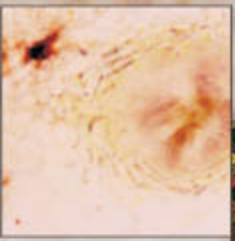

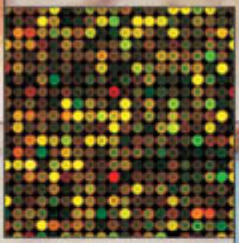

Applications of Plant Biotechnology

In Vitro Propagation, Plant Transformation and Secondary Metabolite Production

Editors
Ashwani Kumar • Sudhir K. Sopory

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Professor Jack Milton Widholm Commemorative Volume

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FOREWORD

It is a pleasure to write a foreword to Applications of Plant Biotechnology: In vitro propagation, plant transformation and secondary metabolite production, dedicated to Professor Jack M Widholm of University of Illinois, USA. I have known Jack for 30 years. He covered a broad program of areas of cell and tissue culture and plant biotechnology starting many new directions and published results to be applicable in many other fields of Botany. Jack is one of the first few cell culturists getting interested in photosynthesis of cultured cells and making significant contribution to establishing the autotrophic cell suspension which were later used to select resistant strains to herbicides like atrazine which are photosynthesis inhibitors. Another significant first contribution was to draw attention to somaclonal variations, or epigenetic influences on cultured cells. This is an area one still does not know yet what consequences this willl have for our understanding of the genetic machinery of plants. In a number of recalcitrant species his lab established systems of somatic embryogenesis, to mention only Glycine and Zea. I could go on and on. The plants he worked with were mainly of agronomic importance. His working at the Department of Agronomy also reflects his bringing up at a farm (as I was myself) and getting in touch early with practical agriculture. We always met and parted as friends, even in spite of discrepancies at the meetings. My best wishes to Jack and his family in the future.

The present book is a compilation of papers written by leading scientists engaged in the field of plant cell and culture and biotechnology. The articles presented in it cover specific aspects of plant biotechnology, with respect to practical applications. Between others, considered are propagation of plants for breeding purposes, gene technology and use of plant cell cultures producing compounds of the secondary metabolism of medicinal interest. These contributions indicate broad acceptance of plant biotechnology and its successful applications. India has made continuous and successful efforts to be in the forefront in the research of this exciting branch of Botany.

I recommend this book for all interested not only in this field, but also to the entire scientific community at large. I extend my hearty congratulations to the editors for producing this book and wish them all the very best for its reception by the audience.

Karl-Hermann Neumann

Prof. JACK MILTON WIDHOLM

I was born on March 11, 1939 in Watseka, Illinois, US and grew up in Ashkum, Illinois, a small town of about 400 people. My father operated the grain elevator and lumber yard in Ashkum which was located in a fertile prairie-formed soil area where farming was the main occupation.

I was an avid gardener growing fruits and vegetables including popcorn. I noticed that small-eared red popcorn could cross with big-earned white popcorn to form big-eared red that I then grew a lot of and showed it at the county fair. I also did experiments to determine the optimal moisture content for popping since my father had a moisture meter at the grain elevator. I also grew many trees and bushes that I am still doing today on our 11 acres of land which has two ponds. I also still grow my own popcorn that I eat almost daily.

After high school I attended the University of Illinois at Urbana-Champaign and received a B.S. in Agricultural Science in 1961. I was offered an assistantship at the California Institute of Technology in Pasadena, California and worked with James Bonner on DNA and RNA isolation and characterization and purified *E. coli* RNA polymerase for the whole lab to use. I received my Ph.D. in Biochemistry in 1965.

During graduate school Jacob and Monod in France were making great discoveries in the area of microbial genetics, enzymology and biochemistry and Phil Filner was a fellow graduate student working with tobacco suspension cultures. It seemed that such cultures would be ideal for doing mutant selection and biochemistry with plants like Jacob and Monod were doing with bacteria.

After one year of postdoctoral work with Bonner, mostly working on his histone-RNA concept, I went to work with a company, International Minerals and Chemical in Libertyville, Illinois. Interestingly they became interested in the idea of plant genetic engineering long before it was possible. After two years (1968) I was offered an Assistant Professor position in the Agronomy Department at the University of Illinois and I am still here.

The first project carried out with Bill Ogren was to attempt to select plant mutants that lacked photorespiration since this process found in C3 plants decreases net photosynthesis greatly. My idea was to grow soybean plants under low CO_2 conditions where plants with photorespiration would lose CO_2 and any mutants would not, so they would survive. When the C4 plant corn, that does not photorespire, was placed in a sealed chamber with soybeans the CO_2 level was pulled down and the soybeans died in a week. Unfortunately no mutants were found after screening about 350,000 mutagenized seedlings and later studies by others with larger numbers of the much smaller Arabidopsis plants were also unsuccessful. No one has been able to alter photorespiration yet.

I also began selection with carrot and tobacco suspension cultures for resistance to toxic amino acid analogs and was successful with tryptophan (Trp), methionine, proline, lysine and phenylalanine analogs. A number of studies were carried out with these lines that provided new information about the control of amino acid biosynthesis in plants. Since in most cases the resistance lines had increased levels of the target free amino acid the idea of selecting mutants with improved nutritional quality or higher secondary compounds related to the amino acid was proposed.

Of all the mutants selected the ones that were resistant to Trp analogs have been studied the most with the first publication in 1972, and we are still doing work with the gene from tobacco that encodes a feedback resistant form of the Trp biosynthesis control enzyme, anthranilate synthase (ASA2). Much of the enzyme and amino acid analysis work was done by Jeff Brotherton who worked in this lab for many years.

We have done selection for resistance or testing for resistance of different cultures with herbicides, fungal toxins, salt, iron-deficiency chlorosis, heavy metals and allyl alcohol. The mechanism of action of a number of herbicides including glyphosate (Roundup) has also been studied by students of Fred Slife in my lab. The selection with glyphosate has revealed that resistance in cultures of many different species is due to amplification of the target gene.

It seemed important to know if various treatments affect tissue culture cell viability so I did a study to find viability stains and found phenosafranin to be excluded from live cells and fluorescein diacetate to be taken up and hydrolyzed to become fluorescent inside live cells. These methods were published in 1972. In later work we also worked with triphenyltetrazolium chloride as a viability stain.

We began initiating photoautotrophic cultures in the early 80s, with the idea of selecting cultures resistant to herbicides like atrazine that are photosynthesis inhibitors to determine if different resistance mechanisms are possible. Mike Horn was successful and suspension cultures of soybean, Amaranthus, *Datura innoxia*, tobacco and cotton were established: These are about half of the good growing cultures in the world and are still in the lab.

For three months in the summer of 1979, I spent a sabbatical in the lab of Ingo Potrykus in Basel working mostly with protoplasts and began protoplast fusion studies using amino acid analog resistant cultures for direct selection. The lab did a number of such studies over the years using different resistances and auxotrophy. Yasuyuki Yamada, Toshiyuki Nagata and Kazuo Nakata visited the lab for about a week during the summer of 1980 to do experiments on protoplast fusion as part of a U.S.-Japan project supported by the National Science Foundations of each country. I visited their labs in 1982.

In my one visit to India I was hosted by J.B. Chowdhury, Haryana Agricultural University, Hisar for about a month. My stay concluded with an international conference in Calcutta on “Crop Improvement Through Tissue Culture”. J.B. worked in my lab on two occasions over the years.

Since Illinois is in the U.S. Corn Belt we did a lot of work on corn tissue cultures in the 1980s and 1990s spearheaded by Dave Duncan. This work included improvement of the culture medium so that regenerable cultures could be initiated from almost all inbred lines and demonstrating high levels of somaclonal variation in regenerated plants. Yuechun Wan was able to induce high levels of chromosomes doubling in haploid anther cultures using colchicine and anti-microtubule herbicides and carried out transformation with type I corn callus. Early work

that showed differential cell wall staining of regenerable and non-regenerable corn callus has been followed up by more detailed cell wall analysis and metabolic profiling. This was lead by Vera Lozovaya in this lab, to show the chemical differences between this callus types that can be diagnostic for plant regeneration ability.

We were the first lab to demonstrate plant regeneration using the wild Glycine species. This regeneration work was started by Toshiaki Kameya, who also did a lot of protoplast fusion, and Usha Barwale later demonstrated good organogenic and embryogenic plant regeneration systems and somaclonal variation in the Glycine max (soybean) regenerants. We are now using an embryogenic system for soybean transformation that is being done by a technician Wei Zhong who has worked with me for many years.

Most of my teaching involved one lecture-lab course “Tissue culture and biochemical genetics of higher plants” that many years ago provided the first introduction to molecular biology for graduate students. Now there are many molecular biology courses. We carried out real experiments in the lab part and published several papers as a result.

The lab is presently still in operation and has recently been able to select plastid transformants of tobacco using the tobacco ASA2 gene as selectable marker and work is under way to do the same with corn that has never been done before. We have also done metabolic profiling of soybean transformed with ASA2 and with cyanamide hydratase to determine how their expression affects plant metabolism. Vera Lozovaya is heading efforts to increase the levels of glyceollin, the soybean phytoalexin, to increase disease resistance. We are also attempting to develop methods to insert genes into the potential biomass plant Miscanthus giganteus.

I feel very fortunate to have been able to be involved in research for over 40 years with such able colleagues who have had most of the ideas and who have done most of the work. I also have many friends both in the U.S. and around the world. My only regret is that there are many interesting projects that were never completed.

I thank my old friend Ashwani Kumar for this great honor.

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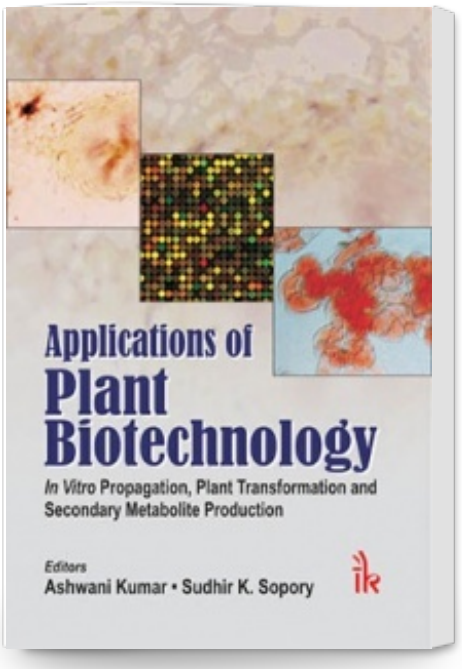

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