EPIDEMICAL AND CUTICULAR STUDIES OF LEAVES

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Abstract

The gross morphological features of leaves are regarded as one of the most important tools in plate taxonomy. They have been used for many years in making taxonomic and phylogenetic inferences. However, for a better understanding of taxonomic relationships, cuticular and epidermal characters of leaves also play an important role. Their use has been on the increase in the recent years.

This review gives a comprehensive account of epidemical and cuticular studies of leaves in taxonomic studies. It also includes a detailed account of their historical background, the methods used for their isolation and their application in taxonomy, palaeobotany/phytological, and recent investigations.

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One of the responsibilities normally assumed to per- form in plate taxonomy is the assemblage of a sufficient body of data about plants to permit con- struction of a more nearly correct phylogenetic ar- rangement of the members of the plant kingdom. This body of data has in the past been based primarily upon characteristics of a gross morphological nature. This has served awfully well in a great many of the plant groups large because morphological structures have been demonstrated to have a genetic basis. With in some families the plants are of such a nature that knowledge of morphologically alone leaves much to be de- sired and has been viewed as a gross morphological basis. The gross morphological characters of the leaf cuticle, however, have been used for identification purposes as long as man has been identifying plants. With in- creased sophistication of classification systems it has become necessary to use more elaborate means of identification. The leaf has not lost its im- portance as a taxonomic character, but it has proven to be more useful when a fuller understanding of all its characteristics are known and appreciated. The epidermal characters, if properly interpreted, are being regarded as important taxonomic tools. They should not be dissociated from supplementing the morphological features which have so long been used in the taxonomic and phylogenetic interpretations. Cuticular studies, such as cuticular frequency, stomatal index, type of stomatal complex, trichome types, and others are helpful in understanding relationships which could not be explained otherwise. Fritsch (1903) be- lieved that the characteristic structure of the stomatal apparatus varied distinctly due to an inherent property in the plant and he considered them to be of great importance in the characterization of which their occurrence, distribution, and relative repre- sentancy are genetically controlled, it should be helpful to utilize these features of the cuticle as taxonomic tools, which may be used in phylogenetic compa- risons.

Stebbins and Khush (1961) rightly remarked that another point "which makes the study of the stomatal complexes a desirable tool in taxonomic research is that genera and even families show great constancy for their possession of a particular complex, yet there is consid- erable variability from one higher taxon to another. Thus, at the borders there are methods of cytology and genetics cannot be applied, this study of cuticular characters in the description of plants is of great value. Schenk (1965a) has indicated that, "a soundly based study of cuticular patterns can be put to a good number of uses besides plant taxonomic and medical research, as it is taxonomic research, and phylogenetic investigations. These include paleobotanical, biochemical, and taxonomic research, and animal foodstuff research."

A review of the literature of cuticular studies and of the epidermal features reveals a wealth of data and great interest in a number of faces concerning these features of leaves. This review does not attempt to cover the ontogenetic or physiologic aspect of the stomatal apparatus, but pertains basically to the tax- onomic application of surface features. According to Stace (1965a), one of the earliest refer- ence to leaf cuticle was the note by Brodie, who in 1842, pointed out that the sand stone is freely broken the epidermis of the fossil frequently peels off. The first significant work on the systematic treat- ment of cuticles was that of Bornemann (1856), in which he described the fossil cuticles of Cydonia. He was probably the first to recognize the uses of the cuticles and explained the relationship between them and the line which the outline of the epidermis is almost always on the cuticular membrane by a network of dark brown lines, which apparently represent the cell walls, but which are much thinner than these have been. These brown lines are to be regarded as part of a homogenous tissue . . . . (translation). One of the earliest systematic studies on the surface features of angiosperms was done by C. T. Andrews (1908) who conducted a series of physiological investigations involving the stomata but made his observations simply by noting the presence of stomata. Scriberder (1908) conducted a series of physiological investigations involving the stomata but made his observations only by noting the presence of stomata. Scriberder (1908) conducted a series of physiological investigations involving the stomata but made his observations only by noting the presence of stomata.

Buccolino and Pulelacci (1902) first employed a collection technique for the morphological impress- ions. The prime use of these impressions was the determination of the stomatal numbers of the leaves of various plants. Nathorst (1907-12) was also one of the early workers on cuticular studies. His "Purpurina" was the first attempt to establish a morphological impression. A series of three or rather lengthy examinations Pulelacci finally concluded that a few questions that in all critical cases it furnishes a definite base for determination. The lower epidermal cells in both species are free, but the free surfaces of the epidermal tissue have been proven in Eocene deposits in Bournemouth, England. Lee and Priestley (1964) performed some rather elaborate investigations concerning the structure and function of the cuticle.

Parkin (1954) took a slightly different approach when he investigated the possible relationships of sta- mota to phylogeny. He quoted Hutchinson's paper con-
cerning phylogenetic classification of flowering plants in which he calls attention to the fact that many arboreal species of flowers are found in the terrestrial plant kingdom and that the pollen of these plants is not significantly different from those found in herbaceous plants. He concludes that the different types of stomata were not significant phylogenetic phenomena.

Bancroft (1875) also made some investigation concerning the phylogenetic significance of the stoma. He noted that the character of the stoma is not significant in the evolution of the plant kingdom, and that it is not significant in the evolution of the seed plant kingdom. He also suggested that the stomatal index is not significant in the evolution of the plant kingdom.

Walcott (1927, 1928, 1930) was one of the early investigators to develop a quick method for removing plant tissues from sections of fossil petrifications. He employed a technique that consisted of placing the sections in a solution of potassium hydroxide, which resulted in the clearing of the petrified tissues. Walcott also investigated the characteristics of the cells and the arrangement of the cells in the plant body when they were subjected to the influence of the potassium hydroxide.

Walcott's investigations were particularly useful in separating the parents and the derivatives from the hybrid offspring. He found that the structural pattern of the cells was preserved in the "cuticle". The form of these cells was determined by the parent plant, and the orientation of cell stretch during the formative period. Therefore, the straight and unbranched parallel cell walls are a direct result of the influence of the potassium hydroxide on the orientation of the underlying vascular tissue, which might suggest that during differentiation the veins exerted a direct directional effect on the orientation of the cells. Walcott indicated that this relationship was a much more important factor than the possibility of orientation being an intrinsic character of the epidermis itself. A clear statement of observational methods was missing from his paper, but it would appear that cleared whole leaves and epidermal strips were used.

Ashby (1932) performed a series of experiments on Larrea tridentata (DC.) Cov. and Ligustrum sp., designed to furnish information concerning water loss by the leaves. As was previously discussed, the data indicate a potential usefulness of univalved leaves, but the light of present information tends to be over optimistic.

One of the most important works dealing with leaf surfaces was that of Salisbury (1872) in which he demonstrated the differences in the cuticle of the leaf of different species. In a series of investigations he made use of what was called a 'reflecting microscope', which was used to study the surface morphology of the leaf surface. This approach allowed the observer to see the cuticle under magnification and to recognize the differences in the cuticles of various species. This work provided a valuable tool for the study of leaf morphology.

Florian's (1931) name is important in the field of cuticle classification due chiefly to his contributions in providing descriptions of the cuticles of many species of modern conifers with particular reference to stomata. In addition, his investigations included descriptions of the cuticles of several species of ferns and gymnosperms.

The French researcher Martin (1831a, 1831b, 1831c, 1831d) conducted an extensive series of investigations on the cuticles of the petals and staminal hairs of Tradescantia. He observed that this system was governed by the walls of these cells. He was concerned about the possible relationship and was preoccupied with the stoma.

The arrangement and orientation of the stoma of several monocots and dicots were extensively discussed by Smith (1935). The suggestions were made that "stomata with a "cuticle" which had the peculiarity of being cut, and which was not maximal thickness. During certain stages of development more "cutin" was produced than was needed to cover the surface of a small area, the cuticle which was produced in the "cuticle". The form of these folds was determined by the parent plant, and the orientation of cell stretch during the formative period. Therefore, the straight and unbranched parallel cell walls are a direct result of the influence of the potassium hydroxide on the orientation of the underlying vascular tissue, which might suggest that during differentiation the veins exerted a direct directional effect on the orientation of the stoma. Walcott indicated that this relationship was a much more important factor than the possibility of orientation being an intrinsic character of the epidermis itself. A clear statement of observational methods was missing from his paper, but it would appear that cleared whole leaves and epidermal strips were used.

Wicks (1935) carried out an investigation involving the relationship of stomatal index and stomatal density in Harmananthus and Brachyopogon in which he point-ed out that "stomatal orientation may be characterized by varying the distance from the base to the apex and from the median line to the margin of the leaf. He also found that the stoma
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species as well as separate duplexes from tetraploids of each species, from both stoma count and stomatal index number. The obtained stoma index by 0.0001 but x is the number of stoma and stomatal cells. They found the major difference in this index as compared to Row-

A more detailed analysis of pollen was performed by Strode (1940) who used light microscopy in his work on the stigma of various species. They found that the stigma of Triticum aestivum had two different types of cells: (1) two guard cells and four well-developed and sharply distin-
guished subsidiary cells; and (2) four guard cells and two well-developed subsidiary cells placed laterally to the guard cells; (4) two guard cells and two subsidiary cells; (5) two guard cells without any subsidiary cells. In the material they examined, they found that the stoma number was constant within a genus and "usually constant within the tribe or family.

Since 1960, leaf epidermis-cul tural studies have appeared to have concentrated in three areas. The first of these has involved the use of specific staining methods and its epidermal features. The second has involved the application of these techniques in a larger number of plant families, and the third has involved the utilization of epidermal cuff information with the results of the published studies involving techniques are the following: Carbery (1960) demonstrated a histological treatment of the leaf epidermis; Loner (1969a, 1969b) developed the use of plastic cover slips to make surface replica; Sornara, Meyer, and Owing (1960) made use of Scotch brand plastic tape to show leaf surface features; Sinclair (1961) introduced the use of Anthrac (1960) herb-

Three conclusions were drawn from the information obtained: (1) two guard cells and four well-developed and sharply distinct subsidiary cells; (2) two guard cells and two well-developed subsidiary cells placed laterally to the guard cells; (3) two guard cells and two subsidiary cells; (4) two guard cells without any subsidiary cells. In the material they examined, they found that the stoma number was constant within a genus and "usually constant within the tribe or family.

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The several works of Stace (1961, 1966, 1965b, and 1966) have added a tremendous amount to "leaf epidermal and cuticular characters". He has stated that real value in the study of phytogeography and phytogeography, in that the leaf characters of certain species are found to be in many phylgeoic and phylgeoic and phylgeoic environments to be of little assistance in the classification of species. However, the degree of differentiation between the characters is often small. In addition, the characters are often influenced by environmental factors such as climate and soil.