Plant molecular biology in India – The beginnings

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In this article we trace the growth of studies in plant physiology and biochemistry in India and their gradual transformation to research in plant molecular biology utilizing the recombinant DNA techniques which began in the eighties. Initially, the universities and institutions in Calcutta and Delhi have played the leading role. Although now there are several centres, and research is being done on many aspects – such as photosynthetic genes and their expression, molecular biology of stress, signal transduction mechanisms and studies on plant genomes – it is clear that in India these studies are still in their infancy. We end this article with a few suggestions and recommendations for all concerned to ponder over with a view to help accelerate research.

IN India, the rise of modern plant biology has been largely associated with a few universities where there were distinguished teachers of plant physiology and biochemistry or of general plant biology though certain research institutes, in particular the Bose Institute, also played a significant role. Around 1950s, there were three major schools of experimental botany. At Allahabad, S. Ranjan, who had returned after a doctorate from Cambridge with F. F. Blackman, founded a group that worked on various aspects of plant physiology. Govindjee, Ravinder Kaur (later Sawhney) and M. M. Laloraya were all members of this group. Govindjee proceeded in 1950s to work with R. Emerson and E. Rabinowitch and became a distinguished authority on photosynthesis. Ravinder Kaur worked with A. Galston and has pioneering contributions on polyamines. However, both went to USA for good. Laloraya worked with K. Thimann (at Harvard) and came back to continue some pioneering work on mechanism of action of plant hormones[1,2]. Laloraya moved to Ahmedabad and then to Indore where his talents were utlized more to build a School of Life Sciences and to administer the newly established university of which he became the Vice-Chancellor. He however continued to devote part of his time to research (and recently advanced a proposal to explain phototropism)[3], but to support work on a broader front he took up studies largely on animal hormones.

At Calcutta, S. M. Sircar, who had worked at the Imperial College of Sciences and Technology, London, had an extended and substantial role in promoting plant physiology and biochemistry. He established a group at the botany department of the university to study seed physiology and plant hormones. In the mid-fifties students like S. P. Sen, B. B. Biswas and others became interested in physiology and biochemistry (plant molecular biology was not born then, but it is these studies that later transformed into this new field). Another person who significantly influenced research was R. N. Singh of Varanasi. Although the botany department of the Banaras Hindu University was then known more for studies in algae and ecology, he came in contact with R. Burris (at Wisconsin) and did some of the first experiments on cell-free nitrogen fixation with blue-green algae. Biswas was attracted to work at Varanasi and produced his first publication in *Nature*[4] before returning to Calcutta to join the Bose Institute. Calcutta had a clear lead in molecular studies in plants in the country.

Nevertheless, an equally significant role in the modernization of plant sciences was played by the late Panchanan Maheshwari, who led a School of Experimental Botany at Delhi University. During his stay at Harvard (1945–1947) when he wrote his well-known treatise on *Embryology of Angiosperms* published by McGraw Hill in 1950, he came in contact with K. Thimann and R. Wetmore and in his later travels with J. Bonner (Caltech, Pasadena), F. Skoog (Madison), J. van Overbeek (Modesto), E. W. Sinnott and A. Galston (Yale), P. White (Bar Harbor) and others. When he moved from Dacca as Head, Department of Botany at Delhi University in 1949, uppermost in his mind was setting up of a school of experimental embryology, tissue culture and reproductive physiology. In 1950s, for some time, he taught plant physiology. He was a great teacher who kept in constant touch with many eminent authorities around the world for exchange of information – and even today his enlivening lectures on photosynthesis, nitrogen fixation and plant hormones are recalled by many. In 1957, when Thimann was visiting the country, he organized the first seminar on 'Modern Trends in Plant Physiology' in India. It is again at this time that the late J. J. Chinoy, also at Delhi University, organized the Indian Society of Plant Physiology (later two more societies, the Society of Plant Physiology and

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Biochemistry and most recently the Society of Plant Biochemistry and Biotechnology were organized primarily through the efforts of S. K. Sinha and S. L. Mehta, respectively, at the Indian Agricultural Research Institute). In the following pages, we give a brief account of pioneering work at different centres, beginning with Calcutta.

Calcutta

At Calcutta University, Sircar did extensive work on the isolation of native growth regulators. Among Sircar's students at Presidency College were S. P. Sen, B. B. Biswas, Bharati Ghosh and Swati Sen-Mandi, all of whom joined the Bose Institute. Indeed, Sircar himself became the Director of the Institute.

From the viewpoint of plant biochemistry and molecular biology, however, the most significant contributions were of Sen and Biswas. Sen, who was the first to move to Bose Institute, headed the radiochemistry group. He began studies on the isolation[5] and mechanism of action of auxins, benefiting by a visit to C. Leopold's laboratory at Purdue in the late fifties, and his group was the first to report stimulation of RNA synthesis by auxin in intact tissues as well as isolated nuclei[6–8]. Sen moved to the newly established Kalyani University sometime around 1970. He continued studies on nuclei of coconut endosperm and one noteworthy contribution was by D. Sengupta, one of his students, on phytochrome regulation of RNA synthesis[9], which assumes new significance in the light of recent demonstration of the presence of phytochrome in nuclei generally and also definitive evidence, obtained by modern recombinant techniques, that phytochrome does have a direct effect on genes. Sen also worked on flowering. But, subsequently, he moved back more to the area of the phyllosphere and nitrogen fixation in which he had done his doctoral work. Thus, it was left to Biswas to develop plant biochemistry and molecular biology at the Bose Institute.

Around the mid-sixties, Biswas who had worked with J. Myers and R. Abrams (USA) returned to India and his group gave fresh evidence for *in vitro*[10] stimulation by auxin of RNA synthesis in isolated coconut nuclei in the seventies. Along with H. Mondal and R. K. Mandal, he also did considerable work on purification of plant RNA polymerases of which two types had been identified in the early 1970s[11]. Another area of research that has engaged Biswas, as also Susweta Biswas, is phosphoinositide metabolism in plants[12–14]. While studying nucleic acid metabolism in the germinating mung bean seeds, their group found a compound that was rapidly labelled with 32Pi. Later identified as myoinositol hexakisphosphate (InsP6), it opened a new chapter in their research and led to the identification of the various enzymes associated with its synthesis. Evidence has been provided for a novel proposal that inositol phosphates, by conversion to ribulose-5-phosphate, can supply energy and phosphate for synthesis of ATP and NADH via interaction with the pentose phosphate pathway during seed germination when oxygen availability is often a limiting factor[13,14]. IP3 receptors were also identified by S. Biswas and her group and it was shown that a different isomer of IP3, inositol 1,3,4 trisphosphate is produced in plants by phytase action, which too can elicit release of endogenously bound calcium[15]. Another topic that has engaged the attention of this group is leaf movements in the *Mimosa* plant. Recently, they found a novel apyrase with a pterin chromophore which is stimulated by blue light[16]. The gene, too, has been cloned and sequenced.

After Biswas left, S. K. Sen oriented the work of the plant molecular biology group more towards biotechnology (his work on engineering *BT* genes in rice was recently published)[17]. One of Biswas' pupils, A. Lahiri-Majumder who has been involved in the studies on inositol phosphate metabolism[18] and now heads the group, recently also worked with H. Bohnert's group in USA[19]. He is now orienting research towards stress molecular biology and further characterizing the role of inositol-1-synthase in generating osmoprotectants such as mannitol.

Turning to the work of other colleagues at the Department of Botany of the Bose Institute, Ghosh has had a group working for long on polyamines in rice and the mechanism of tolerance to osmotic stress. In tolerant varieties, polyamine level is higher. But the most recent contribution of this group is in collaboration with Sengupta (who spent several years at NIH working on animal systems). Employing the new recombinant DNA techniques[20,21], it has been shown that the expression of the gene encoding arginine decarboxylase – an enzyme critical for polyamine biosynthesis – is upregulated under salinity stress. In later work, focusing on the mechanism by which polyamines may work, binding of a protein to the ABA-inducible promoter has been detected by gel-mobility shift assays. Apparently, nuclear extracts of leaves in salt-treated rice plants have higher complex-forming ability which may be due to a role of polyamines in promoting a conformational change. Sen-Mandi has continued studies on loss of seed viability, due to nicks and damage to DNA[22], which she began at Cambridge while working with Daphne Osborne, but a good deal of her current work is on development of DNA markers for seed vigour.

Recently, at the Department of Biochemistry at Calcutta University, Maitreyee Das-Gupta has begun work on Ca^{++}-dependent protein kinases[23].

Plant physiology and molecular biology at Delhi

Studies at the Department of Botany, Delhi University (1960s–1980s)

Continuing about the developments at Delhi, briefly touched upon earlier in the article, many students benefited by the wide contacts of P. Maheshwari and were able to participate in classic researches of that time (for instance the discovery of RNA polymerase in plants and the first experiments on RNA synthesis by isolated chloroplasts as also obtaining some evidence that chloroplasts have DNA of their own)[24,25]. Upon return to India, some of them undertook research as also teaching of plant physiology and biochemistry. Since botany departments in India never had more than one or two specialists in these areas, one had to be an all-rounder to train students in many new emerging techniques and familiarize them with novel concepts. Mohan Ram (who worked with F. C. Steward at Cornell) taught and worked on tissue culture and allied aspects of Plant Growth and Development and, at a later stage, R. C. Sachar (who had worked with R. S. Bandurski at MSU in Michigan and for a few years in the biochemistry departments at AIIMS and at IARI) came back to the university taking over teaching of general biochemistry and established an additional research group (see below).

With many able students that Delhi University attracted, work was undertaken not only on cell and tissue culture, for which facilities had already been developed (this is how anther culture technique for production of haploids was discovered by Sipra Guha)[26], but also on physiology of flowering employing duckweeds which represent the smallest flowering plants and could be easily grown aseptically under controlled conditions[27]. The first experiments in India with *Arabidopsis* – which too can complete its life cycle *in vitro* – were also done in the sixties and include also one of the earliest tissue culture studies of this plant[28]. Since by and large previous work on hormones in India was restricted to study of effects of exogenous applications – a bit irreverentially called the 'spray and pray' experiments – efforts were made to orient research on isolation of native cytokinins[29] and gibberellins and success was achieved in isolating a couple of them that were new to the literature (M. M. Johri participated in some of this work). Studies were also undertaken on promotive effect of auxin on RNA synthesis by nuclei isolated from sources other than coconut milk[30]. Subsequently, work was done by L. Gangwani and J. Khurana on the isolation of cyclic AMP from *Lemna* by HPLC and radioimmunoassay – its presence was confirmed[31], even though the general role in plants remains a mystery. Because of early contacts with H. Borthwick and S. Hendricks, work was also undertaken on isolation of phytochrome in wheat and red-light-induced calcium influx in wheat protoplasts[32]. Several of these later projects were aided by the establishment of a DST Unit for Plant Cell and Molecular Biology in 1983.

While rounding up this account of early researches at Delhi University, mention may be made also of the contributions of Sachar who worked for many years on mode of hormone action[33,34]. For example, evidence was found for the presence in seeds of stored mRNAs coding for poly(A) polymerase, S-adenosylmethionine synthetase and *o*-diphenolase, which were upregulated by gibberellic acid. In the same Department, S. C. Bhatla in collaboration with Khurana, showed a critical role of Ca^{++} ions in development of the moss, *Funaria*[35] and R. Gupta has worked on acetylcholinesterases in legumes[36] and S. Raina on use of DNA markers in biotechnology of trees.

Work at Plant Molecular Biology Department at South Campus

In the eighties, when the recombinant DNA revolution had begun to transform plant biology, some students passing out of the botany department received training abroad as post-docs and this was when research at Delhi University began to take the character of plant molecular biology *sensu stricto*. Indeed, the DST Unit mentioned above was absorbed in a new department, which is the first of its kind in any university in the country. A. K. Tyagi worked with R. G. Herrmann in Germany, collaborating with his group in the isolation and sequencing of the gene for the 33 kDa manganese-binding protein of PSII from spinach that enables oxygen evolution[37] and also the plastocyanin gene[38]. On his return, he started researches on photosynthetic genes and their regulation in *Vigna*, rice and *Arabidopsis*[39,40], some of it in collaboration with Khurana. Phytochrome control has been shown and recently genes such as *psbO*, *psbP* and *psbQ* have been cloned from *Arabidopsis* with the idea of characterizing their promoters and studying mechanisms of their regulation. Work on plastid genes of rice has shown involvement of Ca^{++} and protein phosphorylation in their regulation. The work on promoters is engaging their attention also for tissue specific gene delivery and biotechnology of rice[41].

Khurana who did his post-doctoral studies earlier at the Smithsonian Institution in the eighties and then with K. Poff at MSU, East Lansing isolated the well-known JK 224 mutant[42] (now renamed the *nph* mutant) that has led to the discovery and isolation of the gene coding for the blue light absorbing photoreceptor for phototropism. All these studies have been in the limelight recently because the photoreceptor is a kinase as well. Khurana has also been working on blue-light effects on protein phosphorylation[43] in wheat and isolating mutants for the study of development. Recently, a novel *pho* mutant has been found which flowers in total darkness, even though *Arabidopsis* is a well-known long-day plant[44].

In the same department, A. Grover has been working on stress biology[45,46], employing rice and collaborating also with Elizabeth Dennis (Australia) and T. Hodges (Purdue). His group has found new heat shock proteins in the range of 100–110 kDa that are critical for overcoming stress to high temperature, salinity and also drought. One finding of unusual interest is that the hsp 110 has turned out to be homologous with another recently discovered protein of yeast, hsp 104, pointing to the remarkable conservation of the machinery to overcome stress. Again, in the same department, other groups have been set up by P. Khurana on biotechnology of wheat and gene tagging in *Arabidopsis*, A. Sharma on production of antigens in plants in collaboration with Tyagi and some other institutions, and by I. Dasgupta on the molecular biology of rice tungro virus.

Other studies in South Campus

A large plant biology group also came up at the Department of Genetics, till recently headed by D. Pental (now Director, South Campus), who worked earlier for many years with E. C. Cocking in the UK. Pental's main work is in the area of biotechnology and production of new *Brassica* hybrid lines – in fact, he has also founded a Centre for Genetic Manipulation of Crop Plants funded by NDDB. Using molecular biology methods, his group has studied inter-relationships of cultivated and wild rice, as also *Brassica* species[47]. In the same department M. V. Rajam, who did post-doctoral work at Yale with Galston and has built up an active group on the physiology and biochemistry of polyamines, is now utilizing genetic engineering techniques for manipulating their levels and studying their role[48].

Jawaharlal Nehru University, New Delhi

In the mid-seventies, a plant biology group began to be organized at the Jawaharlal Nehru University (JNU). Among the first appointees were G. Singhal, who had worked with E. Rabinowitch in USA and S. Guha-Mukherjee, who moved to JNU from Delhi University after further post-doctoral work at Michigan State University with R.S. Bandurski and later with J. Varner and J. Scandalios. The group was later joined by S. K. Sopory (from Delhi University) and P. Mohanty (who had worked with Govindjee on photosynthesis). Relevant to our theme is the work on the regulation of enzymes of nitrate metabolism by Sopory and Guha-Mukherjee, where phytochrome control was shown[49,50]. In recent years, Guha-Mukherjee's group has been working towards understanding the molecular basis of cell differentiation. It has been shown that amino acids can influence the morphogenetic potential in *in vitro* cultures. Also, the aspartate kinase gene was cloned and its regulation studied with a view to desensitize the enzyme for feedback initiation and then transfer the 'desensitized' gene in crop plants for increasing levels of essential amino acids[51]. Her group has also been studying satellite and repeat DNA in *Brassica juncea*[52].

Research in plant molecular biology and biotechnology advanced greatly at JNU when A. Datta (who had earlier worked on viruses, later with S. Ochoa on protein synthesis and, still later with E. Nester in USA on T-DNA) started to utilize plant systems for some of his researches. Datta along with one of us (S. K. S.) showed that during early germination in barley, protein synthesis proceeds from stored mRNA and which is blocked by high levels of cytokinins (some efforts were also made to identify a cytokinin-binding protein). In 1990s, he turned his attention to the transfer of a seed protein gene coding for amaranthin from *Amaranthus* to potato and of the oxalate decarboxylase gene from a fungus to tomato to improve the nutritional quality[53–55] – some of these results have attracted a great deal of attention. Another member of the JNU plant molecular biology group is K. Upadhyaya who worked for some time with H. Saedler in Germany. He isolated a gene of one new isoform of calmodulin from *Arabidopsis*[56]. Finally, Sopory, who earlier worked on protoplast culture of potato at Max-Planck Institute in Cologne and then, with the group of S. Roux at Texas, participated in research showing the important role of calcium ions in light signalling. Later, he worked at USDA with A. Mattoo on the turnover of chloroplast proteins and in early 1990s with Herrmann in Germany participating in the discovery along with Tyagi, that in certain photosynthetic genes *cis*-acting elements that activate transcription exist *within* the coding sequence[57]. In the last decade he developed a group on signal transduction and gene expression at JNU. Employing maize coleoptiles, his group showed that the influx of calcium ions is regulated by phytochrome more generally[58]. A number of Ca^{++}-activated protein kinases have been identified[59,60]. In addition to calcium ions, his group has also suggested the involvement of phosphoinositide cycle in phytochrome regulation of a number of genes, like nitrate reductase[49] and *PsaF*. In fact, in collaboration with R. Oelmuller in Germany, it has been found that the light-regulated promoter of *PsaF* has two *cis*-elements, one responsive to Ca^{++} and the other to a lipid such as DAG.

It is in 1989–1990 that the Government of India funded a Centre of Plant Molecular Biology (CPMB) at JNU. Activities of this centre and the special efforts of A. Datta have led to the establishment recently of the National Centre of Plant Genome Research by the Department of Biotechnology, where work has already been undertaken on the sequencing of EST as a preliminary to the total characterization of the chickpea genome.

Finally, a word about the work of two younger colleagues. Although neither are part of the CPMB Centre, B. Tripathi has recently been interested in root–shoot interaction during phytochrome control of growth of a seedling[61]. Neera Bhalla-Sarin has been working on transformation of legumes.

Mumbai

Among other centres, one of the earliest to come up was at the Molecular Biology Group at the Tata Institute of Fundamental Research. M. M. Johri, who took his initial training at Delhi University, then worked with J. Varner (where he observed stimulation of RNA synthesis in isolated pea nuclei by gibberellic acid[62]) and later joined this group in the late sixties when it was headed by O. Siddiqi. Johri has made efforts to develop *Funaria*, a moss, as a model system for addressing questions of molecular biology, as it is essentially a haploid and amenable to mutational analysis. The group has found evidence for a role of both auxin and cyclic AMP in differentiation from the caulonema to the chloronema stage[63,64]. Work has also been done on nitrate reductase and on G proteins and protein kinases that are involved in differentiation. A gene for a novel CDPK has been cloned. The Bhabha Atomic Research Centre (BARC) has also had a large group in plant biology, which began to be organized in early sixties. Relevant to our theme is the initial work of J. Thomas on nitrogenase, later of S. K. Apte and his colleagues on salt stress and mechanisms of osmotolerance in algal systems like *Anabaena* and *Rhizobium* and to some extent in rice seedlings[65,66].

Pune

Spanning more than two decades are detailed investigations undertaken on plant genomes in the laboratory of P. K. Ranjekar at the National Chemical Laboratory in Pune on various plants, largely members of the Gramineae where various kinds of repeated and satellite DNAs have been characterized by reassociation kinetics and related techniques. However, in recent years his research has focused more on developing DNA markers – technology to facilitate a hybrid rice research programme[67,68].

Madurai

At Madurai, A. Gnanam (who worked in his younger days with A. Jagendorf in USA), established a new plant biology group in early 1970s. Gnanam took up duties as a Vice-Chancellor, but in spite of increasing involvement in administration, his group has to its credit some of the first researches in the area of photosynthetic machinery and chloroplast molecular biology, for example, the first *in vitro* systems (from *Sorghum* and later cucumber) for biosynthesis of chloroplast proteins, such as Rubisco and discovery of a new heat shock protein in *Vigna* chloroplasts[69,70]. At present the Madurai centre is headed by K. Veluthambi, an alumnus of Indian Institute of Science, Bangalore and who worked with B. Poovaiah at Washington State University on calcium-induced phosphorylation and later with S. B. Gelvin in Purdue on the biology of T-DNA transfer to host cells[71,72].

New centres

While Delhi and Calcutta had the earliest established and the largest groups in basic plant biology, there are also several other centres of research activity where, many important contributions are being made. In an article like this, justice cannot be done to all these groups. However, a brief account of the more important work of these centres follows.

Other institutions in Delhi (TERI and Jamia)

At the Tata Energy Research Institute (TERI), a plant biotechnology laboratory was organized by V. Jagannathan where Malathi Lakshmikumaran heads a group for use of molecular markers in tree improvement. But she has been also doing some excellent work on 5s RNA and other repeated DNA in various crucifers[73,74]. At the Jamia Milia University, P. Pardha Saradhi (one of Mohan Ram's students), who later worked with Murata in Japan, has been working on the role of osmoprotectants like proline and betaine and raised transgenic plants that are tolerant to stress[75].

Lucknow

P. V. Sane, who in his early days worked with S. Zalik (Canada) and later with R. Park (USA) of the quantasome fame, moved to BARC in Mumbai to work on physical aspects of photosynthesis. In 1980s, he took over the Directorship of the National Botanical Research Institute of the CSIR at Lucknow, where he started a new group on plant molecular biology. Here work was done initially on regulation of enzymes such as nitrate reductase and aspartokinase. But after DBT, funded a special centre within the institute and P. Nath (who obtained his early training in recombinant DNA work at NIH with animal systems) joined, a new group was established concentrating on studies on virology[76] and photosynthetic genes of poplar (*Populus*) – the chloroplast genome is being completely sequenced[77]. However, lately, the plant molecular biology group has diversified its interests in many areas, including work with molecular markers. At the same institute, R. Tuli who earlier worked with J. Thomas at BARC and with R. Haselkorn (USA), has continued studies on cyanobacteria (though most of his work is now on developing *Bt* resistant cotton) – one recent contribution concerns differential expression of genes in *Plectonema* involved in photosynthesis and nitrogen fixation and processes which are temporally separated[78]. Also, at the Department of Botany, Lucknow University, H. N. Verma's group has been doing pioneering work on anti-viral proteins. Collaborating with K. K. Tewari at the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, a gene coding for such a protein has been isolated from *Clerodendron*[79]. The university has also one of the oldest departments in biochemistry, though work with recombinant DNA is still to pick up.

Hyderabad

Moving to centres in south India, one of the largest is now at Hyderabad, at the Central University which was established in the 1970s. V. S. RamaDas (an alumnus of Delhi University, who worked with W. O. James in England and D. I. Arnon in USA) was responsible for organizing the plant biology group of which there are several members now. A. R. Reddy, who in his younger days worked at neighbouring Osmania University with G. M. Reddy on biotechnological aspects, and then with H. Saedler in the Max Planck Institute at Cologne, has recently been occupied with cloning and isolation of chalcone synthase gene in rice and mapping of this locus[80]. The gene is of importance in biosynthesis of flavonoids which are now widely implicated in tolerance to stress. The effect of UV stress has also been studied and genes involved in anthocyanin pathway have been cloned and their regulation studied. R. Sharma is another member, who took his initial training with Sopory and Guha-Mukherjee at JNU, and later with P. Schopfer in Germany and M. Furuya in Japan. He has been interested in photoreceptors and plant growth. One notable discovery is of a gene in tomato, through the mutant approach, that codes for a repressor of phototropism[81]. One of RamaDas' former pupils, A. S. Raghavendra, also on the faculty, has contributed much to stomatal physiology[82]. Finally, in the same department, K. K. Ramaiah is working on translation initiation factor, eiF-2, in wheat[83].

At the Osmania University, G. M. Reddy, headed a DBT Plant Molecular Biology Centre, but here the principal work has been in tissue culture and biotechnology.

Bangalore

The Department of Biochemistry at the Indian Institute of Science, Bangalore has had a long history of researches in the area of enzymology by several pioneering biochemists. But more relevant to our theme is the landmark contribution of J. Padayatty and coworkers[84,85], in particular G. Thomas on cloning of rice histone genes and their transcription. Even though this study was not followed up subsequently, this work, published in *Nature*, is the first in India employing new molecular biology methods. Mention may also be made of S. Mahadevan who during his post-doctoral days at Harvard (with Thimann) discovered the enzyme for conversion of indoleacetonitrile to indoleacetic acid which is one of the principal hormones in plants. Recently, he became interested in following molecular events through which cytokinins can induce haustorial development in *Cuscuta* and employed differential screening for isolating the concerned gene(s)[86,87]. Among other workers, K. Shankara Rao has pioneered use of modern molecular biology techniques for studying embryogenesis in tissue culture[88] and C. Jayabaskaran who worked with T. M. Jacob and then J. Weil in Srasbourg has been studying various aspects of the biology of chloroplast tRNAs[89]. Again, although our account is basically restricted to higher plants, we may mention that in the same department, H. S. Savithri has been leading a group on the biology of plant viruses for many years[90] and R. Maheshwari, on the basis of thermotolerance in thermophilic fungi[91].

To return to higher plants, recently, Usha Vijayraghavan (who worked with E. Meyerowitz at Caltech and is now in the Department of Microbiology and Cell Biology) has done pioneering studies in India, on homeotic genes controlling leaf and flower development in rice and *Arabidopsis*, apart from research with yeasts. Recently, her group has shown that *SUPERMAN*, a gene that maintains the boundary between stamens and carpels, may be conserved between *Arabidopsis* and rice[92].

Chennai

Two new groups in plant molecular biology have come up at Chennai in recent years. At the SPIC Foundation, the expression of ABA and osmotic stress responsive cDNA of rice has been studied by G. Thomas and J. Thomas[93]. The other group is at the well-known M. S. Swaminathan Research Foundation, founded by M. S. Swaminathan after he returned to India upon relinquishing charge as Director, IRRI, Manila. A new research programme recently initiated by A. Parida[94], who moved from Delhi University after his doctoral work with Raina, employs the new molecular biology technologies like RFLPs, etc. for determining the biodiversity of mangroves. The aim is to identify and isolate genes that enable these plants to thrive in saline conditions and possibly transfer them to crops to make them salt-tolerant.

International Centre for Genetic Engineering and Biotechnology, New Delhi

Finally, we may give a brief account of work at the ICGEB. Although the headquarters of this organization are in Trieste, it has played an important role through the plant biology group in the centre located in New Delhi and organized by its first Head, K. K. Tewari (who with S. Wildman in USA was the first to isolate and characterize chloroplast DNA). Chloroplasts have multiple copies of circular DNA and the mechanism of its replication has been an important area of investigation as it can be an important site for engineering foreign genes and enabling production of recombinant products. A partially purified *in vitro* system was developed and factors needed for chloroplast replication were characterized by S. Mukherjee, M. K. Reddy and N. Tuteja[95–97]. Of special interest is the identification of DNA polymerase accessory factors and cloning of a gene encoding a glycoprotein, that stimulates chloroplast DNA polymerase activity[98]. In addition, the mechanism involved in chloroplast division has been studied and a gene encoding the protein FtsZ has been cloned and characterized[99]. Besides the molecular biology of organelle replication, cloning of nuclear genes encoding topoisomerases I and II, and their promoters and the proliferating cell nuclear antigen (PCNA) has been done by Reddy and Mukherjee to understand their involvement in regulation of the cell cycle[100,101]. Recently, Tuteja has cloned the first nuclear helicase from plants and studied the interaction of the overexpressed protein with topoisomerase I[102].

A new line of research work was initiated by Sopory (who moved some time ago from JNU to ICGEB), Reddy and Tuteja on cellular signalling and stress-inducible genes and characterization of their promoters. Their work has led to the identification and characterization of a number of novel protein kinases, including protein kinase C, and cloning of a calcium-binding protein, calnexin, alpha and beta G-protein and phospholipase C[103,104]. Also, a novel gene encoding glyoxalase-I has been cloned and found to be induced in response to salinity and metal stress; when over-expressed in transgenic plants, it imparted salinity stress[105]. Another recent finding relates to transmission of electrical signalling from root to shoot in *Sorghum*[106].

Although we have restricted the scope of this article only to basic aspects, we may mention that, in addition, a procedure for stable and over-expression of foreign genes has been established via chloroplast transformation by V. Siva Reddy. Work on the cloning of *Bt*[107] and other genes by R. Bhatnagar and mapping and cloning of insect-resistance genes[108,109] by M. Mohan and S. Nair are also significant contributions of the centre.

Work at other Institutes

Because of lack of space, we have not covered work on plant virology and plant pathology. But we may just mention that at Baroda, Chattoo (a former Delhi University alumnus who worked with Barbara Hohn in Switzerland) has been engaged on work on pathogens of rice – notable is his contribution on *Magnaporthe grisea* in which novel transposable elements have been identified[110]. Also, we have not covered the extensive work at the state agricultural universities and Indian Agricultural Research Institute which established a National Centre of Plant Biotechnology. All these researches are best discussed in a future article dealing with research in plant biotechnology.

Research in plant molecular biology in India and international effort – Why a big gap, and conclusions

From this brief assay on plant molecular biology work done in India, it is obvious that several laboratories are making great efforts to do research in modern plant biology. Yet, the truth is that most of these efforts have not passed the early stage (of 'pioneering efforts') and it may be a long time before impact of work done in India is felt either here or at the international level. This situation is somewhat in contrast to some other fields, e.g. plant cell and tissue culture in which India achieved worldwide recognition. Why has not then plant molecular biology developed?

The basic reason is plain enough. Work in molecular biology (or modern biology as a whole) is indeed expensive. Apart from the basic instruments and facilities, the pursuit of molecular biology requires high recurring expenditure, internationally of the order of $ 15,000 per student or per research worker! This is because almost every labelled compound, restriction endonuclease or other biochemical required for research has to be imported. For an average research group of about 8 workers with one professor or a group leader, this amounts to an expenditure of about 50 lakh rupees which works out to about Rs 2.5 crores for a 5-year period. Clearly, molecular biology research is out of reach of most laboratories in India.

The establishment of DST and DBT by the Government of India helped generate some financial support for research in plant molecular biology. After a unit was initially created by DST (at Delhi University), DBT took up the responsibility of establishing more centres (a total of 7 were established). Very recently, the Government has funded a large project for sequencing of one part of chromosome 11 of rice, at South Campus of Delhi University and IARI. A larger National Centre of Plant Genome Research has also been established although it will be a few years before its building is constructed and it is fully operational. The main credit for starting plant molecular research centres is due to M. S. Swaminathan (who took the first steps when he was Deputy Chairman, Planning Commission). But, later A. K. Sharma, S. Ramachandran and secretaries following him, including Manju Sharma, have also played important roles.

There is no doubt that the establishment of these centres has gone a long way in bringing modern plant biology in India. However, the main problem is that the centres need to be funded adequately, but they are granted funds for only a 5-year period. Thus, although these centres have brought much more money, than say the UGC could have for an average plant science department, the funding is way below international standards. It has to be emphasized that for work with recombinant DNA technology, the costs are nearly the same as in the UK or USA. We would like to make three recommendations:

1. Support for plant molecular biology centres must increase and in particular, in the universities. Specially, the recurring grants need to be brought up to the international level.
2. Support for centres must be organized on a long-term basis. We give a few examples. The support from the MSU-DOE Plant Research Laboratory, organized in the sixties, at East Lansing in USA, has been arranged by both the US Department of Energy and the Michigan State University. Such is also the case with Carnegie Institute of Plant Biology at Stanford. Similarly, in Germany, all Max-Planck Institutes are set up on a long term basis. In England, the various AFRC institutes and the MRC laboratories in Cambridge have been organized on the long term, although reviews after every five-years are always undertaken.
3. Lastly, there is a need to distinguish between plant molecular biology and biotechnology. It is basic discoveries in science (of which plant molecular biology is one) that feed into research in biotechnology. In a large country like India, it is important that new knowledge is generated here itself rather than our being dependent on research in foreign countries. While biotechnology centres are best organized by DBT or ICAR within agricultural universities and research institutions, the plant molecular biology centres need to be parallely organized mainly in the general universities, and in these centres there should be no immediate pressure to utilize their findings for applied research. Surely, the DST (which has the responsibility for basic research) can join hands with DBT to develop such centres. This way the Government may also attract many of our best minds back to the country, since they want freedom to choose their own problems.

We hope that administrators will recognize the merit of our arguments and ensure that better long-term funding comes for our centres. Otherwise, we will not do any better than train students, the best of whom will continue to settle abroad.

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