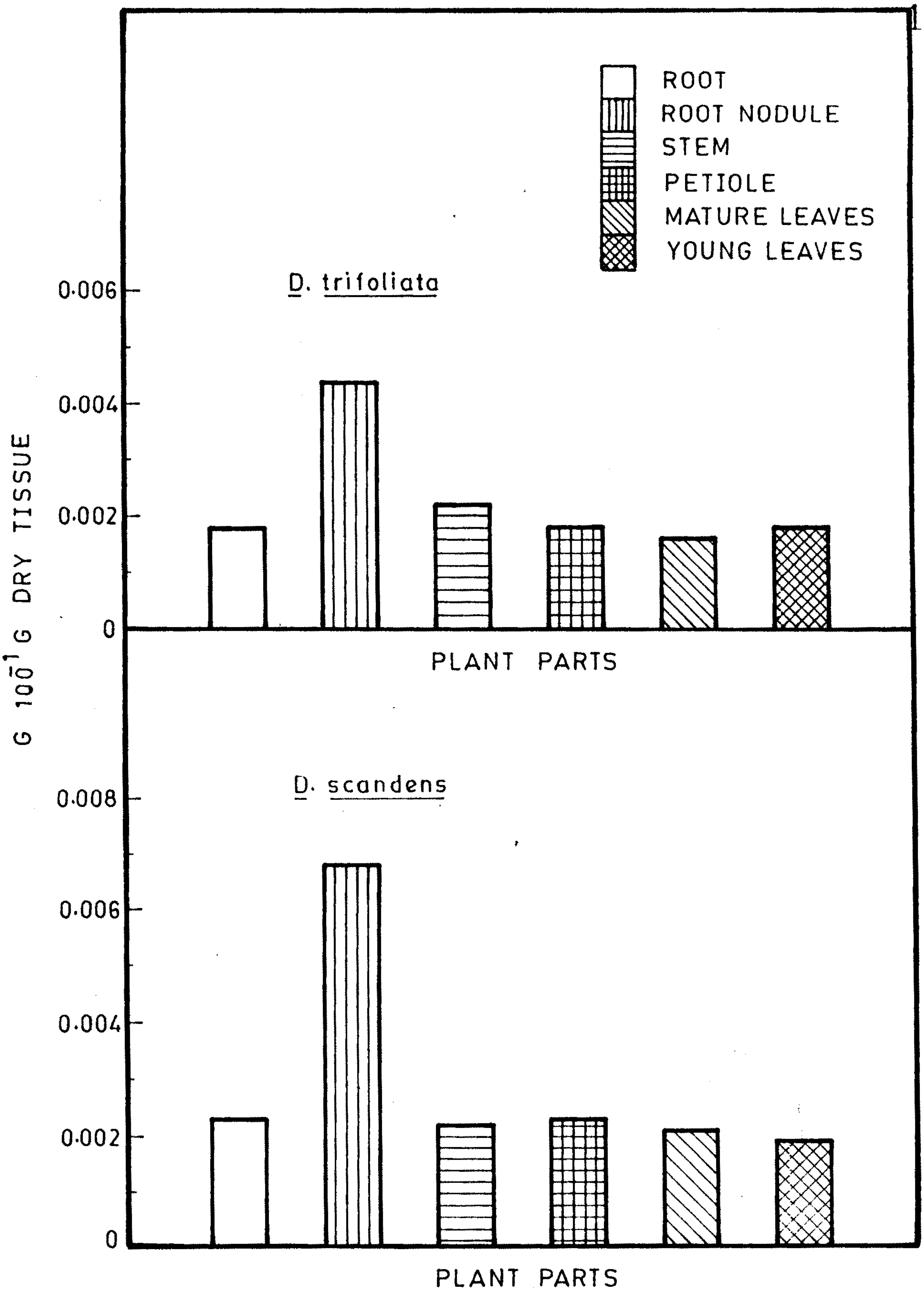


FIG. 17 – COBALT CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

conditions are depicted. It is clear from the figure that the pattern of cobalt accumulation in two species is different. In case of *D. trifoliata*, the cobalt levels are appreciably high in the stem tissue and almost similar in root, petiole, young leaves and mature leaves. On the other hand, in *D. scandens* root and petiolar tissue are rich in cobalt and the values are slightly lower in stem, young leaves and mature leaves. Nodules of both the species have very high levels of cobalt. On the whole there is not much variation in the cobalt level in different plant organs.

Cobalt is required in nitrogen metabolism like molybdenum. Thus it is essential to higher plants for symbiotic nitrogen fixation (Epstein, 1972). Kliewer and Evans (1963) isolated the cobalamin coenzyme B_{12} from the root nodules of legumes and nonlegumes and they showed the close relationship between cobalt supply, the B_{12} coenzyme content of *Rhizobium*, the formation of leghaemoglobin and nitrogen fixation. On the basis of these studies it has been realized that *Rhizobium* and other nitrogen fixing micro-organisms have a absolute cobalt requirement regardless of whether or not they are growing within nodules or regardless whether they rely on nitrogen fixation or are being supplied with mineral nitrogen. In the coenzyme cobalamin (Vitamin B_{12} and its derivatives) Co III is the metal component which is chelated to four nitrogen atoms at the centre of a porphyrin structure similar to that of iron in hemin. According to Dilworth et al. (1979) in Rhizobium, three specific cobalamin dependent enzyme systems exist and cobalt induced changes in their activities are presumably responsible for the relationship between cobalt supply, nodulation and nitrogen fixation in legumes. They are - (a) Methionine synthase, (b) Ribonucleotide reductase : The enzyme participates in the reduction of ribonucleotides to deoxyribonucleotides and therefore in DNA synthesis, (c) Methylmalonyl coenzyme A mutase : This enzyme is involved in the synthesis of heme (iron porphyrins) in the bacteroids and thus in cooperation with the host nodule cells in the synthesis of leghaemoglobin. Under conditions of cobalt deficiency the synthesis of leghaemoglobin is therefore reduced, which affects the nitrogen fixation rate adversely.

In photosynthetic lower plants such as Euglena gracilis it has been noticed by Isegawa et al. (1984) that cobalamin is essential for growth and is localized in various subcellular fractions as well as in the thylakoids of chloroplasts. Thus although the role of cobalt in nitrogen fixation is very well established the participation of this trace element in other metabolic processes of plants is not yet clear.

Kubota and Allaway (1972) reported appreciably higher levels of cobalt (0.15 to 0.27 μg g^{-1} dry matter) in legumes.

Golley and Hollister (Cf Walsh, 1974) reported that the cobalt content in mangrove leaves varied from 0.0056% (Rhizophora brevistyla) to 0.0009% (Rhizophora mangle) while Joshi and Bhosale (1982) reported a cobalt level range 0.00017 to 0.00059% for mangrove leaves. The values recorded in the present investigation for both Derris species are near the low extremity of cobalt contents reported by Golley and Hollister. Very little is known regarding cobalt distribution in different organs of halophytes. Golley and Hollister (Cf Walsh, 1974) noted that the stem tissues of mangroves contained more cobalt than the leaf tissue. Such situation is also seen to some extent in D. trifoliata, but in other species there is not much variation in cobalt level in different plant parts. It is interesting to note here that nodules of both Derris species have appreciable amount of cobalt and these findings are quite understandable in view of key role of cobalt in nitrogen fixation process.

d) Organic constituents :

i) Moisture percentage

The moisture percentage in different parts of D. trifoliata and D. scandens is recorded in (Fig.18a). It is evident from the figure that the nodules of both the species contain appreciable amount of water. It is clear from the Fig.18a that the moisture content in different parts of D. trifoliata is higher than the corresponding plant parts of D. scandens. The young leaves and petioles of D. trifoliata have considerably higher moisture level.

The water status of most of the plant parts is governed by two factors, the extent of water availability and the rate of water loss during transpiration. Many halophytes have been found to have a succulent nature which improves their water relations under saline conditions. The nearly universal increase in water content observed when halophytes are grown on NaCl has been regarded as an adaptive mechanism to dilute potentially toxic levels of Na^+ in the cell sap (Jennings, 1968). Briens and Larher (1982) found a strong correlation between the water and mineral content of 15 salt marsh halophytes collected from the wild. They interpreted the data as suggestive of a close relationship between the water relations of halophytes and their capacity for uptake and storage of mineral ions.

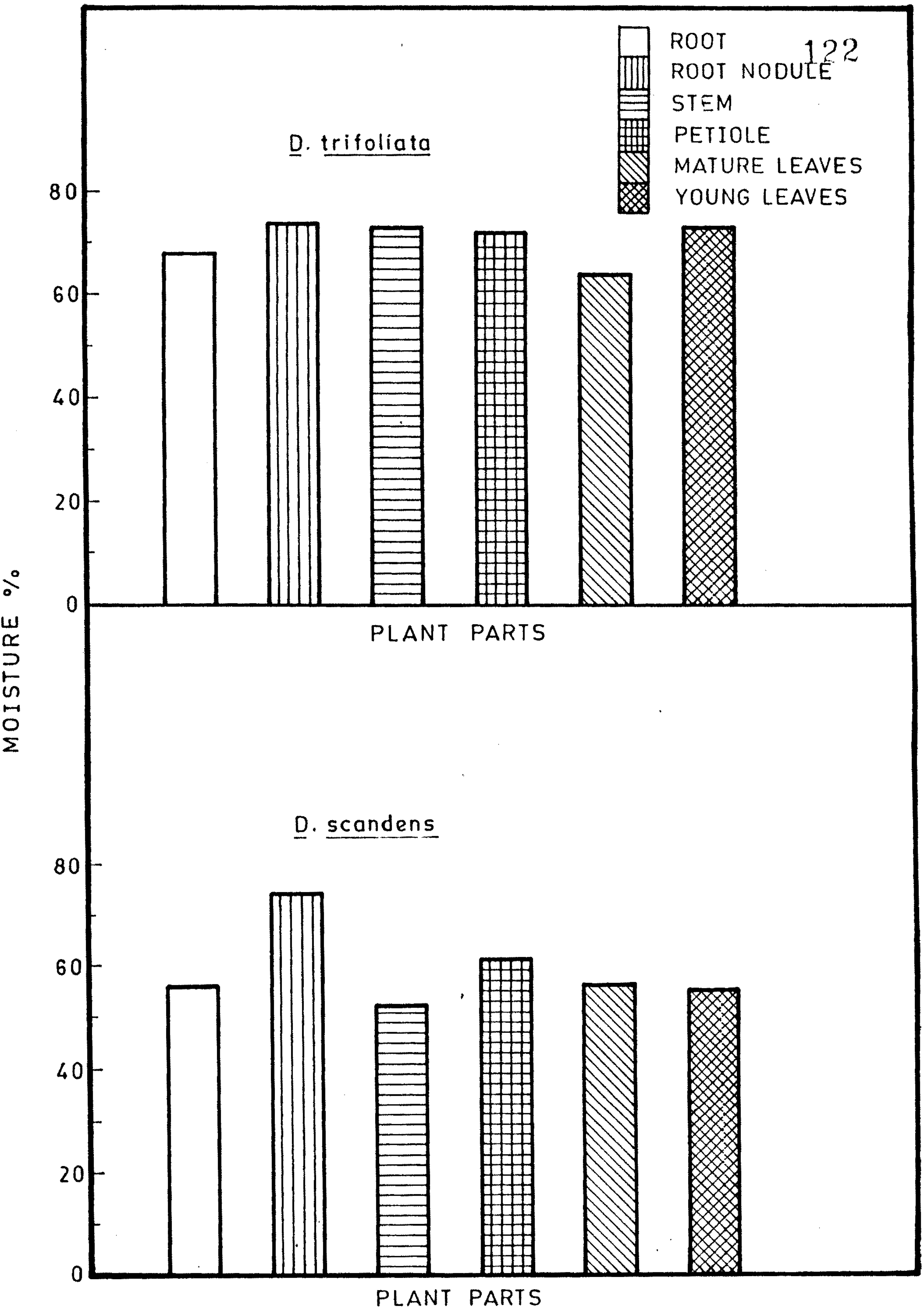


FIG. 18 a – MOISTURE PERCENTAGE IN DIFFERENT PLANT PARTS OF DERRIS SPECIES.

Glenn and O'Leary (1984) noticed that about 18 halophytic species belonging to different families had higher water contents when grown on 180 mol m^{-3} NaCl than on fresh water. In view of these workers the water content and mineral content were coordinated to maintain a constant osmotic potential gradient in some species. These workers observed that the water content (g g^{-1} dry wt) in these species varied from 1.6 to 7.6. The water content (g g^{-1} dry wt) of D. trifoliata mature leaves was found to 1.82. While that of D. scandens leaves was 1.25. Atkinson et al.(1967) observed that the moisture percentage in mangrove Rhizophora mucronata and Aegialitis annulata were 66 to 60% respectively While Amonkar (1977) found 68.31% moisture in green leaves of Salvadora persica. Jamale (1975) found 74.1 % moisture in leaves of Excoecaria agallocha. Thus it is clear that the mangroves also show great variation in leaf moisture content. The moisture percentage of mature leaves of both Derris species is rather low; indicating a near absence of leaf succulence, eventhough the plants are growing under saline condition.

ii) Carbohydrates :

The total sugar level in different parts of *D. trifoliata* and *D. scandens* plants growing under natural conditions is recorded in Fig.18b. It is evident from the figure that the mature leaves of both the species are rich in total sugars. In case of *D. trifoliata* the roots also have appreciable leaves of total sugars, while in case of *D. scandens* this is true with young leaves. The stem tissue in both the cases is low in total sugars.

The analysis of starch content in different plant parts of the two *Derris* species is also depicted in fig.19. It is evident from the figure that there is not much variation in the starch levels in different organs. The root tissue of *D. trifoliata* contains higher amount of starch as compared to the aerial organs. On the other hand, in *D. scandens* more starch accumulation is noticed in the stem tissue. The leaves of both categories of *D. trifoliata* have more starch content than those of the *D. scandens*.

In the biochemistry of plant cell the carbohydrates are of extreme importance because on one side they represent ultimate product of photosynthesis, while on the other side they serve as the main source of energy which is essential for growth and development of plants. In Calvin cycle, first product formed was belongs to simple carbohydrates and

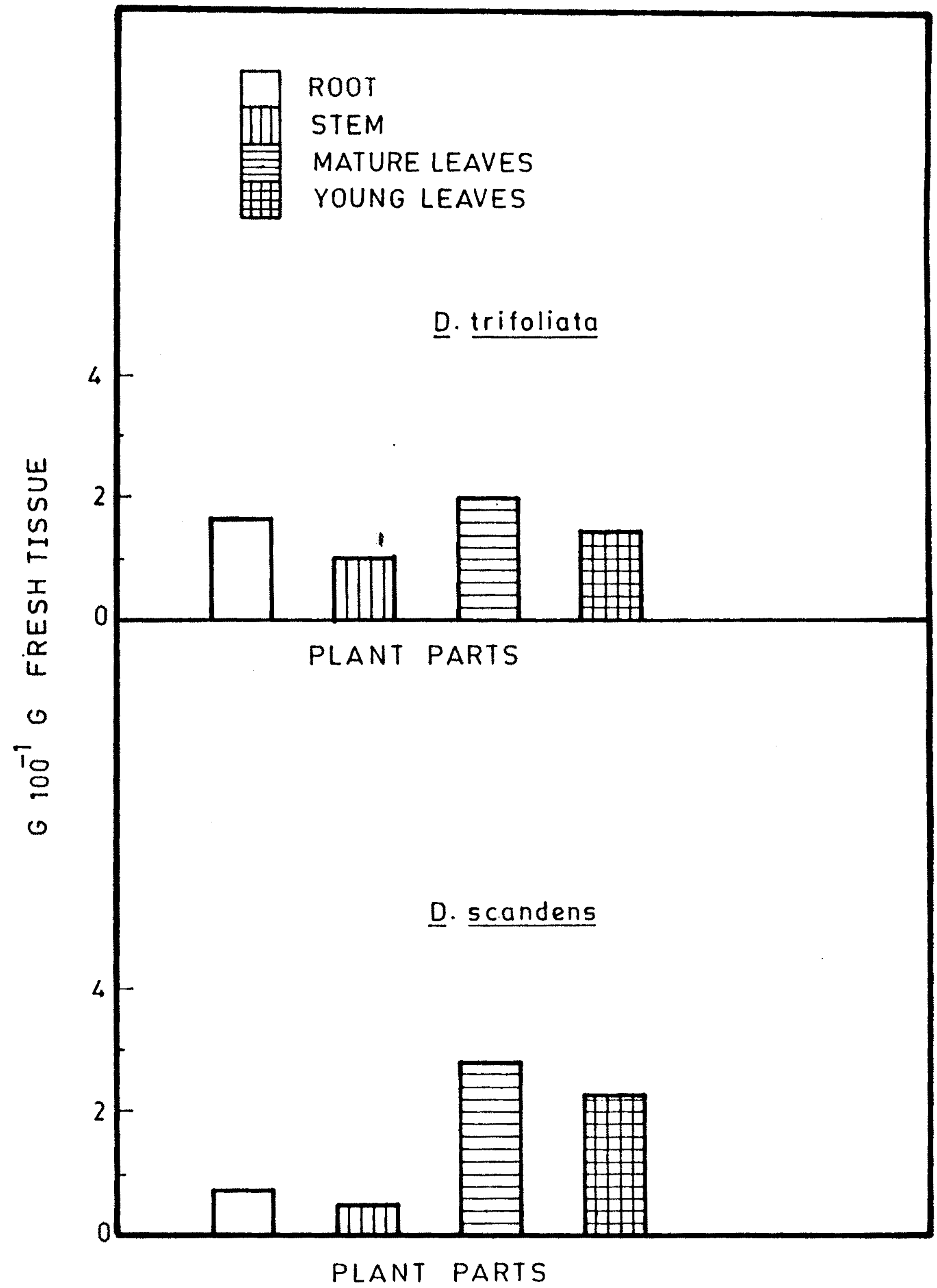


FIG. 18b–TOTAL SUGAR CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

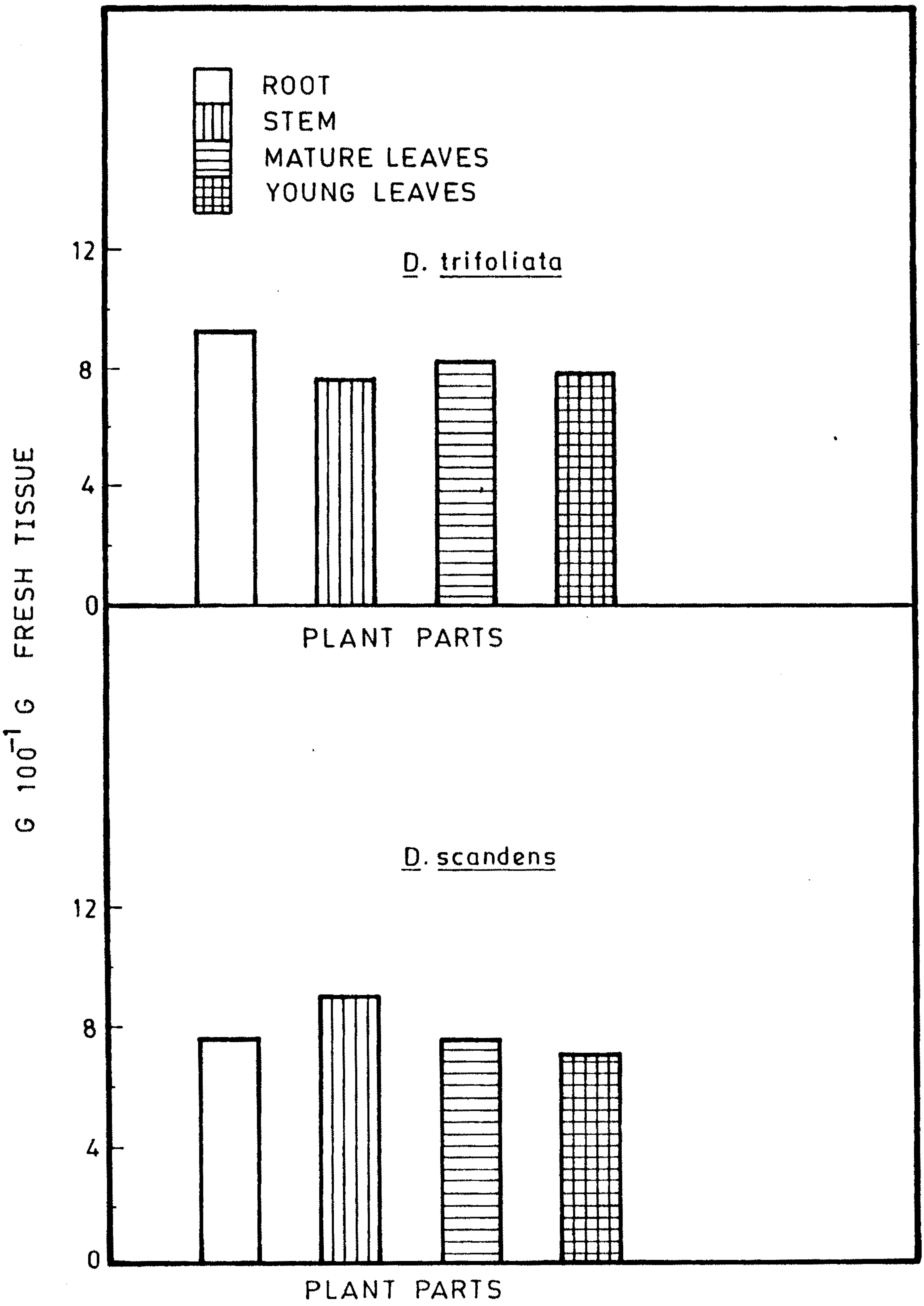


FIG. 19 - STARCH CONTENTS IN DIFFERENT PARTS OF <u>DERRIS</u> SPECIES.

through series of reactions, these are converted into other carbohydrates. Carbohydrates furnish the basic products for the synthesis of several organic substances including cell walls. These organic substances contain hydrogen and oxygen in the same ratio as they occur in water. Sugars are the most important carbohydrates and occur singly or linked, compared to simple sugars (monosaccharides) linked sugars are made up of simple sugars joined together by glycosidic bonds. The number of sugars which are linked by these bonds produce di -, oligo - or polysaccharides. The monosaccharides are classified as tri-, tetra - , penta - or hexoses depending upon the number of their carbon atoms e.g. 3, 4, 5, 6 carbon etc. respectively. Sugars generally take part in metabolism in phosphorylated state which is accomplished by means of ATP. The sugar phosphate bonds are rich in energy and thus activated sugar is involved in metabolic reaction. Among the disaccharides, sucrose is perhaps the most prominent disaccharide in nature. It is almost universally present throughout the plant world and has been detected in all parts of the plant. It represent a major product of carbon dioxide fixation during photosynthesis. Sucrose also is a major component of assimilate transport in higher plants. There are of several types of polysaccharides e.g. fructosans, starch, cellulose, pectin substances. Their monomers are joined together by glycosidic linkages and their polymer chains may even be branched. They are insoluble when have a high molecular weight. Starch is the common storage form of polysaccharide in plants and its polymers are exclusively made up of glucose units. It occurs as grains in the seeds, fruits, leaves roots etc.

The levels of various carbohydrate fractions in the plant tissue are influenced by number of factors among which the rate of photosynthesis and the rate of the respiratory turnover are important. In case of legumes like Derris, the biological nitrogen fixation in nodules creates additional demands on the photosynthesis. There are few attempts to determine the carbohydrate composition of halophytes. As early as 1932, Iljin observed that the photosynthetic parts of halophytes contain relatively little sugar in cell sap ranging from 0.54 to 0.9%. Shetty (1971) reported that the leaf tissue of Acrostichum aureum growing in saline condition had more starch content than that found under nonsaline conditions. Jamale (1975) observed that the total sugar content in mangrove leaves varied from 0.54%(Sonneratia acida) to 0.78 % (Excoecaria agallocha) while the starch content varied from 1.88 % (Sonneratia acida) to 2.76 % (Excoecaria agallocha).

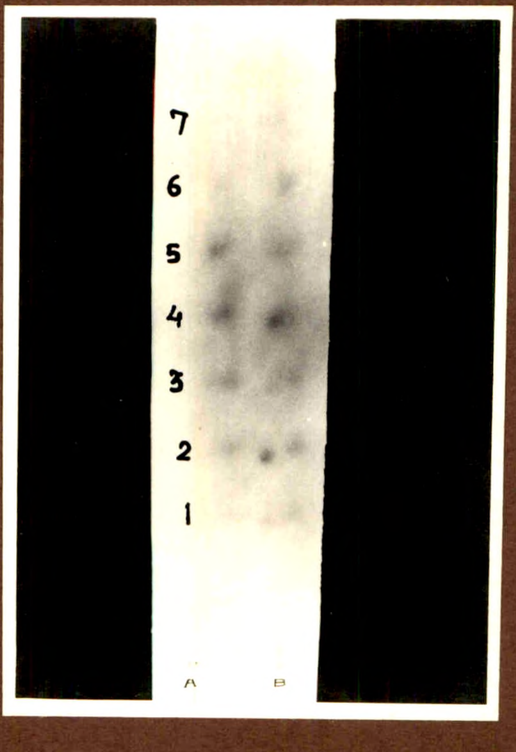
The work of Zahran and Wahid (1982) indicated that the roots of Kochia indica contain 6.295 % total carbohydrate while the shoots had 4.738 % total carbohydrates. Briens and Larher (1982) investigated the levels of carbohydrates in in different organs of sixteen plants collected on coastal marshes. The total soluble carbohydrate was more than 15 % of the dry matter (w/W) in the leaves and the roots of *Agropyron*, *Festuca*, *Puccinellia*, in the stems and the rhizomes of *Atriplex*, *Beta*, *Juncus*, *Phragmites* and *Scirpus*, in the roots of *Beta*, *Halimione*, *Limonium*, *Plantago*, *Spartina* and *Triglochin*. It is also very important quantitatively (7-10 % of the dry matter) in the leaves of *Beta*, *Juncus*, *Phragmites*, *Scirpus*, *Spartina* and *Triglochin* in the stems of *Halimione* and even in the roots of *Atriplex* and *Juncus*. The work of Bhosale *et al*. (1976) indicated that in mangrove leaves the total carbohydrates on a dry weight basis were in a range of 16.73 (*Derris trifoliata*) to 46.87 (*Avicennia officinalis*). Kotmire (1983) observed that in *Avicennia officinalis* leaves, there was 3.16 % starch and 1.13% total sugar while in *Avicennia marina* levels the starch content was 5.04 % and total sugar were 0.59 %. Thus it is clear that there is great variation in the carbohydrate levels amongst different halophytic genera and interspecific differences are also seen. It is evident from our observations that the carbohydrate status of *Derris* leaves is relatively high as compared to the carbohydrate contents of other mangrove species growing in same locality (Jamale, 1975).

It is evident from our studies that the mature leaves of both *Derris* species are quite rich in sugars as compared to all other plant parts. The preliminary paper chromatographic qualitative analysis of the sugar composition revealed that several sugars besides the conventional sugars (sucrose, fructose and maltose) occur in both the species Fig.20. Briens and Larher (1982) reported the leaves of soluble carbohydrates, in different organs of sixteen plants collected on coastal salt marshes. For all the species investigated sucrose, glucose, fructose, ribose, galactose and xylose could be characterized by chromatographic techniques. Maltose was present only in Atriplex, Beta, Halimione, Juncus, Plantago, Spartina and Triglochin shoots. Raffinose was detected in Festuca, Halimione, Juncus, Limonium, Phragmites, Plantago and Scirpus. Rhamnose was abundant in Plantago roots. Ackerson and Younger (1975) suggested that carbohydrate storage is one of the three mechanisms of salinity tolerance in plants. Gorham et al. (1980) observed that the monocot halophytes restrict the entry of inorganic ions and use high levels of soluble sugars to maintain an adequate solute potentials. They further suggested that hexoses rather than sucrose may be involved in osmoregulation in the more halophytic species. Popp (1984) made an exhaustive analysis of low molecular weight carbohydrates (LMWC) occurring in both young and mature leaves of 22 mangrove species from North Queensland, Australia. The cyclitol, pinitol, was the most prevalent compound in all members of the Rhizophoraceae and in the mangrove fern Acrostichum speciosum. In addition, Aegialitis annulata stored chiro-inositol. Another cyclitol, quebrachitol, occurred

Fig.20 Sugar composition of the leaf tissue of Derris species

A paper chromatogram showing free sugars in leaf extracts of A) *D. trifoliata* B) *D. scandens*

2) Sucrose 3) Fructose 5) maltose, 1,4,6 and 7 unidentified sugars.


7
6
5
4
3
2
1
A
B

in Excoecaria agallocha. Mannitol, a polyol, was dominating in Aegiceras corniculatum, Lumnitzera littorea, Lumnitzera racemosa, Sonneratia alba and Scyphiphora hydrophylacea. The sugars sucrose, glucose and fructose were not generally stored in high concentrations (except Xylocarpus granatum, 300 mol.m^{-3} plant water). Those species which contained no cyclitols or mannitol were low in their LMWC content. LMWC concentrations were higher in young leaves than in old ones in nearly all species under investigation.

In the absence of the data on the LMWC composition of Derris leaves and without the knowledge of exact localization of sugars at a cellular level it is difficult to comment on the osmoregulatory mechanism in Derris. However, the high sugar levels in Derris leaves probably indicate participation of sugars in osmoregulation, similar to mangrove species Xylocarpus granatum.

iii) Total Nitrogen :

The total nitrogen contents in different parts of D. trifoliata and D. scandens are recorded in fig.21. It is evident from the figure that leaf tissue of D. trifoliata is rich in total nitrogen; while this situation is seen in case of root tissue of D. scandens. In case of both the species very little nitrogen is found in the stem tissue.

Nitrogen can be regarded as the chemical basis of life,

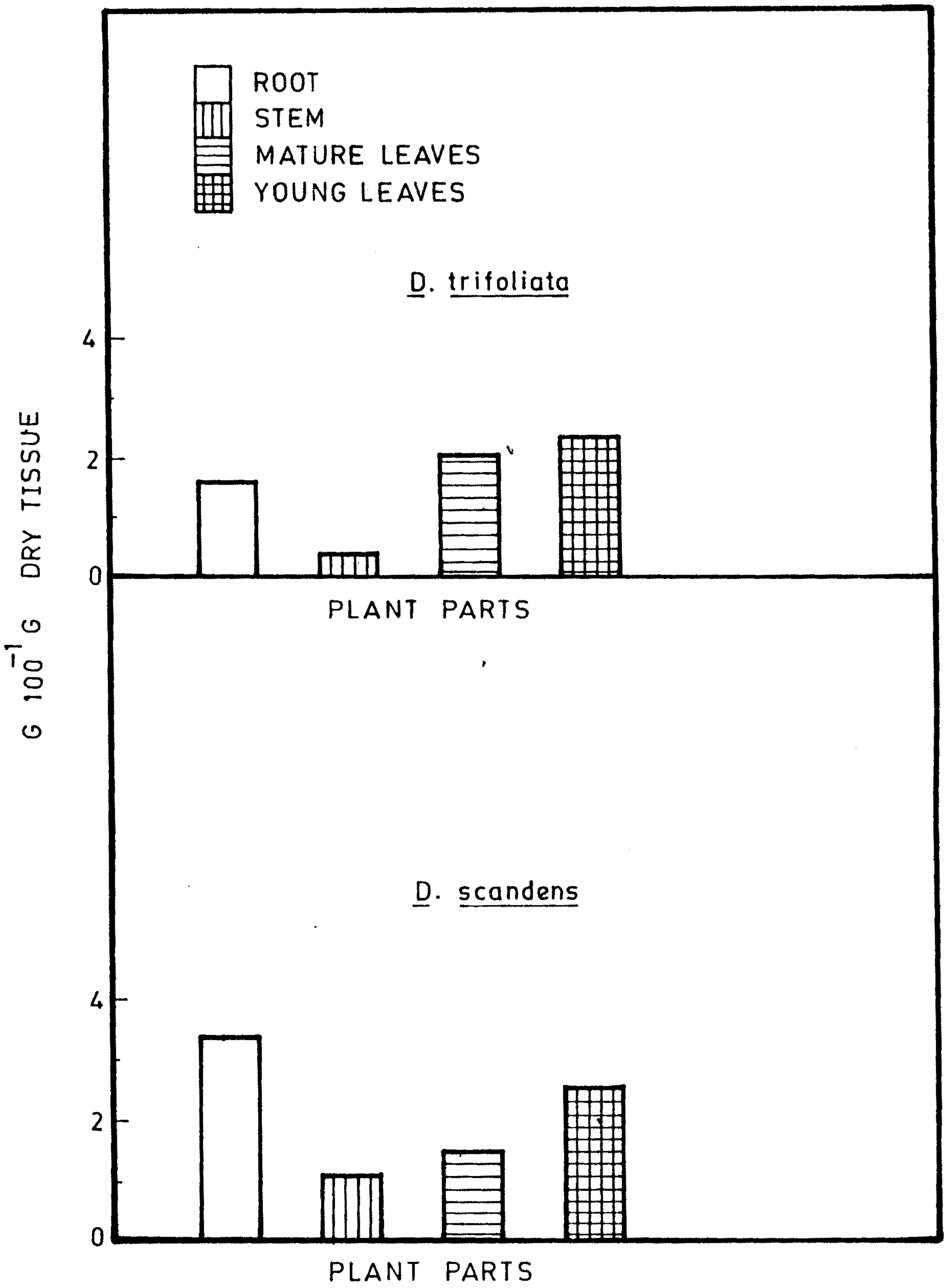


FIG. 21 - TOTAL NITROGEN CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

because it is an essential building-block of proteins without which the life processes are just impossible. Besides proteins, nitrogen is also a component of nucleic acids, pigments and several secondary metabolides. The nitrogen cycle is complex and the organic matter component is dominant. Several bacteria are involved in the conversion of ammonium to nitrate (Nitrobactor, Nitrosomonas and Nitrococcus are the most important), but both forms can in fact be taken up by most plants with equal facility. The leguminous species are having a special advantage over other plants because of their capacity to fix atmospheric nitrogen due to symbiotic association with Rhizobium in their root nodules. Nitrogen exists in several forms in the plant tissue such as protein, free amino acids, nitrate, nitrite, methylated onium compounds, nucleic acids and other metabolides such as nucleotides, pigments and alkaloides. The total nitrogen content of the tissue represents the sum of the nitrogen present in all these fractions and its level in a tissue gives us a broad idea about nitrogen nutrition of the plants. The total nitrogen levels have been determined by several investigators in different halophytic species. Spain and Holt (1980) observed that the total nitrogen content in the mangrove leaves of Queensland varied from 0.78% (Rhizophora stylosa) to 1.85 % (Xylocarpus australasicus). Popp et al. (1984) carried out exhaustive analysis of nitrogenous compounds of 22 mangrove species from Australia. They observed that the total nitrogen content varied from 0.62 % (Excoecaria agallocha) to 2.53 % (Avicennia marina). The work from our laboratory indicated that the total nitrogen content varied from 0.51 % (Excoecaria agallocha) to 6.27 %(Acanthus ilicifolius) and in monsoon the total nitrogen content were higher than other seasons for the most of the mangrove species studied (Jamale, 1975, Joshi and Bhosale, 1982).

In Derris leaves nitrogen content varies from 1.5% to 2.55 % and in the young leaves the nitrogen levels are more than those in the mature leaves. Popp et al. (1984) observed similar trend in the leaves of Ceriops tagal var. australis, Lumnitzera racemosa, Sonneratia alba, Osbornea octodonta, Avicennia marina, Rhizophora stylosa. There are few reports regarding the distribution of nitrogen in different plant parts of halophytes. Spain and Holt (1980) observed that the nitrogen content of the branchwood of the mangrove species was less than that of the leaves. Zahran and Wahid (1982) observed that the root system of Kochia indica had low nitrogen as compared to the shoot system. In the present investigation the lower levels of total nitrogen in the stem tissue as compared to the foliage are noticeable.

Several halophytic species are cultivated for the forage in different parts of the world. We have already seen in Chapter I that the foliage of both D. trifoliata and

D. scandens is utilised as fodder local. Zahran and Wahid (1984) reported that in *Kochia indica* a prospective forage halophyte the crude protein content in the leaf tissue reached a value of 9.75 %. O'Leary *et al*. (1984) observed that the crude protein content in the leaves of forage halophytes *Salicornia europaea*, *Atriplex lentiformis*, and *Atriplex barclayana* were 5.7 %, 16.7% and 11.6% respectively. The analysis of Pasternek *et al*. (1985) revealed that the crude protein levels in a promising halophytic *Atriplex* species ranged from 10.1 to 23.7%. If the crude protein content is computed from the total nitrogen content in the leaves of the two *Derris* species (6.25 X Total nitrogen) we can find that both young as well as mature leaves of *D. trifoliata* have appreciable crude protein content 13.06 to 14.62 %.

iv) Free Proline and Other Amino acids :

The free proline content in parts of two *Derris* species is recorded in Fig.22. It is clear from the figure that the pattern of free proline accumulation varies among the two species. In case of *D. trifoliata* the mature leaves contain highest level of free proline followed by root and stem tissue, on the other hand, in *D. scandens* highest proline contents are seen in the stem tissue followed by root tissue. Both young and mature leaves of this species have far lower free proline levels than the corresponding levels in *Derris trifoliata*.

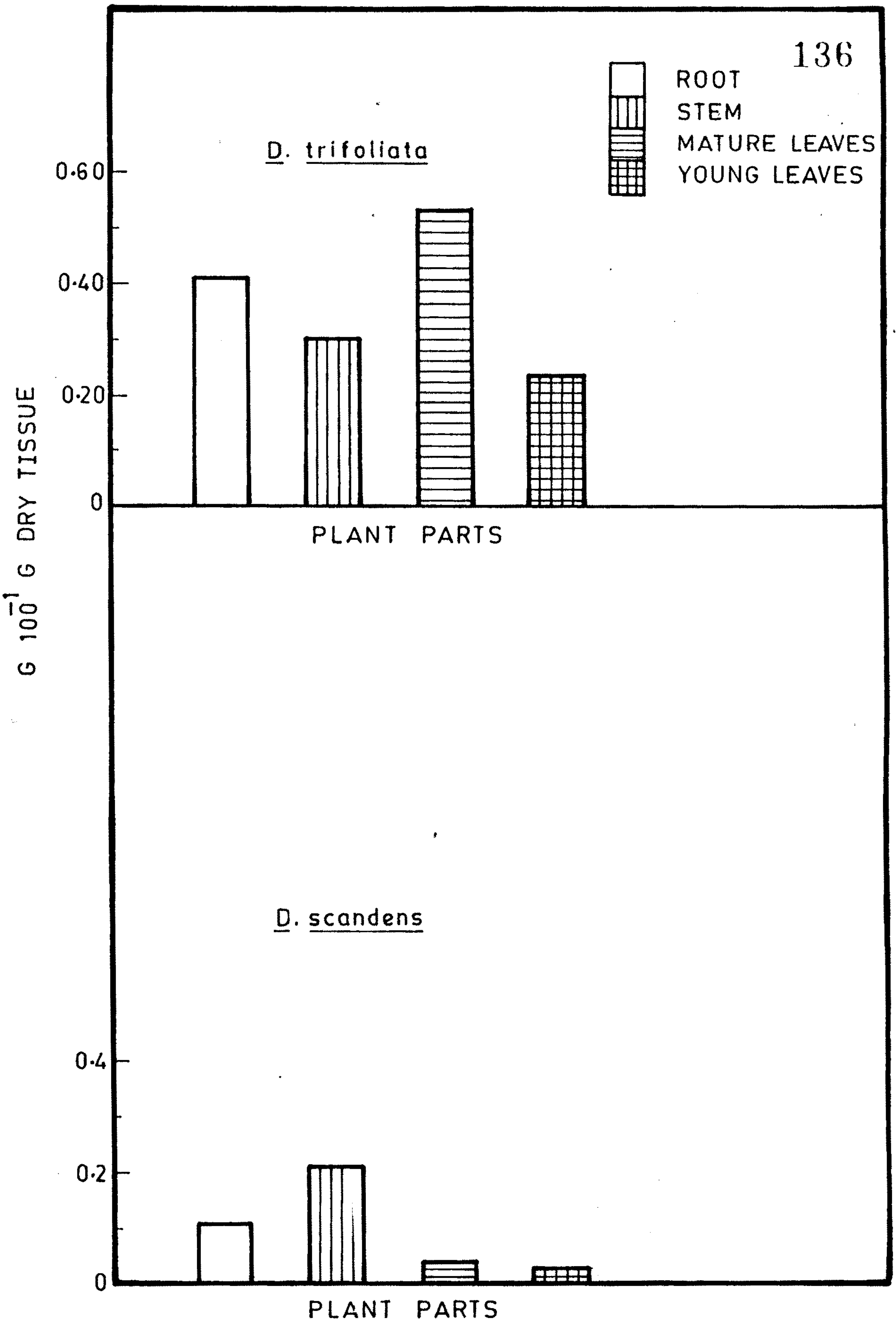


FIG. 22 - FREE PROLINE CONTENTS IN DIFFERENT PARTS OF DERRIS SPECIES.

Among various amino acids occurring in plants the proline has been one of the most popular subject of plant physiological investigations. Proline belongs to glutamate family of amino acids and its precursors are glutamate and arginine. This amino acid is heterocyclic in nature and highly water soluble. Proline is regarded as the only precursor of hydroxyproline which is an essential component of cell wall protein. The interest in this amino acid was aroused due to the fact that there is heavy accumulation of free proline due to different environmental stresses such as drought, salinity, high temperature, low temperature, mineral deficiencies, pathogenesis, and pollution. It is now very well realized that proline plays several important roles under stress conditions. Paleg and Aspinall (1981) suggested that proline acts as a compatible solute regulating and reducing water loss from the cell during situations of water deficit. Proline also serves as a source of carbon and nitrogen for biosynthesis of variety of compounds during a post stress recovery. According to Kathireson (1987) proline also serves a protective role for enzymes. It is indicated by Stewart and Lee (1974) that the proline may serve as a source of solute for intracellular osmotic adjustment under saline conditions. In view of Jefferies (1981) in halophytes proline may act as nitrogen storage compound, the main form of transported nitrogen and a compatible osmotic solute. There are several observations about proline accumulation in halophytic tissues. Stewart et al. (1974) also observed this phenomenon in halophytes. Treichel (1975) found proline accumulation due to salinity stress in *Salicornia fruticosa, Aster tripolium* and *Mesembryanthemum nodiflorum.* Also he added that young leaves contained more proline than mature leaves of *A. tripolium.* Appreciable levels of proline in *Lapidium crassifolium, Artemisia monogyna* and *Triglochin maritima* were recorded by Popp and Albert (1980). High proline levels were also recorded in the leaves of *Spergularia, Triglochin, Zostera* and *Puccinellia* by Gorham *et al*. (1980). Brien and Larher (1982) observed that there was difference in proline levels in different plant parts of the halophytes. They observed high proline levels in *Triglochin maritima, Puccinellia maritima, Agropyron punjens* and *Limonium vulgare*. In all these species leaves had higher proline levels than the root tissue. Waghmode and Joshi (1982) have observed that the proline content varied from 0.015 (*Ceriops tagal*) to 0.156 % (*Lumnitzera racemosa*) in the coastal halophytes from Ratnagiri. Kotmire (1983) reported that the proline content in *Avicennia* varied from 0.005 % to 0.16 %. Recently Popp *et al*. (1984) made a proline analysis of 22 mangrove species from Northern Queensland Australia. They observed high proline accumulation in only three mangrove species; *Aegialitis annulata* (28.5 mol. m^{-3} plant water), *Xylocarpus granatum* (76.0 mol. m^{-3} pw) and *Xylocarpus mekongensis* (56.7 mol. m^{-3} pw). Our observations indicate that the different parts of D. trifoliata have quite appreciable levels of free proline and young leaves contain less proline as compared to mature leaves. Similar situation regarding leaf age was also noticed by Popp et al. (1984) in case of Avicennia, Bruguiera and Hibiscus. According to Popp et al. (1984) in case of mangroves the function of proline include protective effects against both heat inactivation of enzymes and the oxalate destabilization of membranes which are probably also of some importance for mangroves. This protection against heat inactivation may be a distinct advantage since leaf temperatures of mangroves are reported to exceed ambient air temperatures upto 15°C (Clough et al., 1982). Similar situation may also prevail in Derris trifoliata also and under such condition high levels of proline can be regarded as the superior adaptive feature in contrast to D. scandens.

Besides free proline estimation a preliminary qualitative analysis of the free amino acid pool was performed with a descending chromatography and it is depicted in fig.23. It can be seen from the figure that in amino acids Valine and glutamate are prominent in D. trifoliata while cystine, lysine and α-aminobutyrate are prominent in D.scandens.

The excellent work of Joshi et al. (1962) led to the postulation of hypothesis that NH_4 assimilation and amino acid metabolism are integrally involved in plant responses to

Fig.23 Amino acid composition of the leaf tissue of *Derris* species

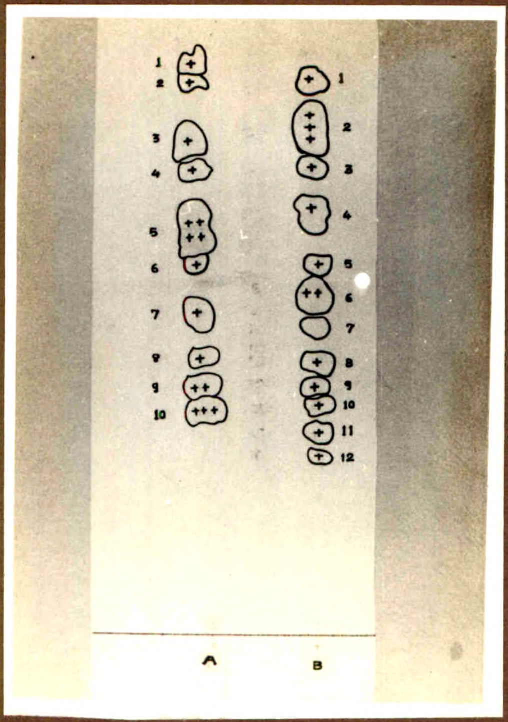
A paper chromatogram showing free amino acids in leaf extracts of -

A) *D. scandens*

1) leucine 2) Isoleucine 3) Valine 4) Tyrosine 5) α-amino butyrate 6) unidentified 7) Alanine 8) Glutamate 9) Arginine 10) Lysine

B) *D. trifoliata* :

1) Isoleucine 2) valine 3) Tyrosine 4) α-amino butyrate 5) unidentified 6) proline 7) Alanine 8) Glutamate 9) Aspartate 10) Lysine 11) Ornithine 12) Cystine


1
2
3
4
5
6
7
8
9
10
1
2
3
4
5
6
7
8
9
10
11
12
A
B

salinity. Their study documented that NaCl shifted the fixation of $^{14}CO_2$ in the dark from organic acids into amino acids for both spinach and a marine plant, Zostera. The latter work at Shivaji University laboratory also demonstrated the predominance of amino acids among the labelled products of photosynthetic carbon metabolism in mangroves and marine algae (Joshi, 1976). Kylin and Quatrano (10) considered accumulation of toxic levels of certain amino acids and NH_4, resulting from derangement of protein synthesis, as a potential mechanism of salt damage. Such reports suggest that a primary plant response to salinity is in amino acid metabolism, specifically key reactions involved with metabolic regulation of NH_4 assimilation. Joshi (1982) analysed the free amino acid composition of leaves of Suaeda nudiflora Suaeda brachiata and Sesuvium portulacastrum. He observed that alanine, aspartic acid, glutamic acid and phenylalanine are dominent in Suaeda nudiflora while aspartic acid, serine and alanine were dominent in Suaeda brachiata. In Sesuvium portulacastrum aspartic acid and glutamic acid were present in large amounts. Pulich (1986) observed that alanine, asparagine and glutamine were the major components of the free amino acid pool in the seagrasses. Recently Popp et al. (1984) made analysis of amino acids in various mangrove species. They observed that glutamate, aspartate, alanine and non-protein amino acid, 4 amino n-butyric acid were the most frequently occurring amino acids in most of the mangrove species. They also observed interspecific difference among the *Avicennia* species with respect to amino acid composition. Thus in leaves of *Avicennia eucalyptifolia* alanine and 4 amino n-butyric acid were dominent while in *Avicennia* marina, arginine, alanine and 4 amino-n-butyric acid were dominent. They also found that serine was dominent, besides alanine, glutamine and 4 amino n-buytaric acid in *Rhizophora lamarckii* while in other two *Rhizophora* species (*Rhizophora stylosa* and *Rhizophora apiculata*) serine was present in relatively very low amounts. In the present investigation also difference in the amino acid composition in the two *Derris* species is evident. Thus the amino acids like cystine, lysine and α-aminobutarate may be playing important role in nitrogen metabolism of *D. scandens* while valine and glutamate may be important in case of *D. trifoliata.* However the further quantitative analysis is essential to arrive at a definite conclusion regarding the amino acid metabolism in this legume species.

V) Titratable Acid Number (TAN)

The titratable acidity status of different parts of the two *Derris* species is shown in Fig.24. It is evident from the figure that there is marked difference in the organic acid content in the two species. In *Derris trifoliata* the aerial parts (in particular young leaves) have very high TAN values as compared to root tissue; while highest TAN values have

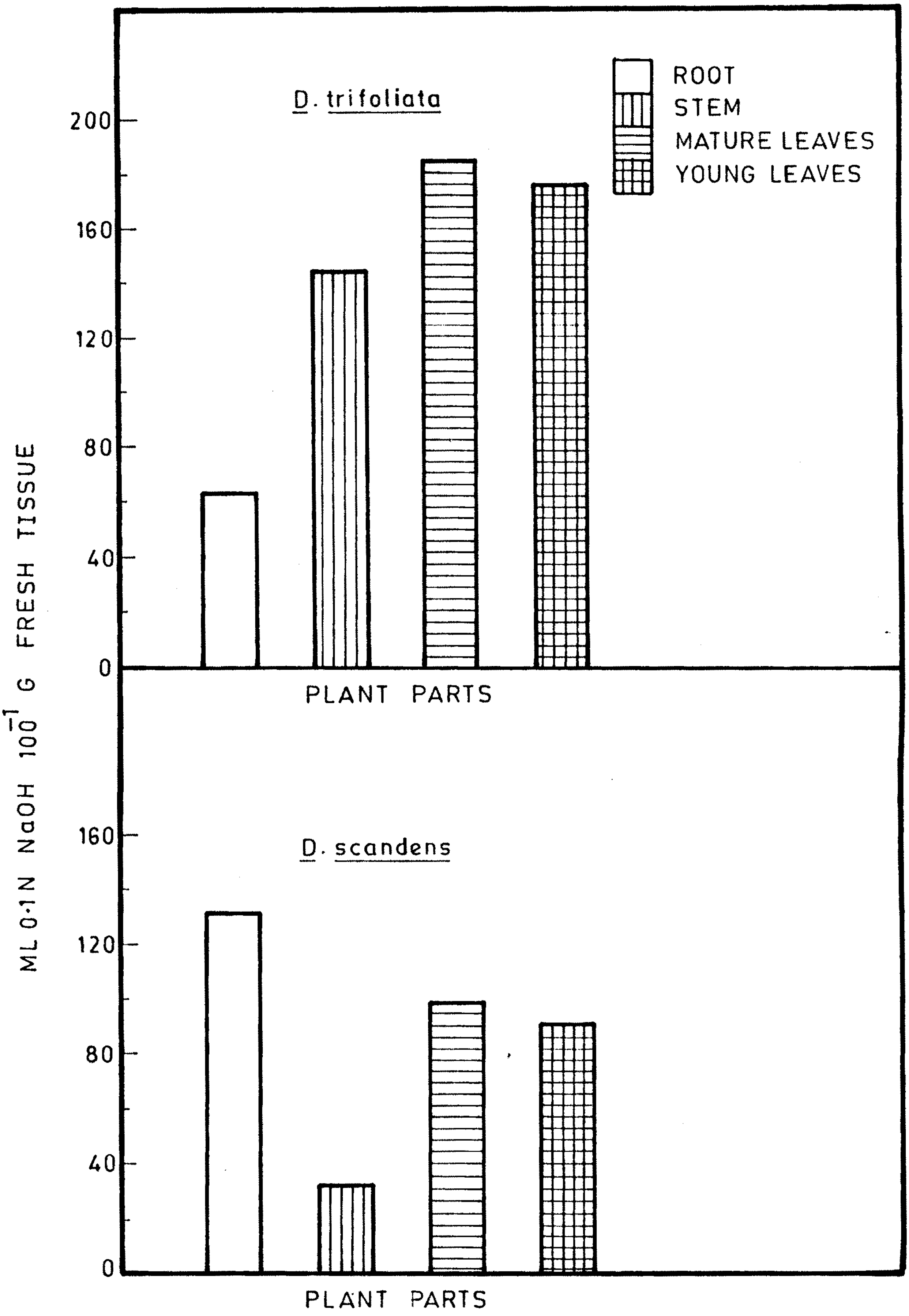


FIG. 24 — TITRATABLE ACIDITY STATUS OF DIFFERENT PARTS OF DERRIS SPECIES.

been recorded in the root tissue of Derris scandens.

TAN values give a general idea about the organic acid level in the plant tissue. Organic acids constitute an important class of metabolides in the cells because they are intermediates of a central metabolic pathway, TCA cycle. Besides the conventional TCA cycles intermediates some times the organic acids like malonate oxalate, glycolate and tartarate accumulate to a high degree in some plant species. Thus they provide carbon skeleton for synthesis of number of organic compounds in the cell. Organic acids have been shown to play a prominent role in osmotic adjustment. Organic acids are accounted for osmotic adjustment only at more severe stress (Jones et al., 1979). According to Strogonov et al. (1970) organic acids are helpful in binding of excess ions in the plant cell regulating the ionic balance of plants, maintaining the electrical neutrality of the cells and finally neutralizing basic compounds. There are few attempts to measure organic acid levels and their probable participation in salt tolerance mechanism in halophytes. Osmond (1962) suggested that oxalates play important role in ionic equilibria in Atriplex. The work of Albert and Popp (1977) revealed that oxalic acid was quite high in chenopodiaceae and caryophyllaceae while in other halophytic species, malate and citrate were prominent. At the same time Rozema (1976) indicated that organic acids play a very small insignificant role in halophytic *Juncus* species. The titratable acidity status of leaves of various mangrove species has been determined in our laboratory. Joshi and Mishra (1970) observed that the TAN of *Clerodendron inerme* was 48.21. Jamale (1975) reported that the TAN values of *Sonneratia acida* and *Lumnitzera racemosa* were 88.12 and 69.52 respectively. Kotmire (1983) observed that the TAN values of *Avicennia officinalis*, *Avicennia marina* and *Thespesia populone* were 123, 100 and 29.07 respectively. It is clear from our observations that as compared to these mangrove species the TAN values of *Derris trifoliata* leaves are quite high; on the other hand, the titratable acidity status of *Derris scandens* is rather low. The very high TAN values in *Derris trifoliata* clearly indicate the participation of organic acids in Osmoregulation in this mangrove species.

vi) Chlorophyll

The chlorophyll contents in the leaves of two *Derris* species are recorded in (Fig. 25). It is evident from the figure that the young leaves of *D. scandens* are having more chlorophylls than corresponding category of *D. trifoliata*. However the mature leaves of both species contain about similar amount of these pigments. The chlorophyll a to b ratio in young ves of both species is very high as compared to that of mature leaves.

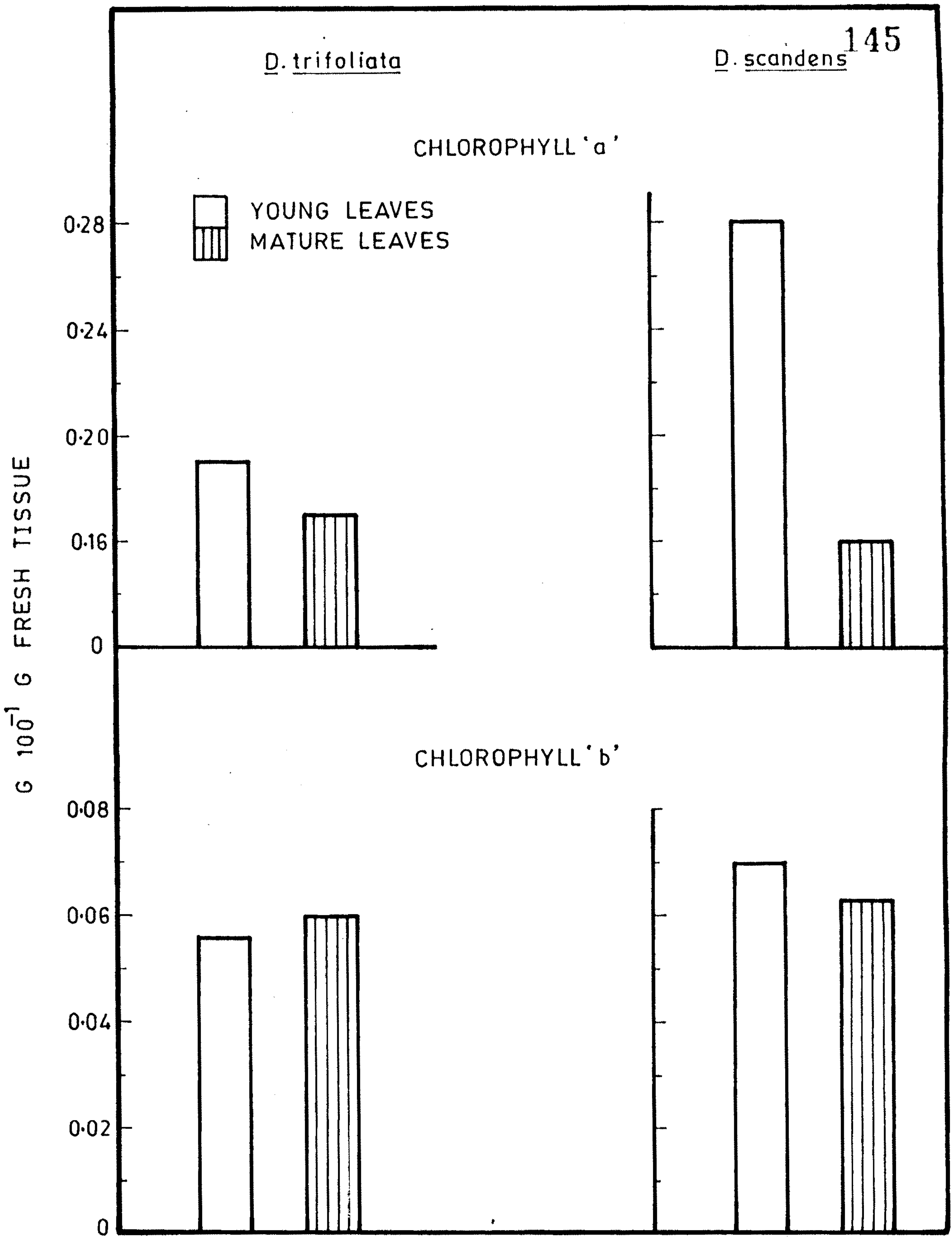


FIG. 25 — CHLOROPHYLL CONTENTS IN LEAVES OF DERRIS SPECIES.

Among the various pigment systems present in higher plants the chlorophylls are perhaps the more important ones because of their key role in the process of photosynthesis which is a basis of for survival of plant life on this planet. Chlorophylls catalyse the conversion of solar energy into chemical energy. The level of chlorophylls in the leaf tissue depends on rate of pigment synthesis on one site and rate of pigment degradation on other site. The chlorophyll contents are also regulated by the leaf developmental stage and in senescent leaves there is marked degradation of chlorophyll. Besides these endogeneous factor the chlorophyll contents are also markedly influenced by environmental factors such as shade, drought, waterlogging, pathogenesis and soil salinity. Waisel (1972) reported that the leaves of *Salicornia* or *Arthrocnemum* plants turn light green when plants are grown in high salt concentrations and such changes in colour was due to a low chlorophyll content. Reduction in chlorophyll content due to the increase in salinity of growth environment has been recorded in a seagrass *Halodule* sp. (*Diplantheral* by McMillan and Monseley (1967). Sathe *et al*. (1986) observed great variation in chlorophyll contents in the leaves of *Avicennia officinalis* at different localities which according to them was due to difference in leaf sodium and potassium values. The chlorophyll contents in the members of coastal ecosystem have been studied by some workers. Jamale (1975) observed that the chlorophyll contents in mangrove leaves varied from 23.26 % mg/100 g (*Sonneratia apetala*) to 190.20 mg/100 g(*Excoecaria agallocha*). Kathiresan (1986) observed variation in chlorophyll content among the three *Rhizophora* species with values ranging from 47 mg/100 g fresh tissue (*Rhizophora mucronata*) to 95 mg/100 g fresh tissue (*Rhizophora lamarckii*). Our observations indicate that as compared to above halophytic species, the *Derris* leaves are rather rich in chlorophyll and there are also interspecific differences. The high levels of chlorophylls may lead to some increase in photosynthetic efficiency of this legumes under saline conditions.

vii) Total Polyphenols

The total polyphenol level in different parts of *D. trifoliata* and *D. scandens* are recorded in (Fig.26). It is evident from the figure that the levels of these compounds in different plant parts are quite higher in *D. scandens* than in *D. trifoliata*. In both the species maximum polyphenolic compounds have been recorded in the stem tissue. In *D. scandens* mature leaves contain more polyphenols than the young leaves. While opposite is true for *D. trifoliata*. The preliminary paper chromatographic analysis of the polyphenols from the leaves of the two *Derris* species does not reveal any major differences and seven phenolic compounds could be detected with UV treatment (Fig. 27).

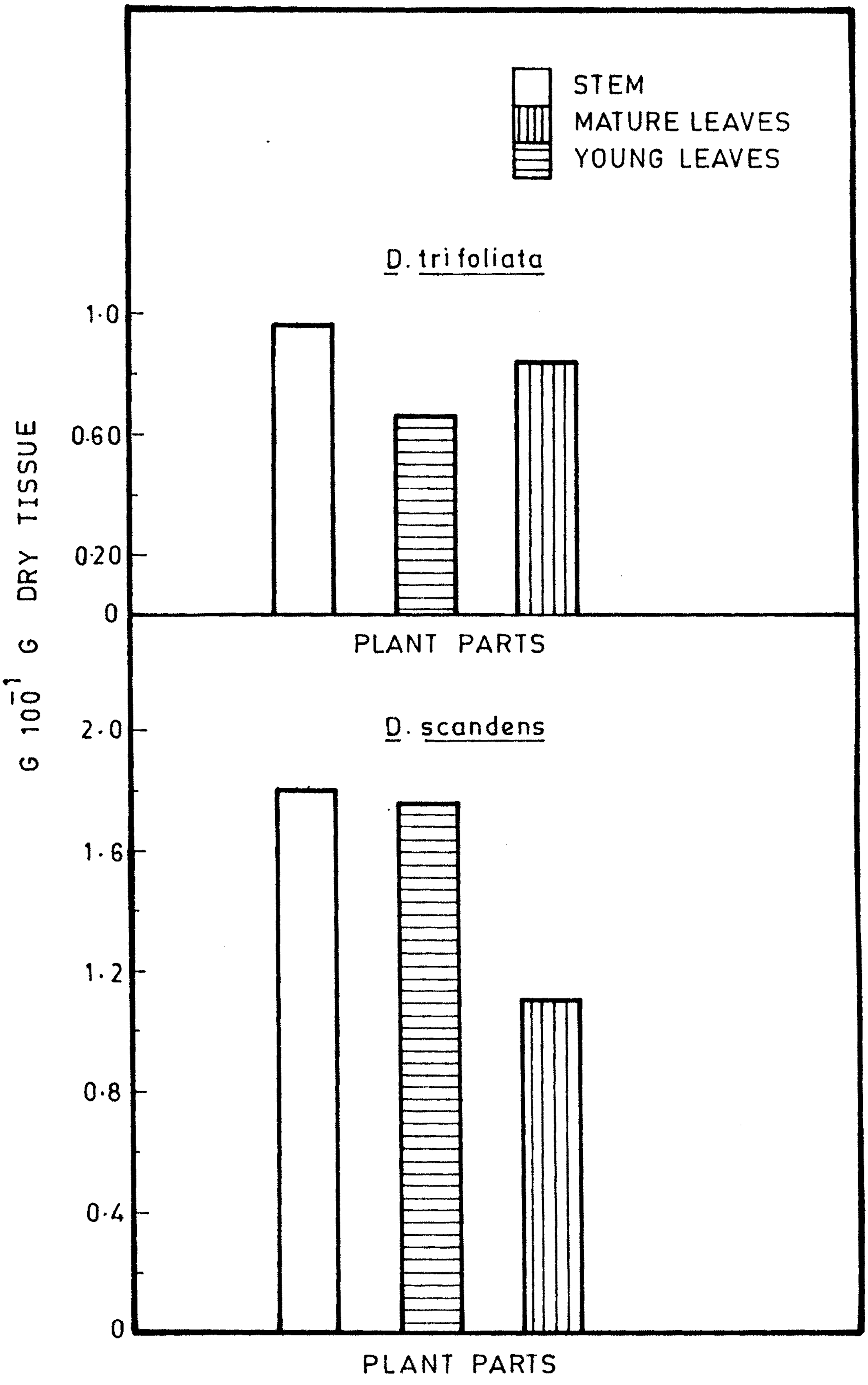
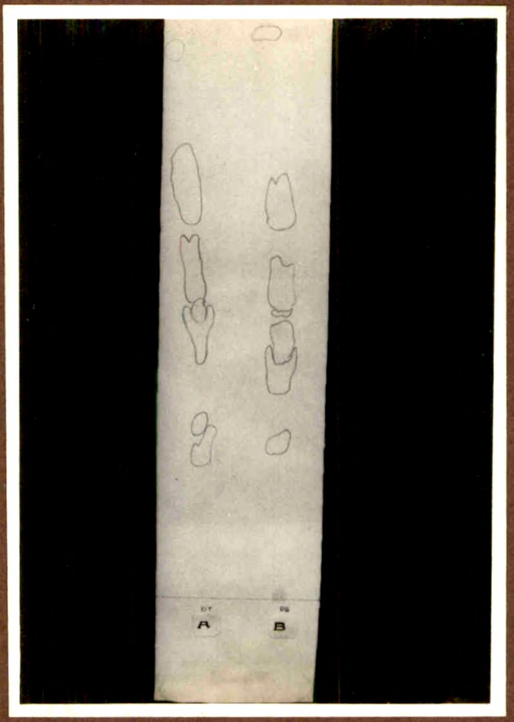


FIG.26 – POLYPHENOL CONTENTS IN DIFFERENT PARTS OF *DERRIS* SPECIES.

Fig.27 Phenolic compounds in the leaf tissue of *Derris* species.

Paper chromatogram showing phenolic compounds in the leaf extracts of -

A) *Derris trifoliata* B) *Derris scandens*


A
B

The polyphenols represent products of secondary metabolism and they are aromatic in nature. Some phenolic compounds have been implicated a role in plant growth regulation process (Galstone, 1969). The secondary metabolides in plants are important in repelling and discouraging predators and pathogens. The tannins are also commercially important for their use in leather industry and ink industry. The mangroves have been regarded as valuable source of tannin which are mainly extracted from their bark. The leaves of mangrove species are also rich in tannins. The extensive work of Jamale (1975) indicated that the polyphenol content in the mangrove leaves varied from 0.96% of fresh tissue (Avicennia alba) to 9.3% of fresh tissue (Rhizophora mucronata) and there were seasonal variations in the phenolic level. His work indicated that the average level of total polyphenols in mangrove leaves is about 3.013% fresh tissue. Kotmire (1983) recorded as high as 22% polyphenols in the leaves of Avicennia officinalis on dry matter basis. Our observations indicate that as compared to these mangroves the level of total polyphenols in the leaves of mangrove associate D. trifoliata is quite low. The extensive qualitative analysis of phenolic compounds in halophytic fern (Acrostichum aureum) by Shetty (1971) has revealed that as many as 27 compounds are present in the leaf extract. As compared to these observations both Derris species are found to show occurrence of only seven phenolic compounds in the leaf tissue (Fig.27). Rotar (1965) determined the levels of total polyphenols in the leaves of a famous tropical pasture legumes Desmodium intortum, He observed that the total phenolic contents range from 3.3 to 8.8 % and these offer reduction in in vitro dry matter digestibility and resistance to decomposition of leaves in the soil. The leaves of both Derris species have been reported to serve as forages and our observations indicate that the low polyphenol level in the leaves could be a positive point in such use. Similarly this will also help in nitrogen mineralization of leaves and leaf litter decomposition in the soil.

The stem tissue of both Derris species contain higher levels of total polyphenols than the leaf tissue. Similar findings have been reported by Jamale (1975) in many mangrove species except Avicennia alba. But the difference recorded by him in the total polyphenol level in the stem and leaf tissue of mangrove leaves is very large which justifies the use of mangrove stem bark in tannin industry. Such situation is not at all evident in case of Derris species.

C) Pod and Seed Analysis and Germination Studies :

The pods of D. trifoliata and D. scandens differ considerably in morphological features (Fig. 28). Pods of D. trifoliata are glabrous, reticulately veined, distinctly

Fig.28 The pods and seeds of <u>Derris</u> species

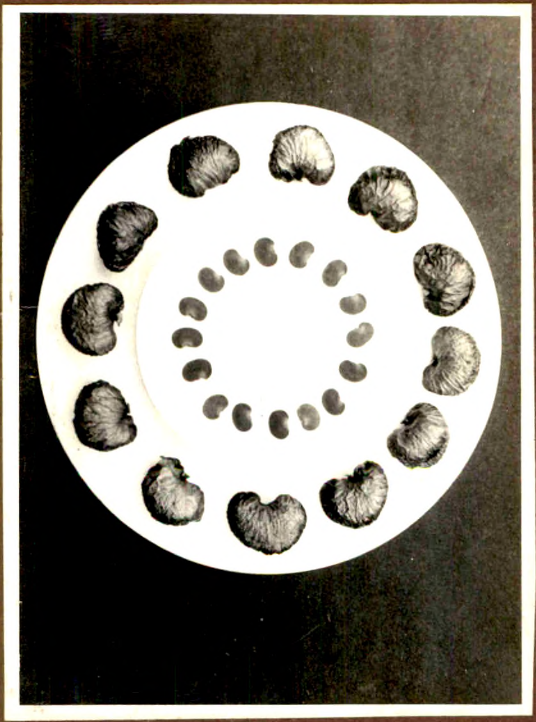
a) <u>D. trifoliata</u> pods b) <u>D.scandens</u> pods

c) i) Outer circle - <u>D. trifoliata</u> seeds

ii) Inner circle - <u>D. scandens</u> seeds.

a

b



c

winged on the dorsal suture. In *D. scandens* these are narrowly oblong, reticulately veined, adpressed with minute, silky hairs and winged on the dorsal suture. Our observations indicate that the seeds also differ considerably in size and other aspects. The seeds of *D. scandens* were relatively very small as compared to those of *D. trifoliata* (Fig.28). This is clearly revealed in the 100 seed weight recorded in Table 4. The seed weight is almost 15 times more in *D. trifoliata*. Thus *D. trifoliata* appears superior to *D. scandens* so far as seed biomass yield is concerned.

The analysis of organic constituents from the seeds of these two species is also recorded in Table 4. It is clear from the table that the seeds of *D. trifoliata* have considerably higher levels of carbohydrates as compared to those of *D. scandens*. The starch is major carbohydrate in both these species. Similar observations were made by Austenfeld (1986) in case of *Salicornia europaea* seeds and Mulik (1987) in seeds of *Acanthus ilicifolius*. It is clear from the Table 4 that nonreducing sugars are predominant on sugar fraction in *D. trifoliata* seeds while reducing sugars predominate in D. scandens seeds. Austenfeld (1986) observed that in seeds of halophytic species *Salicornia auropaea* the nonreducing sugar sucrose accounted for about 42% of the total storage carbohydrates while the reducing sugar glucose and fructose were negligible. The organic acid level as indicated by TAN values appears to be higher in *D.scandens*

Table 4 - Organic constituents* in the seeds of <u>Derris</u> species.

Derris Species	100 seed weight	Carbohydrates			Total nitrogen	Crude protein	Total lipids	TAN	Poly-phenols
		Reducing sugars	Non-reducing sugars	Starch					
D. trifoliata	62.160	0.36	4.07	36.72	3.62	22.63	1.6	21.41	0.60
D. scandens	4.076	2.56	0.39	13.35	4.16	26.00	6.8	58.36	4.48

* Values are expressed as

1) Carbohydrates - g 100^{-1}g

2) TAN - ml. of decinormal NaOH required to neutralize the acids in 100 g of seed tissue

3) Total nitrogen - g 100^{-1}g dry tissue

4) Crude proteins - percent dry weight.

5) Total lipids - g 100^{-1} g

6) Total polyphenols- g 100^{-1} g

seeds than *D. trifoliata* seeds. Similar pattern is also observed for total lipids. Thus both these species are rather low in total lipid contents, as compared to other halophytic species like *Cakile edentula* (51.2%), *Crithmum maritimum* (41.4%) and *Salicornia europaea* (28.0%) as reported by O'Leary *et al.* (1985). At the same time the lipid content of these species is sufficiently within the range 1 to 7.5% of other halophytes (Earl and Jones, 1962). The seeds of *D. scandens* contain higher amount of total nitrogen and crude proteins than those of *D. trifoliata*. Zahran and Wahid (1982) recorded about 21% crude protein in seeds of *Kochia indica.* Earl and Jones (1962) reported that the protein content in halophyte seeds varied from 5.4% (*Atriplex canescens*) to 31.2 % (*Mesembryanthemum crystallinum*). As high as 28.6% protein was recorded in seeds of *Cakile edentula* by O'Leary *et al.* (1985). The protein value of seeds of both *Derris* species is quite appreciable in this context. The seeds of *D.scandens* contain about 7 times, more total polyphenols than those of *D. trifoliata*. Thus leaves as well as seeds of *D. trifoliata* do not appear to be rich source of tannins. The inorganic constituents of podwall and seeds of *Derris* are recorded in Table 5. It is evident from the Table 5 that potassium is the major cation in the seeds of both Derris species followed by calcium and magnesium. In the podwall of both species also potassium is a major cation magnesium is second major cation in podwall tissue of *D. scandens* while calcium occupies a similar position in

D. trifoliata. In seeds of Salicornia europaea, Austenfeld (1986) recorded that potassium was a major cation followed by magnesium and calcium. Higher levels of potassium in seeds of the mangrove species, Acanthus ilicifolius and Sonneratia alba were recorded by Anjal and Bhosale (1985) and Mulik (1987) respectively. It has been demonstrated in several experiments that the chemical composition of fruits and seeds depends on the translocation of nutrients during ripening. The majority of ions are transported to the developing seed through the phloem (Van Die and Willemse, 1980). Only calcium is suggested to be translocated exclusively in the xylem (Mix and Marschner, 1976). Further high K^+ content is essential for the active conformation of many enzymes participating in the intermediary metabolism and biosynthesis of seeds (Clarkson and Hanson, 1980). The seeds of D. trifoliata are quite rich in potassium indicating adequate supply of potassium for the seed metabolism, in this halophytic species under saline conditions. It is evident from the Table 5 that the seeds and podwall contain quite low levels of sodium eventhough both the species are growing under saline conditions. These observations indicate that similar to leaf tissue the seed tissue is also not exposed to toxic effects of sodium. Similar low levels of sodium in the seeds of halophytic species Salicornia europaea were recorded by Austenfeld (1986). and mangrove species Acanthus ilicifolius by Mulik (1987). With Suaeda maritima L. Dum; Yeo (1981) demonstrated that Na^+

was retained in leaf cell vacuoles more effectively than K^+. Furthermore retranslocation studies showed that in contrast to K^+, little Na^+ and Cl^- retranslocated into developing axillaries from other leaves. These data suggest that Na^+ and Cl^- are relatively immobile in the vegetative tissues surrounding the inflorescences contain quite high levels of sodium. In case of Derris species such situation is also only slightly evident in D. trifoliata, where the podwalls have relatively higher level of sodium as compared to seed tissue.

It is evident from the Table 5 that seeds of both Derris species contain appreciable levels of phosphorus as compared to the pod tissue and this may be due to active participation of phosphorus in various metabolic reactions. The seeds of D. trifoliata are richer in iron, whereas the levels of other micronutrients like manganese, copper, cobalt and zinc are quite higher in seeds and podwall of D. scandens. Thus the micronutrient supply to the developing seeds of D. trifoliata is probably restricted due to soil salinization.

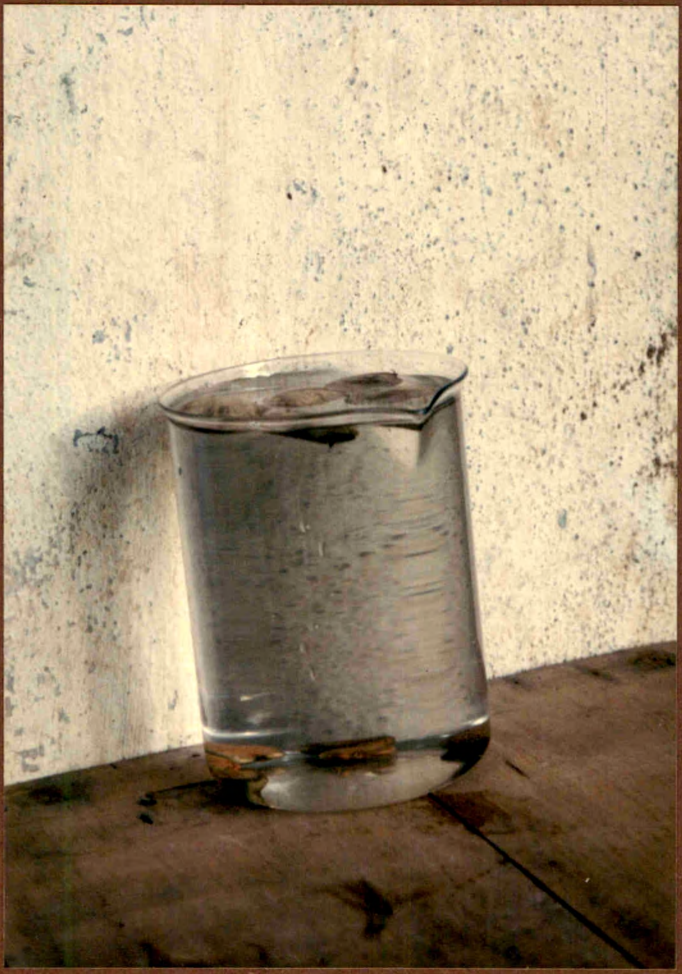
One of the salient features of the Derris species is the presence of winged pods. The presence of such pods appear to be highly advantageous for a species like D. trifoliata which is subjected to periodic flooding of estuary water. As indicated in Fig. 29 the pods can help in floating and dispersal. Such adaptive strategies have been very well recognized in halophyte (Waisel 1972). According to

Table 5 - Inorganic elements* in podwall and seeds of *Derris* species

Derris species	Na^+	K^+	Ca^{+2}	Mg^{+2}	P^{+5}	Fe^{+3}	Cu^{+2}	Co^{+2}	Mn^{+2}	Zn^{+2}
D. trifoliata										
Podwall	0.46	2.44	0.278	0.267	0.0137	0.035	0.0022	0.0010	0.0038	0.0046
Seed	0.35	2.62	0.530	0.282	0.0414	0.032	0.0032	0.0016	0.0040	0.0120
D. scandens										
Podwall	0.20	1.53	0.584	0.755	0.0383	0.029	0.0343	0.0032	0.020	0.0264
Seed	0.29	1.57	1.188	0.436	0.0459	0.108	0.0110	0.0082	0.042	0.0310

* Values are expressed as g 100^{-1}g dry weight.

Fig.29 A photograph showing ability of pods of <u>D. trifoliata</u> to float on water in contrast to seeds.



Waisel (1972) propagules and seeds of most coastal halophytes are well adapted for dissemination by water, accounting for the circumglobal distribution of certain genera or groups of species. Certain tissues of most hydrohalophytes contain large intercellular air spaces which enable them to float for considerable periods of time. *Suriana maritima, Gyrocarpus jacquinii* and *Entada scandens* have air-filled lacunae usually between the cotyledons. *Canavalia obtusifolia* has spongelike intercellular speces in the cotyledons. Whereas *Pancratium maritimum* has an air-containing tissue in the seed coat. In various species of *salicornia,* seeds are buoyed by air bubbles adhering to the seed coat hairs; propagules of *Thespesia populnea* has hollow parts between the fruit and the seeds; *Scaevola lobellia* has aerenchymatous tissue in the endocarp, and *Cocos nucifera* has such tissue in the mesocarp. Fruits of *Halimone portulacoides, Cakile maritima, Crambe maritima,* and of *Suaeda maritima* can float for a long time, becaause they are buoyed by air-containing tissues. Praeger (1913) compiled a detailed list of seed buoyancy capacity of various species. Seeds of approximately 11% of the total 786 species investigated remained buoyant beyond 1 month - most of them marsh or coastal species. It is apparent that the pods of *Derris* also possess such ability.

The preliminary germination study carried out on the two *Derris* species; clearly indicate that there are basic differences in the germination behaviour of the two species.

Thus a seed dormancy due to hard seed coat was recognised in the seeds of *Derris scandens*. This was not found to be overcome by hot water treatment for 10 minutes; only the treatment of the *D. scandens* seeds with concentrated sulphuric acid for 10 minutes brought about significant increase in germination as shown in Fig. 30. In contrast to *D. scandens* the seed coat of *D. trifoliata* was found to be considerably thin and the seeds under field condition were found to germinate even when enclosed in the pod (Fig.31). The preliminary germination study indicated that the germination of *D. scandens* seeds is highly salt sensitive; while that of *D. trifoliata* is relatively unaffected under saline conditions. It was further noticed that in case of germination failure under natural conditions, the plants can adapt the strategy of vegetative propagation as indicated, by the rooting ability on their twigs (Fig. 32).

According to Waisel (1972) vegetative reproduction substitutes for or at least contributes to the reproductive potential of many plants and is certainly true for various halophytic species that are restricted to narrow ecological limits either by the production of disseminules or by their germination. Waisel and Pollak (1969) observed that many halophytic species, e.g. *Arthrocnemum* sp., *Nitraria retusa* and *Salicornia* sp. are capable of forming adventitious roots on their twigs and this ability varies between species and

Fig.30 Effect of concentrated H_2SO_4 treatment on dormancy in Derris scandens seeds

A) Untreated seeds

B) Seeds treated with concentrated H_2SO_4 for 10 minutes.

Fig.31 Seedling emergence from pods of Derris trifoliata

(Fig. 30)

(Fig. 31)

Fig.32 Adventitious roots formation on the twigs of *Derris trifoliata*

during various seasons of the year. Our observations also indicate that the capacity of vegetative propagation through adventitious rooting can help for successful establishment of the climber *Derris trifoliata* under saline conditions.