EPIDERMAL STRUCTURE IN *MARSILEA MINUTA* LINN.

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ABSTRACT

The paper describes the epidermal structures including appendages and the development of the stomata in *Marsilea minuta* Linn. Leaves of various developmental stages were obtained from the plant growing in various environmental conditions, viz. aquatic, semi-aquatic and xerophytic, in the different seasons-summer, rain and winter. External and internal structures of the leaflets in various forms have been studied. Epidermal structures in different parts of the plant, viz. leaflets, petiole, rhizome and sporocarps have been studied in detail in the plants growing under various environmental conditions. Developmental stages of stomata have also been studied. Various stomatal abnormalities have also been noted. Trichome structures are discussed in detail. Importance of epidermal structures within the genus has been discussed.

INTRODUCTION

The genus *Marsilea* has been studied by various workers : MAHABALE AND GORGI (1948), KOLHATKAR (1937, 1957), PANDEYA (1953), MEHRA (1938), MEHRA AND LOYAL (1958, 1960), GUPTA (1957, 1962), JOHNSON (1898), HENDERSON (1933), and MAHABALE (1956) for its morphology of vegetative and reproductive structures including the development of various organs. ALLSOPP (1953a, 1953b, 1955, 1963), ALLSOPP AND SWEYKOWSKA (1960), GAUDET (1964a, 1964b, 1965a, 1965b), and WHITE (1966) had studied experimentally the effect of various physiologically active substances on the various forms of *Marsilea*. Epidermal characters have also been studied on this genus (GUPTA, 1957 & 1962; SHARMA, 1966). Detailed epidermal structures and ontogeny of stomata in *Marsilea minuta* Linn. have not been worked out so far. Another genus *Regnellidium* of this family has been studied by us (TRIVEDI & UPADHYAY, 1980).

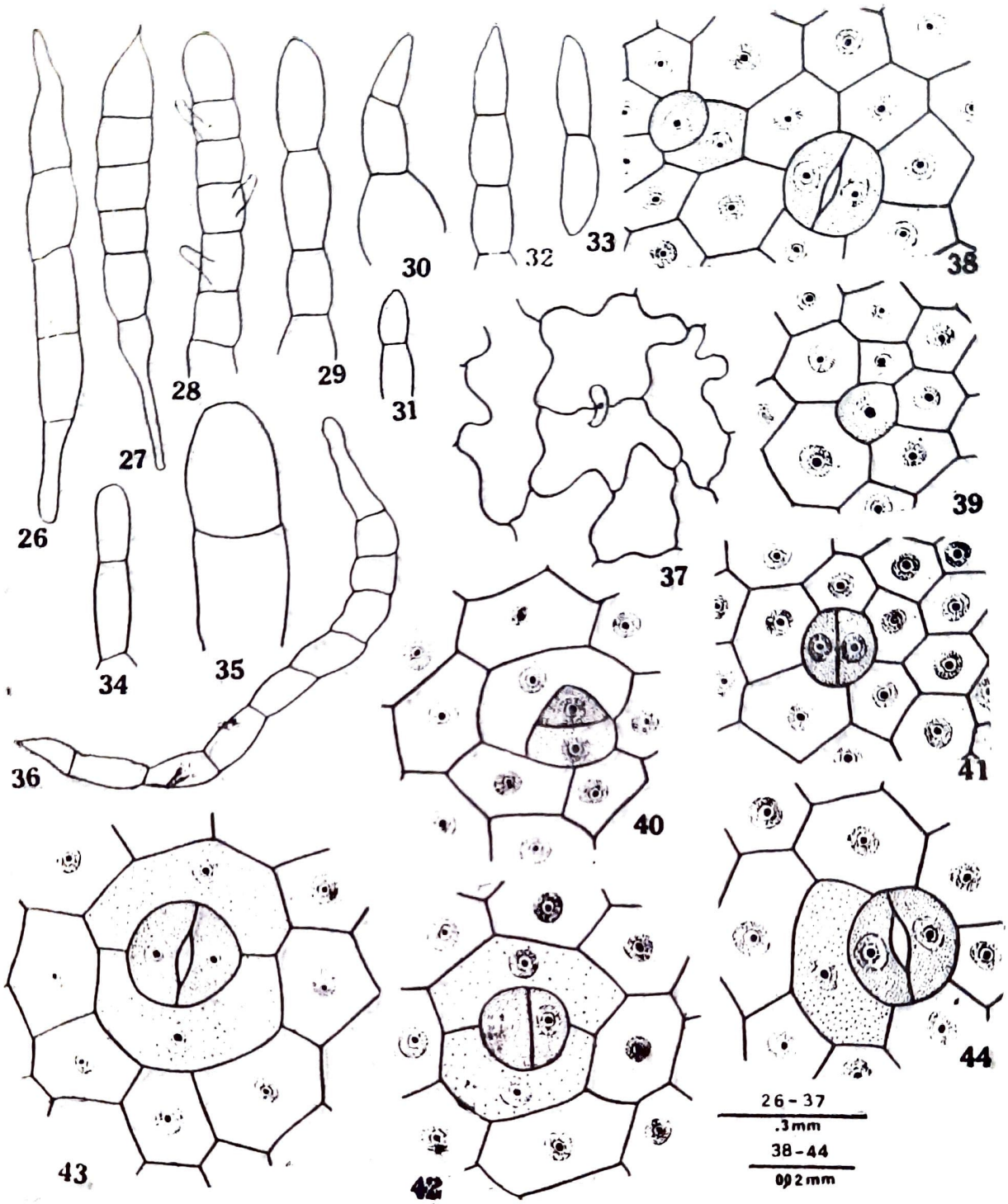
In the present investigation epidermal structures (including their appendages) of *Marsilea minuta* growing under various environmental conditions (hydrophytic-water form; semi-aquatic and xerophytic-land form) have been described. Ontogeny of stomata have also been studied in detail. Terminology used here is the same as suggested by COTTHEM (1970) and FRYNS-CLAESSENS AND COTTHEM (1973).

MATERIAL AND METHODS

Material for the present investigation was obtained largely from the various parts of the Lucknow district and also from the garden of Botany Department, Lucknow University. Large number of plants were collected in different seasons under various environmental conditions. Cuticular studies are based on mature leaves, whereas the developmental studies are based on young and developing leaves. Cuticles were obtained from the fresh leaves. These were stained with aqueous safranin. For the study of ontogeny of stomata, the cuticles were stained with iron-haematoxylin and aceto-carmine.

OBSERVATIONS

Members of Marsileaceae usually grow in water. The family has three genera : *Marsilea* (4 leaflets), *Regnellidium* (2 leaflets) and *Pilularia* (without leaflets), which are



Text-figs. 26—44 ; 26-29 & 36, Different types of trichomes of young parts, 30-35. Different types of trichomes of pinnae, 37. Single guard cell stoma of semi-aquatic form; 38-41. Epidermal cells showing stages of stomatal development of a perigenous type ; 41. Guard-cell mother-cell showing a division; 39-40 & 42-43. Different stages of diacytic stomatal development, 39. Single meristemoid showing prominent nucleus and dense cytoplasm, 40. Stomatal meristemoid showing a division, 42. A guard-cell mother-cell showing vertical division, 43. Mature diacytic stoma showing subsidiary cells at right angle to guard cells, 44. Mature anomocytic stomata.

in semi-aquatic conditions. Leaves are small, with crenulate or deeply lobed margins in plants growing in xerophytic conditions (Text-figs. 71-74). Petioles of these leaves also vary in different conditions. They are quite long in aquatic, medium in semi-aquatic and short in xerophytic conditions (Text-figs. 68-70).

Anatomy of leaflet—Internal structure of the leaflets is also affected in different conditions. In transverse section the leaflet of the aquatic form shows an upper and a lower epidermis; stomata are seen only on the upper surface. Mesophyll tissue is uniform with round parenchyma cells and large intercellular spaces. Endodermis is distinct around the vascular bundles. Xylem is poorly developed with 2 to 4 tracheids and surrounded by phloem. Round the vascular bundles dark-coloured cells, the lignin sacs, are common (Text-fig. 62). In the leaflet of semi-aquatic forms, the stomata are on both the surfaces; the mesophyll is differentiated into palisade and spongy tissue with large intercellular spaces. Xylem and phloem is well-developed and dark-coloured cells round the vascular bundles are less frequent (Text-fig. 64). In xerophytic leaflet cuticle is thick; mesophyll is differentiated into palisade and spongy tissues; palisade cells are completely arranged whereas spongy tissue has small intercellular spaces. The vascular tissue is very well-developed, lignin sacs round the vascular bundles are either absent or very few (Text-fig. 63). The thickness of the cuticle varies in three different forms, but it is quite thick in xerophytic form (Text-figs. 65-67).

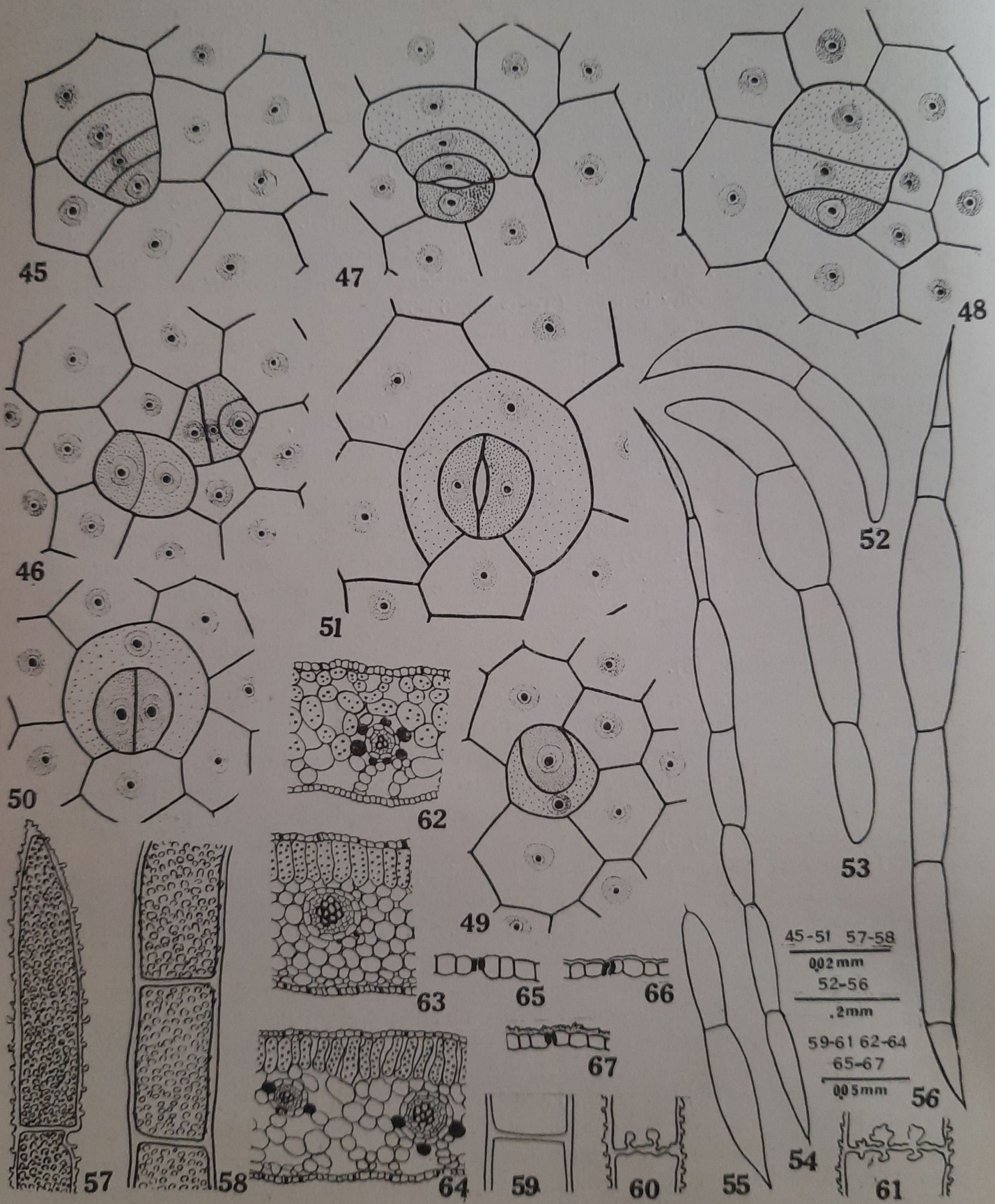
Epidermal cells—There is a good deal of variation in shape, size and thickness of the epidermal cells in different habitat and even in different parts of the same plant. The cells are irregular, thin-walled in aquatic, semi-aquatic and xerophytic forms. Cells are highly irregular and sinuous in aquatic condition (Text-figs. 1-2, 9); medium-sized, irregular and sinuous in semi-aquatic (Text-figs. 3-4) and smaller, irregular and with slightly straight walls (Text-figs. 10-11, 14). It has been noticed that the marginal cells are smaller than those of the remaining cells of the pinnae; they are somewhat elongated and slightly thickened in aquatic, thickened in semi-aquatic and highly thickened in xerophytic forms (Text-figs. 5-7). Epidermal cells that occur on the petiole are elongated and straight walled (Text-fig. 12). Epidermal cells of the sporocarp are hexagonal, rectangular or elongated, quite thick and straight-walled. At the region of raphe epidermal cells show prominent striations in the sporocarps of the plant growing under extreme dry condition (Text-fig. 13).

Stomata—Shape, size, frequency and stomatal index vary in different leaves growing in varied environmental conditions. In the plant growing in aquatic conditions, stomata are fewer in number, larger, irregular, and sparsely distributed, diacytic and anomocytic, occurring mostly on the upper surface, rarely on the lower surface; if present, they show either degenerated stomata or the stomata with comparatively thin-walled guard cells (Text-figs. 1-2, 8).

In the leaflets of the plants growing in semi-aquatic conditions, stomata are irregularly distributed on both the surfaces, smaller and their number comparatively higher than the water form. Stomata mostly anomocytic, rarely diacytic with guard cells slightly thickened (Text-figs. 3-4).

The leaflets of the xerophytic plants show the stomata on both the surfaces but the frequency is higher on the lower surface. The frequency of the stomata in xerophytic forms is quite higher than the aquatic and semi-aquatic forms. Stomata are compactly arranged, smaller, mostly anomocytic and diacytic and the frequency of both the types of stomata are almost similar. Guard cells are thick-walled (Text-figs. 10-11).

Stomata are also present on the petiole of semi-aquatic and xerophytic leaves. They are scattered and are larger than that occurring on the pinnae. It has been observed that some of stomata of semi-aquatic petioles are about two times larger than the stomata of the pinnae (Text-fig. 12).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Abnormal Stomata—Degenerated stomata are commonly seen in the leaflets of the aquatic forms (Text-figs. 2, 8). Stomata with single guard cell are also frequent in the leaflets of semi-aquatic and aquatic forms; such stomata are generally diacytic (Text-fig. 37). Contiguous stomata have been observed in the leaflets of xerophytic forms; these stomata are placed end to end (Text-fig. 17). Exceptionally large stomata—the giant stomata, are seen at places on the petiole of semi-aquatic forms (Text-fig. 12).

Trichomes—Trichomes occur on all parts of the plant. Young parts have numerous crowded trichomes. In older parts they fall off leaving large circular bases. There is a good deal of variation in size and shape of the trichomes in different parts of the plant. Trichomes are generally multicellular and their cells are highly variable. Trichomes occurring on the pinnae are usually 2- celled-sometimes upto 4-celled (Text-figs. 30-35) while those of rhizomatous parts 5 to 14-celled (Text-figs. 26-29, 36) and on sporocarps they are generally long, 3 to 8-celled, rarely 2 to 3-celled (Text-figs. 52-56). Trichomes on the petiole are generally short, 2 to 5-celled and narrow (Text-figs. 18-25). Shape of basal cells and terminal cells also varies. Some of the trichomes possess ornamentation on them (Text-figs. 18-19).

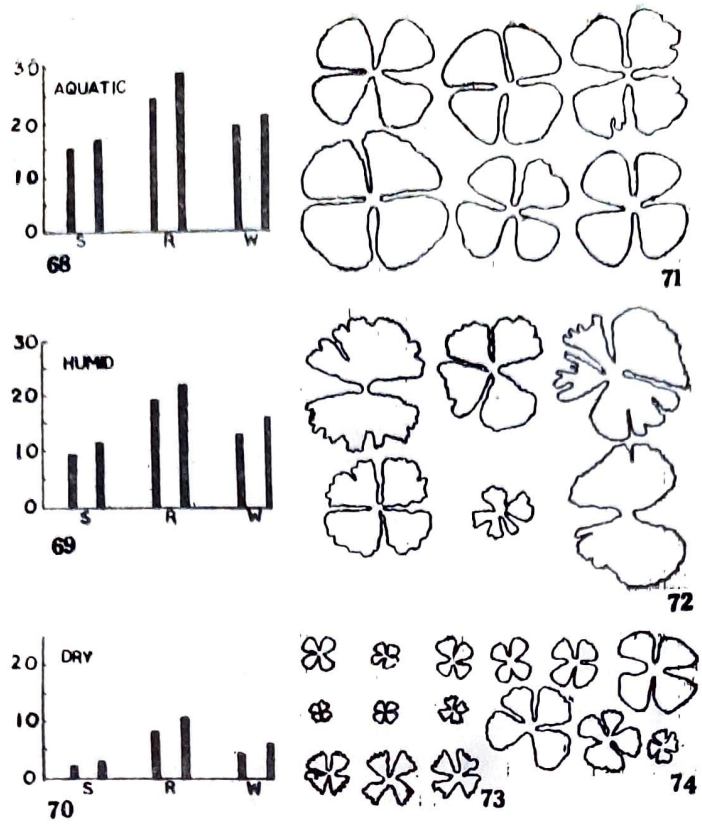
Trichome bases are generally round. They are either one or two-celled (Text-figs. 15-16). The cells round the trichome bases are thick-walled. Trichome bases of the sporocarps are thick-walled and are usually round. Trichomes in aquatic forms are generally long, broad, their cells are quite long, sparely distributed over the various parts of the plant. Trichomes in land forms (xerophytic condition) are usually crowded, short; their cells are narrow and short, sometimes cubical.

Trichomes are generally smooth-walled, but the outer wall of the trichomes occurring on the sporocarp, particularly the plants growing under extreme dry condition, show characteristic patterns, the papillae-like projections (Text-fig. 57). Such trichomes have ornamentation on them (Text-figs. 57-58). Septa of these trichomes are usually transverse and straight-walled (Text-fig. 59), but in some trichomes of sporocarps there are prominent papillae (Text-figs. 60-61).

Ontogeny

Young leaves show many protoderm cells scattered throughout the foliar surface. Some of the protoderm cells divide to form a large and a small cell—"meristemoid" or "stomatal initial".

Text figs. 45—67 ; 45-48. Different stages of anomocytic stomatal development, 45. Stomatal merstemoid showing two parallel divisions, 46. Protoderm cells and stomatal meristemoid showing divisions. 47. A mature stoma showing two parallel subsidiary cells, 48. Stomatal meristemoid showing large cell, subsidiary cell by division, 49-51. Different stages of Eupolo-mesoperigenous stomatal development, 49. Meristemoid showing curved division, 50. Guard-cell mother-cell showing vertical division, 51. Mature stomata showing two guard cells and encircling subsidiary cell which covers about 3/4 of guard cells, 52-56. Different types of trichomes occurring on the sporocarp wall, 57. A trichome of sporocarp wall of xerophytic form showing outer thick papillae and striations, 58. A trichome of sporocarp wall showing smooth walls, 59-61. Different kinds of transverse septa, 59. Smooth transverse septum, 60. Septum showing two small papillae, 61. Septum showing many large papillae, 62. T.S. of leaflet of aquatic form showing mesophyll cells and vascular bundle surrounded by lignin sacs, 63. T.S. of leaflet of xerophytic form showing palisade and spongy tissue, 64. T.S. of leaflet of semi-aquatic form showing palisade and spongy parenchyma with large intercellular spaces, 65-67. Upper epidermal cells of aquatic semi-aquatic and xerophytic leaflets, 65. Epidermal cells not showing any cuticle, 66. Epidermal cells showing thin uniform cuticle, 67. Epidermal cells showing quite thick cuticle.



Text-figs. 68—74 ; 68. Histogram showing the length of petioles in aquatic form in different seasons, 69. Histogram showing length of petioles in semi-aquatic forms in different seasons, 70. Histogram showing length of petioles in xerophytic forms in different seasons (S, Summer ; R, Rainy ; W, Winter), 71. Different types of pinnae of aquatic forms, 72. Different kinds of pinnae of semi-aquatic forms, 73. Different kinds of pinnae of xerophytic forms under extreme dry condition, 74. Different kinds of pinnae of xerophytic forms.

Aperigenous (Text-fig. 38)—The meristemoids function directly as guard-cell mother-cells. The guard-cell mother-cell divides once to form two guard-cells, intervening pore and surrounding epidermal cells which are independently derived from the protoderm.

Anomo-Mesoperigenous—(Text-figs. 44, 45-48)—Meristemoid divides by a wall to form two unequal cells. The small cell functions directly as guard-cell mother-cell or divides second time to form three-celled stage of which the smaller one becomes the guard-cell mother-cell. The guard-cell mother-cell then divides either parallel to second division or at right angle to form the two guard-cells and intervening pore. The stomata formed this way have two guard-cells and one or more subsidiary cells, which are mesogenous in origin and other neighbouring cell perigenous in origin.

Dia-Mesoperigenous (Text-figs. 39-43)—Meristemoid divides to form small cell, the guard-cell mother-cell and a large cell. The guard-cell mother-cell divides to form two guard cells. The larger cell later becomes the subsidiary cell, mesogenous in origin; second subsidiary cell is formed from the neighbouring epidermal cells—periogeneus in origin. The common wall between the two subsidiary cells is at right angle to the guard cells.

Eupolo-Mesoperigenous (Text-figs. 49-51)—Meristemoid divides by a curved wall into a large encircling cell and small guard-cell mother-cell. Guard-cell mother-cell divides by a vertical wall to form two guard-cells. The large encircling cell becomes the subsidiary cell which surrounds the guard-cells on three sides and the other side then is covered by neighbouring epidermal cells.

DISCUSSION

Marsilea minuta is the common species of this genus which grows mostly in different environmental conditions, viz. aquatic, semi-aquatic and xerophytic. Both external as well as internal structures of the vegetative plant body vary in various environmental conditions. Reproductive structures mega-and microsporangia, number of microspores and megaspores and their size are also effected by these factors. We have studied the epidermal structures including their appendages in the leaflets of aquatic, semi-aquatic and xerophytic forms collected in summer, rainy and winter seasons.

The leaves show a good deal of variation in shape, size of leaflets, length of the petioles ; in aquatic and semi-aquatic forms petiole is quite long while in xerophytic forms it is short and about 1/4th of the previous forms. Similarly the pinnae are large with entire margins in quatic forms, gradually smaller and crenulate to deeply lobed in semi-quatic and xerophytic forms. Internal structure of the leaflets are also variable. There are large air spaces and many lignin sacs round vascular bundles in aquatic forms; gradually these air spaces become smaller and fewer. Lignin sacs are absent in semi-aquatic and xerophytic forms.

Epidermal cells vary in different forms; they are irregular with highly sinuous walls in aquatic forms, irregular with sinuous to smooth walls in semi-aquatic and xerophytic forms. Thickness of the cuticle also varies, no cuticle in aquatic forms; thin and thick cuticle in semi-aquatic and xerophytic forms, respectively.

Frequency of the stomata in the leaflets varies in different forms : stomata mostly on the upper surface, frequency very low in aquatic forms ; stomata on both the surfaces, frequency higher on the lower surface, in semi-aquatic forms and stomata crowded on both the surfaces and frequency very high in xerophytic form.

Some abnormal forms such as degenerated, single guard cell, contiguous and giant stomata are recorded. The giant stomata occur on the petiole. The stomata on the petiole are unique as they are almost two to four times larger than the normal stomata occurring on the pinnae. The giant and contiguous stomata are recorded for the first time in this genus.

The morphology of the trichomes also shows good deal of variation. They are usually smooth-walled in aquatic and semi-aquatic forms, but in xerophytic forms they show peculiar papillae and the cells are usually with very distinct striations. Transverse walls of the trichomes usually smooth but in some xerophytic forms which occur on the sporocarp have distinct papillae whose number may be one or more.

Marsilea minuta is usually found growing in water as well as on land and consequently this species is well adapted to water and land. The two forms can be easily distinguished on morphological grounds (Sculthrope, 1967). Land forms have short internodes, branched roots, a few air spaces and more sclerenchyma in vegetative parts. The leaves have long petiole and stomata are distributed on both surfaces of leaflets. Water forms, in contrast, have long internodes, unbranched roots and flexible petioles. There is very little or no sclerenchyma in vegetative parts. There are numerous small and large air spaces in the vegetatative organs. The stomata are restricted largely to upper surface of leaflets.

Gaudet (1964a,b ; 1965a,b) described anatomical distinction and also the different venation patterns in the activity of the marginal meristem of the leaf, both in land and water forms. The typical venation of the land leaf appears to be due to more sustained life of this meristem than in the water leaf. The activity of marginal

meristem seems to be inhibited by etiolation (GAUDET, 1965a). Etiolated leaves resemble land leaves, formed in the presence of light, in having long petioles, and many differ in their venation patterns and in the fact that their leaflets fail to expand. Darkness appears to induce some morphogenetic change which permits conversion of water forms into land forms. The youngest uncoiled leaves of an etiolated submerged plant develop into land leaves on subsequent exposure to light.

Leaflets of water and land forms have been studied by GUPTA (1962), SHARMA (1966) and GAUDET (1964a, 1964b). GUPTA (1962) stated that "the size of stomata is bigger, their number smaller and the epidermal cells more wavy in the xerophytic types than those in aquatic". This statement seems to be incorrect. GAUDET (1964a, b, 1965a, b) stated that "The land forms of the leaf are notable for its heavily cuticularised epidermis with many stomata, whereas in the water forms, leaf epidermis have few stomata but they are lightly cuticularised : a floating leaf has an upper epidermis of the land type and a lower epidermis of water type". Our observations also confirm the findings of GAUDET (1964a,b ; 1965a,b). Our investigation reveals that the stomatal frequency gradually increases, cells are more cuticularised, the leaf lacks air spaces, lignin sacs round the vascular tissue gradually disappear from aquatic, semi-aquatic to xerophytic forms.

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