PRESIDENTIAL ADDRESS

Recent Approaches to the Study of Plant Structure

A. T. GUARD, Purdue University

First of all I want to express my most sincere appreciation to all members of the Academy who have contributed in any way to the work of the Academy during the past year. It would be a pleasure to mention each one by name; however, it would be almost like calling the roll of the membership. Indeed in not a few instances the list would extend beyond the membership. Certainly as one looks back over the work of our Academy and the names of individuals who have ranked high in their respective fields it is not at all presumptuous to say that we have a proud history.

Proud as this history has been, however, this is not a time to look backward. I am especially grateful that there is now an active interest in our organization because I see for it an increasingly important role in the educational development of our state and more particularly in the fields of science in the state.

True, we find competition for our time and efforts in an increasing number of more highly specialized scientific organizations at the national and even the international level, but none of these are designed, nor are they in position to do for the State of Indiana exactly what our Indaina Academy of Science, with its broad type of organization, is able to do. I see for the Indiana Academy of Science both a tremendous opportunity and a stimulating challenge. In view of the fact that we represent every segment of scientific interest in the state, I feel confident that our Academy will be capable and willing to give to Indiana the kind of sound and progressive scientific leadership which she will so much need in the future.

Now to the subject at hand; namely, recent approaches to the study of plant structure. Some fifteen years ago the late Dr. Neil Stevens, then at the University of Illinois, remarked of the biological sciences that they were progressing so rapidly that what is new today will be obsolete within a few years. He went further to say that if there was one area of the subject which might be regarded as definite and fixed it would be the area of morphology and anatomy.

Certainly the anatomy and morphology of organisms has not changed markedly even within historic times. However, the same might be said for other vital phenomena. There is, however, some difference in our approach to an understanding of plant structures—I speak of plants more specifically because this is the field of my special interest and study. That the same is true in biological fields in general, I have no doubt.

As we are confronted with new problems and as we seek to find a more complete understanding of old problems, we naturally look for new techniques and apparatus to investigate these problems. Conversely as new techniques and new apparatus are developed they suggest new problems and new approaches to old problems.

In such a subject as plant anatomy and morphology this is very much the case. It is my purpose to discuss a few of these examples. As many of you are well aware, one of the peculiar characteristics of higher plants is the capability of the root tips and the shoot or stem tips to continue growth almost indefinitely and to initiate primordia, or the beginnings of new organs, such as leaves and branches. More recently studies have been directed toward an understanding of the degree of autonomy possessed by the apex. Is it largely dependent upon the influences of the tissue below, or is the apex largely a self-determining growth center in which the number, form and arrangement of organs originating are determined by influences within the apex itself?

Wardlaw (5) and others have investigated the nature of differentiation of organs here by using the fern *Dryopteris cristata*. Ferns are especially well adapted for this type of study because of several characteristics. While many plants have only two or at most four stages of developing leaf primordia at each apex, the ferns have very many primordia in a wide range of stages of development and the total developing apex is unusually large. Another characteristic which makes these plants suitable for such experiments is that the mature leaves may be removed from the short stem to uncover the primordia. This short stem may then be placed in a properly prepared humid container and allowed to grow during rather long periods of time.

In order to determine the influence of the extreme apex and the adjacent primordia on any one primordium that primordium was isolated by micro-sections made on each side of it. Without going further into the details of the experiment, it can be pointed out that if a primordium close to the tip which would normally develop into a leaf was thus isolated it developed not as a leaf but as a stem. If, however, the primordium was allowed to develop until a definite apical cell was formed, before the isolating cuts were made it did develop into a leaf. Here we see a technique employed which indicates the degree of autonomy of the apex. These experiments also showed that there is a stage—and that very early—in which differentiation has gone beyond the point of no return. Here we see a trend in the study of plant structure. An attempt to discover not merely what is the nature of the mature structure, but also what are the forces which result in the origin and final production of these structures.

The nature of the stem and root apices and of the histogen or histogens into which these apices may be resolved has long been a subject of study and controversy. I shall not go into the very long and controversial interpretation of this problem. It may be of interest, however, to mention some more recent developments along this line. Clowes (2), 1958, has postulated what he terms a "quiescent zone"—a cup shaped region—just inside the root cap; at the very apex of the root proper. His data indicate that these cells are not actively dividing or that their rate of division is much slower than the adjacent regions just back of this area. Here he uses a combination of two of the newer techniques of investigation namely radio-active compounds and the production of DNA as an indication of rate of cell division. On this basis he draws his conclusion of a less active dividing group of cells in the quiescent zone. There may also be a concommitant conclusion that the metabolic activity in this zone is higher than the adjacent region. These conclusions have not met with unanimous agreement as yet; however, they do present some very interesting implications. This condition pointed out by Clowes in the root has also been pointed out by Buvat and others to be present in the stem apex. Here it has been studied somewhat more intently by a number of investigators. Perhaps the French workers have been most aggressive in their investigations. Buvat (1), in particular, has emphasized that there is an apical meristem back of which occurs the initiating ring—meristem. It is this latter which is active in the vegetative growth and where cell division produces new leaf primordia and stem growth while the extreme apical meristem is, as it were, passively carried along. When the change from vegetative to reproductive phases is initiated this more apical group of cells becomes active resulting in the broadening of the apex and the initiation of floral organs instead of leaves. Here again there is not complete unanimity of opinion. There are those who fail to see the distinct areas of the general apex which Buvat and his supporters see.

There are certain basic concepts which have been studied, through the use of tissue cultures of higher plants. One of these is the very significant effect of the apex on plant organization. If one takes a portion of the apex of a plant and grows it on a nutrient medium eventually a root will form and the culture will—if properly cared for—grow into a complete new plant. If, on the other hand, a portion of the plant other than the apex is taken, it will very probably produce a mass of cells which are only a group of homogeneous unorganized parenchymatous cells resembling early stages of what we speak of as wound or callus tissue. If this callus tissue is cultured in the ordinary nutrient medium to which coconut milk has been added, it grows and the cells aggregate until a considerable mass of tissue is formed. Under these conditions there sometimes arises in this mass of tissue, cells which take on the character of xylem tracheids and possibly phloem cells. If such differentiated cells do arise, in some cases this is followed by root formation then shoot formation and eventually a new plant is organized.

These tissue culture experiments may throw considerable light on the importance of the role played by the endosperm in the nutrition of the very young embryo. That coconut milk as well as material from other endosperm tissue used has been shown to be effective in stimulating the embryo may suggest the importance of the endosperm in a role other than merely as a food storage organ for the developing seedling. This sequence of the formation of a mass of tissue followed by a more definite polarity, then the development of root and shoot primordia bears a striking resemblance to what happens in the development of the embryo of a seed.

Wetmore (6) and his students have shown the organizing effect of the apical region on masses of tissue grown in culture. They have grafted buds of lilac in masses of parenchymatous tissue from tissue cultures of the same species. They found that the bud grew and induced the formation of xylem tissue in the mass of homogenous tissue directly below. Here we begin to see some of the basic mechanisms which bring about the organization and differentiation of the stem axis in the plant. We not only see the final organization and morphology of the plant, but also see the interplay of influences which determine the final structure and organization of the plant.

One of the most elusive as well as fascinating problems of the Plant Physiologist is the induction of flowering. The problem of the presence of a florigen if, indeed!, it is a definite substance has long been studied but yet there is no definite answer as to exactly what it is. Here it seems a better understanding of the anatomical similarities and differences of the apex producing leaves and one of the same species producing a flower or a group of flowers may help to solve the problem. Here the work of Buvat and others indicate that there are certain patterns of development which change as the apex changes from a leaf producing apex to a flower producing apex. According to Buvat and others this central area which earlier showed no, or only limited, cell division now becomes more active. As a result of its activity the apex becomes broader and the primordia—of floral organs are produced much more nearly in a cyclic than in a decussate or spiral manner. Not everybody agrees with Buvat and his coworkers, but if this anatomical fact could be firmly established it should promote a better understanding of the physiology of floral induction. Perhaps the number of cells actually involved in floral induction is much less than has ordinarily been thought to be the case. It may be that the magnitude of the inducing substance or substances is much less than has been looked for.

The electron microscope has enabled us to go much deeper into the nature of protoplasmic structure and the nature and sequence of events in the formation of the cell wall. By properly treating the cell walls of plants either chemically or enzymatically, we are able to observe by means of the electron microscope the actual arrangement of these cellulose microfibrils of the cell wall. Frey-Wissling (3) studied this problem several years back and noted that the first microfibrils laid down were in a very irregular arrangement. He also noted that microfibrils laid down later seemed to follow a particular pattern usually parallel and at an oblique angle to the long axis of the cell. He applied the earlier used terms, primary wall and secondary wall respectively to these. As Dr. Nisbet demonstrated in our laboratory this is not in accord with the older and more generally used terms, primary and secondary wall. Indeed!, he found the parallel microfibrils were laid down so close to the tip of the root and so early that to use the arrangement of the microfibrils as a criterion to designate secondary and primary wall would make the terms useless except in a very limited way when studying the sequence of microfibril distribution by means of electron micrographs. It would assign an entirely different meaning to these terms. Already the electron microscope has enabled us to see more intimately the sequnce of events involved in the building up of the cell wall. Eventually it will give us a correct understanding of the nature of the forces and influences which are brought to play upon the formation of the cell wall.

One marvels as they observe the uniformity of development of plants both in form and in the effective timing of the changes which bring about the final development of the individual with such striking regularity. For years almost the entire basis of our taxonomic system was based upon morphological similarity. Obviously this is still one of our mainstays of systematic botany. Genetically we rather casually speak of these characters as gene controlled. Occasionally one of these highly regular growth processes gets out of step genetically—either by mutation or otherwise. It is then they may be studied by comparison of the abnormal with the normal. This is well illustrated by the paper reported by Postlethwait and Nelson (4) on the anatomical cause of wilting in a genetic strain of corn which wilted even when available soil water was high. Their anatomical investigations showed that this condition arose as a result of the delay or failure to mature of the meta-xylem of the intercalary meristem. This resulted in markedly reducing the water supply to the leaves of the plant. This presents an excellent example of the need for correlation of structural development in both time and place. It also offers an opportunity to study what influence results in the normal development.

The paper presented by Miss Clements in the Botany Section indicates one of the approaches in which we are interested in our own laboratory. Here she has compared anatomically a genetic strain of tomato which does not produce axillary buds with a normal tomato. We hope to carry this experiment further and see if we can discover any biochemical difference between the genetic variant and the normal tomato. We believe that with this approach we can obtain a better understanding of the development of a normal plant.

We might continue almost indefinitely reviewing work that has to do with influences both internal and external which are brought to bear on the plant as it attains its final form. This is enough, however, to indicate that with new apparatus and new technique there are many rewarding fields of study in plant form and structure.

References

1. BUVAT, R. 1952. Structure évolution et fonctionnement du méristéme apical de quelques Dicotyledones. Ann. Sci. Nat. Bot. XI. 13 : 199-300.

2. CLOWES, F. A. L. 1959. Apical meristems of roots. Biological Reviews. 34 : 501-529.

3. FREY-WISSLING, A. K., MUHELTHALER and R. W. G. WYCKOFF. 1948. Microfibrillenbauder Pflanzlichen Zellwande. Experientia. 4 :475-476.

4. POSTLETHWAIT, S. N. and O. E. NELSON. 1957. A chronically wilted mutant of maize. Am. Jour. Bot. 44 : 628-633.

5. WARDLAW, C. W. and ELIZABETH G. CUTTER. 1956. The effect of shallow incisions on organogenesis in *Dryopteris aristata*. Ann. Bot., n. s. 20 : 39-56.

6. WETMORE. 1956. Growth and development in the shoot system of plants. Soc. Devel. and Growth, Symposium. 14 : 173-190.