Theoretical and Practical Uses of Plant Cell and Tissue Culture

Recent advances in plant cell and tissue culture techniques have helped this general field of research mature to the extent that increasing usage is being made of the many approaches possible, both for basic biological studies as well as for practical exploitation for agricultural purposes. With the hurdles now cleared of demonstrating totipotency, obtaining suspension cultures of cells and protoplasts, and inducing haploid clones from pollen and anthers, the techniques of microbial genetics are starting to be applied to higher plant systems. The stage is now set for the induction, detection, and identification of desired mutants for the study of biosynthetic pathways and for increasing desirable agronomic characteristics.

To discuss the ways in which all of these techniques now available can be best utilized, a joint seminar by the United States and Republic of China was held in Taipei, Taiwan, on 14 to 22 May 1974. In all, 25 scientific papers were presented, 15 of them by Chinese scientists, 1 by an invited speaker from Japan, and 9 by visiting scientists from the United States. The seminar was under the auspices of the U.S.–Republic of China Cooperative Science Program sponsored jointly by the National Science Foundation (U.S.) and the National Science Council (R.O.C.).

In planning the program, the coordinators from R.O.C. (M. C. Liu) and the United States (L. G. Nickell) concentrated on those areas of research in which the Chinese scientific community had the greatest current interest and for which there is the most promise of immediate increase in research effort among Chinese investigators. The program was divided into ten major topics: differentiation of organized structures, metabolism, pathology, micropropagation of horticultural crops, protoplasts, tree improvement, mutation and selection, products, haploidy, and crop improvement.

The formal sessions were interrupted by a tour of the entire island of Taiwan, site visits being made to research and commercial institutions that conduct (or have plans to begin) experiments on and that practice utilizing plant cell and tissue culture techniques. These site visits included the White Cloud Orchid Farm (Taipei), the National Chunghsing University (Taichung), the Asia Vegetable Research and Development Center (Shan-hua), the Taiwan Sugar Research Institute (Tainan), the Tsengwen Reservoir, and the Biological Research Center of Academia Sinica (Taipei).

A major economic application of plant tissue culture techniques has been in the area of clonal multiplication. T. Murashige (University of California, Riverside) reported on the widespread utilization for herbaceous genera. His discussion included examples of the three principal methods by which clonal multiplication can be attained in vitro: (i) asexual embryo formation in callus and other tissues, (ii) adventitious shoot initiation which are then treated as stem cuttings and rooted to give rise to whole plants, and (iii) enhancement of axillary shoots followed by rooting of shoot cuttings. The major factors discussed were characteristics of the explant, nutrient medium composition, light provisions, and temperature requirements. P. J. Wang (Academia Sinica) and M. C. Liu (Taiwan Sugar Research Institute) discussed the differentiation of potato and sugarcane plantlets, respectively.

In his discussion of the various facets of nitrogen metabolism in plant cell cultures, D. K. Dougall (W. Alton Jones Cell Science Center, Lake Placid, New York) considered (i) the nature of the uptake of materials, (ii) the interconversion of compounds taken into cells, (iii) the regulation of interconversion of small molecules (especially amino acids) synthesized in cells, and (iv) the regulation of the level of proteins. J. C. Su (Academic Sinica) discussed the differences between the callose synthetases from tobacco callus tissue and from the pith tissue from which the callus was derived. K. L. Lai (National Taiwan University) discussed the effects of light on the growth of excised rice roots in culture. His results suggest that the light inhibition of growth is controlled by the photochemical reactions of root metabolism, probably the reversible phytochrome system.

The established practical values and the potential values of plant tissue culture methods for the control, or even elimination, of pathological problems were discussed by A. C. Hildebrandt (University of Wisconsin, Madison). W. H. Chen and L. S. Leu (both from the Taiwan Sugar Research Institute) presented separate papers on methods of freeing sugarcane from the downy mildew fungus disease by the utilization of plant tissue culture techniques.

Two papers were presented on the micropropagation of horticultural crops —one by S. S. Ma (National Taiwan University) on bananas, and one by W. J. Ho (Tainan Seed Service) on *Pleione formosana hayata*, one of Taiwan's most famous native orchids.

The culture of higher plant protoplasts has been sufficiently developed to have become a useful tool for attacking many botanical problems. This utility results both from the presence of a cell membrane that is essentially devoid of cell wall and from their being ideal material for unique experiments on individual cells in culture. A. W. Ruesink (University of Indiana, Bloomington) summarized the work in his own and other laboratories on protoplasts and their surface membrane properties as follows. (i) The surface is resistant to attack by proteases, lipases, and phospholipases; (ii) protoplasts are susceptible to breakage by detergents and certain macromolecules with high pK_a's; (iii) the membrane in most osmotica bears a preponderance of negative changes on its surface; (iv) the outer membrane of a lysing protoplast disintegrates differently in different stabilizing osmotica, suggesting that surface membranes assume different molecular arrangements or charges depending on the external conditions; (v) healthy protoplasts will often regenerate some sort of a wall, but the wall may be abnormal; (vi) protoplasts continue active uptake across their outer membrane; (vii) although most cellulase preparations are optimally active at about *p*H 5 or below, protoplasts once released seem to be most stable at *p*H 5 to 6; (viii) the plasma membranes of adjacent protoplasts can be induced to fuse either intraspecifically or interspecifically; and (ix) the nature of the responses to auxin of the plasma membrane of protoplasts is still controversial. T. C. Tseng (Academia Sinica) discussed the method in which a mixture of three enzyme preparations is used for producing large amounts of intact protoplasts from many Taiwan crop plants, including soybean. Studies with fusion of protoplasts were also discussed. M. C. Liu presented results of similar studies with five species of sugarcane. C. P. Pi (National Taiwan University) discussed his observations with the scanning electron microscope of protoplast formation and cell wall regeneration in *Nicotiana tabacum*.

L. L. Winton (Institute of Paper Chemistry, Appleton, Wisconsin) discussed the use of callus, cell, and pro-

toplast cultures in tree improvement programs. Most of the effort and success has been with poplar stock. The surprising delay in emphasizing work with woody species was discussed by several participants.

Now that (i) cell suspension cultures can be manipulated with considerable ease, (ii) haploid cultures can be established for numerous plants, and (iii) totipotency has been demonstrated for many plants, interest has mounted rapidly in obtaining mutants for the study of biochemical pathways and for crop improvement. The biggest deterrent to rapid development of this specific approach is the lack of selection methods. J. M. Widholm (University of Illinois, Urbana) discussed the mutation and selection techniques now used and the results obtained, emphasizing his own work with the biosynthetic pathways for aromatic amino acids.

After a surge of effort in the 1950's on production of secondary products from plant cell and tissue culture, the total efforts subsided for a decade or more. Recently, interest has again mounted in this area. E. J. Staba (University of Minnesota, Minneapolis) discussed the success in the in vitro production of alkaloids (ergot, opium), steroids (diosgenin), triterpenes (ginseng), and proteins (allergens). M. Misawa (Kyowa Hakko Kogyo Co., Tokyo), presented his results with the production of amino acids (glutamine), enzymes (5-phosphodiesterase), antitumor alkaloid (camptothecine), proteinase inhibitors, and inhibitors of plant virus infections.

The ability to obtain haploid plants from pollen and anthers and to derive diploid plants from them opens experimental approaches previously restricted in biochemical and genetical investigations of higher plants. I. K. Vasil (University of Florida, Gainesville) reviewed experimental androgenesis from its initiation to the present, emphasizing the usefulness of haploids for numerous types of investigation. Haploid rice and sweet potato were discussed by S. C. Woo (Academia Sinica) and H. S. Tsay (National Taiwan University), respectively.

Little effort has been made toward utilizing these techniques with agronomically important crops until the last few years (*1*). L. G. Nickell (Hawaiian Sugar Planters' Association, Honolulu) discussed the potentials of cell and tissue culture for crop improvement. This discussion briefly included meristem culture and embryo culture and emphasized manipulation at the cellular level to obtain new plant materials by asexual methods. M. C. Liu discussed the application of these techniques to sugarcane. P. J. Wang reported on the success in elimination of potato viruses and the subsequent increase in seed potato production in Taiwan.

The final session was devoted to discussion of potential cooperative projects between investigators and institutions in the two participating countries and to specific research which should be emphasized in the immediate future for the benefit of agriculture in Taiwan.

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References

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Isozymes: The Third International Conference

It is now well known that enzymes commonly exist in multiple molecular forms even in a single organism. The recognition of this fact has led to a powerful and integrative concept of contemporary biology, chemistry, and medicine—the isozyme concept. The utilization of isozymes in research has had an impact upon virtually every biological discipline. This impact of the isozyme concept and supporting technology was amply demonstrated at the Third International Isozyme Conference held at Yale University, 17 to 20 April 1974. The conference was supported by grants from the National Science Foundation, the National Institutes of Health, and private sources. Over 400 scientists attended, 70 from 22 foreign countries; 224 formal reports were presented in nine simultaneous sessions.

A plenary session for all attendees was held each morning, followed by the simultaneous sessions for the remainder of each day. The first plenary lecture on the biology of isozymes was presented by Clement L. Markert (Yale University), who first formulated the isozyme concept and who has done much to demonstrate the biological and chemical significance of isozymes. The papers presented at the conference demonstrate that virtually all enzymes can exist in multiple isozymic forms. Researchers studying enzymes have become aware of this, and have described many different varieties of isozymes such as allelic isozymes (or allozymes), multilocus isozymes, and conformational isozymes.

In his opening presentation Markert pointed out some of the significant biological roles that isozymes play, and the means by which isozymes can be used to probe many kinds of processes of biological organization. In addition to emphasizing the role of isozymes in enhancing the biochemical precision of cells, he also pointed out the insights that isozymes have provided into the structure, function, and evolution of genomes. The use of allelic isozymes has permitted the rapid growth of evolutionary genetics in the last 10 years, particularly in investigations of gene flow between populations and in elucidation of the mechanisms of speciation. The multiple locus isozyme systems have provided insights into the mechanisms of gene duplication as well as into the evolution of different catalytic specificities. Furthermore, the multiple locus systems permit the study of the evolution of gene regulation and the control of specificity in gene function. Markert predicted that some of the most promising lines of isozyme research will involve investigations of evolutionary relatedness of genes (allelic and nonallelic), the specific physiological roles played by isozymes, and the role of isozymes in cellular and subcellular architecture. The use of isozymes as gene markers to analyze the genetic and molecular basis of cellular regulatory systems will also continue to increase, he predicted.

The second day's plenary session was presented by Bernard L. Horecker (Roche Institute of Molecular Biology) on the biochemistry of isozymes. He described his rigorous analysis of the multiple locus aldolase isozyme system to illustrate the chemical differences between isozymes, as well as the contemporary technologies used for purification and characterization of isozymes. He, as well as others at the conference, emphasized the increasing utility of affinity chromatography. He also demonstrated that certain isozymes could be generated epigenetically by a secondary modification of an enzyme after initial synthesis. He emphasized that a clear understanding of the molecular basis of isozymes should be obtained before inferences are drawn about biological roles and significance. He also helped focus attention on the fact that, although many studies of isozymes dealt with synthesis, only a few have so far dealt with degradation, which may be as important as synthesis in regulating cellular metabolism.

The plenary session speaker for the third day was Elliot S. Vesell (Pennsyl-