CHAPTER - III

RESULTS AND DISCUSSION

RESULTS AND DISCUSSION

1. Inorganic Constituents :

a) Sodium

The changes in Sodium contents in young, mature and senescent leaves of Agave cantala during three seasons are recorded in Fig.2. It is clear from the Fig.2 that there is only slight variation in sodium content in the three leaf categories in summer season. On the other hand, a marked increase in sodium content in senescent leaves is noticed in rainy season and winter season.

Recently literature has classified the role of mineral elements in the plants according to their biochemical and physiological behaviours rather than their concentrations (macronutrients and micronutrients) in plant tissues. According to Clark (1984) elements required for plant growth have been classified into four major groups : (i) elements that are covalently bonded into constituents or organic matter (C,H, O, N and S); (ii) elements that occur as oxyanions or are esterified with native alcohol groups (P, B and Si); (iii) elements that have nonspecific, osmotic and ionic balance functions plus specific functions in enzyme conformation and catalysis i.e. these elements maintain an ionic identity as free or reversibly bound ions as metal-protein complexes

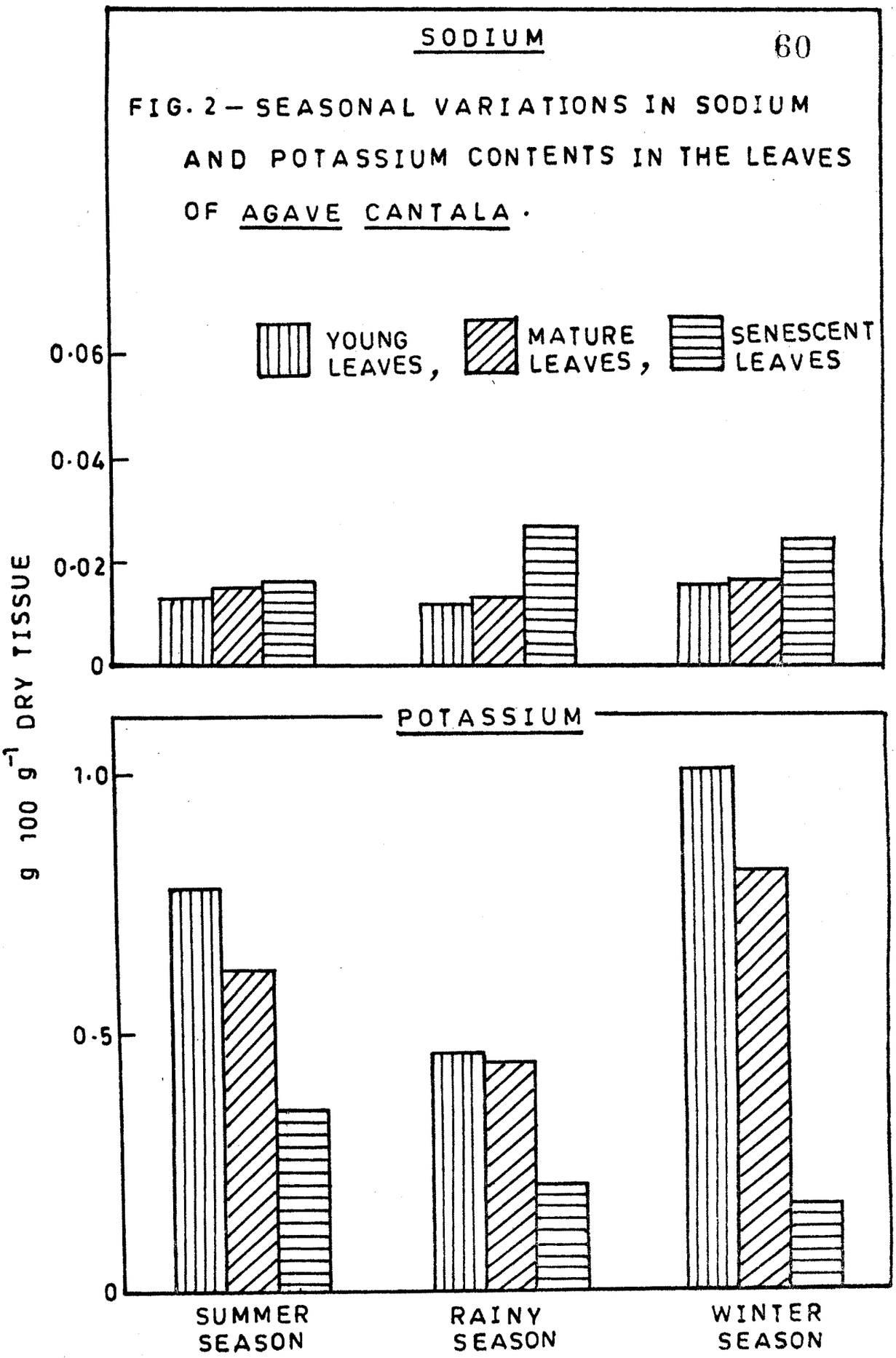


FIG. 2 — SEASONAL VARIATIONS IN SODIUM AND POTASSIUM CONTENTS IN THE LEAVES OF AGAVE CANTALA.

(K, Na, Mg, Ca, Mn and Cl); and (iv) elements that form metalloproteins, are present as structural chelates, and participate in redox reactions or valency changes (Fe, Cu, Zn, Mo and Mn in its function in the photosystem II pathway). From the physiological point of view, this classification better describes the functions of Ca, Mg and Mo in plant processes. Among these essential elements sodium probably represents the most interesting element due to the fact that its exact role in the plant metabolism is still obscure. It is further noticed that plant species differ greatly in their requirement for this monovalent cation.

Thus Harmer and Coworkers (1953) were able to recognise four different categories of plants depending on their sodium requirement - a) plants requiring sodium in the absence of potassium (lucerne, barley, oat, tomato); b) plants showing a low requirement for 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 (celery, beet, turnips and particularly sugarbeet). The halophytes which successfully grow and complete their life cycle in saline environments have a specific requirement for sodium for their various growth processes. However, the same can not be said about majority of the plant species

which are basically glycophytic in nature.

Although no clearcut evidence has been so far presented regarding the role of sodium in various metabolic reactions in the plants, there are reports indicating its involvement in some biochemical processes. Marschner *et al.* (1981) have indicated that sodium can atleast partially replace potassium in some plant processes. The work of Brownell (1979) has clearly shown involvement of sodium in the process of C_4 photosynthesis. Sodium also plays a role in ion transport across the membranes. There are also reports which indicate that sodium causes increase in succulence (Handley and Jennings, 1977) thereby improving water relations in plants. Sodium has been also reported cause of activation of some enzymes (Evans and Sorger, 1966). Nobel and Berry (1985) have however, noticed direct inhibitory effect of sodium on nocturnal acid accumulation in CAM plants.

There is great variation in sodium content in plant ecotypes. Thus in some halophytic species sodium content may be as high as 5% of the dry weight, whereas in other glycophytic plant species normally sodium contents are in the range of 0.1 to 1% of dry weight. In case of succulents there are few attempts to estimate sodium levels. Karmarker (1965) has recorded 1.0 to 2.8% sodium in the leaves of *Bryophyllum pinnatum.* Bartakke (1977) noticed that the leaves of *Aloe barbadensis* contain 0.269 g 100 g^{-1} of drytissue.

The work of Karadge (1981) indicated that sodium content in the leaves of Portulaca oleracea are in the range of 0.1 to 1.4% of dry weight. Nobel and Berry (1985) made a detailed investigation of sodium content in the chlorenchyma of various Agave species (Agave americana, A. deserti, A. fourcroydes, A. lechuguilla, A. salmiana, A. utahensis). Their work suggested that sodium content varies from 0.01 to 0.066 g 100 g^{-1} dry tissue. Our work with Agave cantala shows that in this species the sodium contents in the leaf tissue range from 0.04 to 0.069 g 100 g^{-1} dry tissue.It is further noticed in the present investigations that there are very slight seasonal variations in the sodium contents in leaves of A. cantala. This situation is in contrast to mangrove species, which show distinct seasonal variations in sodium status in accordance with fluctuations in the salt content of the medium (Jamale, 1975).

It is evident from present investigation that sodium contents in the senescent leaves are higher than those of young and mature leaves and this is particularly prominent in the rainy seasons. According to Leopold (1961), leaf senescence performs two primary functions. It permits recovery through retranslocation of the bulk nutrients from the leaves and it brings about shedding of ineffective salt saturated leaves from the plant which serve as the source of substantial quantities of mobile elements which can be

exploited to greater advantage by leaves in more favourably illuminated position. Although this may be true for essential elements like potassium, a marked contrast has been noticed for elements like sodium. Thus Albert (1975) has indicated that the shedding of salt saturated old leaves can be considered as one of the strategies of ion regulation in halophytes.

There are few attempts to trace the fate of this element during leaf senescence in succulents. Ambike and Karmarkar (1975) have also reported an increase in sodium during leaf senescence of Kalanchoe pinnatum. They have suggested that the high sodium accumulation accounts for the corresponding decrease in organic acids. Karadge (1981) found an increase in level of sodium in the senescent leaves of Portulaca oleracea. The accumulation of sodium in senescent Agave leaves however, does not cause any improvement in the water balance (Fig. 6). However, it may be partially responsible for a decline in organic acid status (Fig. 7) as has been suggested by Ambike and Karmarkar (1975).

b) Potassium :

The changes in potassium contents in young, mature and senescent leaves of Agave cantala during three seasons are recorded in Fig.2. From this Fig.2 it is clear that young leaves contain highest level of potassium in winter and summer seasons. In rainy season there is not much

variation in potassium content in the young and mature leaves. In all the three seasons a marked decline of potassium status in senescent leaves is evident.

Potassium is a monovalent cation essential for plant growth for all higher plants and indeed, for all living things except a few microorganisms in which rubidium can substitute for it. The main role of potassium is that of an activator of many enzymes. According to Evans and Sorger (1966) more than 60 enzymes from animals, higher plants and microorganisms require monovalent cations for their maximal activity. Potassium is important in plant metabolism, particularly through its action on process of photosynthesis and respiration.

It has been shown by numerous authors that potassium uptake by the plant cells is closely associated with metabolism, and especially with root respiration. Potassium is also involved in the mechanism of ATP generation. The basic process of energy metabolism - the conversion of radiation energy into chemical energy - is much controlled by the K^+ status of the plant. The beneficial influence of K^+ on phosphorylation has been reported by various researchers using different plant species (Hartt, 1972). Photoreduction (production of NADPH) is also promoted by K^+ (Pfluger and Mengel, 1972). The promoting effect of K^+ on photosynthetic ATP synthesis and NADPH production has a general impact on

various energy-requiring processesin plant metabolism. Potassium plays an important role in maintaining the cell turgor and enhancing the capacity of cells to retain water. In this function K^+ seems to be of particular importance in young tissues. The overall effect of K^+ on the water economy of plants results from the process cited above and probably also from processes that are not yet known or well understood. This beneficial effect of K^+ is of particular importance in practical crop production, since K^+ reduces water losses by transpiration (Brag, 1972), so that more organic matter can be produced per unit water consumed by a crop well supplied with K^+ (Blanchet et al., 1962; Linser and Herwig, 1968).

It has been very well established that potassium plays a key role in the mechanism of salt tolerance and the halophytic plants show a preferential uptake of K^+ under saline conditions. According to Mengel and Kirkby (1980) a high rate of K^+ uptake by root cells depresses osmotic potential in the cells, and this induces water uptake, water transport in to the xylem vessels is also mainly an osmotic process in which K^+ in its function as an osmoticum is very important. Lauchli et al. (1971) have shown that parenchyma cells may accumulate K^+ to a high extent and that K^+ is secreted in to the xylem.

The stomatal movements also depend on the potassium status in the guard cells. Potassium plays a spectacular

role in stomatal opening and closure (Fischer and Hsiao, 1968). The studies of Humble and Raschke (1971) indicated that the increase in turgor in the guard cells associated with stomatal opening resulted from an increase in K^+ concentration in the cells. The K^+ accumulation is associated with an accumulation of malate which appears to be the major anion charge balancing the accumulated K^+. Under light conditions, photophosphorylation seems to provide the ATP required for pumping K^+ in to the guard cells (Mengel and Kirkby (1980). Thus Allaway (1973) under dark conditions found very little amount of malate in the guard cells of Vicia faba. Whereas on exposure to light there was a rapid increase in the malate concentration. Meidner and Mansfield (1968), in their book on stomatal physiology, indicated that there was no evidence that the mechanism for night opening of CAM plant stomata was any different from day opening of other plant stomata. Significant for the understanding of succulent CAM plant stomatal opehing is that Dayanandan and Kaufman (1975) reported K^+ accumulation in the guard cells of Crassula argentea during night opening.

Potassium is known to be very mobile in an upward and downward direction in the entire plant. Ben-Zioni et al. (1971) have suggested that K^+ is significant for the upward translocation of nitrate in the entire plant. The effect of K^+ on phloem transport also has a direct impact on the long-distance transport of nitrogen (Koch and Mengel, 1977), but

not much is understood about a direct beneficial effect of K^+ on phloem loading.

In recent years it has become increasingly clear that hormonal effects may control the movement of nutrients in plants. Pitman (1972) suggested that the uptake of K^+ was regulated by a "feedback" mechanism between roots and shoots, in which the translocation of growth substances may involved. But the mechanism by which such hormonal effects operate in the long-distance transport of K^+ has yet to be established.

Epstein (1972) indicated that maximum or optimum K values in the plant tissues are 1% of dry weight in the land plants. Bartakke (1977) recorded the average value for potassium in Aloe barbadensis as 1.21% of dry weight. The work of Karadge (1981) indicated potassium content can reach the value as high as 1.89% in the leaves of Portulaca oleracea. Nobel and Berry (1985) made a detailed investigation of potassium content in the chlorenchyma tissue of various Agave species (Agave americana, A. deserti, A. fourcroydes, A. lechuguilla, A. salmiana, and A. utahensis). Their work indicated that potassium content varies from 1.27 to 1.78% of dry weight. Our work with Agave cantala indicates that in this species potassium contents in the leaf tissue range from 0.2 to 1.0%. In mature leaves of A. cantala potassium content varies from 0.6 to 0.8%.

These observations indicate that incomparison to other Agave species, A. cantala is rather inferior in potassium accumulation. It is possibly due to the different ecological conditions prevailing the plant growth.

From Fig.2 it is evident that the young developing leaves in all seasons are rich in potassium. It can also be seen that potassium content of senescent leaves in all seasons is lowest indicating its retranslocation from these aging leaves. It appears that during the advancement of senescence K^+ is withdrawn from the senescent leaves and probably retransported to the young developing and actively growing leaves. Withdrawal of this important monovalent cation from the senescent leaves has been reported by many investigators. Thus it has been suggested that this essential element is translocated to the young developing leaves which require K^+ for their growth.

Several reasons have been given for the redistribution of potassium from the aging tissue. According to Loneragan et al. (1976) the redistribution of potassium takes place due to its quick mobility. Kaufman et al. (1970) further speculated that cellular differentiation may a) effectively block any active accumulation of K^+ by vacuoles which obviously are destroyed, b) slow down the processes of protein synthesis by ribosomes and glycolysis which require high concentrations of internal potassium and/or c) causes a change in the pH

which could facilitate the efflux of potassium from the cells.

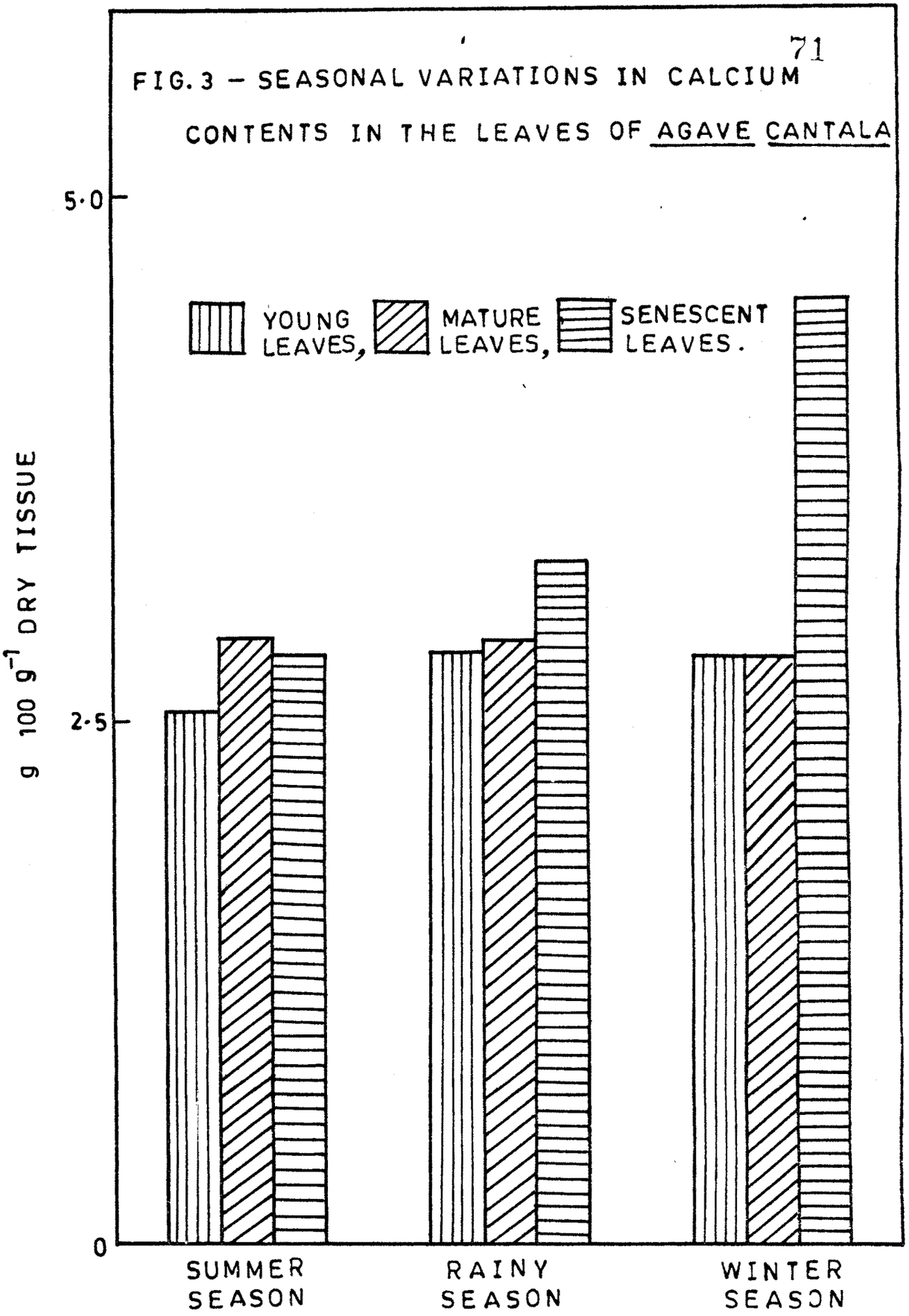
In CAM plants also the leaf senescence is accompanied by similar processes since some workers have observed retranslocation of potassium. Ambike and Karmarkar (1975) reported withdrawal of potassium contents from the senescent leaves. Thombre (1987) also found that the young developing leaves of Aptenia cordifolia and Portulaca quadrifida were rich in potassium as compared with senescent leaves. Our observations indicate that Agave cantala also follows a trend and retranslocation of potassium from the senescent leaves is quite prominent during all the three seasons.

c) Calcium

The changes in calcium contents in young, mature and senescent leaves of Agave cantala during three seasons are shown in Fig. 3. It is evident from Fig.3 that the Agave cantala leaves contain appreciable level of calcium during all the three seasons. There is an increase in calcium contents in senescent leaves during winter and rainy seasons, while there is slight decrease during summer season. There is only slight variation in calcium content in young and mature leaves.

The chemical properties of Ca are relatively complex and Ca^{2+} controls biochemical and physiological functions that are very different from those of other elements. Calcium has a relatively large ionic radius (0.099 nm) compared with

FIG. 3 – SEASONAL VARIATIONS IN CALCIUM CONTENTS IN THE LEAVES OF AGAVE CANTALA



other divalent ions and has a coordination number of six or higher (often seven or eight) to form octohedral complexes. Calcium has the ability to bind with proteins and other macromolecules with low geometrical demand. Calcium coordinates strongly with a variety of ligands and readily substitutes its water of hydration to produce complex cross-linking or bridging of macromolecules. 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.

In plants we can notice different forms of of calcium. It occurs in plant tissues as free calcium, as calcium absorbed to indiffusible ions such as carboxylic, phosphorylic and phenolic hydroxyl groups. It also exists in the form of calcium oxalates, carbonates and phosphates.

The functions of calcium in plants have been related to calcium effects on membranes, cell walls, enzymes and metabolism, phytohormones and with calmodulin (Clarkson and Hanson, 1980). Considerable evidence indicates that an important function of Ca is to stabilize membranes (Clarkson and Hanson, 1980). The influence of Ca^{2+} on ion uptake is pH dependent (Rains et al., 1964). When H^{+} concentrations of external media were high (near pH 4.5), Ca^{2+} had its

greatest effects and counterbalanced the detrimental effect of high H^+ (Marschner et al., 1966). Two general effects appeared under acid conditions : (i) the competitive effects of H^+ on binding reactions of cations and (ii) the direct damage of H^+ on membranes. These and other investigations support the concept that H^+ altered cell membrane properties, including selective ion uptake, can be overcome by Ca^{2+}. Closely associated with selective ion uptake by membranes is the leakiness, or increased permeability, of membranes at low Ca^{2+} levels. Little is known about the biochemical mechanisms involved in Ca membrane responses. The binding of Ca^{2+} with phospholipids, cholesterol, proteins or specially arranged carboxyl groups could possibly after pore radius or conformational structures in membranes (Clark, 1984).

Considerable amounts of Ca are located in cell walls, especially as Ca pectate in the middle lamella, and various concepts on the functions of Ca in cell walls have been developed. They include formation of ionic bridges, increase in cell wall rigidity, increase in hydrophobicity and reduction in water permeability, and cell elongation (Clark, 1984). Microscopic studies of plant tissues containing low Ca show considerable disorganisation of cells (Fuller, 1980). The weakening of cell walls and middle lamella may occur under various Ca deficiency conditions. Many Ca deficiency-related disorders, e.g., fruit and vegetable mealiness, water core, internal breakdown, bitter pit, corking, cracking, tipburn,

and browning can be associated with breakdown and disorganization of the cell walls of plants (Atkinson et al., 1980).

Many enzymes have been reported to be stimulated or inhibited by Ca^{2+}, some of which are α-amylase, esterase, pectinesterase, lipoxygenase, nucleases, protein kinase, pyruvate kinase, polygalactyronic transeleminase, glucose-6-phosphate dehydrogenase, and adenosine triphosphatase (ATPase)(Clark, 1984). A major role of Ca^{2+} appears to be its binding with proteins, nucleic acids, and lipids to affect cell adhesion, membrane and chromatin organization, and enzyme conformation (Clarkson and Hanson, 1980). These Ca^{2+} functions appear to be related to Ca-calmodulin protein complexes. Calmodulin is a Ca-binding protein that is present in many cells. It binds with Ca^{2+} in such a configuration that the Ca-protein complex stimulates many enzymes and physiological processes at Ca concentrations considered to be very low (10-1000 nM).

Calcium uptake in plants is found to be influenced by number of factors such as soil pH, soil temperature, light, other cations, and soil-water potential (Clark, 1984). Once Ca^{2+} passes through the endodermal cells, it enters stele vesseles (xylem and phloem) for longitudinal transport. From early experiments, the concept that Ca^{2+} and other ions move with the transpiration stream by mass movement has prevailed. More recent evidence abounds to indicate that

Ca^{2+} moves in the xylem by ion exchange. As Ca^{2+} moves in the xylem, considerable leakage to adjacent tissues occurs. Adjacent tissues absorb Ca^{2+} according to metabolic utilization. The movement of Ca^{2+} in the xylem from the roots is in the acropetal (toward tops) direction, with little or no Ca^{2+} being moved basipetally (toward root tips)(Marschner and Richter, 1974). Eventhough Ca^{2+} has been reported in the phloem of many plant tissues, the immobility of Ca^{2+} in the phloem has been confirmed by many investigators (Bangerth, 1979; Hanger, 1979).

The optimum value for Ca^{2+} in land plants is 0.5% of dry weight (Epstein, 1972). The work of Bartakke (1977) indicated that average value of calcium is 1.89% for the leaves of Aloe barbadensis. Karadge (1981) reported that Ca content in Portulaca oleracea ranges from 2.5 to 4.4% of dry weight. Nobel and Berry (1985) found the values of Ca contents ranging from 2.3 to 6.11 % of dry tissue in various Agaves (Agave americana, A. deserti, A. fourcroydes, A.lechuguilla, A. salmiana, and A. utahensis). Our work with Agave cantala also shows the values of Ca contents within the range of 2.56 to 4.78 g 100 g^{-1} of dry tissue. If the Ca contents compared with Na and K, A. cantala seems to be calcium rich plant. Our values of Ca contents are near to calcicolus plants. Hence A. cantala can be classified as calcicolus plant.

Similar observations were made by Karmarkar (1965) in

Bryophyllum pinnatum which is a classical example of CAM where Ca contents range from 1.73 to 5.05 %. This shows that Ca can play an important role in organic acid synthesis as well as in CAM. Laetsch (1974) has reported that desert succulents absorb large amounts of Ca and other ions and to balance them organic acids are produced and hence CAM is an ecological necessity. Thus Ca may regulate the CAM in this succulent. In conformative with this opinion Nobel and Berry (1985) observed a highly significant positive correlation between nocturnal acid accumulation and calcium status of chlorechyma in various Agave species. These workers further suggested that high levels for Ca presumably reflect the accumulation of calcium oxalate, which is known to occur in succulent plants (Phillips and Jennings, 1976).

It is evident from present study that calcium shows a tendency of accumulation in the senescent leaves and this tendency is more prominent in winter season. The highly immobile nature of calcium has been documented in several studies. It is noticed that the withdrawal of potassium from older leaves is counterbalanced by redistribution of divalents in opposite directs. Accumulation of calcium in the senescent leaves has been evident in number of studies (Waughman and Bellamy, 1981). According to Clark (1984) calcium immobility in leaf cells may be visualised as Ca^{2+} binding to cell wall and cellular structures. There are few attempts to trace the fate of calcium during senescence in

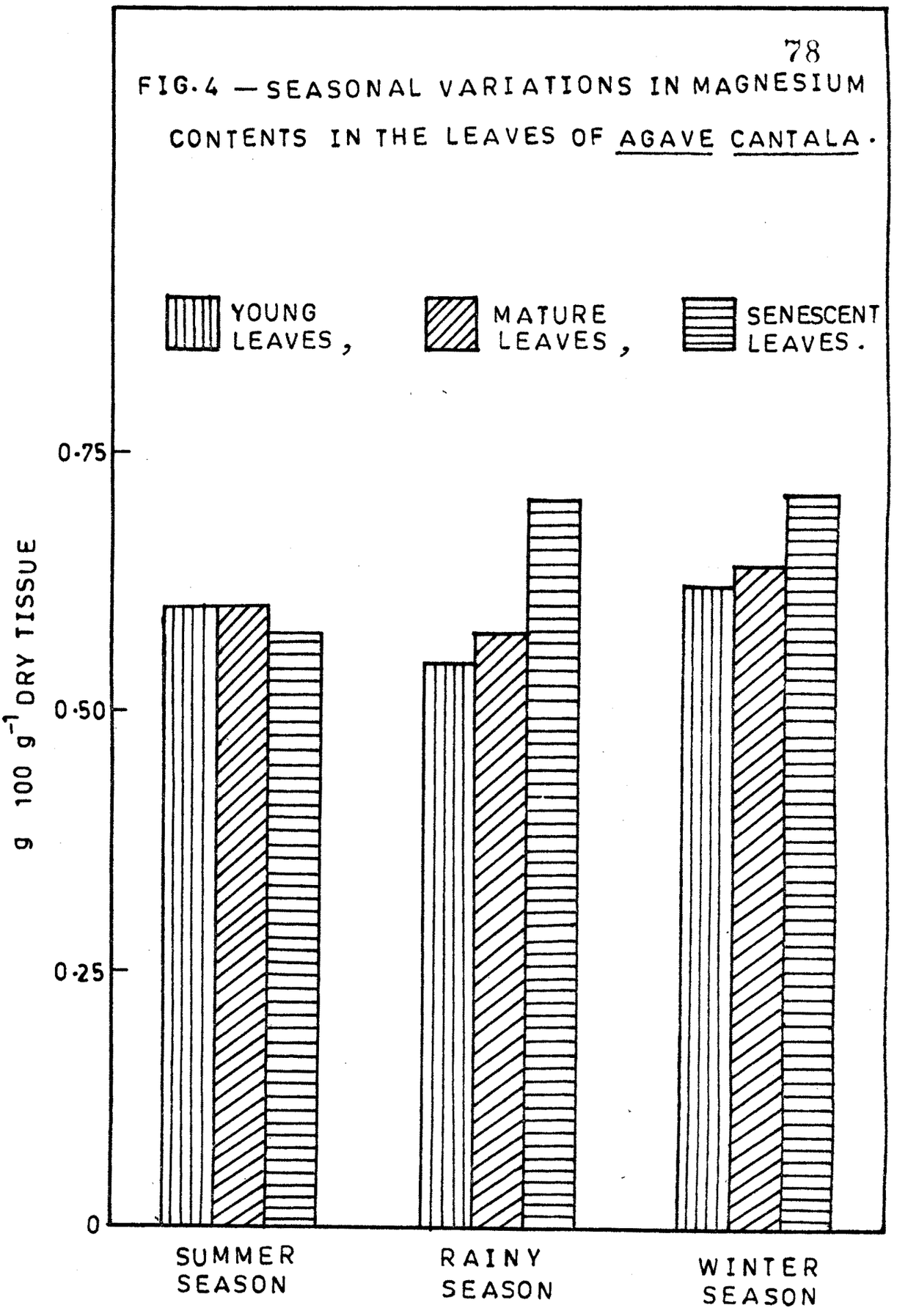
succulents. Karadge (1981) reported that the senescent leaves of Portulaca oleracea shown the calcium contents nearly double from that in the green mature leaves. Similar observations have been made by Thombre (1987) in Aptenia cordifolia and Portulaca quadrifida. Our observations also indicate that the leaf senescence in Agave cantala is accompanied by a marked accumulation of calcium. Our findings further indicate that the environmental factors like temperature, can have a profound influence on calcium accumulation, since a marked calcium accumulation in senescent leaves is seen during winter.

d) Magnesium :

The values of magnesium contents in young, mature and senescent leaves of Agave cantala during three seasons are shown in Fig.4. There is only slight decrease in magnesium content in the senescent leaves in summer season, while there is marked increase in magnesium content in senescent leaves during rainy and winter seasons. Not much variation is noticed in magnesium contents in the young and mature leaves of A. cantala in all the three seasons.

Magnesium in plants is generally absorbed at lower quantities than either Ca^{2+} or K^{+} (Mengel and Kirkby, 1982). It has been suggested by some investigators that Mg^{2+} is actively absorbed by plants, but the evidence for active uptake of Mg^{2+} is not conclusive. Magnesium is generally

FIG. 4 — SEASONAL VARIATIONS IN MAGNESIUM CONTENTS IN THE LEAVES OF AGAVE CANTALA.



absorbed primarily from regions near the root apex, which supports the concept of passive uptake (Russell and Clarkson, 1976). Mulder (1950) reported that high levels of K^+ in the soil resulted in Mg deficiency in the apple leaves. It was also supported by Grimme et al. (1974). They have indicated that high magnesium contents may occur in plants supplied with a low level of potassium nutrition. Hall (1971) also reported very much elevated magnesium levels in calcium deficient tomato tissues. The level of Mg^{2+} in the nutrient medium is also of importance in relation to Mn uptake.

According to Clark (1984) incontrast to Ca^{2+}, Mg^{2+} is very mobile in the phloem and can be translocated from one tissue to another. Magnesium readily moves from roots, stems, cotyledons, primary leaves and secondary leaves to young newly developing leaves if plants are placed in the solutions without magnesium. As fruits and storage tissues are highly dependent on the phloem for their mineral supply, they are thus higher in potassium and magnesium than in calcium (Mengel and Kirkby, 1982). Most of the magnesium salts are highly soluble, often more than 70% of Mg^{2+} in the plant tissues is freely diffusible. Magnesium is associated with diffusible organic anions such as malate and citrate, as well as with inorganic anions (Kirkby and Mengel, 1967). Further they have reported that magnesium is also associated with indiffusible anions including oxalate and pectate.

According to Clarkson and Hanson (1980) of the elements found in the cytoplasm, Mg^{2+} probably has the highest chemical activity. Further they have indicated that considerable Mg^{2+} is bound to polyphosphates (ATP) and to anionic groups of proteins, nucleic acids, and phospholipids. Under some circumstances, Mg^{2+} may contribute to the electrical neutrality of organic compounds such as sugar phosphates, sugar nucleotides and organic and amino acids (Clark, 1984). The most well known role of magnesium is its occurrence at the centre of chlorophyll molecule. Besides its function in the chlorophyll molecule Mg^{2+} is required in other physiological processes. One major role of Mg^{2+} is as cofactor in almost all enzymes activating phosphorylation processes. Mg^{2+} forms 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. Phosphokinases, dehydrogenases, as well as anolases are activated by Mg^{2+}. In these enzymes, however, the magnesium reaction is not specific and Mn^{2+} is often a more efficient activator. A key reaction of Mg^{2+} is the activation of ribulose biphosphate carboxylase. Bandurski (1955) reported that PEP-Case in spinach requires Mg (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 destroyes the ribosomal configuration that is necessary for

protein synthesis (Watson, 1965). The transfer of amino acyls from amino acyl transfer ribonucleic acid (t RNA) to a polypeptide chain appears to be activated by Mg^{2+} (Clark, 1984). In CAM plants, PEP carboxylase the enzyme of CO_2 assimilation during night requires magnesium as a cofactor (Sutton, 1975; Bartakke, 1977).

The content of magnesium in the plant tissues is in the order of 0.5 % of the dry weight (Mengel and Kirkby, 1982). In succulents, however, some workers have noticed quite high levels of this nutrient. Karmarkar (1965) has reported 2.38 % magnesium in the leaves of Bryophyllum pinnatum. Bartakke (1977) recorded magnesium content in the leaves of Aloe barbadensis as 0.935 % of dry tissue. Further observations made by him in enzyme studies in Aloe also suggest activation of PEP-Case, RuBP-Case and MDH by Mg. These enzymes have important role in organic acid metabolism in succulents. Karadge (1981) recorded 0.3% magnesium content in the leaves of Portulaca oleracea. In Aptenia cordifolia and Portulaca quadrifida, Thombre (1987) recorded magnesium content as high as 0.84 % and 0.94 % respectively. Nobel and Berry (1985) studied element levels in chlorenchyma of various Agave species namely Agave americana, A. deserti, A.fourcroydes, A. lechuguilla, A. salmiana, and A. utahensis. They found that magnesium level varies from 0.36 to 0.62 % of dry matter. Our values of magnesium content in Agave cantala (Fig.4) range from 0.55 to 0.7 % of dry tissue. Thus in comparison

to other Agave species A. cantala leaves have capacity to accumulate sufficient amount of magnesium. It is further noticed that this tendency is more prominent in winter and summer seasons rather than in rainy seasons. Our TAN values also support this view.

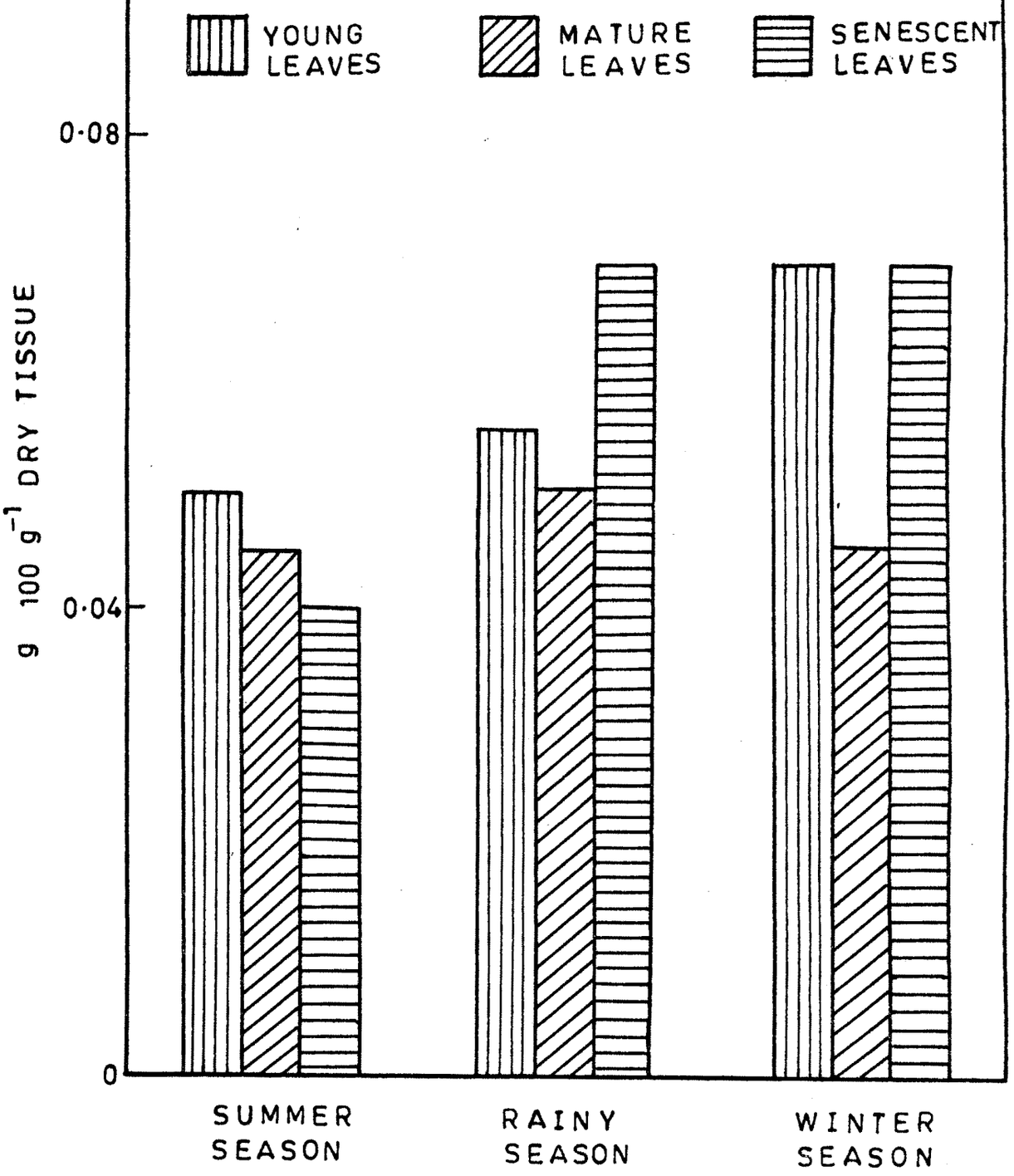
It is evident from Fig. 4 that the pattern of magnesium accumulation in the senescent leaves is not uniform in the three seasons. Thus in summer season we can notice a decline in magnesium level in the senescent leaves while in rainy and winter seasons there is accumulation of magnesium content in senescent leaves. According to Clark (1984) many factors affect Mg^{2+} uptake and accumulation, and plant age and plant part must be considered in evaluating Mg concentrations reported in plants. He found that magnesium remained relatively high in the different plant parts of maize. During grain fill, leaves, tassels, and husks increased, roots and stalks decreased and kernels and cobs decreased slightly in Mg from their initial concentrations. in newly developing tissues. He also found that magnesium concentrations in leaves of various positions on maize plants remained relatively constant but increased with age. He therefore suggested that as long as ample Mg is in the growth medium, most plants have the capacity to absorb, translocate and accumulate sufficient Mg^{2+} in the various plant parts to prevent Mg disorders, especially in harvested fruits and seeds.

In accordance with above view accumulation of magnesium in senescent leaves of sugarcane was recorded by Nimbalkar (1973). While in succulents, Karadge (1981) and Thombre (1987) have noticed a decline in magnesium content in senescent leaves of Portulaca oleracea and Aptenia cordifolia, Portulaca quadrifida respectively. Waughman and Bellamy (1981) made a detailed study of this aspect in mature and senescent leaves of perennial plants. They observed that magnesium level is elevated in the senescent leaves of Iris germanica, Potentilla palustris, Petasites hybridus, Menyanthes trifoliata, Dactylis glomerata, Lilium bulbiferum, Ammophila arenaria, Fagus sylvatica and Crataegus monogyna. At the same time they observed decrease in magnesium level in senescent leaves of Carex lepidocarpa, C. panicea, Sesleria caerulea, Cladium mariscus, Luzyla sylvatica, Juncus inflexus, Carex paniculata, Cerastium tomentosum, Hordeum vulgare, Elymus arenarius, Alnus glutinosa and Convolvulus arvensis. Thus it is obvious that there is inter-specific variatioh with respect to magnesium accumulation pattern during leaf senescence. Our observations further indicate that the seasonal variations also exert influence on this process. Since in the present studies accumulation of magnesium in senescent leaves is seen only in rainy season and winter.

e) Iron :

Fig. 5 records seasonal variations in iron contents

FIG.5 — SEASONAL VARIATIONS IN IRON CONTENTS IN THE LEAVES OF AGAVE CANTALA.



in young, mature and senescent leaves of Agave cantala. It is evident that the senescent leaves have relatively higher level of iron than either mature or young leaves in each season and this is particularly significant in rainy and winter seasons. There is only slight variation in iron content in young and mature leaves of A. cantala.

Iron is an essential micro or trace element. It may be absorbed by plant roots as Fe^{2+} or as Fe chelates. Fe^{3+} is only of minor importance because of the low solubility of Fe-III compounds at the pH of most soils. Fe chelates are soluble and therefore available to roots. However, the uptake of whole Fe chelate molecules is very low. Romheld and Marschner (1981) have stated that rhythm of uptake and translocation of Fe is hormonally controlled, probably from the shoot apex. They further suggested that Fe nutritional status of the plant is transformed into a 'signal' which induces distinct biochemical and morphological changes within the roots causing a fine regulation of Fe supply to the plant. Auxins appear to be involved in this process. According to Ramani (1987) a well regulatory mechanism for iron uptake which depends on iron nutritional status exists in most of the higher plants. With iron deficiency, the tolerant plant species have the capacity to produce certain chemicals 'reductants' in the growth medium. The rate of uptake and translocation of iron to the shoot increases sharply as the reducing capacity increases.

Lingle et al. (1963) noticed that the uptake of iron is considerably influenced by other cations like Mn^{2+}, Cu^{2+}, Ca^{2+}, Mg^{2+}, K^{+} and Zn^{2+}. Heavy metals, in particular Cu and Zn are known to displace Fe from chelate complexes, forming corresponding heavy metal chelates. This may be important in limiting Fe uptake and utilization. Iron is not readily mobile between different plant parts. Green plants deprived of iron soon become chlorotic in the younger plant parts while older tissues remain green. Younger tissues are therefore, dependent on a continuous iron supply in the xylem. Iron is translocated in the xylem appears to be a ferric citrate as a major form (Mengel and Kirkby, 1982).

In the words of Kannan (1987) iron is considered today (as 143 years ago), the most important and the most ubiquitous, and yet the most evading for the plant nutritionists to encounter. Iron has a role in the synthesis of the common precursors of chlorophyll (Kanwar and Randhawa, 1978). Here iron plays some what similar role to magnesium in the porphyrin structure of chlorophyll. They further reported that the most well known function of iron is in iron porphyrin enzymes such as catalase, peroxidase and cytochrome oxidase. It also seems to be concerned with other enzymes such as aldolase and phosphorylase for which iron does not appear to be a co-factor in higher plants. Iron in enzyme systems functions as haem or haemin as a prosthetic group. It is

well known that trace elements are cofactors or activators for more than 33% of microbial enzymes (Dave, 1987). He further indicated that specific association of Fe atoms with haem enzyme increases its activity by an order of a magnitude of 10.

The haem pigments constitute only about 0.1% of the total iron in the plant leaves. The remaining iron is stored largely as a ferric phosphoprotein called phytoferritin and another form of iron occurring in the chloroplasts is ferredoxin. Ferredoxin is a nonhaem-iron protein which participates in oxidoreduction processes 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 (Bennett, 1945; Perur et al., 1961). From the short term experiments on the alga Euglena gracilis it now seems likely that iron is directly implicated in nucleic acid metabolism (Price et al.,1972). The protein and chlorophyll contents get affected by iron supply.

Average values of iron in land plants are 0.011g 100 g^{-1} dry tissue (Epstein, 1972). The values of iron

contents in leaves recorded for CAM succulents are 0.03 % in Bryophyllum pinnatum (Karmarkar, 1965); 0.05 % in Aloe barbadensis (Bartakke, 1977); 0.09 % in Portulaca oleracea (Karadge, 1981); 0.03 to 0.3 % in Aptenia cordifolia and 0.2 to 0.26 % in Portulaca quadrifida (Thombre, 1987). Nobel and Berry (1985) have recorded values of iron contents in different agaves (Agave americana, A. deserti, A. fourcroydes, A. lechuguilla, A. salmiana and A. utahensis). They found that iron contents range from 0.034 to 0.170 % of dry tissue. Our work with Agave cantala indicates the values of iron contents ranging from 0.012 to 0.028 g 100 g^{-1} dry tissue. Thus the iron contents in A. cantala appear to be quite low as compared to other Agave species. At the same time it is in the optimum range recorded for most plant species by Epstein (1972).

The senescent leaves of A. cantala are having higher level of iron in all the seasons than either young or mature leaves. An increase in iron level during leaf senescence has been recorded for some succulents like Portulaca oleracea; P. quadrifida and Aptenia cordifolia (Karadge, 1981; Thombre, 1987). The immobile nature of iron in the plants is very well documented and this may be the probable reason for the increase in iron contents during leaf senescence. However, this increase is unable to prevent the disorganisation of chloroplasts and degradation of chlorophylls which occurs during leaf senescence in most of the plant species. Thus it is quite probable that

iron might be accumulating in some "non-available" form in the senescent leaves. The chlorophyll contents in A. cantala also support above possibility of role of iron which is not influencing the total chlorophyll contents in the leaves of different plants.

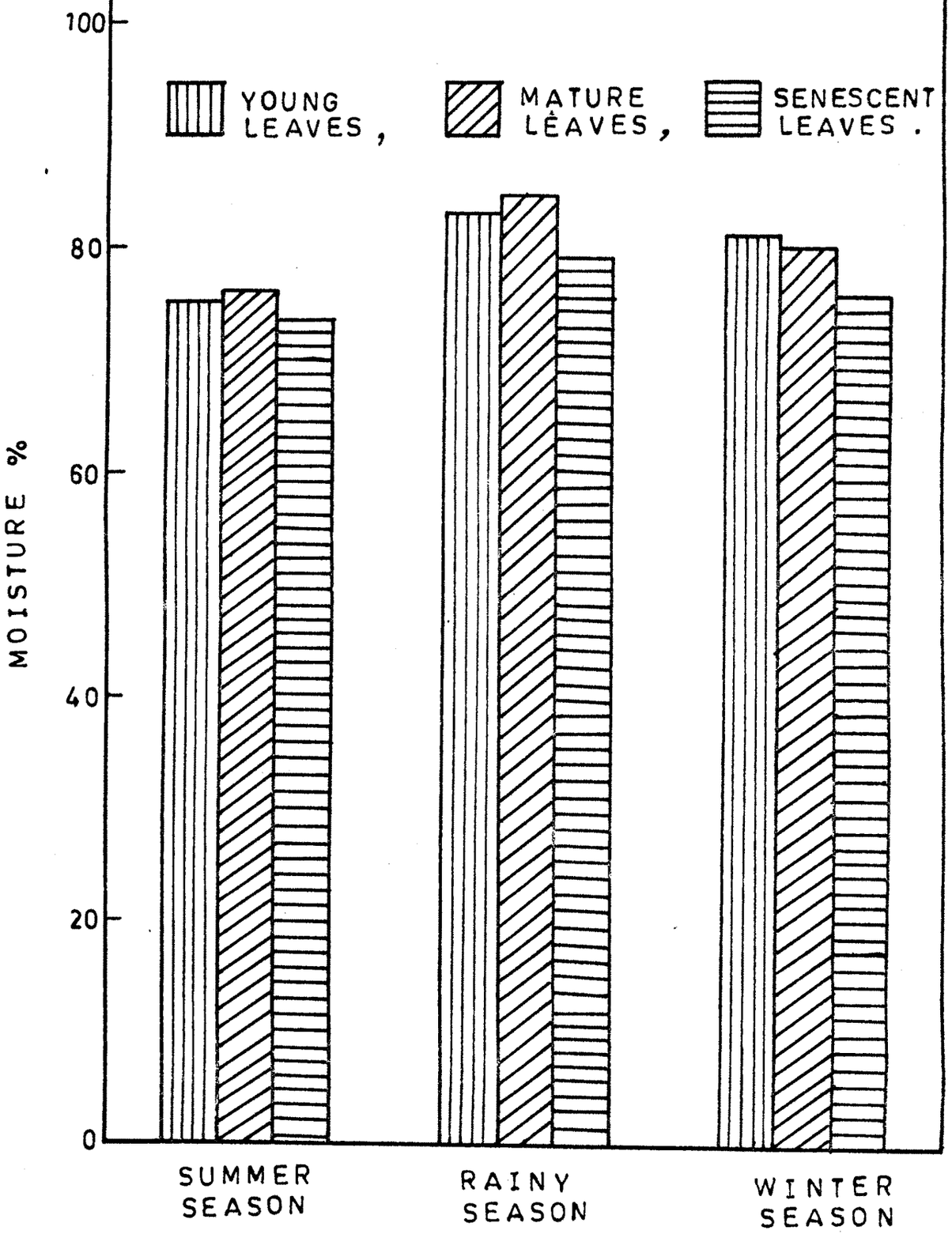
2. Organic Constituents

(i) Moisture Percentage :

Fig. 6 shows the leaf moisture percentage in young, mature and senescent leaves of Agave cantala in summer, rainy and winter seasons. From the figure it is clear that the leaf moisture percentage increases in rainy season, intermediate in winter and comparatively less in summer. In mature leaves maximum moisture percentage is recorded in three seasons than young and senescent leaves.

Water is perhaps the most important fundamental organic constituent in all living organisms. It constitutes about 90% of the total proportion of the protoplasm. The cytoskeleton membrane has got water as the most important component. Water provides aqueous environment for all biochemical reactions catalysed by enzyme proteins. In plants the process of photo-synthesis can not occur without water, because the photolysis of water serves as a medium of transport for various inorganic constituents as well as organic metabolites throughout the plants. Thus it is clear that availability of water is a

FIG. 6 — SEASONAL VARIATIONS IN MOISTURE PERCENTAGE IN THE LEAVES OF AGAVE CANTALA.



crucial factor for plant survival. In xerophytes like Agave the availability of water is really a serious problem because they live in desert conditions, where the rainfall is greatly limited and the rate of evaporation is very high. Thus the plants exposed under such conditions are rather constantly exposed to drought. Naturally the drought tolerance mechanism in the drought resistant plants mainly consists of maintaining an optimum water balance in the plant parts. Maintenance of a favourable water-balance in the leaf tissue is of prime importance because the leaves perform most of the major physiological processes. The example of significant evolution in this direction can be seen in CAM succulents.

The classification of a plant as succulent is based exclusively on morphological criteria, and does not implicate a special taxonomic status. The single morphological criterion which classifies a plant as succulent is the possession of voluminous water-storing tissues resulting in an increase in volume relative to surface area. Thus, a thick, fleshy, juicy habitus results which is envisaged as "succulence", and which results in a form tending towards a spherical shape rather than disc shape typical for most leaves. Hence, succulents are generally characterized by their ability to store relatively large amounts of water. In succulents, all basic organs of the plant can function as water reservoirs. Thus, leaf succulents, stem succulents, and very rately root succulents can be distinguished. CAM plants are only known as members

of the leaf and stem succulents. The storage of water in succulent plant organs occurs in the basic tissues. Those tissues, clearly having the task of storing water, are called water tissues. According to Haberlandt (1918), external and internal water tissues can be distinguished. External water tissues are modifications of the epidermis or subepidermis. They are characterised by very large cells which are often arranged in more than one layer. These cells either lack chloroplasts completely or at best have very few. Internal water tissues can be derived from different types of parenchyma. In leaf succulents, the internal water reservoirs are provided by the mesophyll of the leaf. In stem succulents, the cortex, the pith, or both can contribute to the storage of water. In the case of water tissues derived from the inner part of the leaf mesophyll or the stem pith, they may be free of chloroplasts, as is the case for external water tissues. Contrastingly, in succulents where the internal water tissues are identical with the photosynthetic parenchyma, the water-storing cells contain chloroplasts. This is found either in those leaf succulents where the cells of the total mesophyll or atleast of the outer parts of the mesophyll show both water storage and photosynthesis.

Kluge and Ting (1978) have developed a parameter known as mesophyll succulence (Sm) to decide the succulent or non-succulent nature of the plant, which is as follows :

$$Sm = \frac{\text{water content (g)}}{\text{chlorophyll content (mg)}}$$

The average mesophyll succulence in the tissues of Agave cantala is 6.27, which lies in the range (1.34 to 13.00) described for various CAM species by Kuge and Ting (1978).

Few attempts have been made by Indian workers to determine leaf moisture percentage in CAM succulents. According to Karmarkar (1965) about 93.05 % of moisture is present in leaves of Bryophyllum pinnatum. Bartakke (1977) found 81.28% moisture in the leaves of Aloe barbadensis. Karadge (1981) observed 94% moisture in Portulaca oleracea leaves. The average values of moisture percentage recorded for leaves of Aptenia cordifolia and Portulaca quadrifida are 94.20 and 77.75 respectively (Thombre, 1987). Our observations indicate that the moisture percentage in Agave cantala leaves varies from 73.82 % to 84.47 % in different seasons (Fig. 6), and these values are in agreement of the succulent nature of the plant. In the present studies of A. cantala moisture percentage in the leaves is less in summer, that is due to less water available from the soil. The uptake of water may be increased in rainy season due to its availability. The moderate water supply possibly maintains similar moisture percentage in young, mature and senescent leaves during winter season. Thus in A. cantala leaf moisture percentage may give slight reflection of seasonal variations in climatic conditions to which the

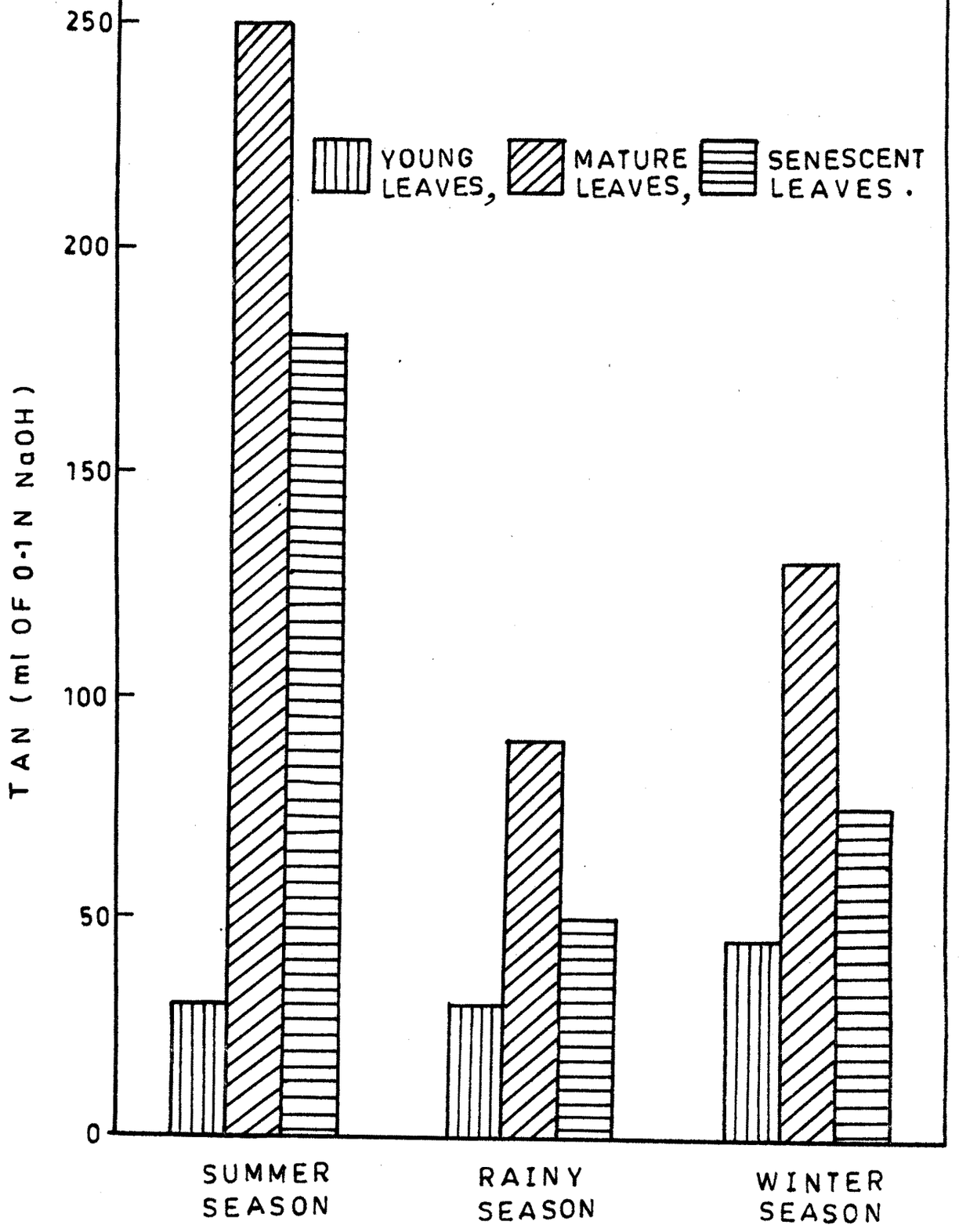
plant is exposed. It is clear from our observations that in all the three seasons there is decline in the moisture percentage of the senescent leaves of A. cantala, although the decrease is not significant. However, this decrease does indicate that the leaf senescence in Agave is accompanied by drying of the old leaves.

(ii) Titratable Acidity (TAN)

Fig.7 records the variations in Titratable Acid Number (TAN) values in young, mature and senescent leaves of Agave cantala during summer, rainy and winter seasons. The TAN was estimated at 12.00 noon in three leaf categories during all the three seasons. From the figure it is clear that the maximum TAN values are recorded in summer season than in rainy and winter. The highest values of TAN are observed in mature leaves in each season. Whereas young leaves show lowest TAN values. The TAN values of senescent leaves are decreased relative to those of mature leaves in each season.

Titratable Acid Number (TAN) gives a broad idea about organic acid status in the plant tissue. Organic acids represent an important class of metabolites in all cells. Generally the tricarboxylic acid (TCA) cycle intermediates are the important organic acids of common occurrence in most of the plant species. These include malic acid, citric acid, isocitric acid, succinic acid, and fumaric acid. The levels of these organic acids fluctuate in various plant tissues under different growth

FIG.7 — SEASONAL VARIATIONS IN TAN VALUES IN THE LEAVES OF AGAVE CANTALA.



conditions due to different physiological states of the plant cell. Thus some of these organic acids enter in storage food while others undergo a rapid metabolic turnover. Besides the conventional TCA cycle organic acids, some plant species also accumulate other organic acids. Thus in some plants especially green algae, glycolic acid, a product of photorespiration, is accumulating. Some plant species like Amaranthus, Oxalis, Pennisetum accumulate large quantities of oxalic acid. Tartaric acid is also stored in some plant species like Tamarindus. It is reported that in Agave americana piscidic acid (p-hydroxy-benzyl-tartaric acid, a dicarboxylic aromatic acid) is accumulated which is also known in the Leguminosae and Liliaceae (Nordal and Ogner, 1964). They further suggested that piscidic acid may play an important role in CAM. The organic acids play several roles in cellular metabolism. They play an important role in maintaining cation-anion balance in the plant cell. The organic acids further provide carbon skeleton for biosynthesis of large number of organic compounds especially amino acids. The organic acids like malic acid has been found to play a key role in stomatal movement. In halophytes like Atriplex organic acids have been reported to play a major role in osmoregulation (Osmond, 1967). The glycolic acid plays significant role in the photorespiration of C_3 plants.

The organic acid accumulation is of special significance in the plants like Agave which possess CAM. Extensive research by Richards (1915), Bennet-Clark (1933 a,b), Wolf (1937), and

then by such investigators as Pucher, Vickery, Thomas, Beevers and Ranson firmly established the relationship between dark CO_2 fixation and organic acid synthesis in succulent plants. Furthermore, during this early period before 1950 it was clear that the organic acids which accumulate during the dark phase, termed "acidification" by the early coworkers, were nearly quantitatively converted to carbohydrates e.g., starch, during the subsequent light or deacidification period. It is now generally accepted that dark fixation of CO_2 is the key reaction in CAM, in which malate is the first and primary stable product of CO_2 fixation. Thurlow and Bonner and Bonner and Bonner (1948) were perhaps the first to show dark $^{14}CO_2$ fixation into malate as well as other organic acids including citrate, isocitrate, fumarate, and succinate. Saltman *et al.* (1957), showed that after 1 min of dark $^{14}CO_2$ fixation, malate had 60% of the ^{14}C, and furthermore, even after 60 min no carbohydrates or phosphorylated compounds were labelled. Since the first stable products detectable by chromatography were malate and aspartate, they deduced that oxaloacetate (OAA) was the first intermediate. The following reaction was proposed :

$$\text{P - enolpyruvate} + CO_2 \longrightarrow \text{oxaloacetate.}$$

subsequently, additional research by Walker (1957) and others suggested that P-enolpyruvate (PEP) was the main substrate for carboxylation. Furthermore, the enzyme, P-enolpyruvate

carboxylase (PEP-Case), discovered by Bandurski and Greiner (1953), was strongly implicated. It was immediately recognised that the reaction catalysed by malate dehydrogenase (MDH) (Walker, 1957) probably was responsible for the reduction of oxaloacetate to malate. It is now generally accepted that the metabolic pathway for the majority of the malate synthesis in succulent plants is :

$$PEP + CO_2 \longrightarrow OAA \longrightarrow \text{malate}$$

Malate synthesized in this way goes on accumulating in the vacuoles during the entire period of darkness and it is subsequently decarboxylated during daytime. Thus the organic acid turnover plays an important role in the metabolism of succulents and due to this reason a great significance is attached to TAN values.

It is general observation that the CAM succulents show relatively very high TAN values than those displayed by non-succulent higher plants. Bartakke (1977) recorded that TAN value for Aloe barbadensis is 36.92 while the TAN value reported for Portulaca oleracea leaves by Karadge (1981) is 82.30. The work of Kluge and Ting (1978) indicated that the succulents like Anacampseros, Xerosicyos, and Peperomia are having TAN values as 150, 100 and 100 respectively. Eickmeier and Adams (1978) noticed that average TAN value in Agave lechuguilla is 66.18. According to Thombre (1987) the average TAN values for Aptenia cordifolia and Portulaca quadrifida are

17.88 and 41.16 respectively. Our observations indicate that the leaves of Agave cantala show a great variation in TAN values, depending on leaf age as well as the season. Thus TAN values range from 25.61 to 253.60 and higher TAN values have been recorded in the summer season than rainy and winter seasons. These observations clearly indicate rapid operation of CAM in summer season. The values of TAN recorded in young leaves are quite low as compared to mature leaves which indicates that organic acids show a tendency of accumulation as the leaves reach maturity. Again we can notice a drop in TAN values in senescent leaves in each season, which probably indicates slowing down of the leaf metabolism during the phase of leaf senescence.

(iii) Carbohydrates

The level of various carbohydrate fractions in the young, mature and senescent leaves of Agave cantala during different seasons is recorded in Fig. 8 and 9. It is evident from the figures that there are marked variations in the level of the carbohydrate fractions in response to leaf age as well as the season. The reducing sugar content varies from 1.005 to 2.42 g 100 g^{-1} fresh tissue and it is always higher than the corresponding level of non-reducing sugar in the leaves. The non-reducing sugar content ranges from 0.098 to 0.665 g 100 g^{-1} fresh tissue. The A. cantala leaves are rich in starch and the starch content ranges from 1.93 to 7.208 g $100g^{-1}$

FIG. 8 — SEASONAL VARIATIONS IN TOTAL CARBOHY-
-DRATE CONTENTS IN THE LEAVES OF
AGAVE CANTALA .

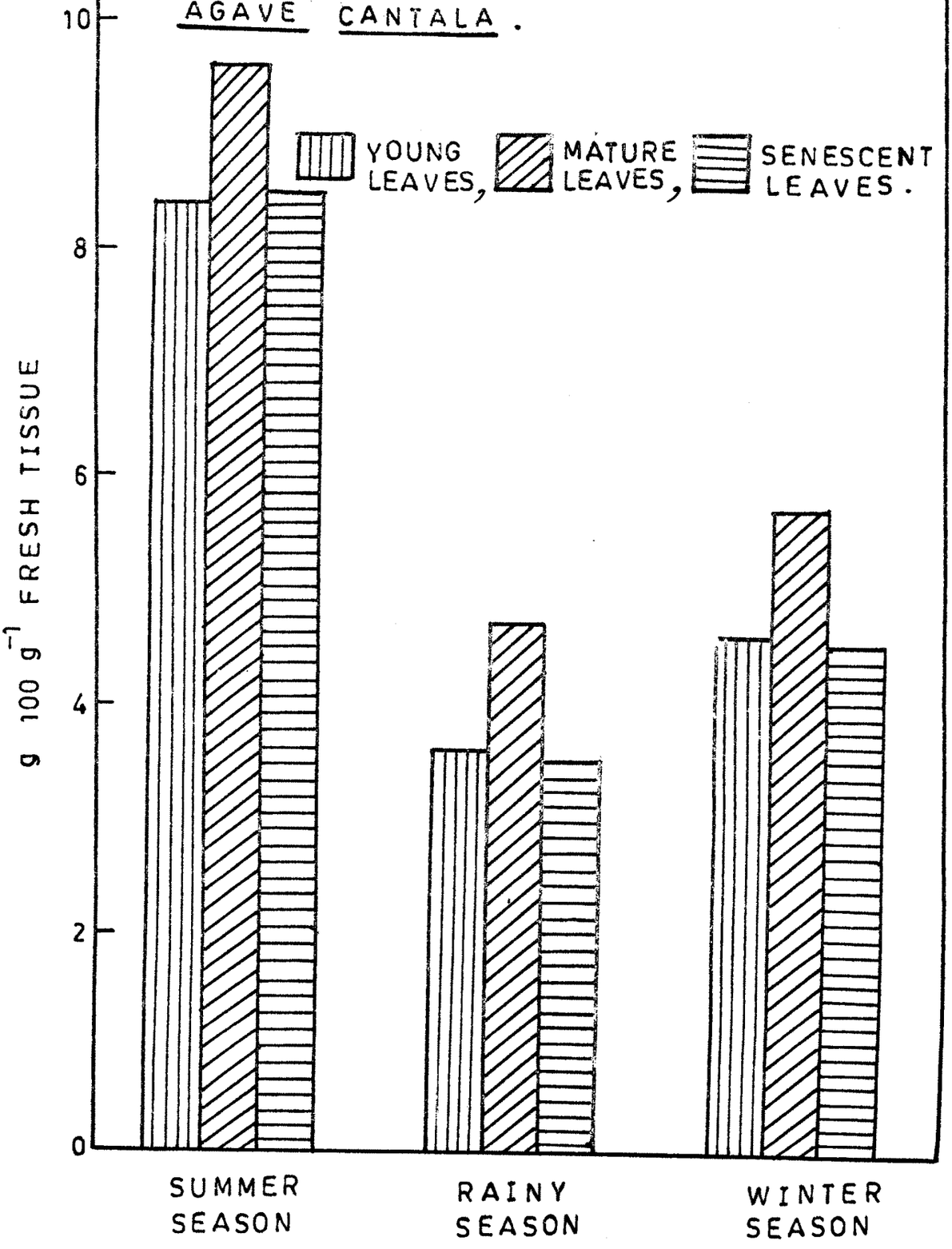
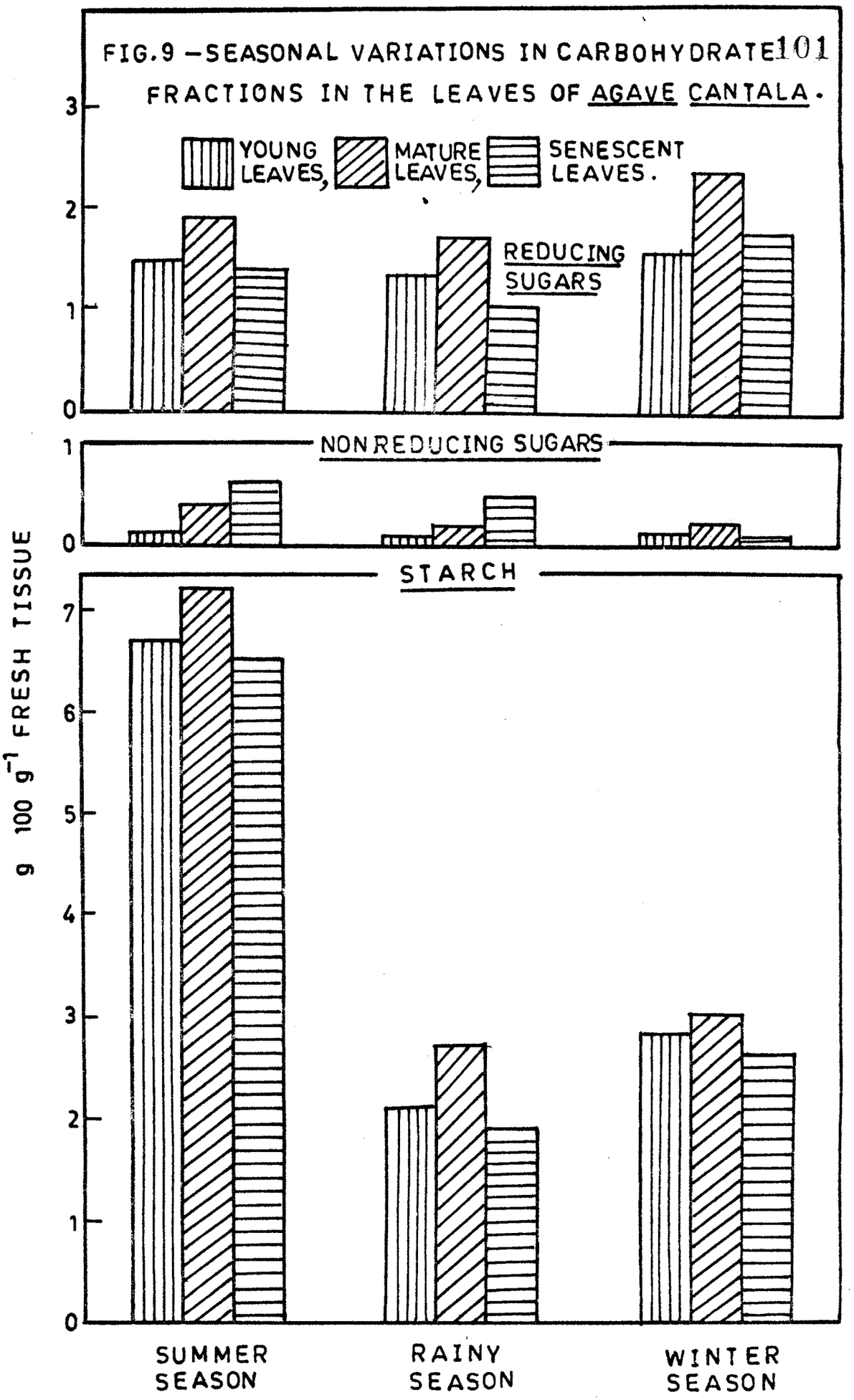


FIG.9 – SEASONAL VARIATIONS IN CARBOHYDRATE FRACTIONS IN THE LEAVES OF AGAVE CANTALA.



fresh tissue. There is great seasonal variation in the carbohydrate fractions especially starch content. Relatively very high levels of starch in young, mature and senescent leaves of A. cantala are recorded in the summer season and this has resulted in marked increase in total carbohydrate contents in this season (Fig. 8). In winter season maximum amount of reducing sugars has been recorded, whereas the non-reducing sugars reach their peak in the summer season. The carbohydrate fractions show increase in amount along with the progress of leaf growth, since in each case the mature leaves record more carbohydrates as against young leaves. In each season there is lowering in the level of starch and reducing sugars in senescent leaves.

The carbohydrates are very important plant constituents because they represent a means of storage of the energy trapped from the sun in the process of photosynthesis, as well as forming the supporting tissues of plants thus enabling them to achieve erect growth, sometimes as high as 400 feet. The carbohydrates are also vitally important in the organic chemistry of the plant, since they provide the carbon skeleton for most of the organic compounds found in plants. Studies reported over the past few decades have shown that many carbohydrates which were previously not considered important in the metabolism of the plants, such as the sugar components of glycosides, do indeed play important roles. The carbohydrates are a group of organic compounds containing carbon, hydrogen and oxygen,

usually in the ratio of 1:2:1. Compounds containing nitrogen and sulfur, and compounds which do not conform to the ratio 1:2:1 for carbon, hydrogen and oxygen are also indeed in the carbohydrate group. The carbohydrates are classified into three large groups, the monosaccharides, the oligosaccharides and the polysaccharides. The monosaccharides are the simple sugar, which do not yield simpler carbohydrates on hydrolysis. The oligosaccharides yield 2 to 8 simpler sugars on hydrolysis. The polysaccharides are complex molecules of high molecular weight which are composed of a large number of monosaccharides joined through glycoside linkages.

The monosaccharides are synthesized in green plants starting with a carboxylation reaction in which D-ribulose-1, 5-diphosphate is the acceptor of CO_2 with the formation of phosphoglyceric acid. By a subsequent series of enzymic reactions, a number of phosphorylated monosaccharide derivatives are formed. Some of these phosphorylated sugars, such as D-glucose-6-phosphate and D-fructose-6-phosphate, are hydrolyzed to the free sugars, which in some instances accumulate in large quantities in plants. The phosphorylated monosaccharides produced during photosynthesis are partially consumed in respiration with the production of energy which is used for numerous metabolic reactions in plants. The monosaccharide phosphates are converted into sugar nucleotides, chiefly UDP-D-glucose, as well as others such as UDP-D-galactose, GDP-D-glucose, and ADP-D-glucose. The sugar moieties

of these nucleotides are interconverted by various specific epimerases and serve as donors of the sugars for the formation of numerous glycosides, oligosaccharides and polysaccharides (Leloir, 1971). Of the monosaccharides, D-glucose, D-mannose, D-galactose and D-fructose are the most widely distributed in higher plants. Glucose is the most common which is found either free or in combination with many important plant substances, such as the oligosaccharides sucrose and raffinose and the polysaccharides starch and cellulose and in many important glycosides. Fructose is the only abundant ketose. It is found free in a pyranose structure and in a combined state as in sucrose and inulin, in the furanose form. It is observed that in some plant species polymers of D-fructofuranose are stored as reserve material rather than starch. In contrast to starch these polysaccharides are of relatively low molecular weight (less than 10,000), are water soluble and do not stain with iodine. Aspinall and Das Gupta (1959) reported that Agave veracruz Mill synthesizes highly branched fructans containing a high proportion of both (2-1)-and (2-6)-linked fructose units. Glucose and fructose form the major component of reducing sugars in higher plants and among these two sugars glucose plays a major metabolic role. This is due to the fact that glucose is a substrate of most important process "glycolysis" in all living organisms. Thus it is obvious that the level of reducing sugars is regulated by genetic as well as environmental factors which

control photosynthesis and respiration. Few workers have estimated reducing sugar contents in succulents. Bartakke (1977) recorded that the leaves of Aloe barbadensis have got reducing sugars in the order of 1.08 g 100 g^{-1} dry tissue. While Karadge (1981) reported that the reducing sugar content in the leaves of semi-succulent Portulaca oleracea varies from 0.12 to 0.23 g 100 g^{-1} fresh tissue. Our observations indicate that the Agave cantala leaves are rather richer in reducing sugar level as compared to these species. It is interesting to note here that the peak of reducing sugar content in different categories of leaves (Young, mature and senescent) of A. cantala is recorded in winter season. This may be probably due to low rates of respiration during night hours when the temperature is relatively low.

Sucrose (α-D-glucopyranosyl-β-D-fructofuranoside) is perhaps the major non-reducing sugar found in higher plants. It is the most widely found disaccharide in nature. It is almost universally present throughout the plant world and has been detected in all parts of plants. It is not only a major photosynthetic product, but also a principal form of storage and translocation of carbohydrates. Sucrose is formed in nature by following different reactions :

uridine diphosphoglucose (UDPG) + fructose-6-phosphate
= uridine diphosphate + sucrose-6-phosphate

sucrose-6-phosphate = sucrose + Pi

UDPG + fructose = uridine diphosphate + sucrose (Miller,1973).

Further he indicated that first reaction is catalysed by sucrose phosphate synthetase, second reaction by sucrose phosphatase and third reaction by sucrose synthetase. Sucrose is hydrolyzed by enzyme invertase into glucose and fructose. The photosynthetic leaf becomes a net exporter of sucrose when it has reached about 1/3 of its final size and continues this process until it attains its maximum size (Miller,1973). According to Milthorpe and Moorby (1969) the capacity of the translocation system for the export does not seem to become impaired until the leaf has reached a very advanced stage of senescence. Besides sucrose other non-reducing sugars are also present in plants, but they occur relatively in small amount. Generally the plants like sugarcane and sugarbeet are regarded as important commercial sources of sucrose. In other plant species particularly succulents the level of non-reducing sugar is low. Bartakke (1977) recorded the non-reducing sugar content as 0.27 g 100 g^{-1} dry tissue in the leaves of Aloe barbadensis, whereas according to Karadge (1981) the level of non-reducing sugars in the leaves of Portulaca oleracea varies from 0.33 to 0.37 g 100 g^{-1} fresh tissue. In case of leaves of Agave cantala the non-reducing sugar content is comparatively high and its ranges from 0.098 to 0.665 g 100 g^{-1} fresh tissue. It is interesting to note that the level of this carbohydrate fraction is highest in the summer months. This may be considered as an adaptive feature, since sucrose is known to play an osmoregulatory role in many cases. Maximov (1929) suggested

that the accumulation of sugars might protect the protoplasm from coagulation and desiccation and the high concentration might prevent visible wilting for a long time in spite of an increasing water deficit. The sugars are effective as protective agent in both cold and drought hardening (Parker, 1972). He further reported that the sugars bind with protein to form protein-sugar complexes and have been found to harden the plant under drought. Vora et al. (1974) reported that the accumulation of sugars under water stress indicates a protective role of sugars. Thus the high level of non-reducing sugars in the summer months may be due to higher photosynthetic efficiency and it may help the plant in tolerating drought as well as high temperature.

Starch is the reserve carbohydrate of most higher plants, where it occurs as granules which are insoluble in cold water and give a characteristic deep blue colouration with iodine. The starch granules are organised structures which vary in both shape (spherical, polygonal, elliptical, etc.) and size (1-100 μ in diameter), these properties being, to some extent, characteristic of the plant species and the maturity of the plant (Manners, 1973). Microscopic examination shows that starch granules consist of a series of concentric layers arranged around a spot, which is generally eccentrically located, called the hilum. On heating in the water, the granules swell and gelatinize, eventually producing an aqueous dispersion or paste, depending upon the concentration of starch.

The temperature at which gelatinization occurs (usually in the range of 60-80°) is also characteristic of the source of the starch. Although starch was formerly regarded as a single polysaccharide, chemical studies clearly established that most starch granules contain a mixture of two polysaccharides (Whelan, 1958). These are usually termed amylose and amylopectin; both are polymers of α-D-glucopyranose. Amylose amounts to 15-25 % of most starches, although certain varieties of waxy cereal starch (e.g., sorghum, maize) contain less than 1 %, whilst other plants (e.g. wrinkled pea, Amylomaize) contain 50-75 %.

The biosynthesis of starch is now very well understood and it is noticed that the sugar nucleotides play a key role in this process. The synthesis of starch during photosynthesis and its accumulation in chloroplasts can be very easily demonstrated in the leaf tissue using simple iodine test. Starch degradation is also an important biochemical process which is mediated through the activity of various amylases, starch phosphorylase and debranching enzymes. According to Manners (1973) the starch content of plant tissues varies considerably; the tubers, roots, seeds and the fruits of some plants may contain between 20-70 % starch (undried basis), whereas in certain leaf tissues, the starch content is transient, and usually does not exceed 1-2 %. The leaves of CAM plants show chloroplasts which may be remarkably rich in starch (Klug and Ting, 1978). According to Karmarkar (1965) in the leaves

of Bryophyllum pinnatum the starch content is 0.999 g 100 g^{-1} dry tissue. Bartakke (1977) reported that starch content in the leaves of Aloe barbadensis is 1.45 g 100 g^{-1} dry tissue. Karadge (1981) indicated that the starch content in Portulaca oleracea leaves ranges from 4.53 to 5.02 g 100 g^{-1} fresh tissue. The starch content in Agave cantala leaves is comparatively very high and the values as high as 7% have been noticed in mature leaves during summer months. In summer season the starch accumulation seems to be more pronounced in young, mature as well as senescent leaves and this may be due to high carboxylation efficiency during this period.

It can be seen from present investigations that there are marked changes in the level of carbohydrate fractions especially starch and reducing sugars during the phase of leaf senescence. Both these components show a decline in senescent leaves whereas no definite trends is noticed with respect to non-reducing sugars. Spencer and Titus (1973) found that the total sugar content of leaves decreases during the onset of senescence. According to Baur et al. (1968) the translocation of hydrolysed carbohydrates from the aged leaves of tobacco takes place to the other parts of the plant. The detached leaves during senescence accumulate large amount of sugars because sugars can not be transported out, while sugars migrate towards roots from attached senescing leaves which causes a decrease in the total sugar content of the senescing leaves (Thimann et al., 1977). Mahapatra and Johnson (1971)

are of the opinion that degradation of starch during senescence takes place in tobacco leaves. Arguelles and Guardiola (1977) have reported that increased amylase activity causes reduction in the total carbohydrate of the senescent leaves. It has been observed by Karadge (1981) that the photosynthetic carbon assimilation in sugars is greatly reduced in senescent leaves of Portulaca oleracea. Lewington and Simon (1969) studied the effects of light on senescence of detached cucumber leaves and they have observed that decreased photosynthetic rate causes a decrease in the total carbohydrate of the senescent leaves. Thus it is apparent that the reduction in starch and reducing sugar content in the senescent leaves may be mainly due to a decline in photosynthetic efficiency during the phase of leaf senescence.

(iv) Total Polyphenols:

Fig.10 records the seasonal variations in the total polyphenol contents in young, mature and senescent leaves of Agave cantala. It can be observed from the figure that polyphenol contents vary from 0.150 to 1.250 g 100 g^{-1} fresh weight. Maximum accumulation of polyphenols is recorded in summer season as compared to rainy and winter seasons in all leaf categories. With respect to leaf age lowest values of polyphenols are seen in young leaves in all the seasons. The high level of polyphenol content is observed in mature leaves during rainy season, while polyphenol accumulation is more pronounced in senescent leaves in summer and winter seasons.

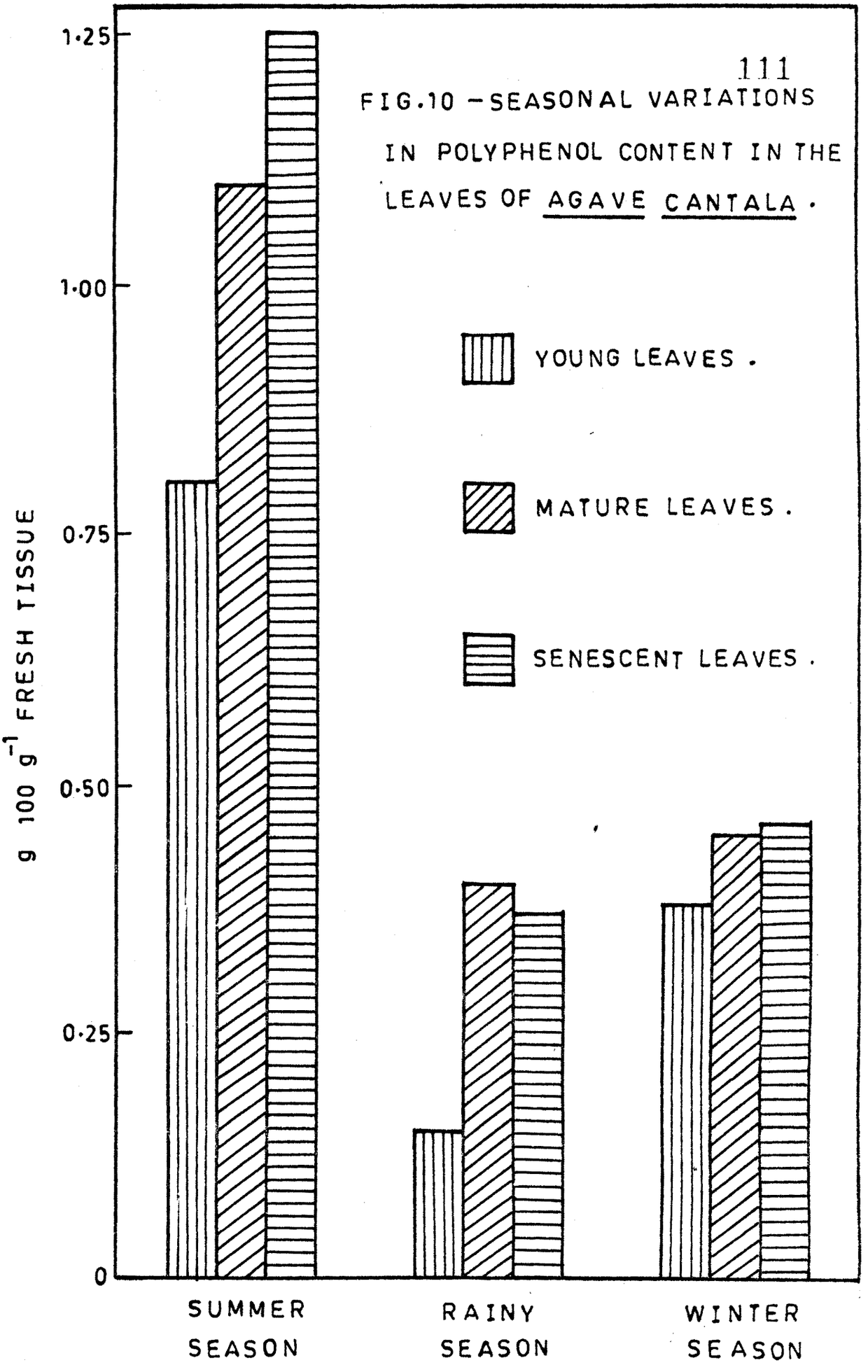


FIG.10 – SEASONAL VARIATIONS IN POLYPHENOL CONTENT IN THE LEAVES OF AGAVE CANTALA .

Polyphenols represent products of secondary metabolism in plants and these compounds have an aromatic ring in their structure. Although these compounds exhibit a wide array of structures basically all compounds except flavonoids arise from a common biosynthetic intermediate, phenylalanine or its close precursor shikimic acid. In the case of the flavonoids one aromatic ring and its C_3 side chain arises from phenylalanin whilst the other arises from acetyl-CoA via the polyketide pathway. Goodwin and Mercer (1983) have recognised following major classes of phenolics : (a) Simple phenols (C_6), (b) Phenolic acids ($C_6 - C_1$), (c) Phenyl acetic acids (C_6-C_2), (d) Hydroxycinnamic acids (C_6-C_3), (e) Coumarins and Isocoumarins (C_6-C_3), (f) Naphthoquinones (C_6-C_4), (g) Xanthones (C_6-C_1-C_6), (h) Stilbenes and Anthraquinones (C_6-C_2-C_6), (i) Ligans and Neoligans $(C_6\text{-}C_3)_2$, (j) Biflavonoids $(C_6\text{-}C_3\text{-}C_6)_2$, (k) Ligins $(C_6\text{-}C_3)_n$, (l) Melanins $(C_6)_n$, and (m) condensed tannins (flavolans) $(C_6\text{-}C_3\text{-}C_6)_n$.

Although the polyphenols represent products of secondary metabolism, it is now very well realised that some phenolic compounds like lignin have a definite structural role, similarly anthocyanins impart attractive colour to flowers. The p-coumaric acid ester is a necessary cofactor in the production of ethylene from methionine. Some phenolic compounds also play a role in growth processes. The work of Sharma et al. (1988) indicated possible involvement of phenolic compounds in stomatal movements. The phenols also play key role in disease resistance process

in plants. Because some phenolic compounds are economically very important e.g. tannins, considerable attention has been paid to the plants which contain large amount of these economically important polyphenols (e.g. Terminalia, Acacia, Cassia etc.). On the other hand other plant species which have low level of phenols, have been paid very little attention in this respect. This is particularly true for succulents. Our observations indicate that the Agave Cantala leaves are also very low in polyphenols, containing about 1% polyphenols. We have already seen that the phytochemical investigations of various Agave species have revealed that the Agave leaves contain number of important aromatic flavonoids as well as glycosides (Chapter I, Section 12 (viii). Our observations indicate that probably the summer season is the best season for extraction of the polyphenolic compounds if at all they are having some economic significance. Such seasonal variations in polyphenols have been evident in the observations of Abu-Mustafa, et al. (1970). These workers observed two maxima in the accumulation of flavonoids and coumarins, one in spring, after buds have opened and shoots start growing and the other in autumn before the inception of dormancy.

In the senescent leaves of Agave cantala a slight elevation in phenol content is seen during summer and winter seasons. These observations are in agreement with the findings of Margna and Margna (1974), Gokhale, et al. (1984), who noticed accumulation of flavonoids and total polyphenols

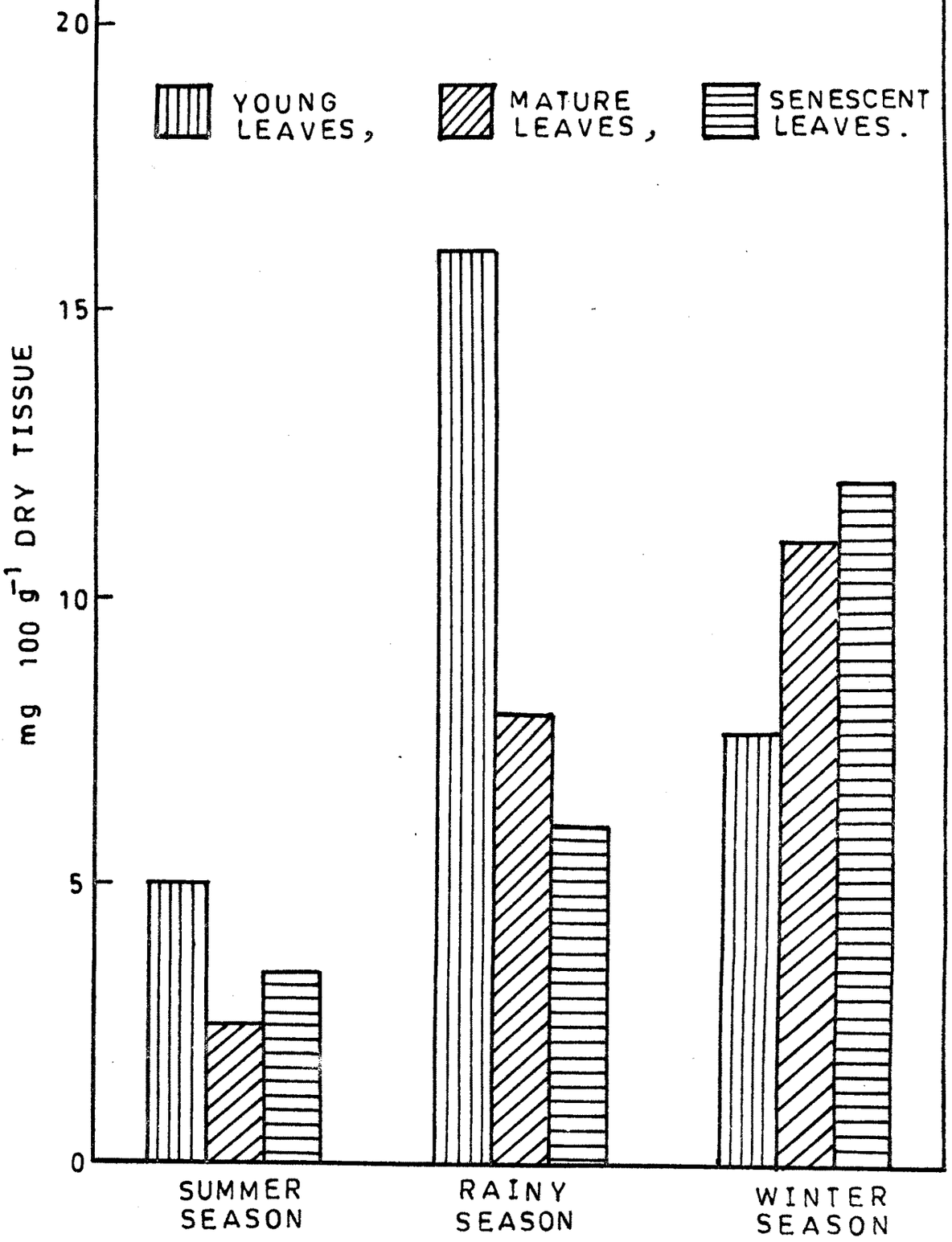
respectively during leaf senescence. The polyphenol biosynthesis is a quite complex process and many other reactions might be contributing to an over all increase in their level during leaf senescence. Thus an increase in phenolics in senescent leaves can be regarded as a general indication of stimulation of secondary metabolism during the terminal phase of leaf life.

(v) Proline

The level of free proline in young, mature and senescent leaves of Agave cantala during different seasons is recorded in Fig.11. The proline content in Agave leaves varies from 2.5 to 16 mg 100 g^{-1} dry tissue. The proline content seems to be more elevated in winter season, whereas in summer lower levels of free proline are seen in all the three categories of leaves.

Considerable attention has been paid to the accumulation of free proline in plants in last fifteen years. Proline belongs to glutamate family of amino acids and it has got heterocyclic structure. Both glutamate and arginine have been found to be potential precursors of proline biosynthesis. There is exhaustive literature available, which indicates massive accumulation of proline in various crop species during drought (Paleg and Aspinall, 1981). It is now noticed that besides drought proline accumulates in higher plants in response to other environmental stresses, such as salinity,

FIG.11 – SEASONAL VARIATIONS IN PROLINE CONTENTS IN THE LEAVES OF AGAVE CANTALA



mineral deficiency, high temperature, low temperature, diseases and air pollution. Several studies have indicated that proline might be playing an important role in the process of drought resistance. Many mechanisms have been proposed to explain this role. The proline accumulation in relation to degree of drought resistance in plants of different ecological habitats, such as semi-aquatic, floating, submerged and mesophytic, has been studied by Mukherjee *et al*. (1982). They assumed that these plants may have different degrees of drought resistance depending upon the ecological condition in which these plants are adapted and these plants may serve as good experimental material for the study of relationship between proline accumulation and drought resistance in plants. They employed mature plants of *Ipomea aquatica* (semi-aquatic), *Trapa natans* (floating), *Vallisneria spiralis* (sub-merged) and *Vigna catjang* (mesophytic) for their study. They observed differential proline accumulation during water stress in leaves of plants growing in different ecological habitats. The trend of proline accumulation in leaves subjected to water stress was as follows : maximal accumulation in *Vigna* (a mesophytic sp.) followed by Ipomea (a semi-aquatic sp.), *Trapa* (a floating sp.) and negligible in *Vallisneria* (a sub-merged sp). Thus it becomes evident from the above observations that proline accumulation is an adaptive character and may be related with the drought resistance property of the species concerned. Most studies of proline accumulation in response to water deficit have been concerned with changes in concen-

tration in the shoot or; more specifically the leaves. However, proline accumulates in all organs of the intact plant during water deficit, although accumulation is most rapid and extensive in the leaves.

It has been suggested that proline might be playing an osmoregulatory role under saline conditions (Paleg and Aspinall, 1981). Palfi et al. (1974) carried out extensive investigations regarding the effects of dehydration on proline accumulation. They observed that not all species accumulate proline under water deficit. These are : Beta vulgaris, Spinacia oleracea, Chenopodium album, Rumex scutatus, Cucurbita pepo, Cucumis sativus, Zea mays, Phaseolus vulgaris, Allium sativum, A. cepa and Lactuca sativa. Among the 60 plants studied majority of herbaceous mesophytic, cultivated plants belonging to families Solanaceae, Leguminosae, Cruciferae, Umbelliferae, Compositae and Gramineae accumulate proline under water deficit. According to them proline increases considerably the amount of strongly bound water in leaves. In addition it is highly water soluble compared with other protein forming amino acids, it is the most stable amino acid as regards resisting 'oxidative' acid hydrolysis and it stores up reducing energy during its formation. According to Savitskaya (1976) proline may be a single source and precursor of hydroxyproline in the structural protein of cell walls, participating in the cell extension process and may serve as energy material for respiration. It also stimulates

absorption of oxygen by plant tissues. Schobert and Tschesche (1978) showed that the proline affects the solubility of various proteins and protects bovine albumin from denaturation by $(NH_4)_2SO_4$ or ethanol. It was suggested that this property of proline may be due to an interaction between proline molecule and hydrophobic surface residues on the proteins, which increases the total hydrophilic area of the associated molecules and hence their stability. If such an interaction occurs in the cytoplasm at the concentrations of protein and proline which occur in the cell, it will be obviously significant in water deficit situations. Thus proline can serve as protector of various enzymes during the period of water stress. Hanson et al. (1979) however, indicated that the build-up of free proline in water-stressed leaf tissue to be a deleterious consequence of internal water deficit, as supported by an apparent association between proline concentration in the tissue and the severity of post-stress "leaf-firing." Their hypothesis contradicted the claim that high proline content in the leaf during stress confers a survival value on the leaf (Singh et al., 1973), or that a high accumulator recovers more rapidly upon relief of stress (Blum and Ebercon, 1976).

The work on proline accumulation in last two decades has been mainly carried out on conventional crop species and other plant species have been paid very little attention in this respect. Atre and Shitole (1986) have observed increase

in proline content in blue-green algae (Lyngbya birgei) in response to salt stress. Obraztsava and Nikifornova (1967) found that drought resistant trees were distinguished by a higher content of free amino acids than the non-resistant ones. Kashid (unpublished work from our laboratory) noticed that the tree species like Eucalyptus, Acacia and Anona differ greatly in their capacity to accumulate free proline and the species like Eucalyptus does not accumulate proline even under the conditions of severe stress in contrast to Anona or Acacia. The absolute free proline content in the leaves of various plant species shows a great variation and concentrations as high as 10% of the total leaf dry weight have been recorded (Stewart and Lee, 1974). In the light of above observations when we study the proline accumulation in succulents, we can notice that very little attention has been paid to this amino acid in metabolism of succulents. Karadge (1981) noticed that the leaves of Portulaca oleracea contain 140 mg of proline 100 g^{-1} dry tissue and he further observed that there is very little change in proline in response to salinity. Cavalieri and Huang (1978) studied proline accumulation in salt marsh halophytes exposed to salinity and found that C_4 grasses had higher threshold values for proline accumulation than C_3 types. They have investigated proline accumulation in eight species of marsh halophytes exposed to salinity. They found that plants did not accumulate proline until a threshold salinity was reached.

Three general patterns were apparent. The C_4 grasses Spartina alterniflora, S. patens and Distichlis spicata at sea water salinity level accumulate proline to 27.4 μ moles g^{-1} fresh weight. Limonium caroliniamum and Juncus roemerianus accumulate proline at salinities greater than 1% with accumulation upto 63.6 μ moles g^{-1} fresh weight at higher salinities. The succulents, Salicornia bigwii, S. virginica and Borrichia frutenscens did not accumulate proline until extremely high salinities were applied. These observations indicate that proline may not be playing any major role in metabolism of succulents. This view is supported by our findings, because very little variations in proline content are evident in leaves of Agave cantala and in summer which is otherwise an adverse environmental factor for most plant species, the proline contents recorded in Agave leaves are very low. It is generally admitted that the extent of proline accumulation reflects the degree of internal water deficit (Tan and Halloran, 1982). Our observations indicate that the proline levels in A. cantala leaves are very low and this may be due to the fact that there is no internal water deficit as such in Agave leaves (Fig. 6) due to their succulent nature.

Very few attempts have been made to trace the fate of proline during leaf senescence. Kao (1981) observed that proline accumulates during senescence of detached leaves of 10 rice varieties. This experiment was performed in the detached leaf segments, therefore, the possibility of proline

translocation is overlooked. Dale and Felippe (1977) reported that during the senescence of barley leaf, the amino acids liberated by proteolysis are transported out to the stem, probably as emides, and thus made available to other organs. Our observations with senescent leaves of A. cantala do not exhibit any definite trend of proline accumulation during leaf senescence and there are possibilities of seasonal variations in this pattern. In summer and winter months there is slight increase in proline content in the senescent leaves, whereas in rainy season the proline content is slightly lower in the senescent leaves relative to mature leaves (Fig. 11). However, these changes are very minor one and they do not signify any major role for proline in the alterations, which occur in nitrogen metabolism during the phase of leaf senescence.

(vi) Pigments

(a) Chlorophylls

The seasonal variations in chlorophyll contents in the young, mature and senescent leaves of Agave cantala are shown in Fig. 12 and 13. From these figures it is observed that chlorophyll a and chlorophyll b are present in maximum amount in mature leaves during all the three seasons than young as well as senescent leaves. The young leaves show less amount of chlorophylls than the senescent leaves during each season. The total chlorophylls (Fig. 13) in young leaves range from 5 to 14 mg 100 g^{-1}, in mature leaves from 23 to 57 mg 100 g^{-1} while in senescent leaves from 17 to 44 mg 100 g^{-1}

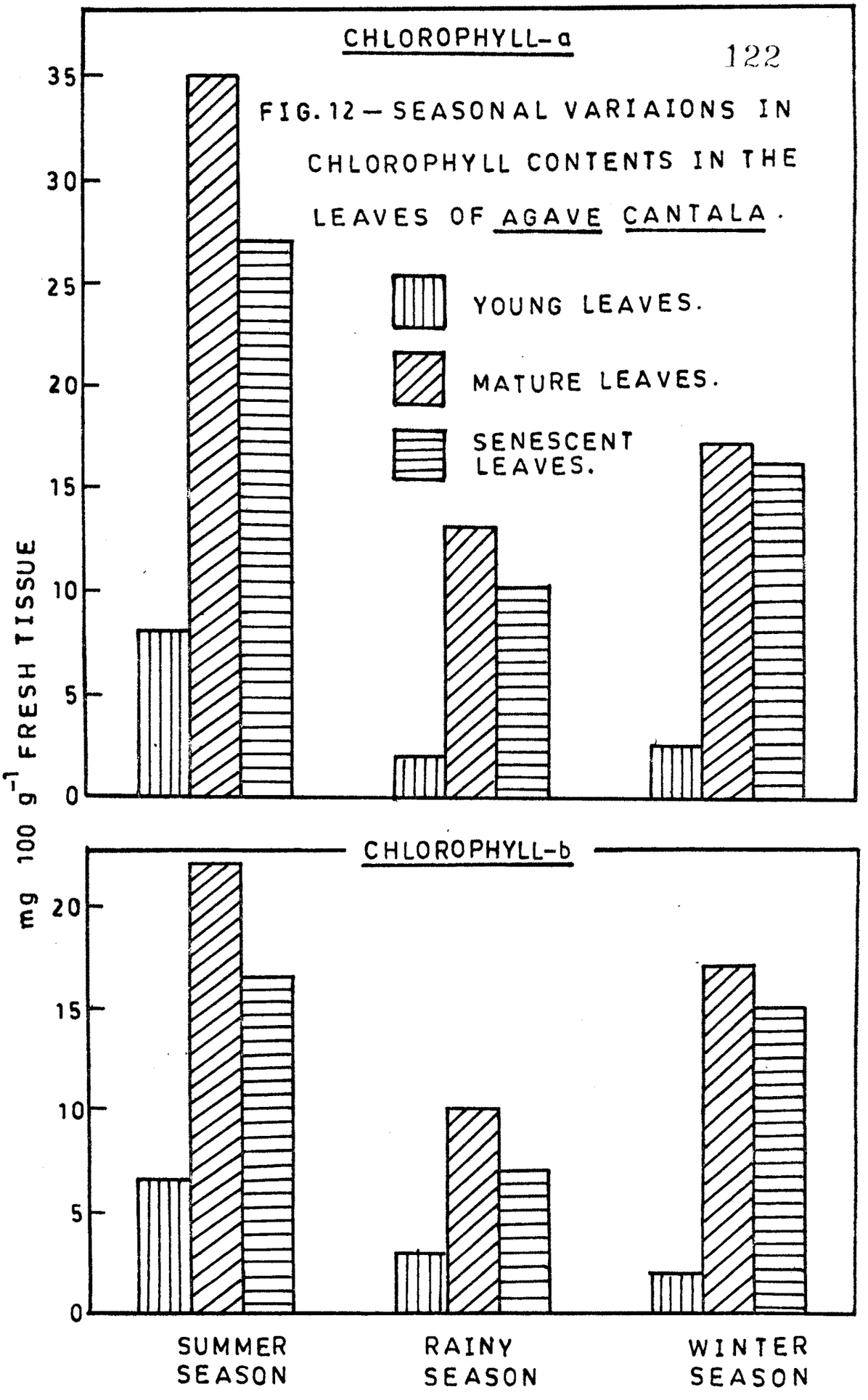
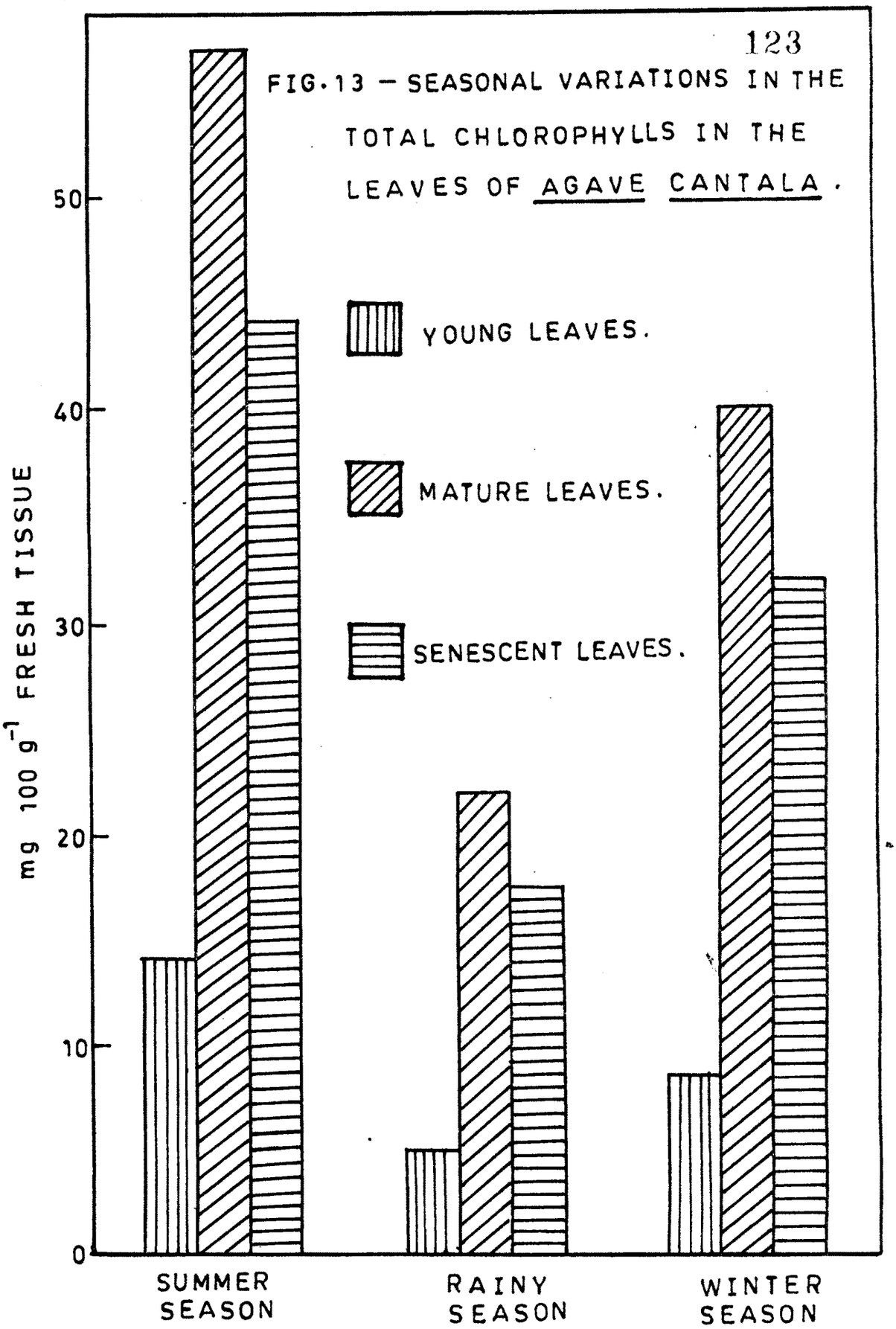


FIG. 12 — SEASONAL VARIAIONS IN CHLOROPHYLL CONTENTS IN THE LEAVES OF AGAVE CANTALA.

FIG.13 – SEASONAL VARIATIONS IN THE TOTAL CHLOROPHYLLS IN THE LEAVES OF AGAVE CANTALA.



fresh tissue. From the figure it also appears that the chlorophyll contents are higher in summer season than either rainy or winter season. Minimum amount of chlorophyll is recorded in the rainy season in all the three leaf categories.

All our food, most of our fuel and many of our fibres are derived directly or indirectly from photosynthesis. As the fossils fuel reserves, which are products of past photosynthesis, are depleted, current photosynthesis may become of even greater important as a source of fuels and organic compounds. The very existence of life on this planet depends mainly on the process of photosynthesis means literally the assembly of a product from raw materials using light. The "light reactions" of photosynthesis encompass light harvesting, i.e. the primary photochemical act, electron transport and photophosphorylation. They occur in or on the thylakoid membranes of the chloroplast. The light reactions of photosynthesis are located in the thylakoids and stromal lamellae. The thylakoids are flattened disc-like vesicles which usually stack to form grana and are interconnected by the stromal lamellae. A single thylakoid disc of about 0.5 μm diameter may contain 10^5 chlorophyll and associated pigment molecules. These pigments include chlorophyll *a* which is ubiquitous in all photosynthetic organisms capable of splitting water in photosynthesis. Chlorophylls are basically the magnesium chelates of closed tetrapyrrole rings derived from phytoporphyrin. The chlorophyll *a* assumes a number of forms

within the thylakoid membrane. These are expressed as changes in absorption/flourescence spectra that presumably depend on the protein molecules with which the chlorophyll molecules are associated and their "solvent" environment (Seely, 1977, Thornber and Alberte, 1977). The chlorophylls absorb in the blue (450 nm) and red (650 to 700 nm) regions of the visible spectrum. Chlorophyll b is almost as wide spread as chlorophyll a but is absent from all the algae except the Chlorophyceae. Two types of chlorophyll c namely c1 and c2, are known. These occur together in the Phaeophyceae, Chrysophyceae and Bacillariophyceae but only chlorophyll c2 is present in the Cryptophyceae. Thus in higher plants like Agave, chlorophyll a is a major pigment involved in harvesting solar energy while chlorophyll b plays rather secondary role. The pigment - protein complexes are organised in to two photo-systems I and II (PSI and PS II) and a light harvesting complex (LHCP), which perform a central role in light reaction. The strength of this pigment-protein-lipid complex in chloro-plast is influenced by number of internal as well as environ-mental factors. The stability of chlorophylls is considered by some workers as an important factor in drought resistance.

The chlorophyll content in the leaves is determined by number of factors. In view of Sestak (1985) the content of chlorophyll in a leaf is the result of balance of a steady chlorophyll synthesis and chlorophyll degradation. The work of Henningsen and Boynton (1974) has indicated that the

chlorophyll accumulation is controlled not only by the rates of processes of chlorophyll biosynthesis or degradation, but also by the formation of chloroplast ultrastructure. The processes such as shading also influence the chlorophyll content due to creation of irradiance gradient. Besides these internal factors the environmental factors such as drought, salinity, mineral deficiency, air pollution also cause a decline in chlorophyll content (Virgin, 1965; Strogonov et al., 1970; Natr, 1975; Tanaka and Sugahara, 1980).

The chlorophyll content in few succulent plants has been investigated. Bartakke (1977) found that the total chlorophylls in leaves of Aloe barbadensis range from 59.87 to 86.77 mg 100 g^{-1} fresh weight, while the leaves of Kalanchoe pinnatum show total chlorophylls to be 45.46 mg 100 g^{-1} fresh weight. In the leaves of Portulaca oleracea total chlorophylls are found to be 96.97 mg 100 g^{-1} fresh weight (Karadge, 1981). Our observations indicate that chlorophyll content in mature leaves of Agave cantala range from 23.19 to 57.94 mg 100 g^{-1} fresh tissue. Our observations further indicate that there are distinct seasonal variations in the total chlorophylls and the leaves harvested in summer show the highest chlorophylls. While in rainy season the chlorophyll content is lowest. This might be related to the light intensity because in rainy season the days are clowdy and the light intensity is low (Table No. 1), while in summer months the light intensity is very high and is probably optimum for photo-

synthetic light reaction. Such seasonal variations in chlorophyll content have been recorded for leaves of Picea sitchensis by Lewandowska and Jarvis, 1977).

The chlorophyll a/b ratios in Agave cantala leaves range from 0.64 to 1.63. It is further interesting to note that these ratios are very low in young, mature and senescent leaves of A. cantala during the winter months and they are high during summer months. In view of Sestak (1966) the photosynthetic rate of the plant is mainly related to chlorophyll a content rather than total chlorophylls. Holden (1973) studied the relative concentrations of chlorophyll a and chlorophyll b in C_3 and C_4 plants. According to him chlorophyll a/b ratio ranges from 3.1 to 5.6 for C_4 dicotyledonous plants, while it ranges from 2.5 to 3.7 for C_3 dicotyledons. He further observed that the average value for chlorophyll b content of C_3 and C_4 grasses was similar on fresh weight basis, whereas, chlorophyll a content was about 30% more in C_4 grasses, however he has not assessed the chlorophyll contents in CAM plants. Bartakke (1977) noticed that in typical CAM succulents like Bryophyllum pinnatum and Aloe barbadensis the chlorophyll a/b ratios are 0.96 and 1.59 respectively. In another succulent species Portulaca oleracea, which is classified as C_4-facultative CAM, chlorophyll a/b ratio was found to be 2.10 (Karadge, 1981). Our observations indicate that in A.cantala the values are similar to Bryophyllum and Aloe. Thus it is apart that the succulents are rather insufficient in

harvesting solar energy as they contain relatively higher amount of chlorophyll b as compared to typical C_3 or C_4 plants. The higher chlorophyll a/b ratios recorded in all the three leaf categories of Agave cantala during summer is rather interesting and it may possibly indicate higher photosynthetic efficiency of A. cantala leaves during summer months. Such seasonal variations in chlorophyll a/b ratios have been recorded by Lewandowska and Jarvis (1977) in the leaves of Picea sitchensis.

It is evident from Fig. 12 and 13 that chlorophyll content shows an increase along with leaf age and it reaches a peak at maturity. The leaf senescence is accompanied by a decrease in chlorophyll content although the decrease is quite insignificant. These observations are in agreement with the comment of Sestak (1985), "the general trend of chlorophyll (a+b) accumulation during ontogeny of each leaf is an increase to a maximum followed by a decrease". Sestak further admits that the rate of increase is usually more rapid than the rate of decrease, especially in leaves formed in the later phase of plant ontogeny, the whole life span of which is larger. The degradation of chlorophyll during leaf senescence has been attributed to the alteration of chloroplast structure as well as increase in activity of hydrolytic enzymes like protease and chlorophyllase. Our observations indicate that the lowering of chlorophylls during leaf senescence in A. cantala is rather a slow process because there is only a

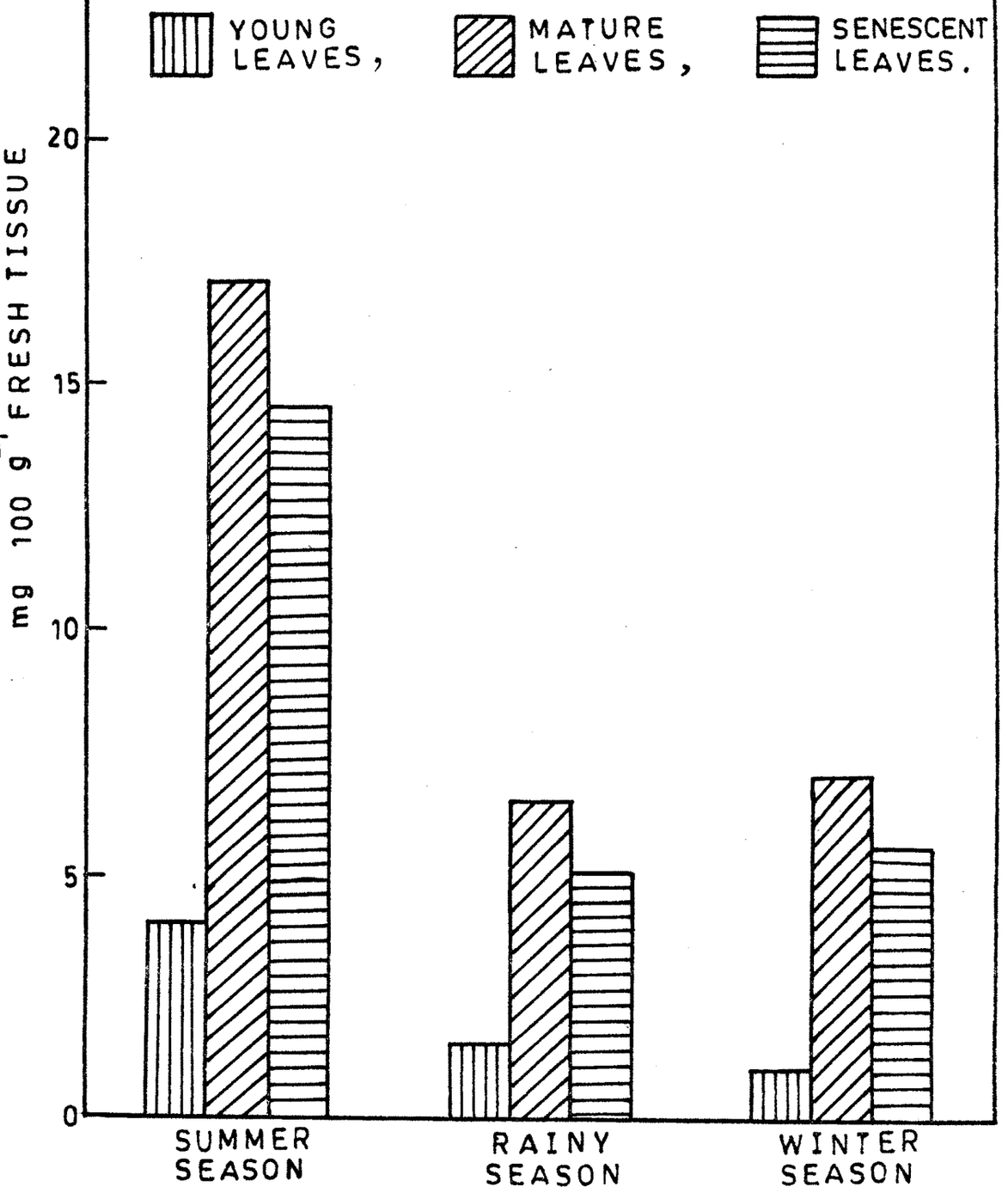
slight decrease of chlorophylls in senescent leaves in comparison to mature leaves. This may be related to longer life span of Agave leaves than other herbaceous species.

(b) Carotenoids

The level of carotenoids in young, mature and senescent leaves of Agave cantala during different seasons is recorded in Fig. 14. It is evident from the figure that the mature leaves contain higher amount of carotenoids during all the three seasons and in summer accumulation of these pigments is more prominent. Carotenoid level is lower in senescent leaves during all the three seasons.

Carotenoids are generally considered as accessory pigments because of their secondary role in the process of photosynthesis. Although their role is rather secondary in comparison to a major role played by chlorophylls in the process of light reaction, many workers have attributed a protective role to these pigments offering photoprotection to chlorophylls against bleaching (Sestak, 1985). They also play a role as antenna pigments, accepting radient energy and transmitting it with some losses to chlorophyll a molecules. Direct participation of xanthophylls in the mechanism of photosynthesis (the violaxanthin cycle participating in oxygen transport, the function of β-carotene in the C-550 absorbance change) has been distributed. The role of carotenoids in the process of photosynthesis has been supported

FIG.14 – SEASONAL VARIATIONS IN CAROTENOID CONTENTS IN THE LEAVES OF AGAVE CANTALA.



by recent findings of the presence of carotenoids in all native chlorophyll complexes in thylakoids, and by their abundance in the chloroplast envelope. Carotenoids may also be performing some other functions because the carotenoids have been detected (i) in non-photosynthetic plant species, e.g. fungi, (ii) in non-photosynthetic tissues or organelles in photosynthetic species, e.g. the petals, anthers, pollen of some flowers, seeds and eyespot of Euglena species. The higher plants have the carotenes, α- and β-carotene and the xanthophylls, lutein, violaxanthin and neoxanthin. During the growth of leaf area and formation of chloroplasts with fully developed ultrastructure, carotenoids are synthesized usually more slowly than chlorophylls and thus the ratio of chlorophylls/carotenoids increases (Sestak, 1985). Because of their secondary role in photosynthetic process, very little attention has been paid to the fate of carotenoids in higher plants. This is particularly true for succulents (Kluge and Ting, 1978). Our observations indicate that the carotenoid content is relatively low as compared to chlorophylls especially in the mature and senescent leaves of Agave cantala. Similarly there is a seasonal variation in carotenoid content and like chlorophylls, these pigments also reach their peak in summer season. Seasonal variations in carotenoid content have been recorded in the leaves of Picea sitchensis by Lewandowska and Jarvis (1977).

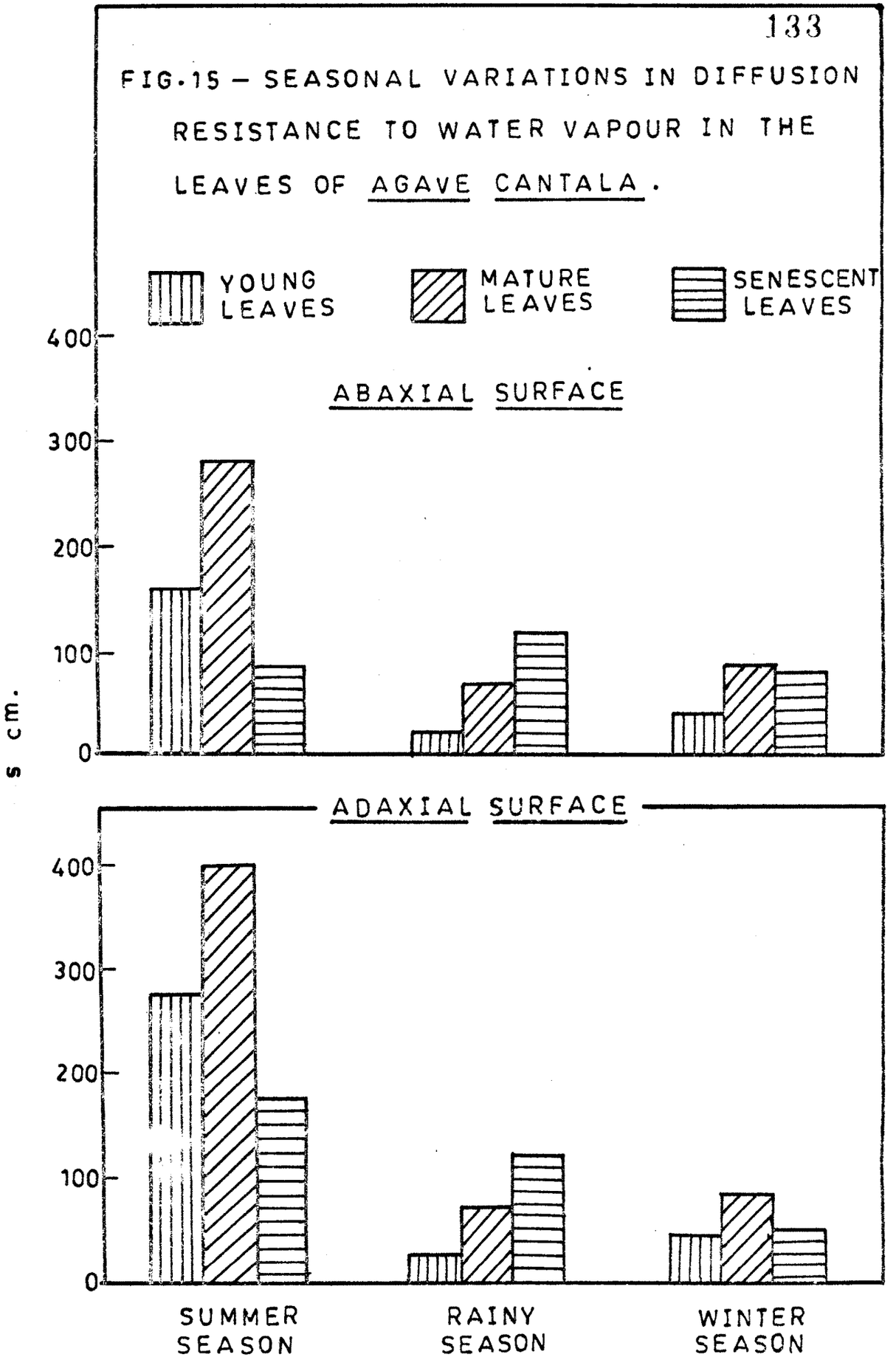
It is evident from our observations that the senescent

leaves of Agave cantala contain less amount of carotenoids than the mature leaves and this trend is seen in all the three seasons. According to Thimann (1980) the fate of carotenoids in leaf senescence evidently needs considerable further study, though it may not be a matter of high priority. According to Sestak (1985) the general trend of content of the sum of carotenoids during leaf ontogeny is similar to that of chlorophylls. Nevertheless, the time course of carotenoid accumulation may be different from that of chlorophyll. Sestak (1985) further admits that the character of ontogenetic changes in carotenoid content depends not only on plant species, but also on plant cultivar and environmental conditions. The work of Matile and Martinoia (1982) has suggested involvement of peroxidase in the catabolism of carotenoids during leaf senescence. Our observations with A. cantala indicate that carotenoid accumulation follows a trend more or less similar to chlorophylls.

3. Study Of Stomatal Behaviour During Different Seasons :

The changes in diffusion resistance to water vapour in the abaxial and adaxial leaf surfaces of the young, mature and senescent leaves of Agave cantala are recorded in Fig.15. It is evident from the figure that the values of diffusion resistance to water vapour are very high in all the three leaf categories during summer months as compared to winter and rainy months. The diffusion resistance to water vapour in young and mature leaves is lower in rainy season as

FIG.15 – SEASONAL VARIATIONS IN DIFFUSION RESISTANCE TO WATER VAPOUR IN THE LEAVES OF AGAVE CANTALA.

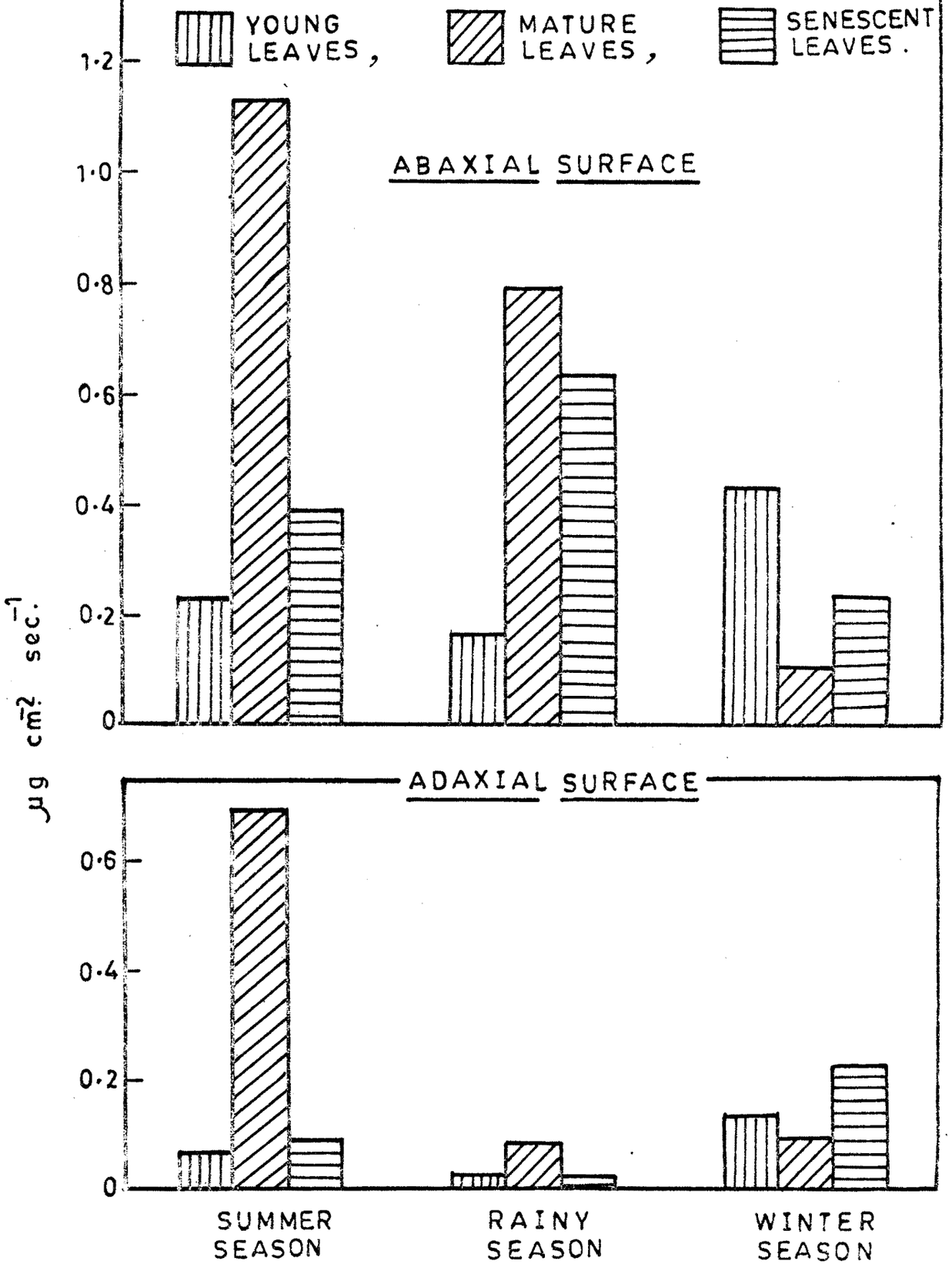


compared to winter season. The senescent leaves show lower values of diffusion resistance to water vapour in winter than the values exhibited by these leaves in rainy season. The mature leaves of A. cantala show higher values of diffusion resistance as compared to the young leaves in all the three seasons indicating greater closure of stomata during day time. The diffusion resistance to water vapour in senescent leaves does not show any definite trend during the course of leaf senescence in different seasons.

The transpiration rates from the abaxial and adaxial leaf surfaces of young, mature and senescent leaves of A. cantala during different seasons at 12.00 noon are recorded in Fig.16. It is evident from the figure that in young and mature leaves the transpiration rates are little higher during the period of rainy season, intermediate during winter season and very low during the summer season. The transpiration rate in the senescent leaves does not show any definite trend which can be correlated with season. It is further noticed that in all the three leaf categories (i.e., young, mature and senescent) of Agave cantala leaves, the higher transpiration rate is shown by abaxial leaf surface than adaxial leaf surface in all the three seasons.

The water relations and the metabolism of land plants depend on the diffusion of water vapour and of gases through the stomatal pores, which occur on their aerial parts. The most important feature of the land plants above the evolu-

FIG·16 — SEASONAL VARIATIONS IN TRANSPIRATION RATE IN THE LEAVES OF AGAVE CANTALA.



tionary level of Anthoceros is that the true stomatal pores can change from the wide open to an apparently completely closed condition. These stomatal pores are situated between two specialised epidermal cells called the guard cells which bring about opening and closing movements of the stomata by changing their dimensions and shapes. The succulent plants have fewer stomata distributed over exposed surfaces than most mesophytic, non-succulent plants. Virduin (1949) reported an average stomatal density of about 10,000 cm^{-2} in mesophytes. On the other hand, Ting et al. (1967a) observed that the number of stomata cm^{-2} on abaxial leaf surfaces of Agave americana and A. deserti was 2,100 and 2,000 respectively, whereas the number of stomata cm^{-2} on adaxial leaf surfaces of A. americana and A. deserti was 2,100 and 1,800 respectively. Génerally the dimensions of the stomata of succulent plants do not differ from mesic plants. According to Kluge and Ting (1978) the stomata of succulent plants are slightly or not at all sunken, but are associated with a deep substomatal cavity because of the presence of a multilayered hypodermis below the epidermis.

There are very few investigations about the processes of opening and closing of stomata. However, some recent investigations have shown that the mechanisms involved in opening and closing are of particular interest. Some authors have suggested that opening and closing do not depend upon a simple reversal of the same reaction and there is a process

involved in the opening which is not necessary for its maintenance. Raschke (1975 a, 1976) has reviewed the mechanism of stomatal opening. According to him the physical driving force for stomatal opening is increased turgor of the guard cells relative to adjacent cells, which brings about expansion and subsequent opehing. The energy-dependent accumulation of solutes is responsible for creating osmoticum for water uptake causing there by hydrostatic pressure or turgor. It is also known that CO_2 can regulate stomatal aperture. Increasing CO_2 results in a decreased aperture. Abscissic acid is necessary for CO_2 sensitivity (Raschke, 1975b). Light may also act as a signal for stomatal opening but according to Raschke (1975 a) light reduces CO_2 and it is the CO_2 and not light which controls stomatal opening, light does not seem necessary for stomatal opening. The stomatal movements are greatly affected by the environment. However, the aperture is not always determined by the external factors prevailing at the time of observation. The plants retain a considerable measure of control through their endogenous rhythms. Besides light and CO_2, relative humidity and temperature also influence stomatal behaviour (Meidner and Mansfield, 1968).

Nishida (1963) carried out the most extensive investigations of stomatal behaviour in members of the Cactaceae, Crassulaceae and Liliaceae. Some Crassulacean members growing under normal conditions, such as Bryophyllum calycinum, Kalanchoe blossfeldiana and Cotyledon peacockii showed stomatal

opening at night and closure during the day, while others like Sedum verticillatum showed the usual daytime opening. He further reported that night opening began rather slowly after sunset and throughout the night there was a gradual progression to a wider aperture. On the other hand, after sunrise the stomata initially opened further, but after two or three hours they closed and remained closed for the rest of the day. Many Crassulacean members are known to be capable of a larger scale fixation of CO_2 at night, resulting in accumulation of organic acid in the cell vacuoles. Nishida (1963) obtained evidence of some correlation between organic acid content and the opening of the stomata. In Sedum verticillatum although stomata did not open at night, he observed little diurnal fluctuation in acidity in that species, while in case of Bryophyllum daigremontianum he found a large accumulation of acids at night accompanied with stomatal opening. Levitt (1967) has explained the stomatal behaviour at night in succulents in the following manner. Initially, CC_2 fixation in the dark leads to stomatal closure by decreasing cell pH, however, acids accumulate in the vacuoles to such an extent which bring about an increase in osmotic pressure, causing there by opening. Meidner and Mansfield (1968) indicated that there was no evidence for the mechanism of night opening of CAM plant stomata which was quite different from day opening of other plant stomata. Day opening of stomata is a response to reduced CO_2 because of photosynthesis, while in succulent

plants the reduced CO_2 comes about by dark CO_2 fixation with nocturnal opening accompanying the Crassulacean Acid Metabolism. In view of Kluge and Ting (1978) the night stomatal opening of CAM plants is initiated by reduced partial pressures of CO_2, because of dark CO_2 fixation. Potassium, Malate and probably other organic anions accumulate and reduce osmotic potentials; which results in water uptake, increased turgor and stomatal opening.

The rate of water vapour loss from a leaf is not different from the rate of evaporation from an open water surface of the same size as the leaf, although the combined pore area of the open stomata amounts to only 0.5 to 2.0 % of the total leaf area when both epidermis are taken into consideration. This 'efficiency' of stomatal pores for gaseous diffusion depends on their size and on their distribution in the epidermis. It becomes necessary to examine the nature of gaseous diffusion through small pores, as the loss of water vapour from a leaf, as well as the entry of CO_2 into a leaf, occurs by diffusion. The rate of diffusion is proportional to the difference in vapour density between the leaf air space system and the atmosphere; and inversely proportional to the resistance of the diffusion path. The diffusion resistance experienced by water vapour taking exit from the leaf is largely proportional to stomatal aperture and represents the variable resistance in the non-metabolic part of the transpiration path way. Its value is a function of the light flux

density, leaf temperature, CO_2 concentration, leaf water potential and leaf-air humidity deficit. The rate of transpiration of a leaf under given metrological conditions depends upon its total diffusion resistance to water vapour which consists of two separate terms namely the stomatal and cuticular resistance. The leaf resistance varies enormously from minimum value when the stomata are fully open to a maximum value which may be large or nearly infinite when the stomata are closed. The leaf resistance is considered as a dynamic quantity which may change continuously with environmental conditions for a given leaf. The many quantitative studies of the stomatal resistance of succulent plants conducted by Ting et al. (1971) have revealed that because of few stomata and long apparent substomatal diffusion path lengths and minimum stomatal resistances tend to be larger than for mesophytic plants. For example, the work of Ting et al. (1967 b) with Kalanchoe blossfeldiana, under a variety of temperature conditions, minimum resistance estimates were observed ranging from 2 to 10 s cm^{-1}, while the studies of Szarek et al. (1973) have shown resistance minima rarely below 2 s cm^{-1}, and on the average 5 to 10 s cm^{-1}. In the opinion of Neales et al. (1968) in all cases resistance can exceed 100 s cm^{-1} and may be as much as several hundred. In case of Agave cantala we could not measure the diffusion resistance during night hours. Hence it is not possible to make estimations about the minimal diffusion resistance

(which indicates highest rates of gas exchange as well as transpiration) in this species. However, the highest value of diffusion resistance recorded at 12.00 noon is 406.5 s cm^{-1} during summer months. These values are quite high as compared to maximum values of stomatal resistance recorded for Kalanchoe daigremontiana and K. blossfeldiana as 140 s cm^{-1} and 75 s cm^{-1} respectively (Ting et al., 1967 b). In present studies the resistance values in rainy season are comparatively very low which are still higher than the minimal values of resistance recorded for the succulent species. This indicates that Agave stomata are partially open during rainy season during daytime and they are very slightly open during daytime in winter and summer months. The transpiration readings also support this view.

Although majority of studies indicate that there is predominent stomatal opening, gas exchange and transpiration process during the dark period in most of the succulents; there are few reports which indicate that stomata remain open during daytime in few cases under peculiar environmental conditions. Shreve (1916) found that the stomata of cacti were more open at night than in the day, thus accounting for the greater night transpiration in cacti despite a much greater evaporative demand during the day. James (1958) recorded stomatal opening at night and closing during the day for many succulents like cacti, but Opuntia growing in Britain showed the normal behaviour of opening during the day and closing at

night. Ting et al. (1967b) have shown that under certain temperature regimes (e.g., 26°C during the day, 21°C at night) the stomata of Kalanchoe blossfeldiana were much more widely open during the day than at night. There was some opening during the night but this did not approach the level achieved during the day, and the pattern of stomatal behaviour resembled that of a mesophytic except that night opening began earlier. In case of Kalanchoe blossfeldiana Queiroz (1974) also observed that stomata were open during day and closed at night. Osmond (unpublished) studied CAM in Opuntia inermis at various sites in Eastern Australia during different months. He observed typical CAM patterns of CO_2 exchange and stomatal resistance in this species in natural stands and these patterns undergo clear seasonal modifications. During March (Australian fall), substantial CO_2 was fixed in light and CO_2 fixation was highest at the beginning of the night. During November (Australian summer), stomatal resistance remained high throughout the day and during the first half of the night while only later in the night did stomata open and net dark CO_2 fixation occur. The amounts of malic acid accumulated were correlated with the intensity of dark CO_2 fixation during the seasons. Regarding Agave deserti Hartsock and Nobel made very interesting observation in 1976. These workers observed that this CAM plant can be converted to predominently daytime stomatal opening and C_3 photosynthesis by increased watering. Our observations with Agave cantala indicate that

since the leaves show some transpiration during daytime at 12.00 noon, the stomata are at least partially open during daytime in this species. In view of Kluge and Tinge (1978) the afternoon opening could be the result of a reduced partial pressure of CO_2 after organic acid depletion and photosynthetic reduction of endogenously produced CO_2 in succulents. According to Noble and Hartsock (1979) the temperature seems to exert a direct influence on stomatal opening for Agave deserti independent of water vapour concentration drops and CO_2 levels, the effect being greater when the plant is in the CAM mode than in the C_3 mode.

The ecological conditions have been found to be an important factor in determining the stomatal behaviour, gas exchange and transpiration rate in succulents, and seasonal variations have been also reported (Kluge and Ting, 1978). It is difficult to comment on the seasonal variations in stomatal behaviour of Agave cantala based on the present data because due to experimental limitations only day time records were made. The transpiration rates recorded during daytime (i.e. at 12.00 noon) in A. cantala leaves range from 0.02 to 0.430 $\mu g\ cm^{-2}\ sec^{-1}$. These values are very low when compared with the average daily transpiration rates (including night hours) of succulents which are in the range of 16 to 83 $\mu g\ cm^{-2} sec^{-1}$ (Kluge and Ting, 1978). Further the values appear to be far lower than the transpiration rate recorded for other Agave species namely A. deserti by Nobel and Jordan

(1983) which is 97 μg $cm^{-2}sec^{-1}$. Thus the transpiration rates recorded in the present investigation do not allow us to make any conclusion regarding the overall transpiration and stomatal behaviour during different seasons. At the same time it is evident from our observations that the daytime transpiration is greatly reduced during summer months. Thus indicating a favourable situation for total operation of CAM.

4. Crassulacean Acid Metabolism Studies :

(i) Study of diurnal fluctuations in carbohydrates during different seasons :

The changes in level of various carbohydrate fractions during different hours of day in leaves of Agave cantala in different seasons are recorded in Fig. 17, 18 and 19. It is evident from the figure (Fig. 18) that there is continuous increase in the level of reducing sugars along with the progress of the day in rainy season and winter season while during night hours (12.00 night) there is decline in this level. In summer season the reducing sugar content is very slightly increased in the period of 12.00 noon to 6.00 p.m., but there is a sharp decrease in the level of reducing sugars. The non-reducing sugars also follow a similar trend during summer season (Fig. 18). However, in rainy season there is continuous decrease in the level of non-reducing sugars along with the progress of the day. The pattern of non-reducing sugar accumulation in winter season is not uniform although

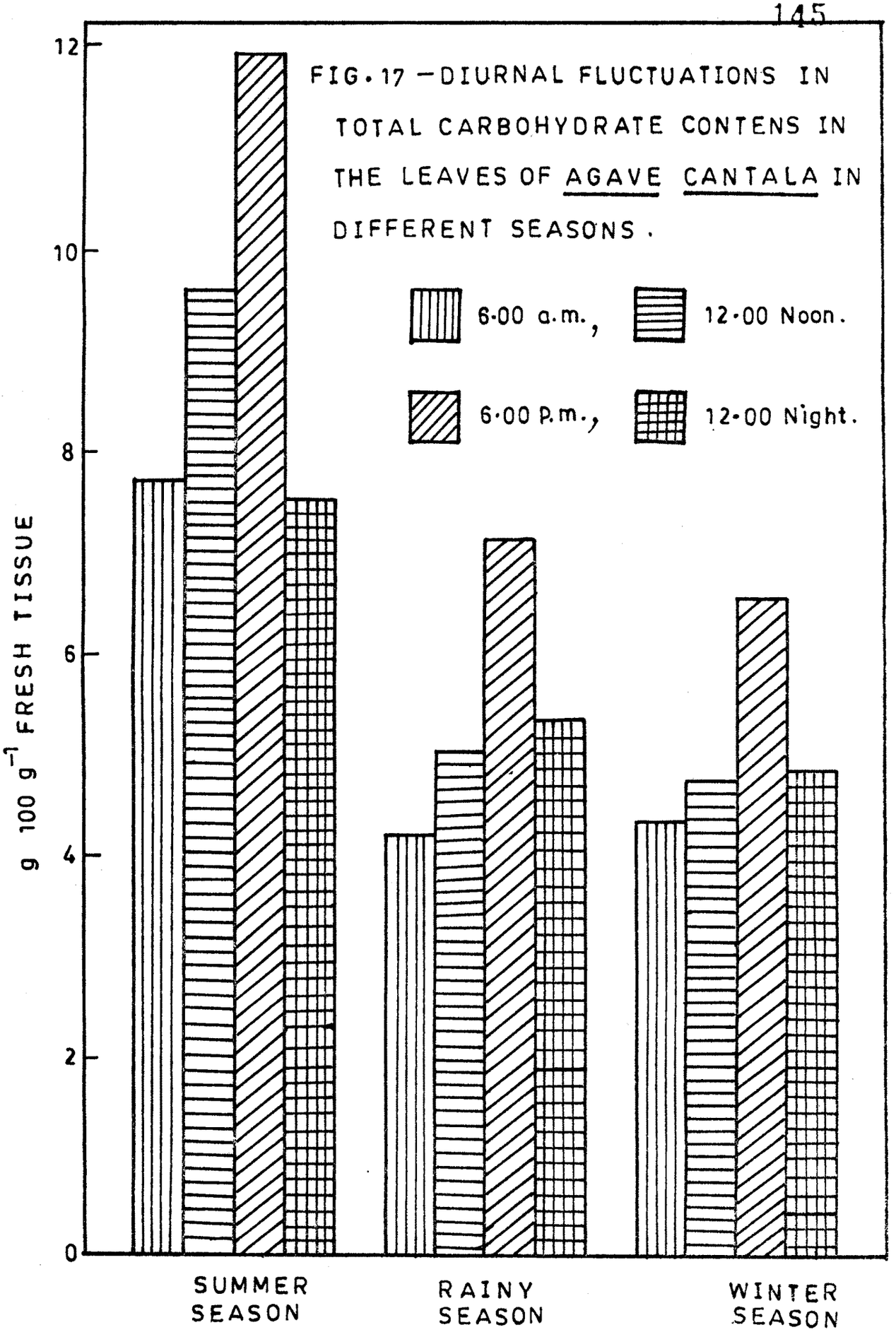


FIG. 17 — DIURNAL FLUCTUATIONS IN TOTAL CARBOHYDRATE CONTENS IN THE LEAVES OF AGAVE CANTALA IN DIFFERENT SEASONS.
6·00 a.m.,
12·00 Noon.
6·00 P.m.,
12·00 Night.
g 100 g⁻¹ FRESH TISSUE
0
2
4
6
8
10
12
SUMMER SEASON
RAINY SEASON
WINTER SEASON

FIG. 18 — DIURNAL FLUCTUATIONS IN REDUCING AND NON-REDUCING SUGARS IN THE LEAVES OF AGAVE CANTALA IN DIFFERENT SEASONS.

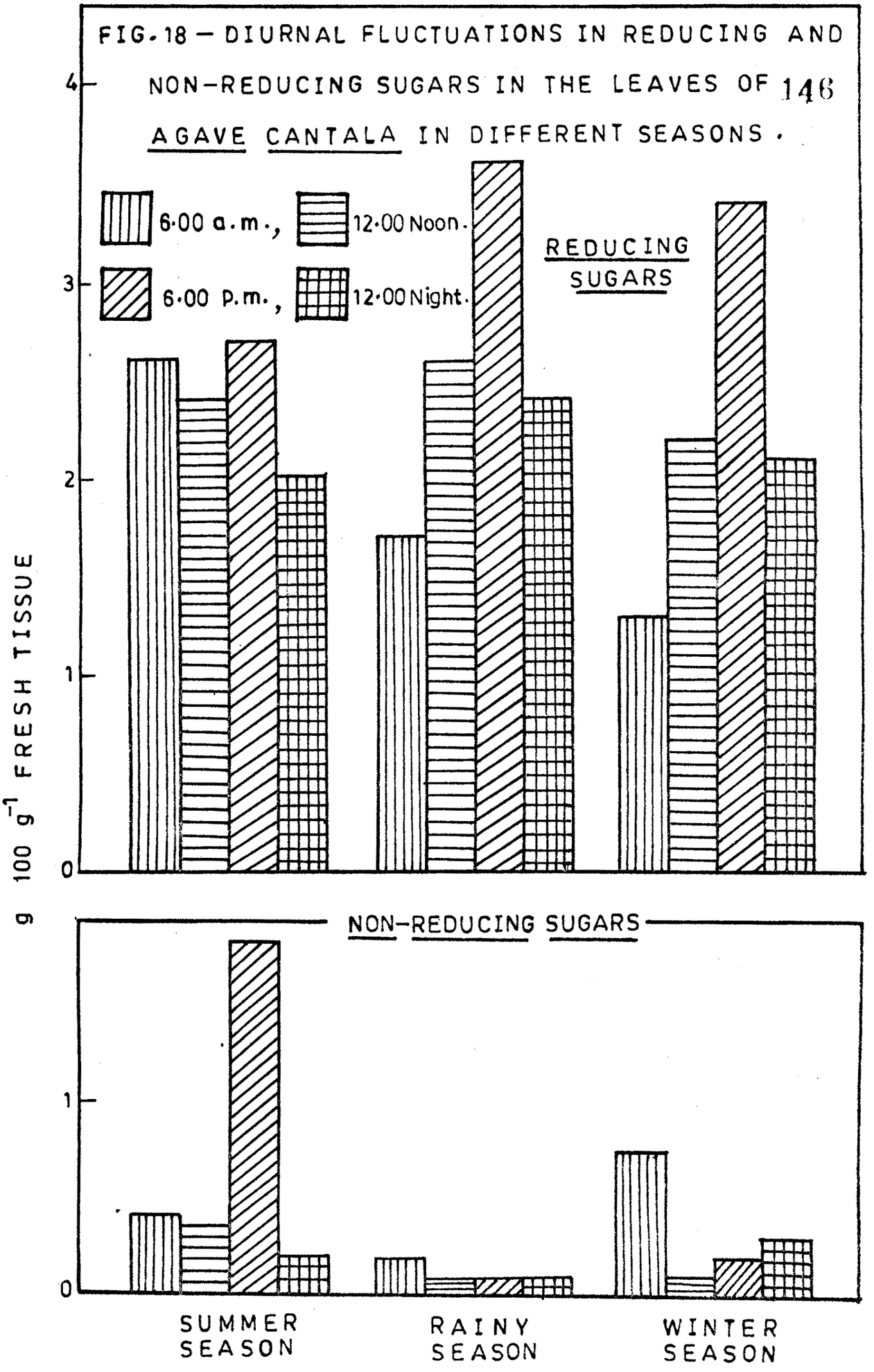
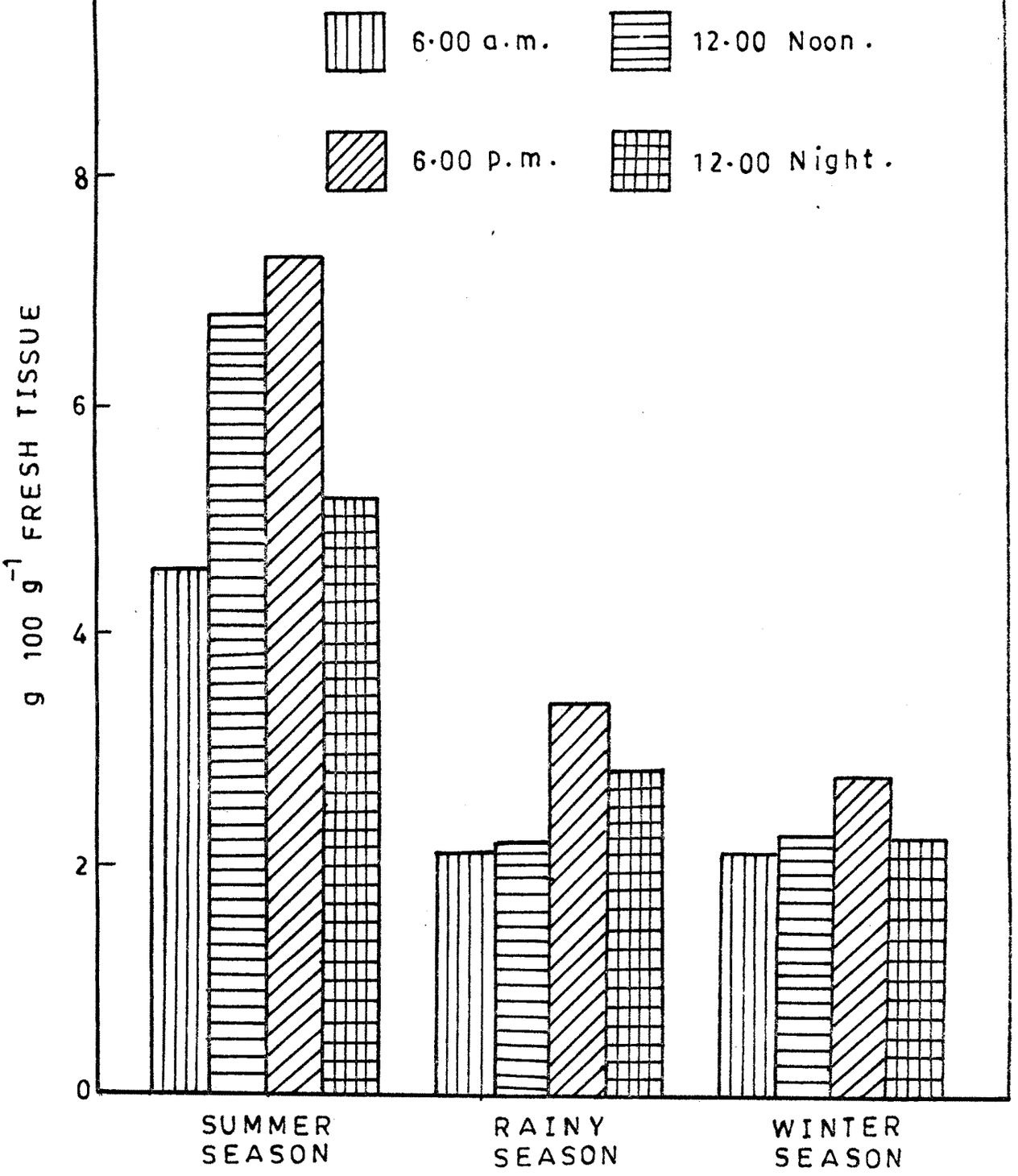


FIG. 19 — DIURNAL FLUCTUATIONS IN STARCH CONTENTS IN THE LEAVES OF AGAVE CANTALA IN DIFFERENT SEASONS.



a marked lowering in non-reducing sugar content is seen, when the values are compared with morning (6.00 a.m.) readings. It can be seen from the figure (Fig. 19) that the leaf starch content goes on increasing along with the progress of the day and this is followed by a decrease in starch level during night hours (12.00 night). The starch contents are highest during summer months and their fluctuations are quite significant during these months. As a result of fluctuations in these carbohydrate fractions we can notice the changes in total carbohydrate fractions during different hours of the day in different seasons (Fig. 17).

According to Kluge and Ting (1978) the plants can be regarded as having CAM if they exhibit the following criteria: (1) The malic acid content of the photosynthetic tissue fluctuates in a diurnal rhythm with accumulation of malic acid during the night period and disappearance during the day. (2) The carbohydrate content also fluctuates with malic acid, but, with inverse phase. (3) The plants having CAM fix atmospheric CO_2 during the night, whereas during the day, a depression or cessation of net CO_2 uptake occurs. An inverse rhythm of stomatal opening is seen in CAM plants with respect to non-CAM plants (i.e., stomatal opening occurs at night rather than during the day light period). Thus it is clear that besides stomatal behaviour and malate metabolism, the carbohydrate metabolism is also an important facet of CAM

plants. The CO_2 fixation during a dark period results in the vacuolar accumulation of malic acid and at the same time decrease in levels of stored carbohydrate content. On the other hand, during a light period there is a rapid and marked decrease in stored malic acid. According to Walker (1962) NADP-dependent "malic enzyme" participates in the decarboxylation and consumption of malate during deacidification. The equation is as follows :

$$\text{Malate} + NADP^{+} \xrightarrow{Mg^{2+}} NADPH_2 + \text{Pyruvate} + CO_2.$$

Dittrich (1975) observed also NAD-dependent "malic enzyme" in CAM plants. The role of pyruvate or other 3-carbon fragments in the light is more important than in the dark. Varner and Burrell (1950) have found that when leaves of Bryophyllum calycium were exposed to light after a period in which $^{14}CO_2$ was fixed in the dark, labelled carbon translocates from acids to carbohydrates. The experiments conducted by Ranson and Thomas (1960) have clearly indicated that malate is not converted directly to carbohydrate. Moyse et al. (1958) and Champigny et al. (1958) have reported an increase in radioactivity during light hours, in insoluble compounds, sugars and phosphorylated sugars and amino acids. Kunitake and Saltman (1958) have indicated that in light due to RuBP-Case activity PGA is formed and it is further converted into carbohydrates in light reactions in CAM plants. In the opinion of Moyse et al. (1958) in light as well as in the

dark the equilibrium of the reaction PGA ⟷ PEP is towards the formation of PEP which makes unlikely the direct reutilization of PEP in the synthesis of sugars. The studies of Champigny (1960) suggested that malate may be degraded to CO_2 completely prior to carbon entering carbohydrates. The work of Haidri (1955 a,b) infiltrated pyruvate-2-^{12}C into Kalanchoe leaves and distribution of carbon in hexoses indicated that pyruvate entered carbohydrates by a reversal of glycolysis and the data also suggested that some pyruvate was oxidised to CO_2 and then incorporated into carbohydrates. Ting (1971) measured carbohydrate formation in Opuntia from malate in light and noticed that in the light about 50% of ^{14}C was located in carbohydrates which was formed by decarboxylation of malate. Denius and Homann (1972) have indicated that in Aloe arborescence malate synthesized and stored during dark is mobilized during light and then oxidatively decarboxylated to pyruvate. They further have suggested that in Aloe species, the respiration may be important in production of energy for a conversion of accumulated pyruvate into carbohydrate and perhaps for the transport of malate out of the storage pool in the vacuole. Depending upon the gas exchange measures they have rejected the supposition for complete break down of malate to CO_2 and water via TCA cycle constitute an integral part of deacidification process. According to them the role of respiration may be important in providing energy for conversion of accumulated pyruvate to

carbohydrates and transport of malate out of storage pools.

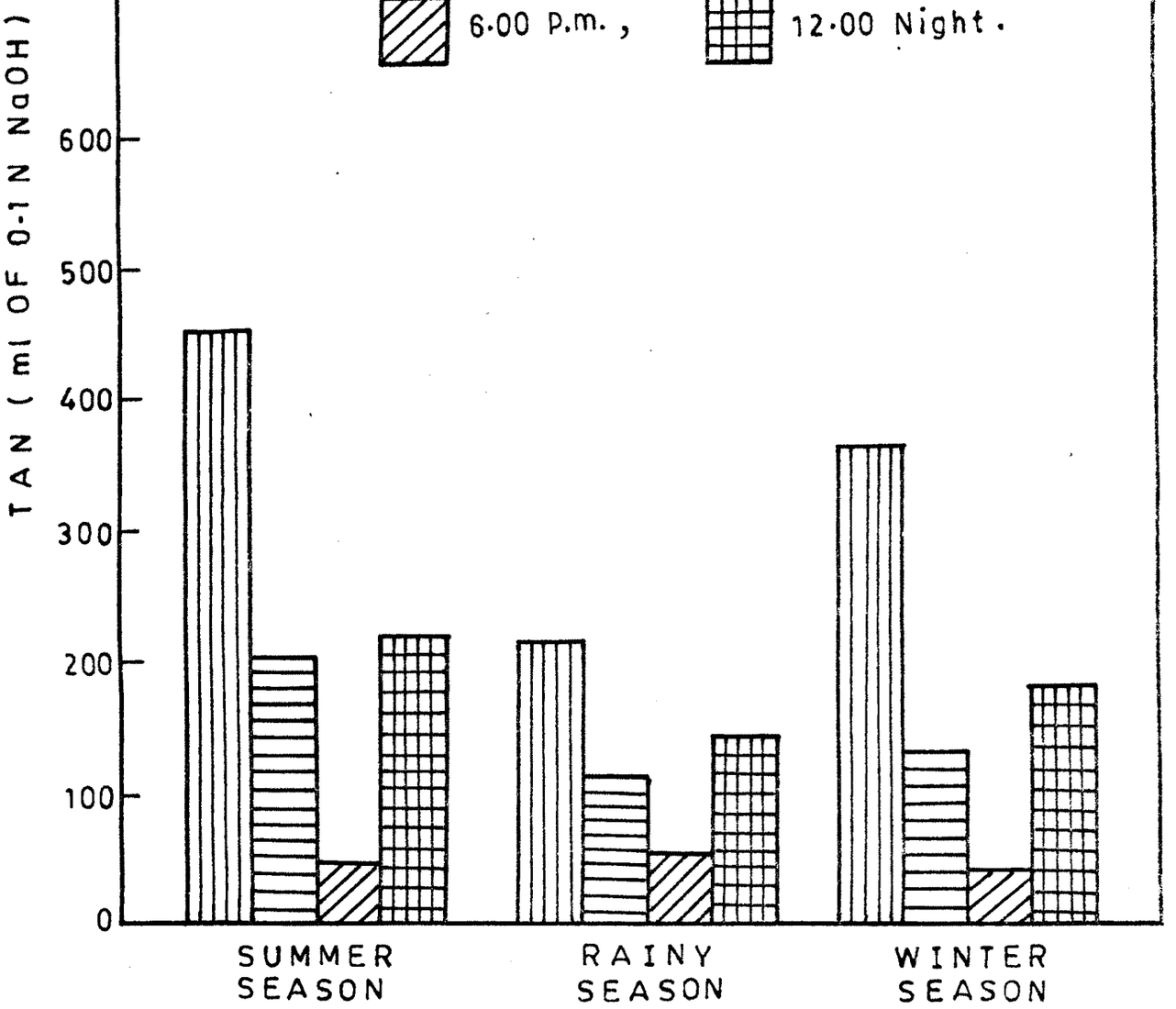
Based on above findings Kluge and Ting (1978) concluded that (1) malate is not converted to carbohydrates directly, (2) 3-carbon fragment can be directly converted to carbohydrates, (3) some of the 3-C fragments can be oxidised to CO_2 which then may converted to carbohydrates by photosynthesis. Thus these workers suggested that pyruvic acid can give rise to a 3-C compound which is converted directly to carbohydrates by reversal of glycolysis. The CO_2 generated by decarboxylation of malate is fixed by RuBP-Case and through the operation of Calvin cycle also significant amount of carbohydrates are synthesized. The energy requirement for the carbohydrate synthesis is fulfilled by photosynthetic phosphorylation. It is assumed that in the dark the stored starch or other glucan is hydrolysed to sugar phosphates which are metabolised to PEP via glycolysis and perhaps to some extent by pentose phosphate cycle. Thus as a result of these synthetic and degrative reactions during different hours of the day the diurnal fluctuations in carbohydrates are noticed in succulent plants. Review of literature indicates that not much attention has been paid to the diurnal fluctuations in carbohydrates as compared to diurnal fluctuations in the titratable acidity or pH values in the succulents. This may be probably due to the fact that the nature of carbohydrate reserves is different in different succulent members. Secondly assessment of diurnal fluctuations in pH

or titratable acidity is rather easier than that of carbohydrates. Our observations with Agave cantala leaves indicate that there are definite changes in the level of reducing sugars and starch during different hours of the day. The pattern is of general increase in level of these components during light hours followed by a rapid decline during dark period. The non-reducing sugars however, do not show any definite trend which may be due to translocation of sucrose to other plant parts. The diurnal fluctuations in the content of reducing sugars are more prominent during rainy season and winter while the diurnal fluctuations in starch content are more significant during summer season.

(ii) Study Of Diurnal Fluctuations In TAN Values During Different Seasons :

Diurnal fluctuations in the TAN values in mature leaves of Agave cantala during different seasons are recorded in Fig. 20. It is evident from the figure that the titratable acidity is at its peak at 6.00 a.m. in each season whereas the lowest TAN values in corresponding seasons are recorded at 6.00 p.m. The values of TAN are intermediate at 12.00 noon and 12.00 night respectively in each season. It is clear from the figure that the most significant fluctuations in titratable acidity are recorded in the summer season followed by winter season whereas in rainy season the fluctuations in TAN values are of lower magnitude. The oscillations observed in organic acid content in A. cantala leaves are more prominent than those observed in case of carbohydrate contents (Fig.17).

FIG·20 – DIURNAL FLUCTUATIONS IN ACID CONTENTS (TAN) IN THE LEAVES OF AGAVE CANTALA IN DIFFERENT SEASONS.



The first observation of the diurnal fluctuation of organic acids in succulent plants is rather difficult to assess. According to Osmond (1978) M. Grew was the first to realise acid accumulation in a succulent plant (Aloe) in 1682. Since then the observations regarding accumulation of organic acids during dark and their subsequent utilization during the day hours have been common in the literature pertaining to CAM plants. The diurnal fluctuation in titratable acidity has been now regarded as one of the most clear indications of operation of CAM. Review of literature indicates that the CAM activity is detected in number of Agave species such as A. americana, A. deserti, A. lechuguilla, A. utahensis, A. virginica and A. fourcroydes. Eickmeier and Adams (1978) have determined delta acidity (morning titratable acidity minus evening titratable acidity) values of A. lechuguilla. They found that these values varied between 24.6 and 78.3 $\mu eq\ g^{-1}$ fresh weight. These values are quite low when compared to the values of delta acidity recorded for Agave cantala leaves in the present investigation which vary from 160.35 to 407.41 $\mu eq\ g^{-1}$ fresh weight. Thus the operation of CAM appears to be more efficient in A. cantala rather than A. lechuguilla. Neales (1975) studied the patterns of gas exchange in succulent plants and classified them into three categories namely 'Non-CAM', 'Weak-CAM' and 'Full-CAM'. According to him the 'Full-CAM' is typically displayed by A. americana. Our observations indicate that similar to A. americana, A. cantala

also shows 'Full-CAM' behaviour, since in all the three seasons marked diurnal fluctuations in CAM behaviour are noticed.

Before 1955, most of the studies of CAM were directed towards the understanding of the biochemistry of acid metabolism. It is very well realised only in last thirty years that the environmental factors significantly influence the behaviour of CAM in succulents. This effect is found to be mediated through influence of environmental factors on stomatal as well as biochemical components of the CAM plants. Moyse (1955) observed that malate does not accumulate in the dark in the absence of O_2. Avadhani (1957) also fully supported this observation. Ranson and Thomas (1960) reported that no appreciable acid accumulation occurred under anaerobic conditions. Kluge and Ting (1978) concluded that O_2 is necessary for dark acidification, but high concentrations of O_2 do not significantly inhibit. According to them light deacidification may be more closely tied to oxygen tension; oxygen inhibits malate consumption. It is now well established that CO_2-free air inhibits nocturnal malic acid synthesis and accumulation (Kluge, 1968 and 1969). This effect is probably due to the lack of substrate for CO_2 dark fixation. According to Wolf (1960) the deacidification in the light was slightly stimulated in CO_2-free air. In the opinion of Bonner and Bonner (1948) and Thomas and Ranson (1954) at night enrichment of the atmosphere with CO_2 increased the rate of synthesis

and the final level of malic acid. They further found that a CO_2 concentration of 2% was optimal and there was no enhancement of nocturnal malic acid synthesis with respect to normal air (0.03 % CO_2) in 10-15 % CO_2. Concentrations higher than 20% were clearly inhibitory if compared with normal air. Finally, at 70%, nocturnal malic acid accumulation was completely prevented. From the experiments of Bruinsma (1958) it is well established that 30% CO_2 prevents deacidification in the light. Nishida (1977) reported inhibition of deacidification in 5% CO_2. Nobel and Hartsock (1986) studied short-term and long-term responses of Agave deserti to elavated CO_2. They found that increasing the ambient CO_2 level from 350 microlitres per litre to 650 microlitres per litre immediately increased daytime net CO_2 uptake about 30% while leaving nighttime net CO_2 uptake of this plant approximately unchanged. A similar enhancement of about 30% was found in dry weight gain over one year when the plant was grown at 650 microlitres CO_2 per litre compared with 350 microlitres per litre. It has been also known that CO_2 metabolism of certain CAM plants e.g., the short-day plant Kalanchoe blossfeldiana responds to photoperiod (Bode, 1942). The work of Gregory et al. (1954) has shown that CAM activity (as measured by dark CO_2 fixation) was induced by short days and prevented by long days or light interruption of long nights.

It is generally well established that low night

temperature and high day temperature are correlated with enhanced CAM activity. According to Neales (1975) the accumulation of titratable acidity in the leaves of CAM plants is generally decreased by high and favoured by low night temperatures. Such type of behaviour has been shown by Agave americana (Neales, 1973). Thus in view of Neales (1975) these findings substantiate the claim that CAM is an adaptation to environments of cool nights. In contrast to low night temperature the warm days are found to favour CAM (Joshi and Bartakke, 1974; Crews et al., 1976). In view of Queiroz (1968) the time of day at which PEP carboxylase and malic enzyme were maximum and minimum was a function of the thermoperiod. According to Osmond (1978) the malate consumption during the day is accelerated by increased light intensities.

It is also reported that the higher the light intensity during deacidification phase, the lower the minimum value of malic acid reached. The mineral nutrition has been also found the influence on CAM process. Salinity has been found to favour CAM in Aloe barbadensis and Portulaca oleracea (Bartakke, 1977; Karadge, 1981). The work of Nobel and Berry (1985) indicated that in case of six Agave species namely A. americana, A. deserti, A. fourcroydes, A. lechuguilla, A. salmiana and A. utahensis the nocturnal acid accumulation was positively correlated with levels of nitrogen, boron and calcium in the chlorenchyma tissue. The water relation of CAM plants is of considerable importance in understanding the adaptations of

these plants of xeric habitats. It is observed that CAM maintains a positive carbon balance or at least prevents a negative balance even in extended periods of drought. The daytime gas exchange of both Bryophyllum daigremontianum (Kluge and Fischer, 1967) and Agave americana (Neales, et al., 1968) are greatly reduced in plants deprived of water. The nocturnal phase of gas exchange is much less affected, thus the positive carbon balance of the plant is maintained and water loss greatly reduced. Under drought, 98% of the total net CO_2 assimilation of an Agave americana plant (in a 16 hr/8 hr light/dark regime) takes place in the 8 hr dark period, when the plant approaches the 'Sper-CAM' condition. This suppression of daytime gas exchange in droughted plants has been observed by Bartholomew (1973) in the Crassulacean plant, Dudleya farinosa, growing in the field. Szarek et al. (1973) have shown that in Opuntia basilaris under drought, gas exchange by day and night is minimal with very high diffusive resistances (600 s cm^{-1}) throughout the day/night cycle. Eickmeier and Adams (1978) studied effect of watering frequency on fluctuations in titratable acidity in Agave lechuguilla. They observed that the delta acidity values were slightly higher when there was high water stress at 35°C night temperature. On the other hand, these values were higher when the watering frequency was high and night temperatures were 15°C. Thus these observations indicate a possible interaction of temperature and water status. In Agave salmiana Nobel and Meyer (1985) observed that drought reduced nocturnal increase

in titratable acidity, e.g., it decreased 30% after 7 days of drought, 58% after 14 days and 96% after 28 days of drought.

Since there is marked variation in temperature, light intensity and rainfall pattern during different seasons of the year; it is quite understandable to notice alterations in intensity and pattern of CAM during different seasons of the year. There are few attempts to study influence of seasonal variations on titratable acidity in succulents. Bennet-Clark (in Wolf, 1960) studied diurnal fluctuations in titratable acidity during different months of the year in Sedum praealtum. He obtained maximum delta acidity (105 μeq g^{-1} fresh weight) in months of July, August and September; intermediate delta acidity (62 μeq g^{-1} fresh weight) during April, May, June, October and November and minimum delta acidity (40 μeq g^{-1} fresh weight) in December, January, February and March. From these observations he concluded that generally, maximum amplitude of malic acid rhythm is found during the summer months. Bharucha and Joshi (1957, 1958) have studied the Crassulacean Acid Metabolism in Aloe vera and Bryophyllum calycinum at Bombay. They observed per cent increase in TAN over the evening TAN value in Aloe vera as 474 in monsoon and 855 in winter. In Bryophyllum calycinum per cent increase in TAN was found to be 1137 in monsoon and 3959 in winter. They reported that the magnitude of diurnal fluctuations varies from season to season and it is highest in winter and lowest in monsoon. They concluded that these seasonal variations

can be attributed to prevailing environmental factors.Szarek (1974) studied CAM behaviour with respect to titratable acidity in Opuntia basilaris in its natural habitat at the Boyd Deep Canyon in California (USA) during different months of the year. He reported maximum delta acidity (85 $\mu eq\ g^{-1}$ fresh weight) in August followed by February and March (72, 65 $\mu eq\ g^{-1}$ fresh weight). Minimum delta acidity (30 $\mu eq\ g^{-1}$ fresh weight) was recorded in June whereas it was near the minimum value (32 to 35 $\mu eq\ g^{-1}$ fresh weight) in July, September, October, November, December and May. Intermediate delta acidity (55 $\mu eq\ g^{-1}$ fresh weight and 40 $\mu eq\ g^{-1}$ fresh weight) was noticed in January and April respectively. Based on these observations he concluded that amplitude of acid rhythm in Opuntia basilaris occurred when the plant water potential was high, and the lowest amplitudes could be observed when plant water potential was low. It is evident from the above reports that the pattern of changes in CAM behaviour during different seasons is not uniform in the above succulent plants. This may be due to the fact that the sites of experiments are different in three investigations mentioned above and there is every possibility of differences in climatic conditions at the three sites which can cause alterations in CAM pattern.

Kolhapur, the site chosen for present investigation shows three distinct seasons namely rainy season, winter and summer with respect to climatic pattern. In all these three

seasons various environmental parameters are moderately balanced and generally do not attain extremes (Table No.1). The summer season is not too hot to curtail life activities of most of the perennial plant species. The same can be also said about the winter season which is not too cool to totally affect plant life. These climatic conditions are very well reflected in the oscillations of TAN values observed in the present investigation. Thus in summer months the nocturnal accumulation of acids as well as the potential of deacidification during daytime is very high. This is followed by winter. In rainy season the oscillations of titratable acidity are rather low which may be possibly due to some gas exchange occurring during daytime. According to Kluge and Ting (1978) the maximum amplitude of the malic acid rhythm found during the summer months is probably due to the fact that during the summer CAM plants deacidify during the day to a lower level and reach a higher malic acid level during the dark period than during rest of the year. Thus climatic conditions prevailing in the region of western Maharashtra appear to be quite congenial for the operation of CAM and maintenance of positive carbon balance in Agave cantala and hence this region appears to be quite suitable for cultivation of this economically important species of Agave as a supplementary crop.