CHEMOTAXONOMIC OBSERVATIONS ON THE GENUS *Cenchrus* L.

by

Vandana Mehta

(L-86-BS-82-M)

Thesis

submitted to the Punjab Agricultural University
in partial fulfilment of the requirements
for the degree of
MASTER OF SCIENCE
in
BOTANY

Department of Botany
College of Basic Sciences & Humanities
PUNJAB AGRICULTURAL UNIVERSITY
LUDHIANA-141004

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To

My

Parents

CERTIFICATE I

This is to certify that this thesis entitled "Chemotaxonomic observations on the genus Cenchrus L." submitted for the degree of M.Sc. in the subject of Botany (Minor subject: Biochemistry), to the Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by Vandana Mehta (L-86-BS-82-M), under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

(Dr Gurbaksh Singh)
HEAD OF THE DEPARTMENT

30.12.88
(Dr. S.K. Sachdeva)
MAJOR ADVISOR
Associate Professor
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PAU, Ludhiana.

CERTIFICATE II

This is to certify that the dissertation entitled, "Chemotaxonomic observations on the genus Cenchrus L." submitted by Vandana Mehta (L-86-BS-82-M) to the Punjab Agricultural University, Ludhiana, in the partial fulfilment of the requirements for the degree of M.Sc. in the subject of Botany (Minor subject: Biochemistry) has been approved by the student's Advisory Committee after an oral examination on the same in collaboration with an External Examiner.

14.3.89

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Major Advisor

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31 MAR 1989

(Dr. B.S. Dhillon)
Dean, Postgraduate Studies

ACKNOWLEDGEMENTS

I express my deep sense of gratitude to my major advisor Dr. S.K. Sachdeva, Associate Professor, Department of Botany, Punjab Agricultural University, Ludhiana, for his excellent guidance, constructive criticism and inspiration during the course of present investigation. I am extremely obliged to him for theinnumerable hours he spared out of his busy schedule during the compilation of the manuscript.

I am thankful to Dr. O.S. Singh, Professor-cum-Head, Department of Botany for providing necessary laboratory facilities.

My sincere thanks are due to the members of my advisory committee Dr. C.P. Malik, Professor, Research Coordinator, Department of Botany, Dr. J.S. Arneja, Biochemist and Dr. D.S. Bhatia, Senior Botanist, for their keen interest and valuable suggestions.

I am grateful to Dr. Sukhwinder Kaur, Assistant Professor, Department Plant Pathology and Dr. Shashi Sharma for their help. My sincere thanks are due to Jolly, Neelam, Aman, Pushp, Mukti, Rajwans and a galaxy of other friends for their cooperation and cheerful company.

Last, but not the least, this project would have remained on paper if my affectionate parents, Sanju and Didi, had not given me constant inspiration and encouragement for the fulfilment of present academic mission.

Vandana Mehta

(Vandana Mehta)

CONTENTS

CHAPTER I

INTRODUCTION

The genus Cenchrus L. belongs to the tribe Paniceae of the sub-family Panicoideae of Poaceae. The genus is represented by 25 species distributed in the tropical and warm temperate regions of both the hemispheres. It is one of the most highly evolved genera of the Paniceae (Bews, 1929; Hitchcock, 1935). The characteristic spiny involucre is a highly developed structure (Brown, 1948). Seven species of the genus are reported from the Indian subcontinent (Bor, 1960). Though majority of the species of this genus grow as troublesome weeds yet the three most prevalent species in Punjab plains that are exploited for fodder purposes are Cenchrus ciliaris L. (Buffel grass), C. setigerus Vahl (Birdwood grass) and C. pennisetiformis Hochst et Steud (Cloncurry Buffel).

Cenchrus ciliaris L.

It is a tussocky perennial species with long slender bristles plumose on the thickened basal portion. Also from the base arise a number of smaller bristles. Various cultivars of this species have been identified (Nicholson et al.,1985). This grass is a valuable fodder especially for hay. It is also planted as a lawn grass.

Cenchrus setigerus Vahl

It is a tussocky summer growing perennial species. It flourishes in sandy or light textured soils. The fascicles range in colour from straw to purple and are easily identified by their short stiff teeth like deltoid bristles of uneven length, which are connate for 0.7 to 2.8 mm from the base. This grass also serves as excellent green forage.

Cenchrus pennisetiformis Hochst et Steud

It is also a tussocky summer growing perennial species. The tidy shaped dark purple or straw coloured fascicles closely resemble those of C. ciliaris, but can be easily identified by the involucre bristles being connate for 1.0 to 3.0 mm above the base. This is extremely valuable fodder grass as it remains green during the dry season, and cattle eat it with avidity.

These species are not only widely distributed but also highly variable cytologically. A large number of cytological races have been reported in these species e.g. C. ciliaris with 2n=18, 36, 36+0-2B, 40, 44, 50, 52 and 54; C. setigerus with 2n = 34, 36 and 54 and C. pennisetiformis with 2n=35, 42 and 54 (Cf. Fisher et al., 1954; Nath and Swaminathan, 1957; Singh and Godward, 1960; Patil et al., 1961; Mehra et al., 1968; Federov, 1969; Ramaswamy et al., 1969; Vij and Chaudhary, 1981).

This speaks very well of cytological dynamism prevailing in these taxa. Apart from morphological characteristics, such cytological races have been observed to differ from one another in biochemical characteristics also (Sachdeva and Bhatia, 1979 and 1980; Sachdeva and Kals, 1981; Sachdeva et al.,1986; Kumar, 1987; Bala, 1988). A perusal of literature reveals that no comprehensive attempt from chemotaxonomic point of view has been made on Indian species of this genus. It was thus thought worthwhile to make extensive collections of C. ciliaris, C. setigerus and C. pennisetiformis from plains of Punjab, work them out cytologically and evaluate them biochemically in respect of amino acids, soluble sugars, total soluble proteins, ascorbic acid content and flavonoids. It was also intended to study flavonoid and protein patterns. Such studies have been observed to be helpful in the identification and in elucidating the interrelationships among taxa.

The main objectives of the present study were:

1. To collect the three species from different localities of Punjab plains and to grow them under uniform nursery conditions in the botanical garden of the University.

2. To record the chromosome numbers in the collected taxa from the study of meiosis.

3. To undertake a detailed study of morphological and anatomical features of these taxa.

4. To analyse and record variability in respect of total soluble proteins, major sugars, total free amino acids, individual amino acids, total favonoids, ascorbic acid, content alongwith flavonoid and soluble protein patterns.

CHAPTER II

REVIEW OF LITERATURE

The literature on different aspects concerned with the present study has been reviewed under the following headings:

2.1 Morphology and Anatomy

- 2.1.1 Morphology
- 2.1.2 Anatomy

2.2 Karyological information

2.3 Phytochemistry and Biosystematics

- 2.3.1 Carbohydrates
- 2.3.2 Amino acids
- 2.3.3 Flavonoids
- 2.3.4 Ascorbic acid
- 2.3.5 Proteins

2.1 Morphology and Anatomy

2.1.1 Morphology

Plant taxonomy is basically dependent upon the external morphological characters. Both vegetative and reproductive characters are important, but, ingeneral, less attention has been paid to vegetative characters because of their plastic nature. However, there are instances where vegetative characters

proved more valuable as compared to reproductive characters (Stace, 1980). Ramaswamy et al.(1969) on the basis of morphological characters such as height, number of tillers, hairiness, vigour, density of spikelet, leaf length/breadth, internode length/thickness, peduncle length/thickness and bristles length etc. identified two cytotypes with 2n=36 and 54 in Cenchrus setigerus and five cytotypes with 2n=34, 36, 44, 54 and 56 in C. ciliaris. Henderson (1976) found that stem and leaf width were useful characters in separating Sisyrinchium montanum and S. septentrionale from other north western taxa. Tripathy et al.(1977) observed taxon characteristic dentations on the margins of leaves in different races of Cynodon dactylon. Anwar (1980) observed flag leaf length to be of taxonomic value in Oryza sativa. Ebinger and Jimmy (1975) studied culm morphology of 194 grass species and found culm characteristics to be of much taxonomic significance.

Bor (1960) classified different species of Cenchrus based on similarities and differences in the morphological characters of the inflorescence. On the basis of fascicle morphology Nicholson et al.(1985) distinguished Cenchrus ciliaris and C. pennisetiformis. They also identified nine cultivars of C. ciliaris on the basis of fascicle and plant morphology. de Wet et al.(1984) on the basis of inflorescence morphology could differentiate five races in cultivated finger millet (Eleucine coracana L.). Berg (1985) suggested taxonomic and ecological

implications of spikelet structure in Panicum australience, a member of Poaceae. Busey (1986) employed the size of inflorescence and the spikelet as valuable taxonomic characters to identify the various St. Augustine grass cultivars.

Morphological data, especially when taken in conjunction with data from other sources has helped systematics to derive concreteconclusions. According to Merchant (1967) species and hybrids of Spartina townsendii complex could be separated by a combination of a few well defined morphological characters and by stomata and pollen grain characteristics. Sachdeva and Bhatia (1978), Sachdeva and Kals (1981) and Bala (1988) observed that morphological differences, stomatal size and pollen size have correlation with increasing level of ploidy and could be used in identification of different cytotypes in the Cynodon dactylon, Dactyloctenium aegyptium and Setaria verticillata complexes.

2.1.2 Anatomy

Anatomical characters provide an extensive data of systematic value. Carlquist (1961) stated the leaf to be anatomically the most varied organ that possessed many anatomical features of potential taxonomic significance. In the grass family Poaceae, leaf anatomical features have proved to be of great assistance in delimitation of taxa (Metcalfe, 1960; Ellis, 1976). Leaf epidermis and leaf cross-section characters such as the disposition of sclerenchyma, the

arrangement and form of vascular bundles, the differentiation of epidermal long cells and short cells and the form and distribution of silica bodies and various types of trichomes and papillae have played a major part in the modern reclassification of many taxa at all levels (Stace, 1980).

The taxonomic value of leaf epidermal studies has been the subject of attraction for many taxonomists. Gendel (1976) worked out the anatomical details of leaf of Aegilops mutica (Poaceae) and opposed its inclusion in the genus Triticum as T. tripsacoides based on simple morphology. A comparison of leaf anatomy of A. mutica with other representatives of Aegilops indicated that the characteristics common to all Aegilops spp. were also present in A. mutica differing only with respect to stronger development of the sclerenchyma. So he advocated its retention in the genus Aegilops. Jellings and Leech (1984) examined the variations in over 20 leaf characters including morphological, anatomical and cellular features in nine Triticum genotypes at three levels of ploidy. Analysis of these characters showed that the major components of the variations were related to the ploidy level, the genotypes of the species and cultivars examined.

Brown (1975) reported that nature and characteristic features of kranz cells could be used as important taxonomic characters. But, characterization of the anatomy or physiology of taxon may be incorrectly represented by basing conclusions

on the bundle sheaths of only large and small veins. Dangler(1985) compared the leaf blade anatomy of seven C_4 grasses with a C_3 species Festuca arundinacea and supported Brown's opinion.

C_3 and C_4 species of the family Gramineae are separated on the basis of differences in interveinal distances between adjacent vascular bundle sheaths (Crookston and Moss,1974; Hattersley and Watson,1975; Hattersley,1984).

Cohen et al.(1982) studied the leaf anatomy and stomatal characteristics of four tall fescue selections and found no difference among selections for per cent cross sectional area of vascular bundle and mesophyll tissues. Russell and Evert (1985) studied the leaf vasculature in Zea mays L. and suggested linear relationships between leaf width and total bundle number. Sharma and Slam (1984) suggested that leaf epidermal characters were of little diagnostic value either at specific or sub-specific levels in Dactyloctenium. However, in Digitaria these were observed to be sufficiently discriminatory at both specific and sub-specific levels (Sharma et al.,1985).

Tan and Dunn (1975) found that stomatal frequency and size could be used in Bromus inermis to identify the cultivars and varietal differences at various levels of ploidy. Cultivars with fewer stomata were found to have larger stomata, longer and wider leaves, greater dry weight per tiller and were at higher ploidy levels.

Stiff and Powell (1974) studied the stem anatomy of 20

cultivars of tuft grass genera Cynodon, Zoysia, Agrostis, Poa, Stenotaphrum, Festuca and Digitaria. Three types of stems were distinguishable with single-ring, multiple-rings and with compounds rings. Govindarajalu (1976) investigated 13 species of Scripus and found that all these taxa could be segregated into two primary groups based on 2 or 1-layered condition of bundle sheaths in the culms. Sachdeva and Bhatia (1979) observed that in Cynodon dactylon the diploid, triploid and tetraploid forms could be distinguished on the basis of presence of starch grains in the different tissues of stem. Sachdeva and Kals (1981) observed that significant differences in the cortex and sclerenchyma could be used to distinguish the three cytotypesof Dactyloctenium aegyptium. Chakaravarty and Verma (1965) based on the stem anatomical characters shown by the genera belonging to the two tribes, Paniceae and Andropogoneae, of the same group confirmed the earlier classification based on the external morphology.

Bor (1960) in his monograph on grasses remarked that classification of grasses presented by him was based wholly on morphological characters or party on those characteristics modified by anatomical and cytological data which are by any means adequate. Anatomical features of different grasses may, therefore, be helpful in their identification and classification.

2.2 Karyological Information

Karyological studies have been an important tool in the

elucidation of biosystematic problems. The characteristics of chromosomes and their behaviour during cell division and gamete formation have provided a great deal of relevant information. They furnish diagnostic characters in classification and provide an insight into genetic and evolutionary processes leading to speciation. Three characteristics of chromosomes that have yielded maximum information are chromosome number, chromosome morphology and chromosome behaviour at meiosis.

Chromosome number along with data from other sources has been an important taxonomic character (Raven, 1975). Different species of Cenchrus show intraspecific variation in chromosome number. C. ciliaris shows a series of cytological races. Darlington and Wylie (1955) reported 2n=34 in this species. Fisher and others (1954) found four cytotypes with 2n=32, 36,40 and 54. Nath and Swaminathan (1957) discovered two more cytological races with n=22 and 26. Mehra (1968) reported still another type with 2n=18. Vij and Chaudhary (1981) reported n=18 and 18+0-2 B's in Cenchrus ciliaris in the Chandigarh area. According to them predominant occurrence of the cytotype n=18 of C. ciliaris suggests its stability in the area. In C. setigerus three chromosomal forms (2n=34, 36 and 54) have been reported (Fisher et al.,1954; Darlington and Wylie,1955; Ramaswamy et al.,1969). Singh and Godward (1960) reported 2n=42 in C. pennisetiformis and Patil et al.(1961) reported 2n=35 and

54 in this species. Fisher et al.(1954) suggested the basic number 9 and the forms with 2n=32 and 40 reported by him, were regarded as aneuploids. However, Vij and Chaudhary (1981) suggested the dibasic nature of this genus with n=9 and 17.

Apomictic forms have been reported in various species of Cenchrus (Fisher et al.,1954; Bray,1978; Sherwood et al., 1980). Read and Bashaw(1960) pointed out the significant role of apomixis in the speciation of C. setigerus and C. ciliaris.

Shanthamma and Narayan (1976) reported that the apomictic forms of C. ciliaris and C. glaucus show univalents, multivalents, laggards, irregular distribution, bridge fragments and polyads and 53 and 49 per cent pollen fertility respectively. The irregularities in chromosomal behaviour at meiosis and the variation in chromosome number resulting therefrom in the progenies was observed to be maintained in the populations due to aposporous and facultative apomixis (Fisher et al.,1954).

2.3 Phytochemistry and Biosystematics

A number of early taxonomists laid emphasis on the need of chemical evidence in taxonomy (Swain,1963; Alston and Turner, 1963; Harborne, Boulter and Turner, 1971; Heywood, 1973). The advent of chemosystematics is linked to the development of newer and powerful analytical techniques combining speed and simplicity which enabled reasonably rapid surveys of plant extractives. Different techniques such as chromatographic

techniques, electrophoresis and spectrophotometry etc.have been employed to generate data of taxonomic value. Niklas (1976) and Niklas and Gensel (1976,1977,1978) extended such chemical analysis to paleozoic vascular plants in relation to their taxonomy. Also attempts have been made to evaluate chemical evidences by statistical methods. The approach has been called numerical chemotaxonomy (Runemark,1968; Weimarck, 1972; Crawford and Darn, 1974).

The chemical constituents which are of great help in chemotaxonomic studies are amino acids, flavonoids, proteins and enzymes, fatty acids and essential oils. Virtually every chemical component found in plants has been used in chemical comparison of taxa at various taxonomic levels (Smith,1976; Tanksley and Orton,1983; Harborne and Turner, 1984; Martin et al.,1985).

2.3.1 Carbohydrates

Variation in carbohydrate contents due to non-genetic factors and quantitative and qualitative changes associated with varying environmental and developmental conditions has discouraged chemotaxonomic evaluation of carbohydrates. Early work on this aspect was mostly concerned with variation in starch grains. Reichert (1919) suggested the possible use of carbohydrate evidence in systematics. Characters of starch grains have since proved their value in several groups, notably

in the Gramineae (Hubbard, 1948; Stebbins, 1956). Blackman (1921) emphasized the use of carbohydrates data in phylogenetic studies but little critical work was done for many years.

Macleod and McCorquodale (1958) in a survey of oligosaccharides in leaves of the Gramineae suggested that the commonly occurring raffinose was replaced in Bromus by simple fructans and in others like Festuca and Lolium by raffinose isomer. Crowden et al. (1969) observed Umbelliferose to be a universal storage sugar of Umbelliferae. Meier and Reid (1982) found that Umbelliferose is also present in Araliaceae and Pittosporaceae and thus added further chemical evidence linking these families with the Umbelliferae. They also suggested that members of Campanulaceae, Compositae, Gramineae and some taxa of Liliales could be distinguished on the basis of significant differences in storage carbohydrates.

Elizabeth and Margaret (1971) studied the low molecular weight carbohydrates and water soluble polysaccharides metabolized by the dedophorales and concluded that water soluble polysaccharides of Urospora were quite unlike those of dedophorales, which occupies a somewhat precarious taxonomic position in the dedophorales. Stinard and Nevins (1980) showed that non-cellulosic β-glucans were present widely in grasses but did not occur in any related monocotyledonous groups.

Sachdeva and Kals (1981) observed that the three cytological races of Dactyloctenium aegiptium accumulated different levels of glucose, fructose and sucrose at inflorescence emergence stage. Such differences might have arisen due to different genetic controls. Sachdeva and Bhatia (1979, 1980) noticed that the leaves of three cytological races of Cynodon dactylon accumulated different levels of glucose, fructose and sucrose at inflorescence stage. They ascribed this to the differential activity of acid invertase. Bala (1988) observed similar differences in different cytotypes of Setaria verticillata and S. tomentosa.

2.3.2 Amino acids

Taxonomic significance of aminoacids in angiosperms has been studied by many workers. Alston and Irwin (1961) studied the free amino acid profile in the five species of Cassia. Among the species examined, there was definite but only moderate variation in free amino acids present and the differences were predominantly quantitative. Bell (1962) has shown that about 50 species of genus Lathyrus could be subdivided into five main groups on the basis of associations of amino acids within the seeds. Fowden (1958) recorded the occasional occurrence of δ acetylornithine, α-aminoadipic acid, β-alanine or pipecolic acid as useful taxonomic markers in the grasses examined. Taira (1968) found that amino acid patterns in grass seed proteins are consistent with the modern classificatory views on grasses.

Reddi and Phipps (1972) studied amino acids in grass tribe Arundinelleae and found the observed patterns of variations unrelated to taxonomic groupings and considered the utility of such components as "taxonomic noise". However, Watson and Creaser (1975) observed that festucoid and chloridoid grasses differed on the basis of quantitative variation in the levels of leucine and alanine than other grasses having higher levels of glycine and lysine.

Vaidyanath and Reddy (1980) investigated that the amino acid profile of different species of Oryza, wild as well as cultivated species, helpful in the evaluation of their patterns of evolution and origin. Krishnamurthy and Subramanian (1978) observed the nature and composition of free amino acids to study the chemotaxonomic affinities among Leersia and Oryza species on one hand and amongst the three species of Oryza on the other. They showed that three species of Oryza were more inter-related than were Leersia and Oryza.

Sachdeva and Bhatia (1979) and Sachdeva and Kals(1981) observed significant qualitative and quantitative variations in amino acid composition in different cytological races of Cynodon dactylon and Dactyloctenium aegyptium complexes respectively.

Yeoh and Watson (1981) observed that the variations in amino acid patterns of 121 species from 72 grass genera were extremely consistent with taxonomic grouping. They observed

patterns of pooids, chloridoids, eupanicoids, andropogonoids and danthonoids were clearly distinguishable from one another. Yeoh and Watson (1982) working with leaf amino acid profiles of grasses found that danthonoids reveal intermediate patterns between chloridoids and panicoids.

Yeoh et al.(1984) on the basis of leaf amino acid profiles of Leguminosae distinguished Mimosoideae from Papilionoideae and Caesalpinioideae. Thus free amino acid studies have provided a meaningful taxonomic data.

2.3.3 Flavonoids

Flavonoid analysis have contributed much towards the elucidation of plant relationships than any other secondary plant product primarily because of their universal occurrence in plants, easy isolation, great structural variations and biochemical stability. Literature on this subject is quite vast. Important works include those of Hegnauer (1962-73), Alston and Turner(1963), Swain (1963,1975), Batesmith (1968), Harborne and Swain(1969), Harborne (1975,1977, 1979) Dell and McComb(1978), Crawford(1978), Swain et al.(1979), Crawford and Giannasi(1982), Phillipson(1982), Harborne and Turner (1984), Denford (1984).

The role of flavonoid chemistry is very well appreciated when our conventional morphological and cytological criteria fail completely to solve the taxonomic riddles (McClure et al., 1966), Alston and Turner (1963) could identify different species of Baptisia on the basis of a number of species-specific

flavonoids. McClure and Alston(1966) identified one hundred and eightysix classes of Lemnaceae representing worldwide collection of twentytwo taxa.

Horborne and Williams(1976) studied the flavonoid patterns in leaves of different groups of grasses and identified them upto species level. Williams et al.(1971) identified the flavonoid pigments in eleven monocotyledonous families dividing them into four groups. Different families were identified on the basis of special constituents e.g. aurones formed characteristic features of sedges, positively charged flavones of palms and anthocyanins of Gramineae. The presence of luteolin, tricin and glycoflavones in the families Cyperaceae, Palmae and Gramineae supported their taxonomic alignment. Further, palms were found to differ from related sedges and grasses by having their flavones and glycoflavones as salts in association with potassium disulphate (Harborne and Williams, 1971).

Frost, Harborne and King (1977) studied the five chemical races of cultivated barley (Race, A, B, B_1, B_2 and S) and observed the flavonoid patterns in S race to be original and others to be evolved from it. Bekele (1983) supported the already established patterns in barley based on numerical analysis of the various flavonoid races.

Interspecific hybrids show additive profiles i.e.certain

parental species specific compounds are found together in hybrids. Such profiles have been observed to be useful in some instances for documenting the autopolyploid and allopolyploid nature of taxa (Smith and Levin, 1963; Levy and Levin, 1971, 1974; Levy, 1976; Murray and Williams, 1973; Sachdeva and Bhatia, 1980; Soltis et al., 1983). Williams et al. (1983) noticed that sometimes parental compounds could not be detected in the hybrids.

Crawford (1970) distinguished two varieties of Coreopsis mutica by studying the chromosome number and flavonoid chemistry. Variety Leptomera having chromosome complement 2n=56 produced only flavones in its leaves whereas variety mutica had chromosome number 2n=11[illegible] and was observed to produce flavones, flavonones and anthochloros. Sachdeva and Bhatia(1980), Sachdeva and Kals(1981), Kumar (1987) and Bala (1988) observed the flavonoid patterns to be cytotype specific.

Harborne et al.(1985) surveyed 170 Australian species of Cyperaceae belonging to 35 genera and observed the presence of highly characteristic flavonoid patterns in leaf and inflorescence. Sulphuretin was found for the first time in this family.

Julkunen-Titto(1986) screened the leaves of 15 Salicaceous species for total phenolics, condensed tannins, catechins and phenolic glycosides and could distinguish each willow species on the basis of its leaf phenolics glycosides.

Nageshwar et al.(1987) studied the distribution pattern of different phenolic acids in ten taxa of tribe Amherstieae (Caesalpinioideae). He found a fair degree of relationship among the taxa and suggested that the tribe was an homogenous assemblage.

2.3.4 Ascorbic acid

The value of ascorbic acid taxonomic marker has been doubted by some workers, nevertheless, any clearly definable areas of variation and differences in the distributional pattern would be of taxonomic interest.

Jones and Hughes (1983) recorded ascorbic acid content of the leaves of 213 species belonging to 60 angiosperm families. They found highly significant differences between different families. A relatively low concentration of ascorbic acid was observed to be characteristic of certain families e.g. Boraginaceae, Compositae, Rubiaceae while a high concentration of others e.g. Ericaceae, Euphorbiaceae and Primulaceae. Thus the Compositae regarded by most taxonomists as "advanced" taxon had a significantly lower level of ascorbic acid concentration. Cronquist (1968) and Takhtajan(1969) found that highest amount were associated with families of more or less intermediate evolutionary location, such as the Euphorbiaceae and Primulaceae. In the Gramineae ascorbic acid content analysed from four different species e.g. Avena fatua, Dactylis glomerata, Holcus lanatus and Phleum pratense did not furnish any taxonomically useful information.

Sachdeva and Bhatia (1980) observed differences in ascorbic acid contents among diploid, triploid and tetraploid forms at inflorescence emergence stage in the flag leaves of Cynodon dactylon and found triploid forms contained almost double the quantity of ascorbic acid present in the diploid and tetraploids. Sachdeva and Kals (1981) found that different cytotypes of Dactyloctenium aegyptium show variation in ascorbic acid contents. Apparently quantitative differences among the taxa need more studies to ascertain their utility as chemotaxonomic markers.

2.3.5. Proteins

Ubiquitous occurrence of proteins in plant tissues and the variations found in different species of the same genus, even different parts of the same plant, have led to their use for taxonomic comparisons (Cronquist, 1976; Kloz et al.,1960; Gibbs, 1963). They sometimes show developmental variations (Robinson and Brown, 1952; Scandalios,1974).

Variations in the proteins have been best studied through electrophoretic banding patterns. Johnson and Hall (1965) conducted electrophoretic studies in wheat proteins and statedthat a homology between the bands of different species, based on similarity in migration velocity provides a criterion of genetic affinity from which evolutionary relationships may be inferred.

Hart and Bhatia (1967) showed that within the genus Nicotiana the leaf proteins of each species exhibited a distinct pattern. Johnson and Hall (1965), Johnson et al.(1967) and Johnson(1972) provided data in support of the theory that hexaploid Triticum aestivum originated as a result of hybridization between the diploid Aegilops squarrosa (DD) and the tetraploid cultivar T. dicoccum (AABB). The resulting triploids, through doubling of chromosomes gave rise to the fertile hexaploid T. aestivum (AABBDD).

Gel electrophoretic procedures were also found tobe useful to differentiate diploid, tetraploid and hexaploid taxa in the wild barley species, Hordeum murinum (Booth and Richards, 1978). Vardhan et al.(1987) studied the seed protein profiles of diploid and autotetraploid green gram (Vigna radiata L.) and found that diploid and polyploids showed uniform protein pattern and represented a small segment of the genetic variability.

Siddiq et al.(1970) studied the intervarietal relationships of three subspecies of Oryza sativa L., namely "indica", japonica" and "javanica" through the electrophoretic patterns of soluble proteins. They found that "japonica" and "javanica" showed higher percentage of slow mobility proteins than "indica" which suggested that the two were of later origin as compared to "indica". Monteiro et al.(1932) noticed great varietal differences in the protein content of 14 varieties of Indian millet (Setaria italica). However, Bala (1988) observed minor differences in four Indian varieties of Setaria italica.

Nicholson et al.(1985) analysed electrophoretically the caryopsis proteins of Cenchrus pennisetiformis, C. setigerus and 9 cultivars of C. ciliaris which were identified on the basis of differences in fascicle and plant morphology and in the biochemical components of caryopsis. They suggested cathodic gel electrophoresis of the seed proteins as the most successful method for cultivar and species identification. Rao and Sharma (1985) made an assessment of protein spectrum of different genotypes of Hordeum and observed that variation of electrophoretic banding patterns between different cultivars could be utilized as a distinct criterion in the identification of different species or cultivars of Hordeum vulgare.

Burston and Tischler (1980) demonstrated qualitative differences in the electrophoretic patterns of soluble proteins of two cultivars of Bermuda grass. Salinas et al.(1982) compared gliadin protein patterns in hexaploid wheat cultivars. Using both starch and polyacrylamide gels they analysed the 38 cultivars of Triticum aestivum and one of T. spelta.

Ladizinsky and Hymowitz (1979) suggested that morphologically indistinguishable cultivars and species of all common crops could be identified by gel electrophoretic procedures. Bulinska-Rodomska and Lester (1985) observed that seed protein electrophoresis confirmed the existence of polymorphism among hexaploid populations of Festuca arundinacea. Both, protein patterns and morphological results suggested that F. pratensis

and F. arundinacea should retain independent specific status. High protein homology of these two species with F. gigantea pointed towards phlogenetic links between these taxa.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis has been used successfully to identify cultivars of barley and wheat (Shewry et al.,1978a,b) and Evans (1983) recognised the use of SDS PAGE to differentiate Cicer milkwetch (Astragalus cicil L.) cultivars. Barratt (1980) could identify the cultivars of Vicia faba by SDS PAGE of seed globulins. Ferguson and Grabe (1986) identified 28 cultivars of perennial rye grass (Lolium perenne) by presence or absence of specific bands through SDS-PAGE procedures aided by band intensity ratio calculated from densitometer scans. All the cultivars were found to be differentiated by their unique banding pattern. Seed proteins of Agropyron junceum complex was studied by Moustaka et al.(1986) using isoelectric focussing. There was variation only in the intensities of some particular bands.

CHAPTER III

MATERIALS AND METHODS

The three species of Cenchrus L. were collected from plains of Punjab. These were grown under uniform nursery conditions in the botanical garden of the Punjab Agricultural University, Ludhiana. Samples of representative plants from each taxon were selected for morphological anatomical, cytological and biochemical studies.

3.1 Cytological Studies

To record the chromosome numbers in the collected taxa, emerging inflorescences were fixed in Carnoy's fixative (6:3:1, v/v) absolute alcohol: chloroform: glacial acetic acid) for 24 h and then stored in 70 per cent ethanol. Slides were prepared by anther squashes in 2 per cent acetocarmine stain. Slides were made permanent using butanol-acetic acid series and finally mounted in euparal. Pollen fertility was estimated from the ability of pollen to stain with acetocarmine stain. Only well filled dark-staining pollen grains were taken to be fertile.

3.2 Morphological Observations

Ten mature plants were selected to study morphological characters. The vegetative characters studied were: plant height, internode length, number of leaves per culm, leaf blade

length, leaf blade breadth and leaf-sheath length. The floral characters studied were: spike length, spikelet length, outer glume length and inner glume length, lemma length, palea length, anther length, style and stigma length and colour, and fertile pollen size. Camera Lucida drawings for glumes, lemma, palea, style and stigma papillae, anther and fertile pollen were drawn.

3.3 Anatomical Studies

Leaves and culms collected at the same developmental stage were fixed in FAA(1:1:18, v/v Formaldehyde: glacial acetic acid: 70 per cent ethanol) for 48 h. They were then taken out, washed with water and boiled in 2 per cent KOH in order to soften the tissues. The subepidermal tissue was removed with the help of a razor blade. The epidermal peeling, thus, obtained, was mounted in 10 per cent glycerine. Data were collected from such temporary unstained preparations for stomatal size and frequency.

Transverse section of leaf and internode were cut with a sharp razor blade. These sections were stained with safranin-fast green combinations and mounted in DPX mountant.

3.4 Biochemical Determinations

Three to five leaves from top of the erect culms were collected just at the time of emergence of the inflorescence, for biochemical estimations. For electrophoretic studies proteins were extracted from seeds.

3.4.1 Total soluble proteins (Lowry et al.,1951)

Reagents

Reagent A - 2% Na_2CO_3 in 0.1N NaOH

Reagent B - 0.5% $CuSO_4.5H_2O$ in 1% sodium potassium tartarate

Reagent C - Prepared by mixing 50 ml of Reagent 'A' with 1 ml of Reagent 'B' shortly before assay.

Reagent D - Folin-Ciocalteu's (folin phenol) reagent diluted with water in 1:1 ratio.

Extraction

A known amount of fresh leaves were homogenised with 80% acetone to remove chlorophyll and centrifuged at 5,000 g for 15 min. The residue was given washings with 80 per cent acetone and centrifuged. To the residue 5 ml of 15 pef cent trichloroacetic acid (TCA) was added and kept at 4°C for 24 h. The extract was then centrifuged at 5,000g for 15 min and supernatant was discarded. The precipitated proteins in the residue was dissolved in 2 ml of 0.1N NaOH and centrifuged. The process was repeated and final volume of the supernatant made 5 ml with 0.1N NaOH.

Estimation

To 0.1 ml aliquot of protein suspension(in NaOH) 5 ml of reagent 'C' was added in a test tube andallowed to stand for 10 minutes. Then 0.5 ml of reagent 'D' was added followed by immediate stirring. The reaction mixture was kept at room temperature for 30 min for complete development of the colour.

Absorbance was read at 570 nm against the blank prepared by substituting water in place of protein extract.

The protein content (mg/g fresh weight) was calculated from the standard curve made by using different concentrations of bovine serum albumin (BSA).

3.4.2 Estimation of total soluble sugars and free amino acids

Extraction

A known amount of dry leaves was homogenised with hot 80 per cent ethanol. The extract was centrifuged at 3,000g for 15 minutes. Both supernatant as well as the residue were retained. The process was repeated and the last wash was tested by using anthrone reagent until it did not give any positive reaction for soluble sugars. The pooled supernatants (after making a known final volume) were used for the estimation of total soluble sugars and free amino acids.

3.4.2.1 Total soluble sugars

(A) Colorimetric method (Loewus, 1952)

Reagents

(A) .2 per cent anthrone in ethyl acetate

(B) Cold conc. H_2SO_4

Estimation

To 0.2 ml aliquot of the extract prepared under section 3.4.2, 0.5 ml of reagent 'A' was added and the mixture was kept at room temperature for 5 min. 5 ml of reagent 'B' was added

slowly with constant shaking. The tubes were swirrled gently until ethyl acetate had hydrolysed as indicated by the floc of anthrone which appeared. The absorbance of the greenish brown colour so obtained was read at 620 nm against the blank (80 per cent ethanol).

Total soluble sugar content was calculated (mg/g dry weight) from the standard curve prepared by using glucose.

(B) Chromatographic method (Shallenberger and Moores, 1957)

Extraction

A known amount of the dry leaf material was homogenised in hot 70 per cent ethanol and centrifuged at 3,000g for 10 min. This process was repeated three times to ensure complete extraction. The supernatants were pooled and final volume adjusted to 10 ml with 70 per cent ethanol. It was evaporated to dryness in a china dish on a boiling water bath. The residue was eluted with 2 ml of 20 per cent ethanol and again centrifuged. The clear supernatant, so obtained, was stored in a deep freeze for further analysis.

Chromatographic separation

0.3 ml aliquot of the above extract was chromatographed on Whatman No.1 chromatographic paper. The chromatogram was developed in n-butanol: acetic acid glacial: water (4:1:1, v/v) solvent system and then removed from the chamber and air dried at room temperature for 24 hr.

The chromatogram was sprayed with aniline reagent which was prepared by mixing the following solutions (5:5:1, v/v)

i. 4 per cent aniline in n-butanol

ii. 4 per cent diphenylamine in n-butanol

iii. 10 ml conc. ortho-phosphoric acid

The reagent was filtered to get a clear solution. The chromatogram was then air dried at room temperature and later activated at 90°C for 10-15 min. Light brown to dark brown spots were identified with the help of reference spots of known sugars and marked with lead pencil. Each marked spot was cut into small pieces and eluted in 5 ml of 20 per cent ethanol for 1-2 h. To 0.2 ml of aliquot 1 ml of 5 per cent phenol and 4 ml of conc. H_2SO_4 were added slowly with constant shaking. Absorbance was read at 480 nm against the blank as 70 per cent ethanol.

The content of different sugars was calculated (mg/g dry weight) from the standard curve prepared by using reference spots of authentic sugars (50-200 µg).

3.4.2.2 Free amino acids

(A) Colorimetric method (Lee and Takahashi, 1966)

Ninhydrin reagent

It wasprepared by mixing three solutions A,B and C in the ratio of 5:12:2 and pH adjusted to 6.0 with sodium citrate.

Solution A - 1 per cent ninhydrin in 0.5M citrate buffer (pH 5.4)

Solution B - pure glycerol

Solution C - 0.5M citrate buffer (pH 5.5)

Estimation

0.2 ml of aliquot was taken from the ethanol extract prepared under section 3.4.2. To it 3.8 ml of ninhydrin reagent was added and thecontents were shaken vigorously and heated in boiling water bath for 12 min. The tubes were cooled under running water to room temperature. The absorbance of blue colour was read at 570 nm against the blank prepared by replacing 80 per cent ethanol in place of the ethanol extract.

The free amino acid content (mg/g dry weight) was calculated from the standard curve prepared by using glycine.

(B) Chromatographic method (Consden et al.,1944)

The extract prepared for the chromatographic analysis of soluble sugars was used for the determination of individual free amino acids.

Chromatographic separation

0.2 ml of the extract was chromatographed on Whatman No.1 chromatographic paper. The chromatogram was run in the first solvent system consisting of n-butanol: acetic acid glacial: water (4:1:1, v/v) and removed from the chamber when the solvent front had reached the 5 cm mark at the other end, and then

dried overnight at room temperature. The chromatogram was turned at right angle and run in second solvent system comprising phenol: water: ammonia (80:20:1 w/v/v). It was then removed from the chamber and dried at room temperature overnight. The chromatogram was sprayed uniformly with 2 per cent ninhydrin in n-butanol and dried at room temperature, and later activated at 80°C for 10 min. Different amino acids were identified from their specific Rf values and by comparing with the standard ones. Spots were cut into small pieces and eluted in 5 ml of 70 per cent ethanol. Absorbance of the light blue colour so obtained was recorded at 570 nm against the blank as 70 per cent ethanol.

Individual amino acid content (mg/g free amino acids) was calculated from the standard curve prepared by using reference spots of glycine and proline with different concentrations.

3.4.3 Ascorbic acid (Aberg, 1958)

Extraction

A known amount of fresh leaves were cut into small pieces and homogenised thoroughly in a mortar and pestle with 0.4 per cent oxalic acid and acid washed sand at the rate of 5 ml/g tissue. Filtered through two layers of cheese cloth and centrifuged the filterate at 1,000 g for 20 min. The final volume made to 6 ml with 0.4 per cent oxalic acid.

Estimation

Ascorbic acid was estimated by visual titration method

based on the reduction of 2,6-dichlorophenol indophenol dye (DCPIP). Following solutions were recuired for its estimation.

Ascorbic acid standard solution: 0.2 per cent of ascorbic acid solution was prepared in 0.4 per cent oxalic acid to represent 0.2 mg/ml of ascorbic acid.

Indophenol solution: To 150 ml of water added 50 mg of sodium 2,6-dichlorophenol indophenol and heated in a boiling water bath to dissolve the dye. Added 42 mg of $NaHCO_3$. Cooled the solution to room temperature and final volume made to 200 ml with water and stored in refrigerator.

Standardization: The indophenol reagent was standarised before use. For this, 5 ml of the standard ascorbic acid solution was taken in a titration flask and titrated against the indophenol dye, until the solution became pink.

Estimation of the unknown: Pipetted out 5 ml of oxalic acid extract in a titration flask and titrated against the standardised indophenol reagent until the solution became pink.

Ascorbic acid content in the extract was calculated by using the formula:

$$\frac{I \times S \times D}{A \times W} \times 100 = \text{mg ascorbic acid/100 g tissue}$$

where:

I = ml of indophenol used in titration
S = mg of ascorbic acid reacting with 1 ml of reagent
D = Volume of the extract in ml
A = aliquot titrated in ml
W = weight of the sample in g

3.4.4 Flavonoids Determination

(A) Total flavonoids (Balboa et al., 1974)

Extraction

A known amount of the dry leaf material was homogenised in petroleum ether to remove the chlorophyll and a number of other non-flavonoid constituents. The residue was extracted in 5 ml of 1 per cent HCl in methanol and centrifuged at 1,500g for 10 min. The procedure was repeated. The supernatants were pooled and evaporated to dryness on a boiling water bath. The dried residue was dissolved in 5 ml of water and centrifuged at 2,000g for 10 min to obtain a clear extract. The final volume was made to 10 ml with water.

Estimation

Reagent : Aluminium chloride (anhydrous) 0.1M aqueous solution

Out of the above extract 5 ml was taken and evaporated to dryness at 60°C. To it, added 10 ml of aqueous aluminium chloride solution and centrifuged at 3,000g for 15 min. The optical density of the clear extract so obtained was recorded at 420 nm against the blank as aqueous aluminium chloride solution.

Flavonoids were determined (mg/g dry weight) from a standard curve prepared by using different concentrations of rutin.

(B) Chromatographic method

Extraction

Dried leaf material (2 g) which had been finely ground

in a pestle-mortar, was extracted at room temperature with cold 95 per cent methanol. The extraction was carried out for three days. The residual plant material was removed by centrifugation at 5000 rpm for 10 minutes. The supernatant was evaporated to dryness on a boiling water bath. The sticky green residue was dissolved in 1 ml of methanol, centrifuged and kept in a refrigerator for further use.

Chromatographic separation

Flavonoids were separated using two dimensional descending paper chromatography. 0.2 ml of the extract was spotted on Whatman No.1 chromatographic paper with the help of a micropipette. The spots were dried and run in two solvents:

i) n-butanol: acetic acid: water: 4:1:1, v/v

ii) 15 per cent acetic acid

After developing with the Ist solvent, the chromatograms were dried and then run in the second solvent. The chromatograms were dried at room temperature. Tentative identification of flavonoids was made by noting the colour reactions with ammonia, ammonia + UV light and UV light only. Based on the colour reactions and Rf values of the spots, individual groups of flavonoids were identified.

All spots which were detected by this procedure were circled with a lead pencil and chromatograms were treated with the spray reagent. 20 per cent (w/v) sodium carbonate solution

was sprayed uniformly on the papers. The flavonoids gave fluorescence when seen under UVlight and the intensity of the flavonoids spots was increased after spraying.

3.4.5 Polyacrylamide gel electrophoresis (PAGE) (Davis, 1964)

PAGE was employed for soluble proteins

Stock solutions

(a) Running Gel/Lower Gel

Solution A : 36.600 g of Tris (tris hydroxy methyl amino methane) and 0.32 ml of TEMED (N',N', N', N'-Tetramethyl ethylene) were dissolved in 48 ml of 1N HCl and total volume made to 100 ml with glass distilled water. The pH of the solution was adjusted at 8.9. The solution was filtered and stored in refrigerator.

Solution B : 28 g of acrylamide and 0.735 g of N',N'-Methylene bisacrylamide were dissolved in 100 ml of distilled water. Filtered and stored in refrigerator.

Solution C : This solution was prepared fresh. 0.14 g of ammonium persulphate in 100 ml of water.

(b) Stacking Gel

Solution D : This solution was prepared fresh. 0.56 g of ammonium persulphate dissolved in 100 ml of water.

Solution E : 5.98 g of Tris and 0.48 ml of TEMED were dissolved in 48 ml of 1N HCl and total volume made 100 ml with water. Filtered and stored in refrigerator.

Solution F : 10.5 g acrylamide and 2.5 g of N',N'-methylene bisacrylamide were dissolved in 100 ml of water, filtered and stored in refrigerator.

(c) Tris-Glycine Buffer (pH 8.3)

6 g of Tris and 28.800 g of glycine were dissolved in 1000 ml of water.

(d) Preparation of Bromophenol blue solution

This was prepared by dissolving 10 mg of Bromophenol blue in 1 litre of 0.05M phosphate buffer.

Extraction of soluble proteins

Seed extracts were prepared by grinding weighed samples, using an ice-cold mortar and pestle in a medium containing 0.062M Tris-HCl buffer (pH 7.6), 5 mM 2-mercaptoethanol, 4 per cent polyvinylpyrrollidone (PVP) and 5 mM ethylene-diamine tetra-acetate (EDTA) for solubilizing proteins. The tissue to buffer ratio was 1:2.5 (g/ml, w/v). All operations were carried out at 4°C. Cell suspension paste was filtered through two layers of muslin cloth and centrifuged at 15,000 g for 20 min at 0°C. The supernatant, so obtained, was used for protein analysis. Protein concentration was determined by the method of Lowry et al.(1951).

Preparation of sample

Prior to electrophoresis samples were taken in test tubes and to each tube 1-2 drops of 40 per cent sucrose and 10 ul of 0.002 per cent bromophenol blue solution were added. The latter

acted as a tracking dye.

Preparation of gels

Stock solutions were removed from refrigerator and permitted to warm at room temperature before use.

Running gel (10% Acrylamide, small pore gel)

Three solutions A, B and C were mixed in the proportion of 1:1:2 by volume respectively. The mixture thus obtained was then poured into 10 cm long corning gel tubes with the help of glass dropper. After filling the tubes upto 7.5cm mark, the mixture was carefully overlaid with a few drops of water to ensure a flat miniscus. After polymerization of the gels (20-30 min), water was removed and stacking gel mixture (about 1 cm length) was overlaid.

Stacking gel

Solutions D, E and F were mixed in the proportion of 1:1:2 by volume respectively. The stacking gel was then overlaid very carefully with few drops of water to ensure a flat miniscus. After polymerization (15-20 min) water was removed and gel surface was rinsed with electrophoresis buffer.

Sample loading and electrophoresis

The sample 50 µl (150 µg protein) was carefully loaded on to the gel surface using a micropipette. Electrophoresis was carried out after loading the sample. 2 mA current was applied per rod gel until the sample entered the resolving gel

and raised to 3 mA constant current per rod gel until the tacking dye reached close to the bottom of the gel. Subsequently, the gel tubes were taken out from the apparatus and emptied of gel columns. The columns were removed pouring water along inner wall of the tubes by means of a hypodermic syringe.

Staining of proteins

Gels were stained for 2 h in 0.1 per cent amido black in 7 per cent glacial acetic acid and were destained in 7 per cent acetic acid glacial.

Analysis of gels

For visual detection of bands, the gel column were placed on white tracing paper and brightly illuminated from the opposite side, by tube fitted X-Ray viewing screen. Position of tracking dye and individual bands was marked on the paper and tracings, thus, completed with respect to the marker dye front was calculated, as the ratio of the distance travelled by a band to the distance travelled by the marker on the gel column. Size and staining characteristics of individual bands were recorded and the band was classified accordingly. Various categories thus raised are represented. In order to facilitate inter-taxa comparisons, representations of all the gels columns were made to a common scale of 8 cms. Characteristics of size, staining and Rm of the individual bands were utilized in these comparisons. Bands displaying identical characteristics

have been numbered alike and are taken to represent identical chemical species. Broad and intensely differentiated bands were taken to indicate a greater quantity of concerned chemical species whereas narrow and faint bands indicated low quantity.

CHAPTER IV

RESULTS

The results are presented under the following headings:

I. Cytological Observations

II. Observations on Morphological Characteristics

III. Observations on Anatomical Characteristics

IV. Observations on Biochemical Parameters

I. Cytological Observations

Two morphotypes were collected in each of the three species. Whereas the two morphotypes (C-I, C-II) in *C. ciliaris* turned to be cytotypes with 2n=32 and 36, the morphotypes of *C. setigerus* (S-I and S-II) and *C. pennisetiformis* (P-I and P-II) exhibited the same chromosome number of 2n=34 and 36 respectively. The data regarding the chromosome number and percentage of pollen fertility in respect of different taxa is presented in Table 1, Plate 1; Figs. 1-5, Plate 2; Fig.6. In all these taxa, in addition to bivalents, a varying number of quadrivalents, octavalents were often observed. At Anaphase I and Anaphase II laggards were quite common. These multivalents and laggards affected the pollen fertility adversely which was 60-65 per cent in *C. setigerus*, 55-65 per cent in *C. pennisetiformis* and comparatively still lower in *C. ciliaris* (40-45%). The fertile

Plate 1. Figs. 1-5. Meiotic stages of different taxa of three species, C.ciliaris (C-I), C. setigerus (S-I, S-II) and C. pennisetiformis (P-I, P-II)

Fig.1. C-I cytotype, 2n=32 at Metaphase-I

Fig.2. S-I form, 2n= 34 at Anaphase-I

Fig.3. S-II form, 2n=34 at Diakinesis

Fig.4. P-I form, 2n=36 at Anaphase-I

Fig.5. P-II form, 2n=36 at Anaphase-I

Plate 1

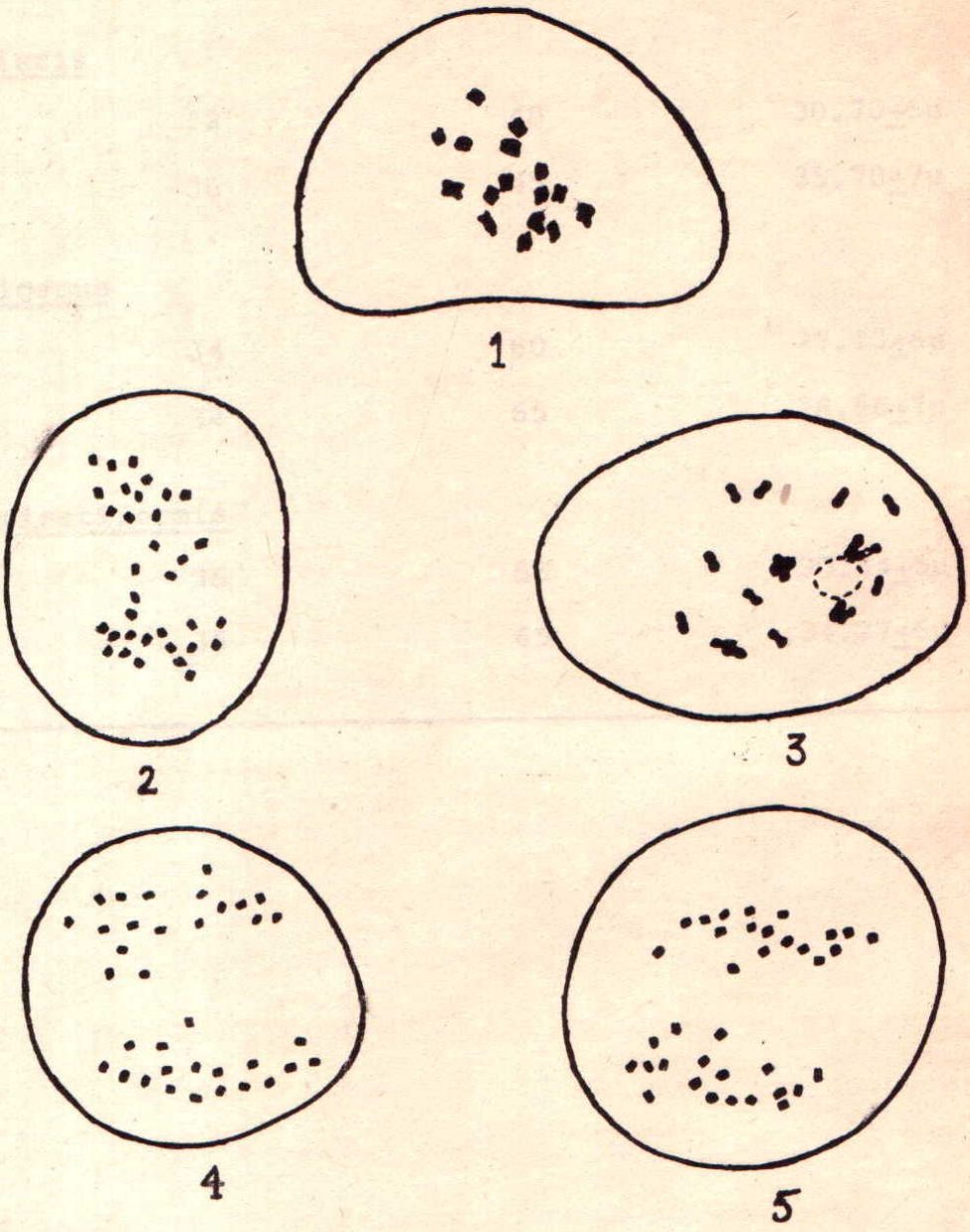


Table 1. Chromosome number, pollen fertility and pollen size in different taxa of three species of Cenchrus L.

Taxa	Chromosome number (2n)	Pollen fertility (%)	Fertile pollen size (µm)
C. ciliaris			
C-I	32	40	30.70±5µ
C-II	36	45	35.70±7µ
C. setigerus			
S-I	34	60	27.13±6µ
S-II	34	65	28.56±7µ
C. pennisetiformis			
P-I	36	55	30.34±5µ
P-II	36	65	34.27±6µ

pollen size in the two cytotypes of C. ciliaris and C. pennisetiformis varied considerably being larger in C-II and P-II forms but in case of C. setigerus there was not any significant difference.

II. Observations on Morphological Characteristics

The data regarding the morphological traits of the six taxa belonging to three species are presented in Tables 2,3 and 4, Plates 2 and 3; Figs.7-9 and Plate 4.

Cenchrus ciliaris: The C-II form was much robust as compared to C-I form. The internodal length, leaf blade length and width, leaf sheath length and number of leaves per culm were observed to be of higher magnitude in C-II form. Inflorescence was cylindric and 3.5-5.6 cms in C-I form and 7-11 cms in length in C-II form. Outer involucral bristles, that were purple in colour lent purple tinge to the overall inflorescence. Inner involucral bristles were 2-3 times as long as spikelet and were connate at the base only. One inner involucral bristle was quite conspicuous being much longer than the rest. Spikelets that were usually 3 or rarely 1 in each involucre, varied in length in both the forms and were yellow/straw coloured. Lower glume was very small, 1-nerved and hyaline. Upper glume was comparatively very large. Lower lemma was sterile. Upper lemma and upper glume were similar in appearance. Anther length, style and stigma length were observed to be highly variable in the two forms.

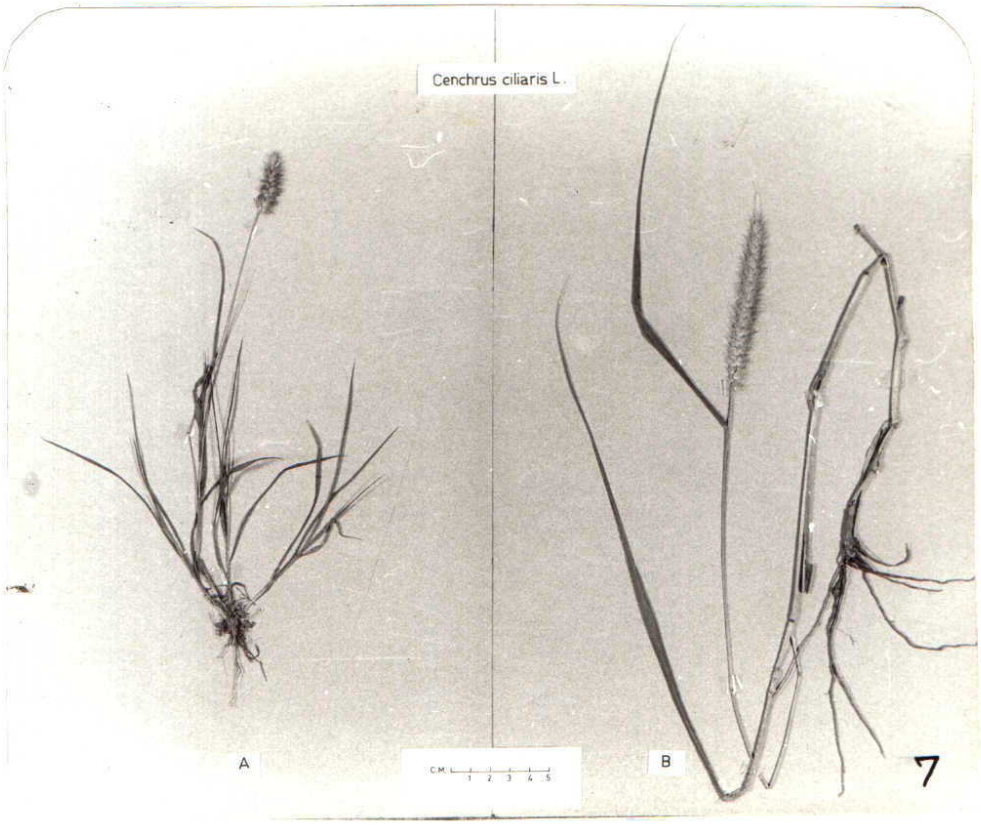
Plate 2. Figs. 6,7

Fig.7A. Cytotype C-I

Fig.7B. Cytotype C-II

Fig.6. C-II cytotype, 2n=36 at Anaphase-I showing a laggard.

PLATE 2



Cenchrus setigerus: The height of S-I form varied from 20-40 cms and in S-II form it ranged between 50-90 cms. Internode length was observed to be related with the height of plant. Leaf blade length and width, leaf sheath length and number of leaves per culm were more in S-II form as compared to the S-I form. Spikes were purple in colour in mature plants. However, in young plants these varied from green to straw coloured. Involucral bristles were erect, stiff and were joined at the base to form a cup-shaped structure. The length of involucre was small as the large involucral bristles were lacking. The spikelets were usually 3 in each involucre. Length of spikelet, glumes, lemma and palea was observed to be less in S-I form as compared to the S-II form. Spikelets were purplish yellow.

Cenchrus pennisetiformis: The height of P-I form ranges from 15-40 cms and P-II form varies from 50-80 cms. Internode length, leaves per culm, leaf blade length, leaf blade width and leaf sheath length were found to be of lower magnitude in P-I form as compared to P-II form. Spikes of P-I form were very small varying from 3-5 cms. Whereas those of P-II form varied from 9-11 cms. Spikes were purplish black in colour. Inner involucral bristles joined upto 3 mm to form a cup shaped structure. Outer involucral bristles were very large. Length of spikelet, glumes, lemma and palea was observed to be less in P-I form as compared to P-II form.

Plate 3. Figs. 8,9

Fig.8 C. setigerus, A, S-I form; B, S-II form

Fig.9 C. pennisetiformis, A, P-I form; B, P-II form

PLATE 3

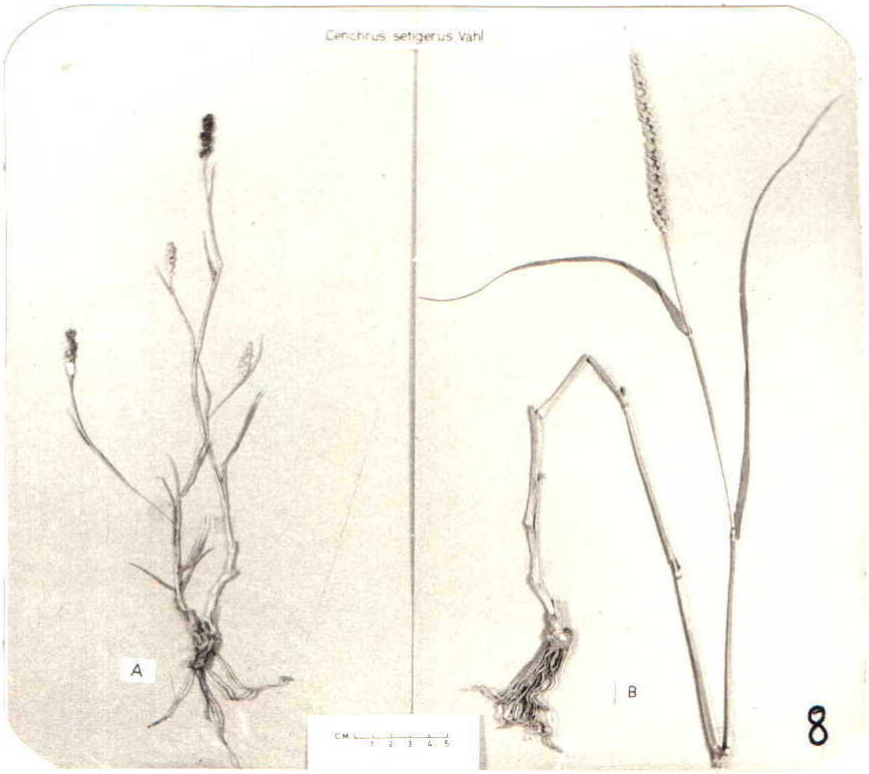


Plate 4.

Spikelet morphology of different taxa of three species, *C. ciliaris* (C-I, C-II); *C. setigerus* (S-I, S-II); *C. pennisetiformis* (P-I, P-II)

Ogl = Outer glume
Igl = Inner glume
Olem = Outer lemma
Ilem = Inner lemma
Pal = Palea
OV = Ovule
Sg = Stigma
St = Style
An = Anther
Pn = Pollen

PLATE 4

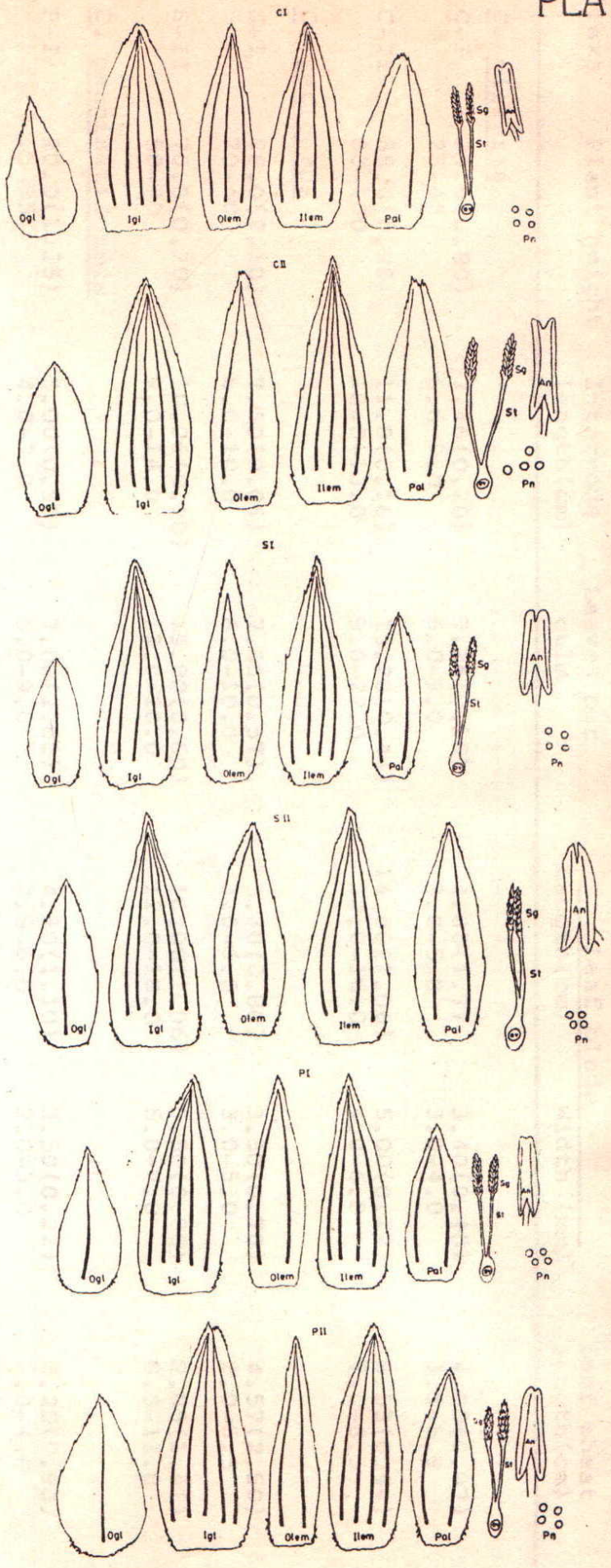


Table 2. A comparison of some of the morphological characters (vegetative traits) in taxa of different species of genus Cenchrus L.

Taxa	Plant height	Internode length(cm)	Leaves per culm	Leaf blade		Leaf sheat length(cm)
				Length(cm)	Width (mm)	
C. ciliaris						
C-I	33.8(0.80) 25-40	7.46(0.20) 6.0-9.0	7.26(0.50) 6.0-9.0	4.00(1.7) 3.0-5.0	3.40(0.40) 3.0-4.0	4.00(0.07) 3.5-5.9
C-II	85.0(0.90) 60-100	11.8(0.16) 10.0-13.0	15.20(0.44) 9.0-22.0	14.80(1.06) 12.0-16.0	5.00(0.22) 4.0-6.0	8.53(0.44) 7.0-8.5
C. setigerus						
S-I	28.0(2.10) 20-40	7.50(0.06) 6.0-10.0	7.66(0.57) 6.0-10.0	5.30(0.80) 3.0-6.0	3.50(0.70) 3.0-5.0	4.57(2.50) 2.0-6.5
S-II	70.0(0.20) 50-90	10.06(0.20) 9.0-14.0	15.80(0.60) 12.0-20.0	16.00(1.06) 12.0-18.0	5.50(0.80) 5.0-7.0	8.85(1.41) 8.5-11.0
C. pennisetiformis						
P-I	20.0(1.25) 15-35	5.00(0.50) 4.0-7.0	7.00(2.80) 5.0-9.0	4.50(1.10) 2.5-6.0	2.50(0.22) 2.0-3.0	3.20(0.90) 2.0-4.0
PII	65.0(2.50) 50-80	9.23(0.10) 8.0-12.0	14.50(0.10) 8.0-20.0	12.00(1.20) 8.0-14.0	4.00(0.90) 4.0-6.0	7.75(1.00) 7.5-10.5

Values shown are mean(standard deviation) range of 10 measurements.

Table 3. A comparison of some floral traits in taxa of different species of genus Cenchrus L.

Taxa	Spike		Involucre length(mm)	Spikelet		Length of involucralglume	
	Length(cm)	Colour		Length(mm)	Colour	Lower(mm)	Upper (mm)
C. ciliaris							
G-I	4.85(0.12) 3.5-5.6	Purplish yellow	10.00(0.24) 7.0-12.0	3.50(0.47) 3.0-3.8	Yellow	2.43(0.20) 2.0-2.4	3.64(0.30) 3.2-3.8
C-II	9.05(0.15) 7.0-11.0	Purplish yellow	12.80(0.08) 11.0-15.0	4.00(0.37) 3.5-4.0	Yellow	2.65(0.09) 2.5-2.8	3.92(0.09) 3.8-4.0
C. setigerus							
S-I	4.47(0.40) 2.5-5.0	Purple	3.50(0.10) 3.0-4.0	3.60(0.23) 3.4-4.0	Purplish yellow	1.86(0.13) 1.7-2.0	3.45(0.28) 3.3-3.8
S-II	10.70(0.20) 8.0-14.5	Purple	5.00(0.00) 4.0-6.0	3.75(0.38) 3.5-4.5	Purplish yellow	2.30(0.23) 2.0-2.5	3.70(0.26) 3.5-4.0
C. pennisetiformis							
P-I	3.50(0.22) 3.0-5.0	Purplish black	8.67(0.12) 7.0-10.0	3.34(0.25) 3.5-4.0	Purple	2.38(0.07) 2.2-2.5	3.30(0.15) 3.0-3.5
P-II	10.00(0.25) 9.0-11.0	Purplish black	9.5(0.00) 8.0-12.0	3.83(0.37) 3.7-4.5	Purple	2.68(0.09) 2.5-2.8	3.8(0.18) 3.7-4.0

Values shown are mean (standard deviation) of 10 measurements.

Table 4. Comparison of some floral traits in taxa of different species of *Cenchrus* L. (continued from Table 3)

Taxa	Length of lemma		Length of palea of lower lemma(mm)	Anther length (mm)	Style and stigma length (mm)
	Lower (mm)	Upper (mm)			
C. ciliaris					
C-I	3.42(0.05) 3.4-3.5	3.68(0.21) 3.5-4.0	3.42(0.10) 3.3-3.5	2.55(0.48) 2.0-3.0	3.92(0.25) 3.2-4.6
C-II	3.85(0.14) 3.7-4.0	4.35(0.18) 4.2-4.5	3.82(0.18) 3.6-4.0	3.27(0.28) 3.0-3.5	4.56(0.38) 3.3-5.0
C. setigerus					
S-I	2.98(0.09) 2.8-3.0	3.6(0.16) 3.5-3.8	3.0(0.30) 2.8-3.5	2.60(0.43) 2.0-3.0	3.80(0.16) 3.5-4.0
S-II	3.85(0.23) 3.5-4.0	4.29(0.24) 4.0-4.5	3.80(0.21) 3.5-4.0	3.20(0.41) 2.5-3.5	4.35(0.36) 3.8-5.0
C. pennisetiformis					
P-I	3.33(0.25) 3.0-3.5	3.84(0.28) 3.57-4.0	3.12(0.38) 2.5-3.5	2.50(0.46) 2.0-3.0	3.46(0.22) 3.0-4.0
P-II	3.43(0.10) 3.3-3.5	3.89(0.13) 3.7-4.0	3.56(0.36) 2 9-4.0	3.22(0.42) 3.0-3.5	4.20(0.42) 3.5-5.0

Values shown are mean (standard deviation) rangeof 10 measurements.

III. Observations on Anatomical Characteristics

a. Leaf stomatal frequency per unit area and stomatal size is presented in Table 5. In all the taxa stomatal frequency per unit area was more on the abaxial as compared to adaxial side. Maximum stomatal frequency was observed in the C-I form of C. ciliaris and minimum was found in the P-II form of C. pennisetiformis. The frequency was observed to be more in C-I S-I and P-I forms as compared to C-II, S-II and P-II forms respectively. The reverse was true of stomatal size. The C-I, S-I and P-I forms had smaller stomata compared to C-II, S-II and P-II forms respectively.

b. Transverse section of leaf (Plates 5,6 and 7)

Typically the leaf anatomy exhibited following characters in the three species:

Upper surface of leaf showed prominent ridges and furrows. On the lower side were present ribs that were not so distinctive. Some cells of the epidermis were extended as spiny projections. In the furrowed region thin walled, large bulliform cells were noticed. A gradation between vascular bundle size was quite evident and based on this criterion they could be classified into four types. Large vascular bundles were of basic type with well developed xylem and phloem tissue. Sclerenchymatous girdles were observed on both abaxial and adaxial side of these. The two types of intermediate sized vascular bundles had poorly developed xylem and phloem tissues and were

Table 5. A comparison of stomatal frequency and stomatal size in taxa of different species of Cenchrus L.

Taxa	Stomatal frequency per mm^2		Stomatal size (µm)
	Abaxial	Adaxial	
C. ciliaris			
C-I	59.86(0.46) 58-62	27.53(0.06) 25-30	28.50x14.25
C-II	49.67(0.01) 45-53	23.46(0.01) 22-26	33.25x16.15
C. setigerus			
S-I	52.27(2.32) 51-55	28.90(0.02) 30-34	29.45x15.20
S-II	43.66(0.30) 42-47	26.80(0.63) 24-28	33.82x17.57
C. pennisetiformis			
P-I	45.20(0.02) 44-47	24.90(0.30) 22-26	28.97x14.72
P-II	40.40(0.00) 39-42	22.40(0.14) 20-25	33.63x16.53

accompanied by sclerenchyma only on the abaxial side. However, the fourth type consisting of very small bundles were not accompanied by sclerenchyma on any side. The keel was moderately conspicuous containing one large vascular bundle accompanied by some small bundles on both the sides. Each vascular bundle was surrounded by a sclerenchymatous bundle sheath, which was surrounded by another bundle sheath of thin walled parenchymatous cells possessing chlorophyll. Mesophyll cells were radiating from all around the vascular bundles. In C. ciliaris, sclerenchymatous girdles on the abaxial side connect epidermis to bundle sheath. However, in C. setigerus and C. pennisetiformis, two to three layers of large parenchymatous cells separated the bundle sheaths from the sclerenchymatous girdles. Leaf of C. pennisetiformis showed quite prominent ridges and furrows which were quite conspicuous in P-I form. The cytotypes of C. ciliaris and morphotypes of C. setigerus did not reveal any significant departures from the typical trend.

c. Culm structure (Plate 8; Figs.10-15)

Transverse section of internodal portion at the same developmental stage in different taxa exhibited following characters:

The stem was observed to be flattened on one side and convexly curved on the other side in all the taxa. Epidermal cells were covered over by cuticle. The cortical portion was demarcated into outer and inner cortex by the intervening zone

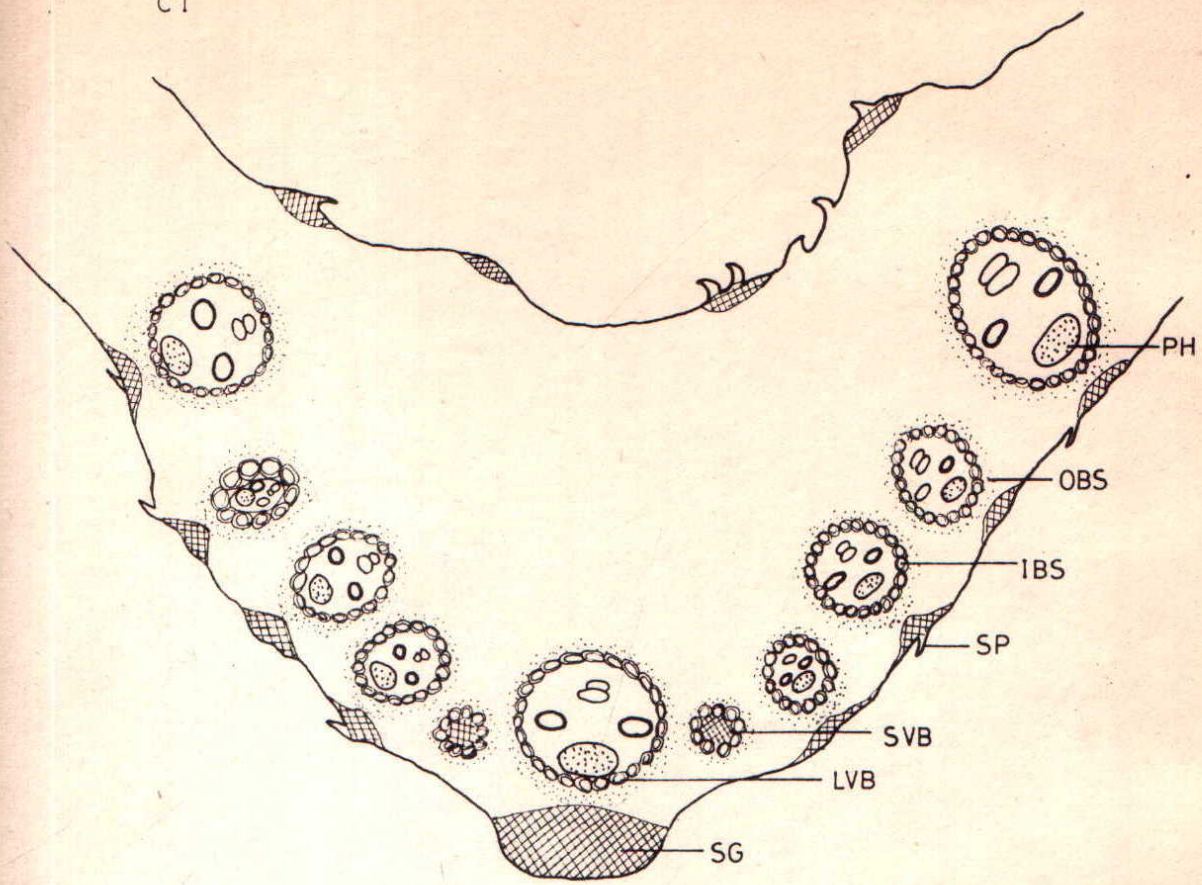
Plate 5. Camera lucida drawings of the leaf anatomy of *C. ciliaris* (C-I, C-II)

Abbreviations; Plate 5, 6 and 7

EP = Epidermis
SP = Spiny projections
BC = Bulliform cells
SG = Sclerenchymatous girdles
SVB = Small vascular bundle
LVB = Large vascular bundle
OBS = Outer bundle sheath
IBS = Inner bundle sheath
PH = Phloem
MXV = Metaxylem vessel

PLATE 5

C I



C II

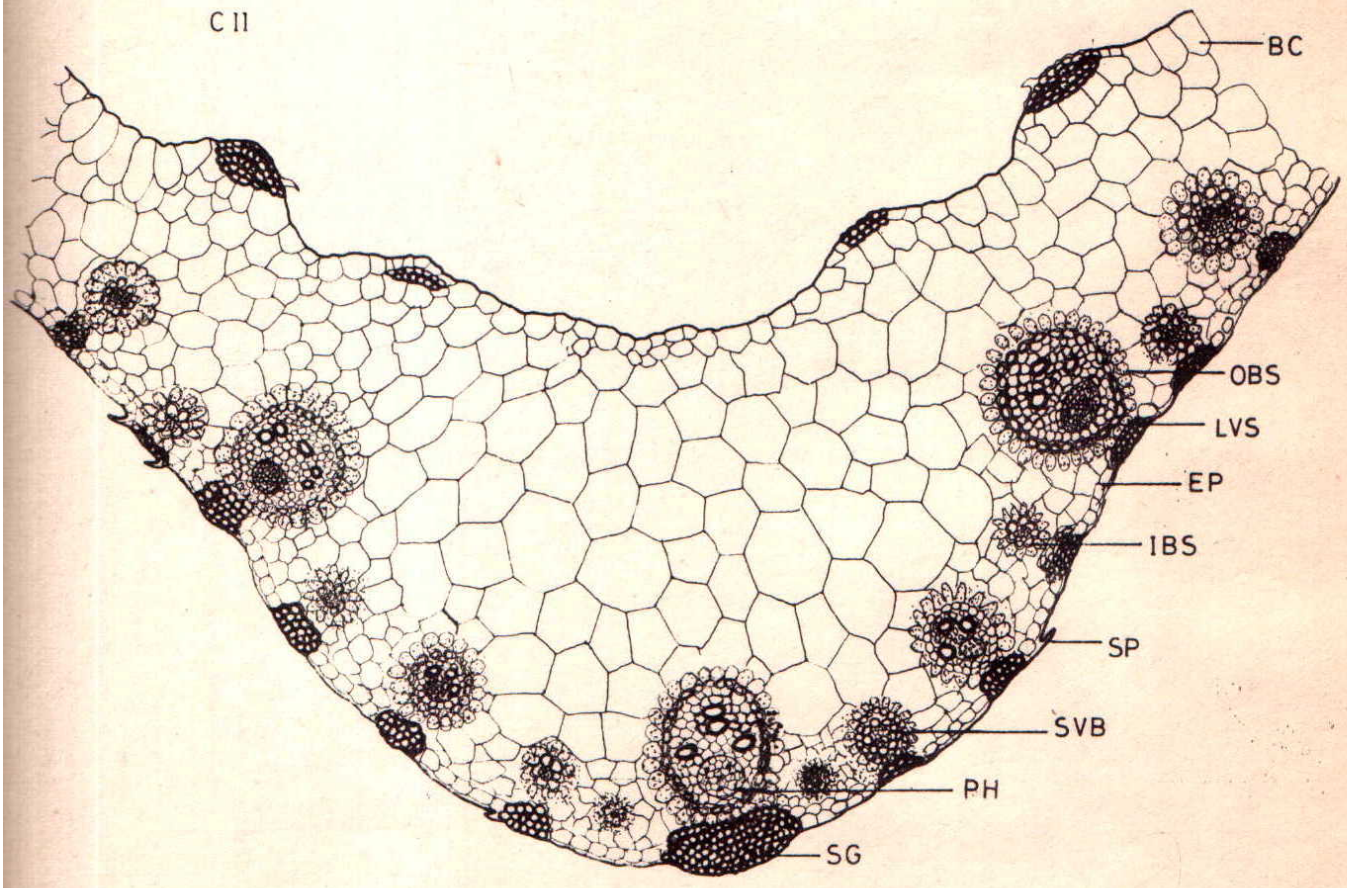


Plate 6. Camera lucida drawings of the leaf anatomy of *C. setigerus* (S-I, S-II)

PLATE 6

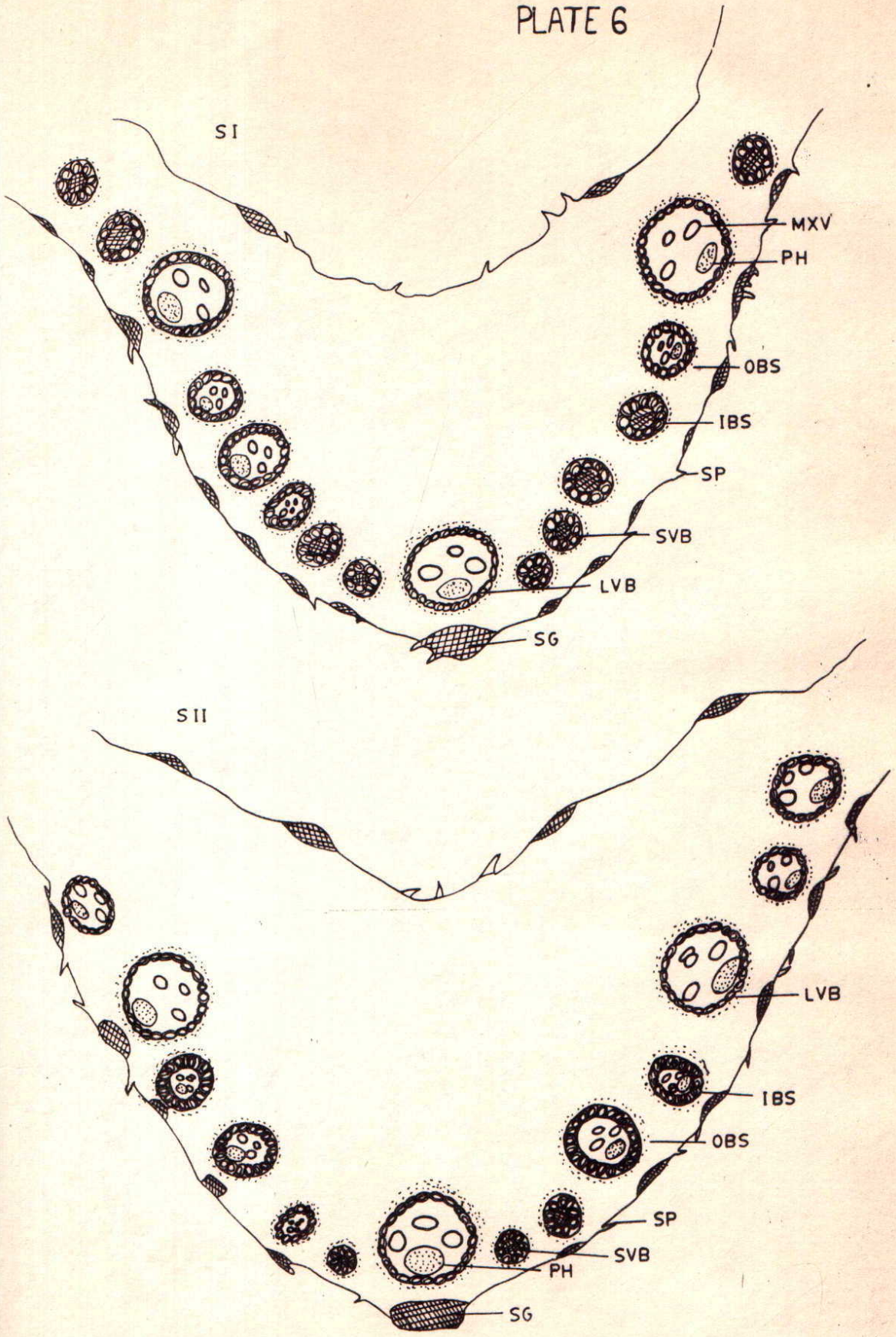
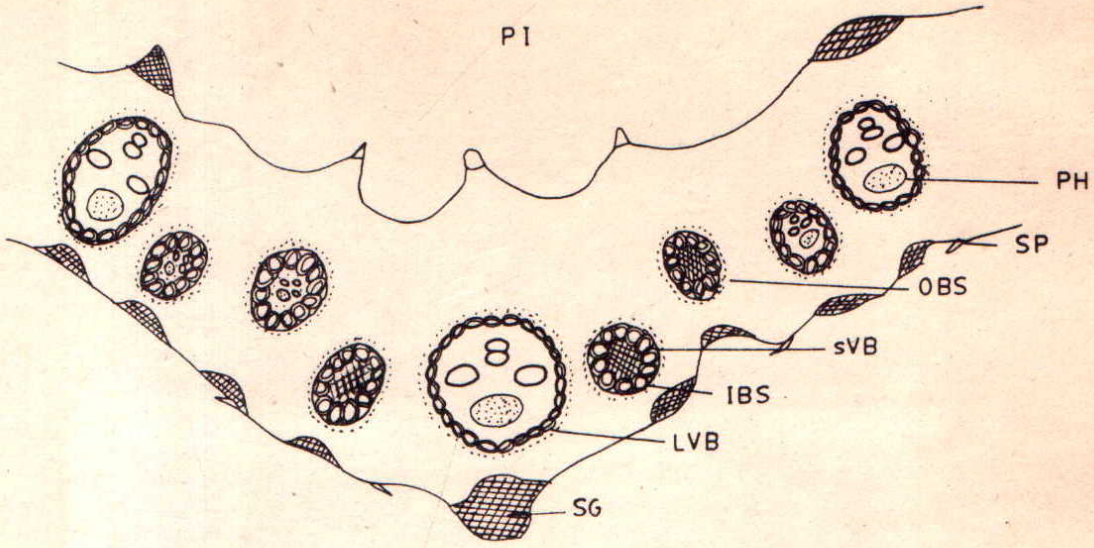


Plate 7. Camera lucida drawings of the leaf anatomy of C. pennisetiformis (P-I, P-II)

PLATE 7



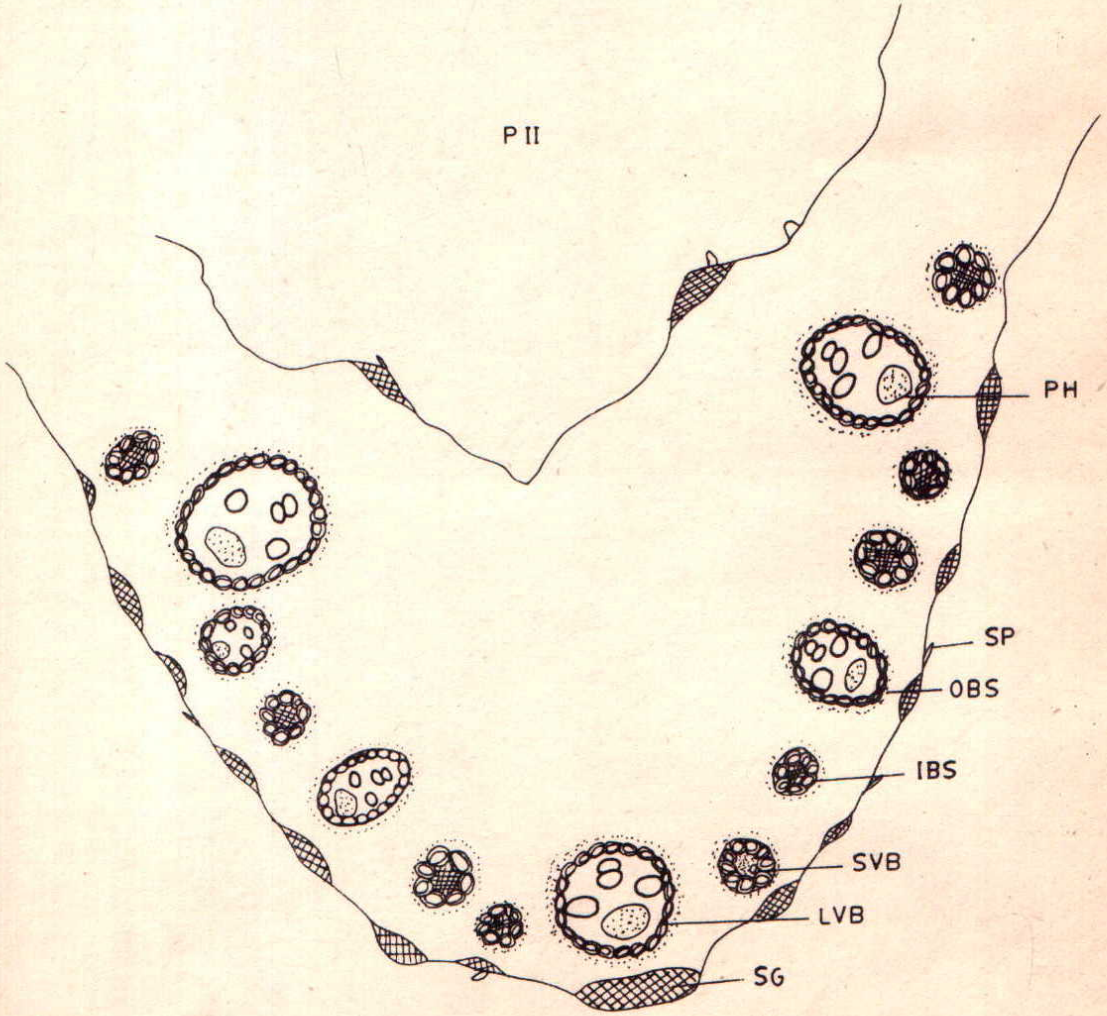


Plate 8. Figs. 10-15

Culm anatomy in T.S. of different taxa of three species, C. ciliaris (C-I, C-II), C. setigerus, (S-I, S-II) and C. pennisetiformis (P-I, P-II)

Fig.10 C-I cytotype

Fig.11 C-II cytotype

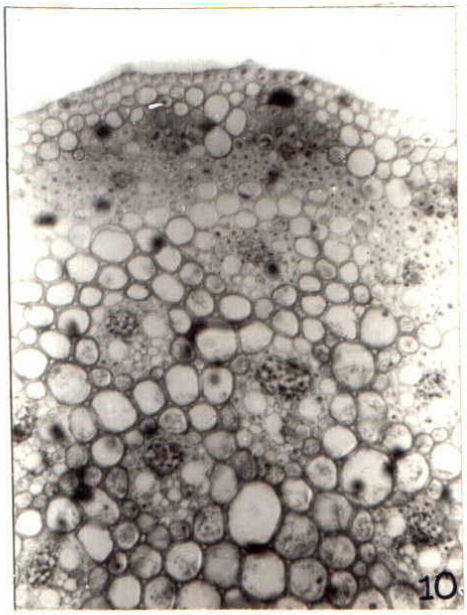
Fig.12 S-I form

Fig.13 S-II form

Fig.14 P-I form

Fig.15 P-II form

PLATE 8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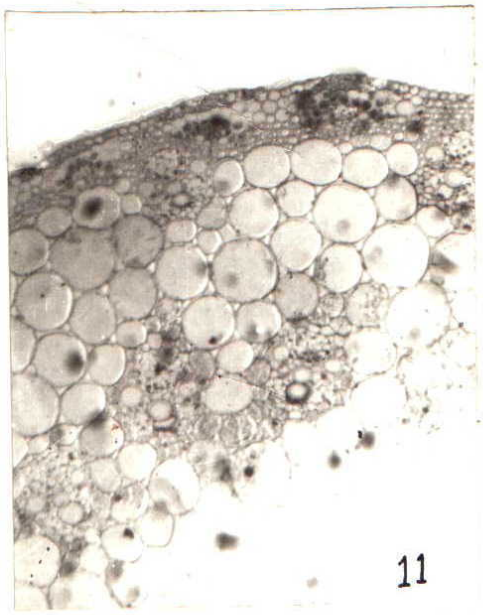
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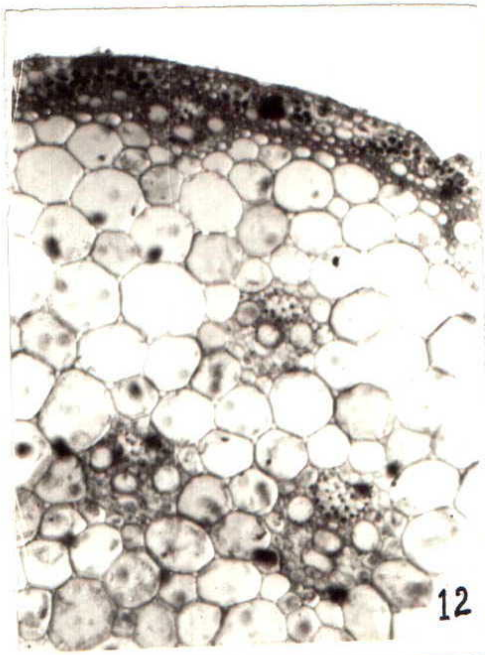
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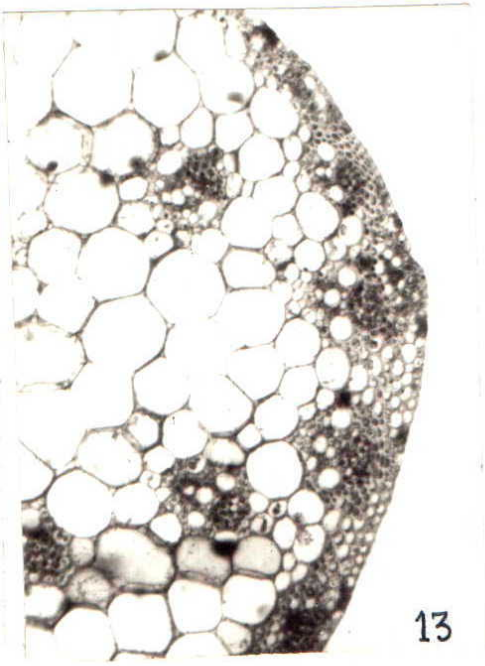
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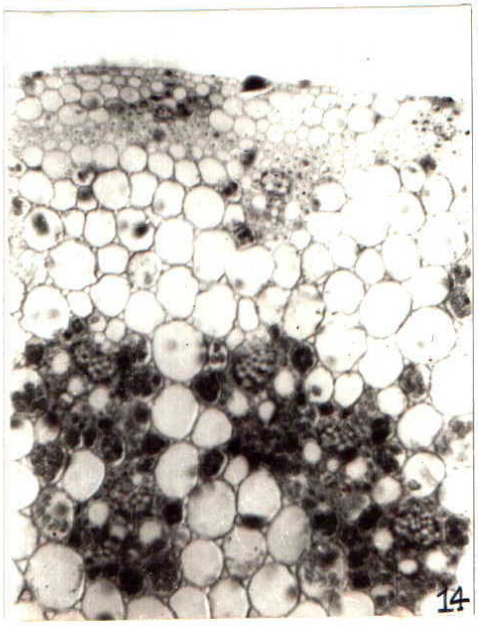
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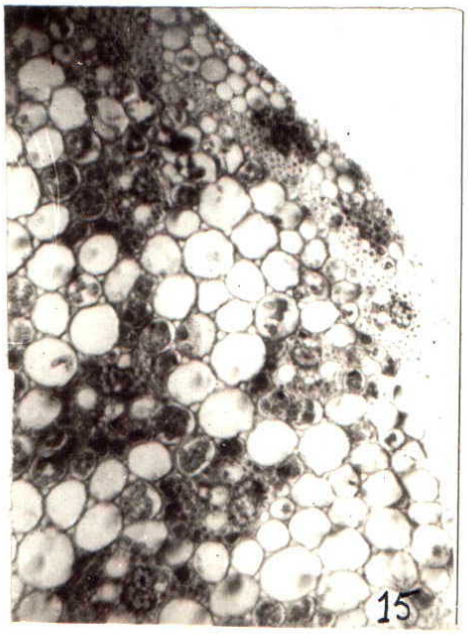
15

of mechanical tissue. This distinction, however, was much obscured in C. setigerus where outer cortex was not so well developed. The vascular bundles located in the outer cortex were small and lacked well developed xylem and phloem tissue. These vascular bundles were surrounded wholly by radiate chlorenchyma. Vascular bundles of the ground tissue were round and larger in size. They were observed in three ill-defined circles, thus presenting a somewhat scattered distribution. In cytotypes of C. ciliaris, the cortical region was densely packed by vascular bundles. The vascular bundles were sparsely arranged in C. setigerus while an intermediate condition prevailed in morphotypes of C. pennisetiformis.

IV. Observations on Biochemical Parameters

a. Total soluble proteins

The two cytotypes of C. ciliaris and morphotypes belonging to C. setigerus and C. pennisetiformis differed significantly from each other. The C-II form of C. ciliaris, S-II form of C. setigerus and P-II form of C. pennisetiformis were comparatively richer than their counterparts of the same species. Maximum amount of soluble protein was noticed in S-II form of C. setigerus while C-I formof C. ciliaris showed the minimum levels of these.

b. Total free amino acids

Maximum content of total amino acids was found in C. setigerus even though the two forms of this species differed

Table 6. Comparison of different biochemical parameters in taxa of different species of *Cenchrus* L.

Taxa	Total soluble proteins (mg/g fresh weight leaf)	Total free amino acids (mg/g dry weight leaf)	Total soluble sugars (mg/g dry weight leaf)
C. ciliaris			
C-I	27.76(0.08)	91.99(0.03)	5.36(0.01)
C-II	31.74(0.02)	81.87(0.02)	4.95(0.01)
C. setigerus			
S-I	30.04(0.08)	124.19(0.16)	4.44(0.14)
S-II	34.77(0.03)	105.79(0.08)	4.06(0.16)
C. pennisetiformis			
P-I	29.47(0.09)	101.19(0.02)	5.24(0.10)
P-II	32.12(0.06)	88.318(0.05)	4.76(0.09)

Values shown are mean(±S.E.) of three determinations.

considerably from each other in this respect (Table 6). The cytotypes of C. ciliaris and two morphotypes of C. pennisetiformis also differed considerably from each other in the amount of total free amino acids. At specific level, the minimum amounts were noticed in C. ciliaris.

Individual amino acid composition

Individual amino acid composition in taxa of different species is presented in Table 7.

i. Acidic amino acids and amides

Aspartic acid and asparagine: Maximum amount was found in C. ciliaris and minimum in S-I form of C. setigerus. Percentage of this amino acid in the three forms, C-I, S-I and P-I was comparatively lower than in the C-II, S-II and P-II forms, respectively.

Glutamine and glutamic acid: Maximum amount of this amino acid was found in two forms of C. ciliaris which had comparable amounts. The minimum amounts were noticed in two morphotypes of C. setigerus.

ii. Basic amino acids

Histidine: Maximum amount of this amino acid was recorded in C. pennisetiformis. In C. setigerus and C. ciliaris, however, almost similar amounts were observed.

Lysine: Almost similar amounts of this amino acid were observed in three species. Low concentrations of lysine were observed

in C-I, S-I and P-I forms as compared to C-II, S-II and P-II forms respectively.

Arginine: Minimum percentage of arginine was observed in C. pennisetiformis. Comparable amounts were observed in C. ciliaris and C. setigerus.

iii. Aromatic amino acids

Phenylalanine: Almost similar amounts of this amino acid were observed in different species.

Tyrosine: Maximum percentage was observed in P-II form of C. pennisetiformis. The minimum concentration was noticed in S-I form of C. setigerus. The remaining forms exhibited nearly comparable amounts.

Tryptophan: Maximum amount was observed in two forms of C. setigerus, followed closely by taxa of C. ciliaris and C. pennisetiformis.

iv. Sulphur-containing amino acids

Cysteic acid and cystine: Minimum amounts were observed in S-I form. The remaining forms were slightly richer though they showed nearly comparable levels.

Methionine: Maximum concentration was observed in C. ciliaris. Amount was lower in C-II, S-II and P-II forms as compared to C-I, S-I and P-I forms respectively.

v. Acidic, neutral and basic amino acids

Glycine and serine: Very high amounts were observed in the two

forms of C. setigerus. However, in C. ciliaris and C. pennisetiformis almost similar amounts were observed. In S-I, C-I and P-I forms amounts were comparatively less than the S-II, C-II and P-II forms respectively.

Threonine: Maximum amount was observed in P-II form of C. pennisetiformis and minimum in two forms of C. ciliaris. The remaining taxa revealed intermediate and comparable levels of it.

Alanine: Maximum amount was observed in S-II form and minimum in P-I form of C. pennisetiformis. The remaining taxa exhibited almost comparable levels.

Valine: Maximum amount of valine was estimated in C. ciliaris and minimum in C. setigerus.

Leucine and isoleucine: There was no substantial difference in the concentration of leucine and isoleucine in taxa of the three species.

Proline: Proline was observed in maximum amount in P-II form of C. pennisetiformis closely followed by S-II form of C. setigerus and P-I form of C. pennisetiformis. Minimum amount was observed in two forms of C. ciliaris.

Hydroxyproline: Very low concentration of this amino acid was observed in three species. However, C. pennisetiformis exhibited higher amount followed by C. setigerus and C. ciliaris.

d. Level and composition of soluble sugars

Almost comparable amounts of soluble sugars were observed

Fig.16. Standard chromatogram of amino acids

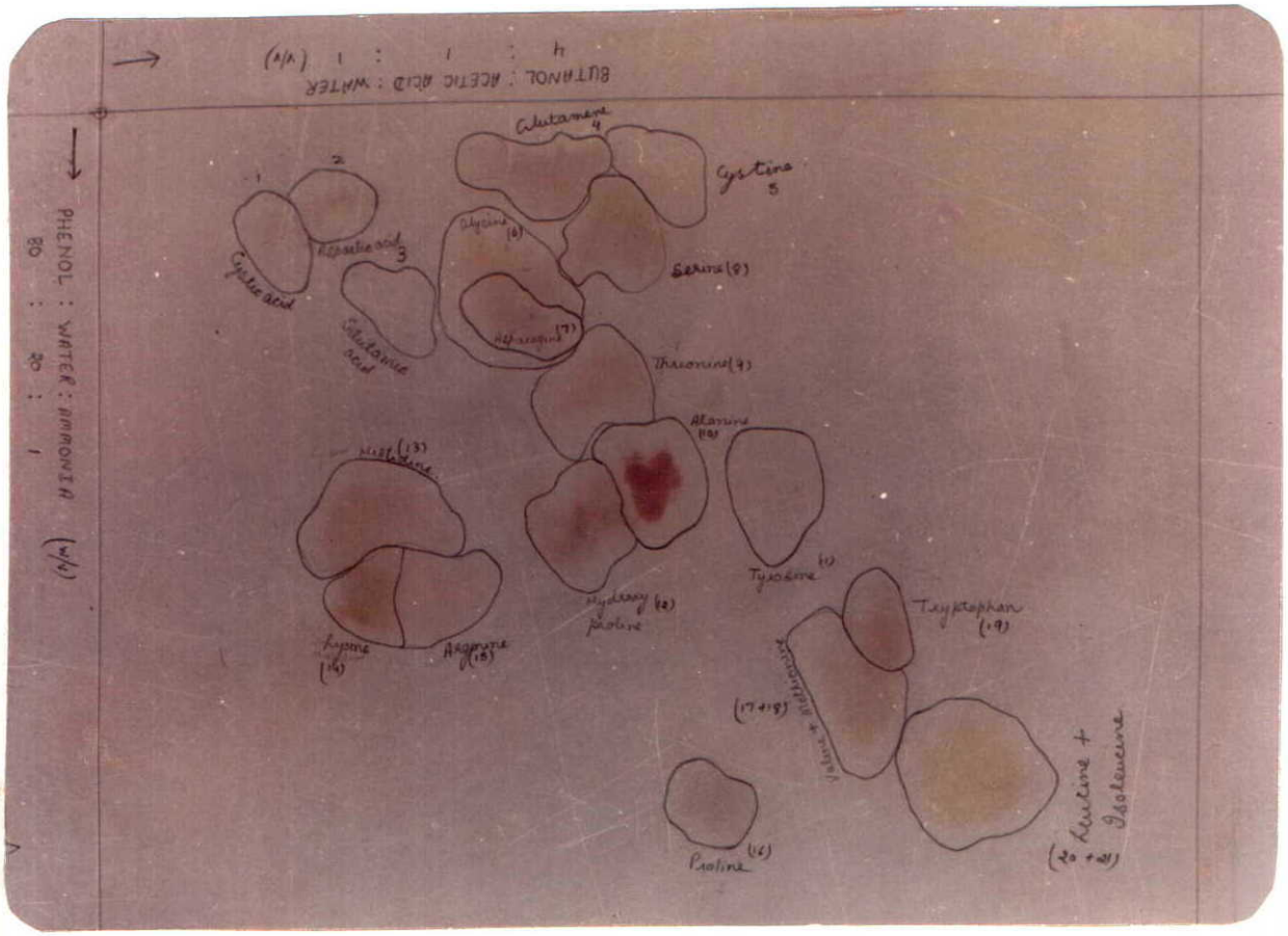
BUTANOL : ACETIC ACID : WATER
4 : 1 : 1 (v/v)
PHENOL : WATER : AMMONIA
80 : 20 : 1 (w/v)
Glutamine 4
Cystine 5
Cystic acid
Aspartic acid 3
Cysteine (6)
Serine (8)
Hydroxyproline (7)
Sulphonic acid
Threonine (9)
Glycine (10)
Alanine (11)
Methionine (13)
Tyrosine (14)
Tryptophan (19)
Hydroxy Proline (12)
Lysine (16)
Arginine (15)
Valine + Methionine (17+18)
Proline (17)
Leucine + Isoleucine (20+21)

FIG 16

Table 7. Amino acid composition (g% total amino acids) in taxa of different species of Cenchrus L.

Taxa	*Cys +Cys	Asp	Ser +Gly	Glu +Gln	His	Lys	Arg	H-Pro	Thr	Pro	Ala	Tyr	Trp	Val+ Me	Phe	Ileu+ Leu
C. ciliaris																
C-I	2.23	4.04	10.67	11.87	3.73	2.60	3.20	0.70	6.02	4.80	10.02	8.82	7.68	11.21	4.60	6.37
C-II	2.50	4.50	9.84	11.20	3.56	3.10	3.30	0.82	6.87	5.25	10.85	9.25	7.95	11.01	4.78	6.54
C. setigerus																
S-I	1.85	3.13	13.07	8.48	3.50	2.48	2.82	0.90	7.95	5.85	9.49	7.54	8.54	10.13	4.62	6.47
S-II	2.48	3.60	14.08	7.90	3.74	2.95	3.02	1.00	8.20	6.20	11.69	9.28	9.27	8.21	4.50	6.61
C. pennisetiformis																
P-I	2.16	3.38	10.46	9.92	4.02	2.17	2.15	1.29	7.79	6.08	9.14	8.87	7.43	10.56	4.55	6.55
P-II	2.30	3.79	8.84	9.25	4.13	2.87	2.09	1.34	8.84	6.51	10.40	10.87	9.23	9.18	4.48	6.63

*Cys= Cysteic acid, Cys =Cystine, Asp = Aspartic acid, Ser = Serine; Gly = Glycine, Glu = Glutamic acid, Gln = Glutamine, His = Histidine, Lys = Lysine, Arg =Arginine, H-Pro= Hydroxy-L-proline, Thr = Threonine, Pro = Proline, Ala = Alanine, Tyr = Tyrosine, Trp = Tryptophan, Val = Valine, Met = Methionine, Phe = Phenylalanine, Ileu = Isoleucine, Leu = Leucine

in the six taxa of the three species (Table 6,8). It was, however, observed that sucrose content was maximum in C-I form of C. ciliaris followed by C-II form of the same species. The two forms of C. pennisetiformis and S-I form of C. setigerus showed similar levels of it whereas the amount of sucrose was comparatively much lower in S-II form of C. setigerus. Glucose level was maximum in C-I form followed by P-I form of C. pennisetiformis. The remaining taxa had almost comparable amount. Fructose content was maximum in C. pennisetiformis followed by S-II form of C. setigerus. The S-I form of C. setigerus and the two forms of C. ciliaris exhibited comparable levels of fructose.

e. Ascorbic acid content

The data presented in Table 9 shows that almost similar concentrations of ascorbic acid content were observed in three species. However, concentration in C-I and S-I forms were slightly lower than C-II and S-II forms respectively.

f. Total flavonoids

The data shown in Table 9 revealed that almost comparable amount of total flavonoids are present in taxa of C. ciliaris, C. setigerus and P-II forms of C. pennisetiformis. The P-I form of C. pennisetiformis, however, depicted slightly lesser amounts of these.

g. Flavonoid pattern

The two dimensional paper chromatographic patterns of

Table 8. Sucrose, glucose, and fructose content (mg/g dry weight) in taxa of different species of *Cenchrus* L.

Taxa	Sucrose	Glucose	Fructose
C. ciliaris			
C-I	1.74	1.69	1.41
C-II	1.37	1.08	1.66
C. setigerus			
S-I	0.95	0.99	1.62
S-II	0.66	0.87	1.87
C. pennisetiformis			
P-I	1.06	1.28	2.12
P-II	0.99	1.16	2.08

Table 9. Ascorbic acid content (mg/100 g fresh weight) and mg/g flavonoid contents in taxa of different species of *Cenchrus* L.

Taxa	Ascorbic acid (mg/100g fresh weight)	Total flavonoid content(mg/g dry weight)
C. ciliaris		
C-I	48.66(0.10)	1.53(0.12)
C-II	52.22(0.2)	1.79(0.16)
C. setigerus		
S-I	50.00(0.01)	1.62(0.18)
S-II	52.00(0.00)	1.86(0.09)
C. pennisetiformis		
P-I	46.87(1.00)	1.03(0.08)
P-II	48.06(0.80)	1.55(0.15)

Values shown are Mean(±S.E.) of three determinations

flavonoids of different taxa of three species are shown in Figs.17,18 and 19. A total of 22 spots were observed in different species. Ten spots (all black) were observed to be common to all the taxa of three species. Species specific spots in C. ciliaris (spot 18) and C. setigerus (spot 10) were observed. The presence of spot No.2 and absence of spot No.3 characterised the species C. pennisetiformis. The C-II form could be distinguished from C_I form by the presence of spots 14 and 19 and by the absence of spot 8. The S-II form of C. setigerus was distinguishable from S-I form by the presence of an additional spot 22. Likewise, morphotype P-II could easily be identified by the presence of spot 20. The three spots 4,6 and 11 were conspicuous by their absence in C. ciliaris. Similarly, the two species C. ciliaris and C. setigerus were observed to share spot 3 which was absent from C. pennisetiformis. The spot 20 which was noticed to be present in both the forms of C. ciliaris was also observed in the P-II form of C. pennisetiformis. The Rf values and colour reactions of individual spots under visible light, NH_3 fumes, UV light and UV + ammonia are tabulated in Tables 10, 11 and 12.

h. Electrophoretic analysis of proteins

The seed protein patterns are presented as zymograms in Fig.20. A total number of nine to thirteen bands were observed in different species. These patterns werehelpful in distinguishing the three species and different taxa of these.

Fig.17. Flavonoid patterns of two cytotypes C-I and C-II of C. ciliaris

FIG. 17 Cenchrus ciliaris

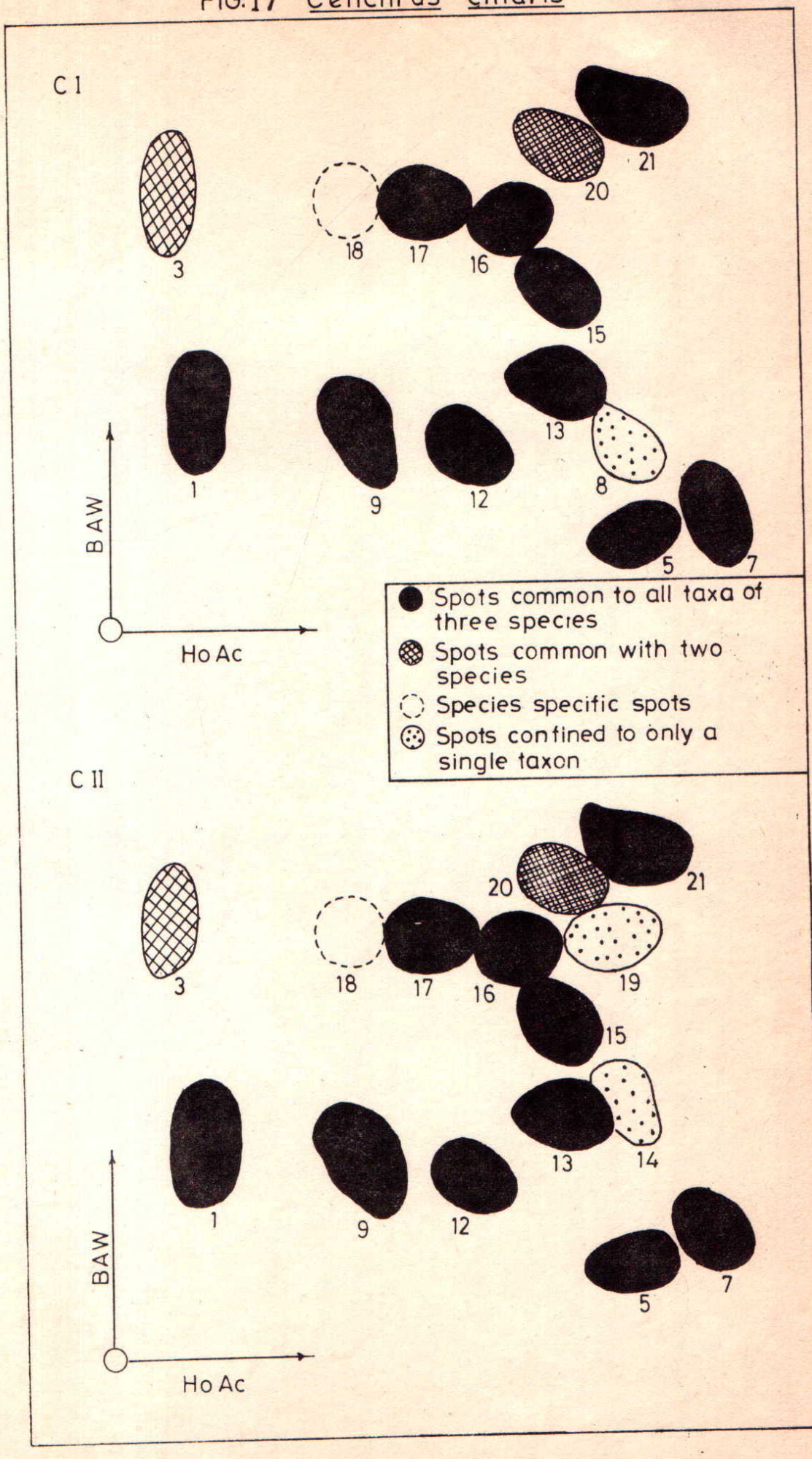

Fig.18. Flavonoid patterns of two forms S-I and S-II of C. setigerus.

FIG.18 Cenchrus setigerus

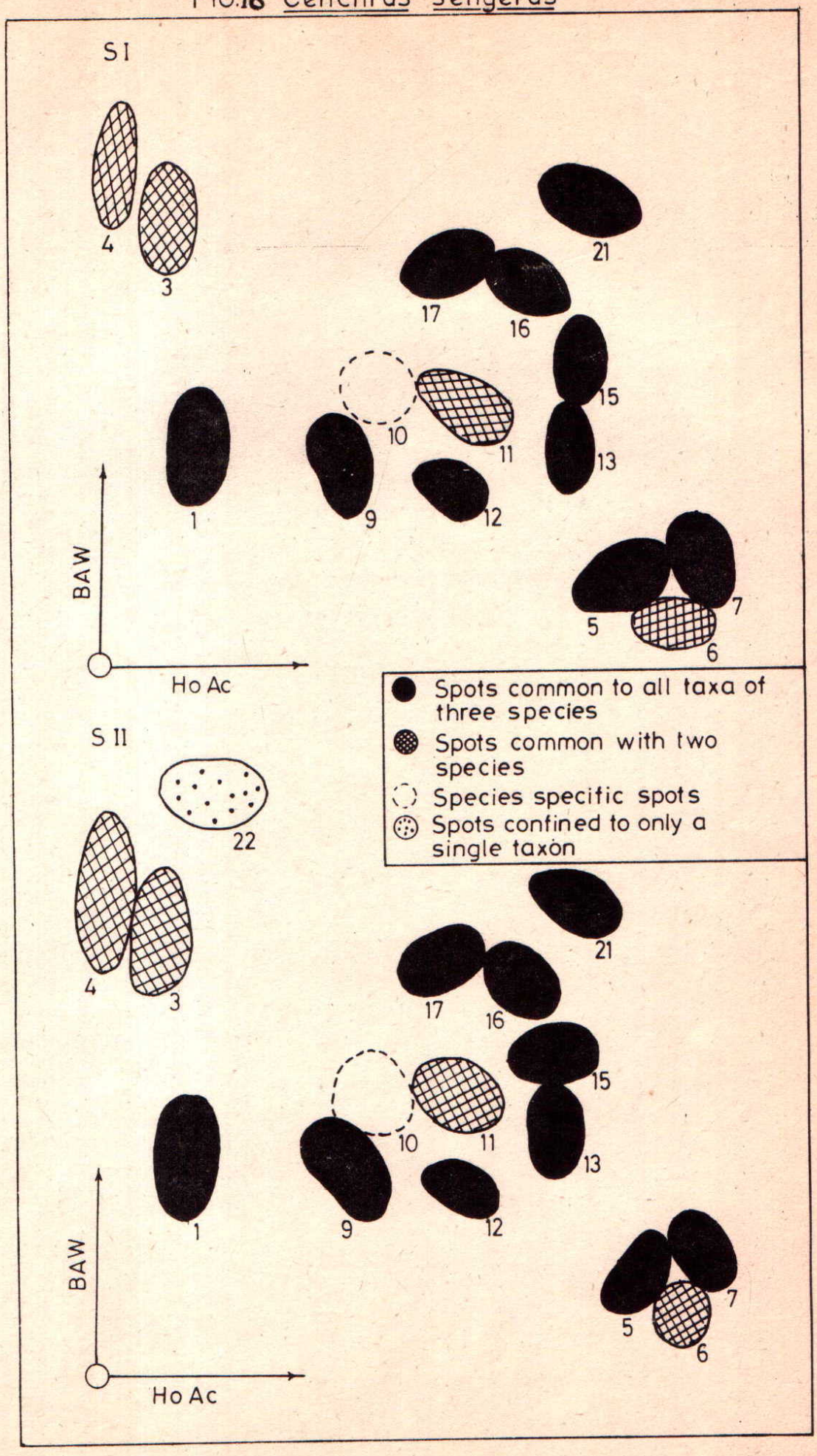

Fig.19. Flavonoid patterns of two forms P-I and P-II of *C. pennisetiformis*

Fig 19 Cenchrus pennisetiformis

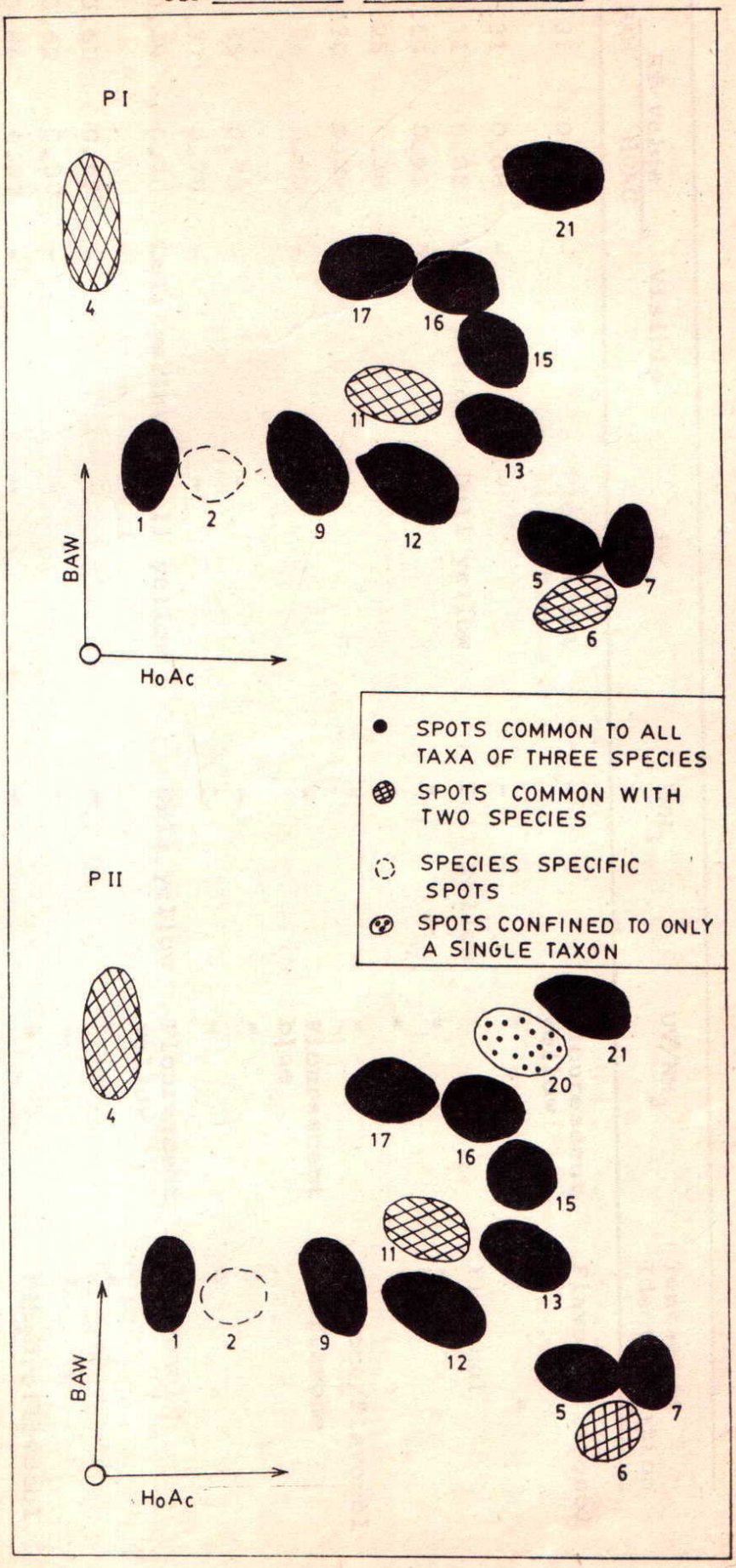

Table 10. Rf values, colour reactions and tentative identification of flavonoids of *C. ciliaris* .

Spot No.	Rf value BAW	Rf value HOAC	Visible	UV	NH_3	UV/NH_3	Tentative Identification
1.	0.31	0.13	-	Flourescent light blue	-	Flourescent yellow	Flavone/flavonol
3.	0.31	0.08	-	-	-	"	" "
5.	0.11	0.82	Dull yellow	Dull yellow	Dull yellow	"	Flavonol
7.	0.19	0.93	"	"	"	"	"
8	0.25	0.38	"	"	"	"	"
9	0.30	0.39	-	-	"	"	Flavone/Flavonol
12	0.26	0.55	-	-	-	Flourescent blue	Isoflavone
13	0.35	0.70	-	-	-	"	"
14	0.37	0.78	-	-	-	"	"
15	0.49	0.70	Dull yellow	Dull yellow	Dull yellow	Flourescent yellow	Flavonol
16	0.60	0.63	-	-	-	"	"
17	0.62	0.50	-	-	-	"	"
18	0.66	0.37	-	-	-	"	Dihydroflavonol
19	0.62	0.78	Dull yellow	Dull yellow	Dull yellow	"	"
20	0.70	0.70	-	-	-	"	"
21	0.75	0.81	Dull yellow	Paleyellow	Dull yellow	"	"

Table 11. Rf values, colour reactions and tentative identification of flavonoids of C. setigerus

Spot No.	Rf value BAW	Rf value HOAC	Visible	UV	NH_3	UV/NH_3	Tentative identification
1	0.31	0.13	-	Flourescent light blue	-	Flourescent yellow	Flavone/Flavonol
3	0.65	0.25	-	-	-	"	"
4	0.75	0.08	Yellowish green	Deep purple	-	Dull brown	" "
5	0.11	-	Dull yellow	Dull yellow	Dull yellow	Flourescent yellow	Flavonol
6	0.08	0.90	"	"	"	"	"
7	0.19	0.93	"	"	"	"	"
9	0.30	0.39	-	-	"	"	Flavone/Flavonol
10	0.40	0.40	-	-	"	"	Flavonol
11	0.41	0.54	-	-	-	Flourescent blue	"
12	0.26	0.55	-	-	-	"	Isoflavone
13	0.35	0.70	-	-	-	"	"
15	0.49	0.70	Dull yellow	Dull yellow	Dull yellow	Flourescent yellow	Flavonol
16	0.60	0.63	-	-	-	"	"
17	0.62	0.50	-	-	-	"	"
21	0.75	0.81	Dull yellow	Pale yellow	Dull yellow	"	Dihydroflavonol
22	0.85	0.16	-	-	-	Flourescent blue	Isoflavone

Table 12. Rf values, colour reactions and tentative identification of flavonoids of C. pennisetiformis.

Spot No.	Rf value BAW	Rf value HOAC	Visible	UV	NH_3	UV/NH_3	Tentative identification
1	0.31	0.13	-	Flourescent light blue	-	Flourescent yellow	Flavone/Flavonol
2	0.31	0.25	-	"	-	"	" "
4	0.75	-	Yellowish green	Deep purple	-	Dull brown	" "
5	0.11	0.82	Dull yellow	Dull yellow	Dull yellow	Flourescent yellow	Flavonol
6	0.08	0.90	"	"	"	"	"
7	0.19	0.93	"	"	"	"	"
9	0.30	0.39	-	-	"	"	Flavone/Flavonol
11	0.41	0.54	-	-	-	Flourescent blue	Flavonol
12	0.26	0.55	-	-	-	"	Isoflavone
13	0.35	0.70	-	-	-	"	"
15	0.49	0.70	Dull yellow	Dull yellow	Dull yellow	Fluorescent yellow	Flavonol
16	0.60	0.63	-	-	-	"	"
17	0.62	0.50	-	-	-	"	"
20	0.70	0.70	-	-	-	"	Dihydroflavonol
21	0.75	0.81	Dull yellow	Pale yellow	Dull yellow	"	"

The variations observed in each species are mentioned below:

C. ciliaris: Twelve bands in C-I and thirteen bands were observed in C-II form. All the twelve bands depicted by C-I form, though slightly variable in their intensity, were observed to be present in C-II form which had in addition a cytotype specific bands in the zone 'C'(arrow).

C. setigerus: Eleven bands were observed in both the forms of these, ten bands had the same mobility and, by and large, the same intensity. The two forms differed, however, with respect to positioning of one band (arrows)which was morphotype specific.

C. pennisetiformis: Nine and eleven bands were observed in P-I and P-II forms respectively. All the nine bands present in P-I form were also revealed in P-II form which in addition exhibited two morphotype specific bands (arrows).

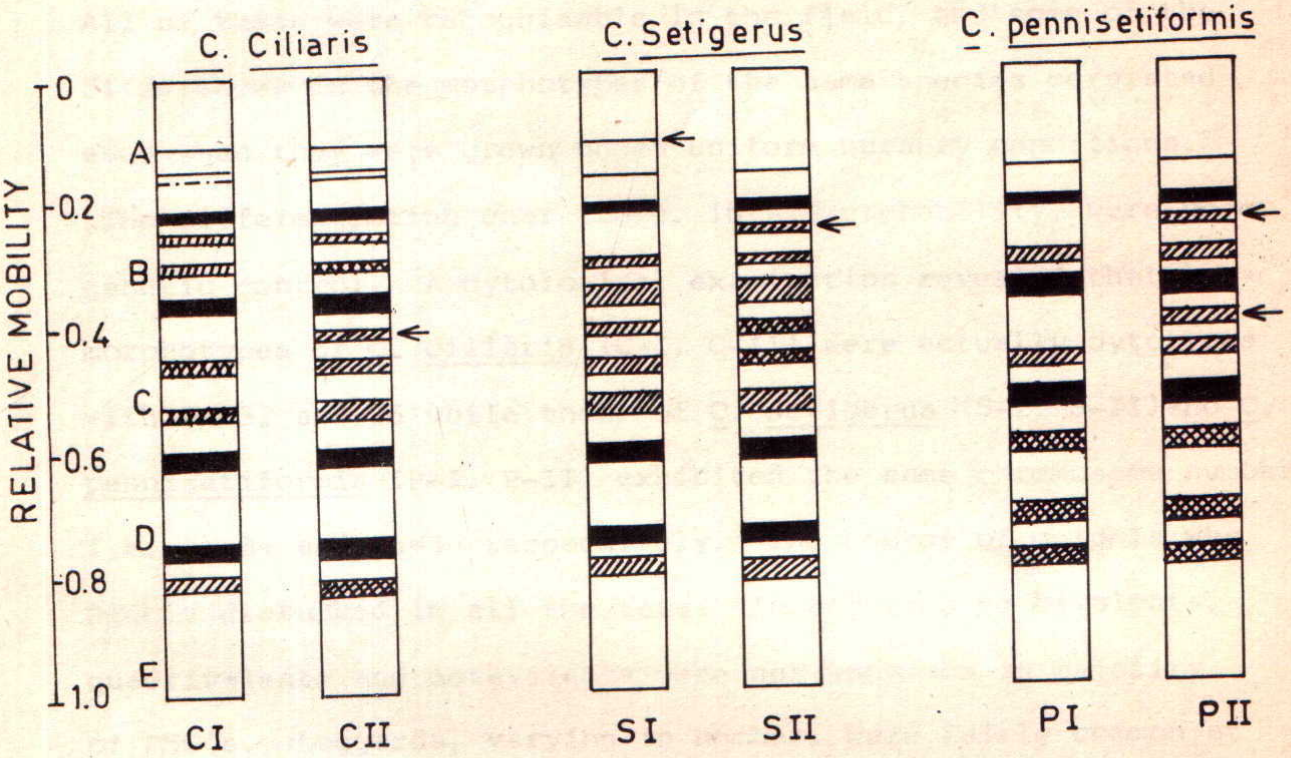

FIG. 20 ZYMOGRAMS OF ELECTROPHORETIC BANDING PATTERNS OF TOTAL SOLUBLE PRDTEINS IN TAXA OF THREE SPECIES OF CENCHRUS L.

BAND INTENSITY INDEX

STRONG

MEDIUM

WEAK

CHAPTER V

DISCUSSION

Two morphotypes in each of the three species of Cenchrus ciliaris, C. setigerus and C. pennisetiformis were observed. All of these were recognisable in the field, and some of the differences in the morphotypes of the same species persisted even when they were grown under uniform nursery conditions. The differentiating characters, in all probability, were under genetic control. A cytological examination revealed that morphotypes of C. ciliaris (C-I, C-II) were actually cytotypes with 2n=32 and 36 while those of C. setigerus (S-I, S-II) and C. pennisetiformis (P-I, P-II) exhibited the same chromosome number i.e. 2n=34 and 2n=36 respectively. The course of meiosis was highly disturbed in all the taxa. In addition to bivalents, quadrivalents and octavalents were not uncommon in majority of PMC's. Laggards, varying in number, were fairly common at A-I and A-II. This irregular distribution of chromosomes affected the pollen fertility adversely and to a greater extent in C. ciliaris than in C. setigerus and C. pennisetiformis. The fertile pollen size did not prove useful, in a fool proof way, in the identification of any of these taxa, though slight variations were noticeable in cytotypes of C. ciliaris and morphotypes of C. pennisetiformis. However, usually well

marked size differences in pollen size have been observed to accompany differences in chromosome numbers (Sachdeva and Bhatia, 1979; Sachdeva et al.,1986). Such an indistinction in C. ciliaris may be due to the fact that these cytotypes were not at different ploidy levels.

A comparison of the morphological traits showed that comparatively stout or robust forms (C-II, S-II and P-II) in all the three species always had more of internodal length, leaf blade length and width, leaf sheath length and number of leaves per culm. No significant departures were observed in floral traits, except length of the spikes, length of lemma and palea. The length of the spike was observed to be much more in C-II, S-II and P-II form compared to their counterpart taxa. However, the length of the lemma and palea was observed to be more in C-II and S-II forms than C-I and S-I forms. No variation in these characters was noticed in morphotypes of C. pennisetiformis.

Leaf stomatal frequency per unit area and stomatal size were useful enough in the identification of taxa of the three species. Stomatal frequency per unit area was observed to be more in C-I, S-I and P-I forms as compared to C-II, S-II and P-II forms respectively. The reverse was true of stomatal size, being more in the robust taxa. Broadly speaking, anatomical details of the leaf were quite similar except that in C. ciliaris, sclerenchymatous girdles on the abaxial side connected epidermis to bundle sheaths while in C. setigerus and C. pennisetiformis, bundle sheaths of vascular bundles were separated

from epidermis by two to three layers of large parenchymatous cells. The taxa of C. pennisetiformis could, however, be distinguished from those of C. setigerus by quite prominent ridges and furrows which were, particularly, quite conspicuous in P-I form. Anatomical details of culm structure, as seen in transverse section, were also similar to a larger extent. Though cortical portion was demarcated into outer and inner cortex by the intervening zone of mechanical tissue in all the three species, the distinction was much obscured in C. setigerus where outer cortex was not so well developed. Further, in cytotypes of C. ciliaris, the cortical region was densely packed with vascular bundles but they were sparsely arranged in C. setigerus while C. pennisetiformis exhibited an intermediate condition.

The biochemical evaluation of taxa revealed considerable variations in the various biochemical characters studied. Some of the variational patterns were observed to be of quite helpful in identification of taxa and in pinpointing the biochemical superiority of some of these.

The amount of total soluble proteins varied greatly in the six taxa of the three species. The C-II form of C. ciliaris, S-II form of C. setigerus and P-II form of C. pennisetiformis were comparatively richer than their counterparts of the same species. Maximum level of soluble proteins was noticed in S-II form of C. setigerus and minimum in C-I form of C. ciliaris.

Variations in amino acid complement have been observed to be of great taxonomic value (Bell, 1962; Byers, 1971; Watson and Creaser, 1975). Of particular interest are qualitative variations (Sachdeva and Bhatia, 1979 and Sachdeva and Kals, 1981). Presently no qualitative variations were noticed. Quantitative variations of relatively different magnitudes were, however, observed. In C.ciliaris, whereas cytotype C-I was observed to be richer in the amount of serine, glycine, glutamic acid, glutamine, valine and methionine, the C-II contained higher amounts of aspartic acid, lysine, threonine, alanine, tyrosine and tryptophan. Except for a bit of higher levels of glutamic acid, glutamine, valine and methionine in S-I form, the S-II form of C. setigerus exhibited higher levels in most of the rest of the amino acids. The P-I form of C. pennisetiformis exhibited higher levels of serine, glycine, glutamine, glutamic acid, valine and methionine. The P-II form was observed to be richer in the amount of remaining amino acids except arginine, hydroxproline, phenylalanine and isoleucine and leucine which revealed comparable levels of these.

Regarding level and composition of soluble sugars, it may be mentioned that almost comparable amounts were noticed in the six taxa. Sucrose content was observed to be maximum in C-I form of C. ciliaris followed by C-II form of the same species. The S-I form of C. setigerus and two forms of C. pennisetiformis revealed similar levels of it whereas the amount of sucrose was comparatively much lower in S-II form of C. setigerus. Glucose

level was highest in C-I form followed by P-I form of C. pennisetiformis. The rest of taxa had almost comparable levels. Fructose content was maximum in C. pennisetiformis followed by S-II form of C. setigerus. The S-I form of C. setigerus and two forms of C. ciliaris showed comparable amounts of fructose. Such variations in the amounts of sucrose, glucose and fructose have been observed to be related to the activity of the enzyme acid invertase (Ricardo and Rees, 1970; Maclachlan et al.,1970; Shukla et al.,1973; Sachdeva and Bhatia, 1979).

Analysis of ascorbic acid content revealed almost similar levels of it in C-I, S-I, P-I and P-II. The C-II and S-II forms were slightly richer. At cytotype level, C-II cytotype with more of chromosome number was definitely richer than C-I cytotype. However, there does not exist any correlation between the amount of ascorbic acid and levels of ploidy (Sachdeva and Bhatia, 1980).

Two dimensional paper chromatographic patterns of flavonoids showed a total of 22 spots in the presently investigated taxa. Of these, ten spots were observed to be common to all the taxa. Not only the three species but even various forms of these could be distinguished from one another without any ambiguity. The two species C. ciliaris and C. setigerus exhibited species specific spots. The species C. pennisetiformis could easily be recognised by the presence of spot 2 and by the absence of spot No.3. In C. ciliaris, the C-II

form was distinguishable from C_I form by the presence of spots 14 and 19 and by the absence of spot 8. In C. setigerus, S-II was identifiable by the presence of an additional spot 22 which lacked in S-I form. Likewise, in C. pennisetiformis, morphotype P-II could easily be recognised from P-I by the presence of spot 20. The utility of flavonoid patterns, without any doubt, is considerable. They have contributed much towards the identification of taxa and elucidation of plant relationships. Though for such analysis the identification of compounds has usually been stressed upon for its importance in understanding the evolution, yet useful information has been gathered even from unidentified chromatographic spots (Grant, 1968; Harborne, 1975). Such studies have doubtlessly provided great assistance in distinguishing cytotypes of a species with limited inter-cytotype morphological variability (Sachdeva and Bhatia,1979; Sachdeva and Kals, 1981; Sachdeva et al.,1986; Bala,1988).

Electrophoretic analysis of seed protein patterns revealed a total of nine to thirteen bands in taxa of three species. The observed patterns were discriminatory enough to distinguish unambiguously the three species as well as different taxa of these. The cytotypes C-I and C-II exhibited twelve and thirteen bands respectively. Though all the twelve bands noticed in C-I form were also observed to be present in C-II, yet the latter cytotype was distinguishable by an additional cytotype specific band in the zone 'C'. Both forms of C. setigerus exhibited eleven bands, of which, ten bands had

comparable mobility. The two forms, however, differed with respect to the location of one band which was morphotype-specific. The two forms P-I and P-II of C. pennisetiformis displayed nine and eleven bands respectively. All the nine bands present in P-I form showed their presence in P-II form also, though the latter exhibited an additional morphotype specific band. The differences, observed presently, in number, electrophoretic mobility and intensity of bands paralleled differences noticed generally in varieties of many taxa, for example, soybean (Payne and Koszykovski,1978), Digitaria (Hayward and Hacker,1980), Stenotaphrum secundatum (Green et al.,1981), Zea mays (Cardy and Kannenberg,1982), Kentucky bluegrass (Wu et al.,1984), Hordeum (Rao and Sharma, 1985), rye grass (Ferguson and Grabe,1986) and Agropyron junceum (Moustakas et al.,1986).

CHAPTER VI

SUMMARY

Two morphotypes in each of the three species, Cenchrus ciliaris (C-I, C-II), C. setigerus (S-I, S-II) and C.pennisetiformis (P-I, P-II) were collected from plaints of Punjab. Intermorphotype differences were observed to persist even when these forms were grown under uniform nursery conditions reflecting, thereby, genetic control of differentiating characteristics. The morphotypes of C. setigerus and C. pennisetiformis revealed the same chromosome number of 2n=34 and 2n=36 respectively. However, in C. ciliaris, the two morphotypes turned out to be cytotypes with 2n=32 and 36. The course of meiosis in all the taxa was highly disturbed. Varying numbers of multivalents at M-I and laggards at A-I and A-II were quite common. This affected the pollen fertility adversely. The increase in height was always observed tobe accompabied with an increase in internodal length, leaf blade length and width, leaf sheath length and number of leaves per culm. No significant departures were observed in floral traits except with regard to length of the spike and length of lemma and palea. Leaf stomatal frequency per unit area and stomatal size were highly discriminatory in the identification of taxa. Stomatal frequency per unit area was observed to be more in C-I, S-I and P-I forms as compared to

C-II, S-II and P-II forms respectively. The reverse was true of stomatal size. Though broadly similar in structural details, the leaves in taxa of C. ciliaris had sclerenchymatous girdles on the abaxial side that connected epidermis to bundle sheaths, in C. setigerus and C. pennisetiformis bundle sheaths were separated from epidermis by two to three layers of parenchymatous cells. The taxa of C. pennisetiformis were distinguishable from those of C. setigerus by the possession of prominent ridges and furrows which were highly developed in P-I form. Whereas the cortical portion of the culm was well demarcated into outer and inner cortex, such distinction was obscured in C. setigerus. In cytotypes of C. ciliaris, the cortical region was densely packed with vascular bundles but these were sparsely arranged in C. setigerus with intermediate condition prevailing in C. pennisetiformis.

Biochemical evaluation of taxa was undertaken in respect of soluble proteins, free amino acids, soluble sugars, ascorbic acid content and flavonoids. In addition, flavonoid and electrophoretic soluble protein patterns were also studied. The amount of soluble proteins varied greatly in different taxa. The C-II form of C. ciliaris, S-II form of C. setigerus and P-II form of C. pennisetiformis were comparatively richer than their counterparts of the same species. Maximum level of soluble proteins was noticed in S-II form of C. setigerus. In analysis of free amino acids no qualitative variation was encountered. Quantitative variations of relatively different magnitude were, however,

noticed. In C. ciliaris, whereas cytotype C-I was observed to be richer in the amounts of serine, glycine, glutamic acid, glutamine, valine and methionine, the C-II contained higher amounts of aspartic acid, lysine, threonine, alanine, tyrosine and tryptophan. Except for a bit of higher levels of glutamic acid, glutamine, valine and methionine in S-I form, the S-II of C. setigerus exhibited higher levels in most of the rest of the amino acids. The P-I form of C. pennisetiformis exhibited higher levels of serine, glycine, glutamine, glutamic acid and valine and methionine. The P-II form was observed to be richer in the amount of remaining amino acids except arginine, hydroxyproline, phenylalanine and isoleucine and leucine which revealed comparable levels of these.

In all taxa, comparable amounts were noticed in respect of level and composition of soluble sugars, Sucrose and glucose content was observed to be maximmm in C-I form of C. ciliaris. Fructose content was maximum in C. pennisetiformis. In ascorbic acid content, C-II and S-II forms were comparatively richer than other forms. Flavonoid and electrophoretic patterns of proteins were observed to be highly discriminatory. Based on these, not only the different species but even the morphotypes pertaining to these could be distinguished without any ambiguity. The differences observed presently in number, electrophoretic mobility and intensity of bands paralleled differences noticed generally in varieties of cultivated taxa.

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* Original not seen.