Rice Biotechnology – Report on the Third International Rice Genetics Symposium October 16–20, 1995, Manila, Philippines

The Third International Rice Genetics Symposium was held at Manila, Philippines from October 16–20. The symposium was organized by the Rice Genetics Cooperative, International Rice Research Institute (IRRI), Los Banos and supported by the Rockefeller Foundation. IRRI has been organizing the rice genetics symposia every five years since 1985. In the last 12 years the Rockefeller Foundation has been intensively supporting rice biotechnology research with an investment of 65 million dollars. These funds have supported rice biotechnology research in the Asian countries as well as encouraged the advanced plant molecular biology laboratories in Europe and USA to take up research on rice. Further close linkages between the laboratories in the developing and developed countries have been established. As a result, rice plants are now grown in glass houses, with the backdrop of the Alps, in Switzerland and rice research is carried out at Cornell and Wisconsin Universities in USA and at John Innes Centre, Norwich and the University of Birmingham (UK). Japan has invested heavily in the Rice Genome Research Programme. China has a modest Rice Genome Programme besides a large number of laboratories engaged in rice biotechnology. This investment in rice has resulted in the blooming of rice biotechnology. This was clearly visible in the symposium with over 500 participants and over 300 oral and poster presentations. A marked shift towards molecular biology approaches was evident in comparison to the previous two symposia held in 1985 and 1990. In fact, at the concluding session, the participants were reminded by Ralph Riley that molecular biology only provided additional, new tools to be used by the plant breeders to facilitate their work of developing more productive, stress-tolerant cultivars with better enduse qualities.

Papers were presented in the areas of rice genome characterization, genetic transformation of rice, molecular tagging and map based cloning, gene isolation characterization and expression, quantitative trait loci (QTL) mapping, molecular cytogenetics, molecular analysis of cytoplasmic male sterility, genetic diversity in pathogen populations, genetics of morphological, physiological, disease resistance and quantitative traits. Gene mapping and transformation emerged as the two dominant areas of current world-wide interest in rice biotechnology.

Among the cereals, rice happens to have the smallest genome size and this has made it an ideal material for molecular biology studies and biotechnology. Restriction fragment length polymorphism (RLFP) technology was applied initially in the rice genome mapping but now polymerase chain reaction (PCR) based markers are being increasingly used. It was reported that 1904 markers consisting of cDNA clones as well as RAPD markers have been mapped covering a distance of 1590 cM. Integration of cytological, conventional and RFLP linkage maps was reported resulting from correct orientations of the maps with respect to each other.

Synteny (similar arrangement of genes along the chromosomes) between rice and wheat chromosomes was reported earlier. This has been further confirmed now and extended to barley, maize, sorghum, sugarcane, foxtail millet, pearl millet, oats and ryegrass. This makes the cloned and mapped genes of rice extremely useful for isolating and mapping genes in other cereals and should provide a quantum leap for molecular biology of other cereals.

Conversion of cloned RFLP markers of the Cornell University – IRRI genetic map to sequence-tagged sites (STS) was reported. The use of STS facilitates the breeding programmes by way of speed, convenience, reliability and relatively low cost as the PCR methods are used for analysis. Marker-assisted breeding would greatly facilitate selection for insect and pathogen resistance and in pyramiding of resistance genes.

Early results on association between molecular markers and quantitative trait loci (QTL) which determine the components of yield and ultimately the yield were reported. Such markers can assist in evaluation of the germplasm, selection of parents-to-be used in hybridization programme, prediction of hybrid vigour and in making selections.

A large number of reports were presented on the transfer and expression of alien genes into rice providing resistance to insects, viral, bacterial, and fungal diseases and nematodes. Reliable *Agrobacterium* mediated and gene gun methods have been developed. For insect resistance synthetic, codon optimized Cry 1A(b), Cry III A, from *Bacillus thuringiensis*, soybean trypsin inhibitor, cowpea trypsin inhibitor, potato proteinase inhibitor (pin 2), snow drop (*Gallanthus nivalis*) agglutinin (GNA) and ribosome in activating protein; for virus resistance, rice yellow mottle virus coat protein, hoja blanca virus (RHBV) and tungro virus, chitnase and cercosporin B gene for fungal diseases and herbicide resistance *bar* gene; and the gene for glycene betaine synthesis to provide salinity stress tolerance have been transferred to rice. Stability and expression of some of the transferred genes have been checked in the progenies upto the fifth generation. Time from the transformation event to obtaining the transformed plants and harvest of seeds from such plants have been considerably shortened. Approximately 11 weeks to obtain plantlets and less than a year for harvesting seeds were reported. Transformed plants are under glass house evaluation and are expected to move out into the field shortly. Insertion of single copy of the gene at one site, or multiple copies at one site as well as single or more than one copies at multiple sites have been observed.

In one presentation, transfer of rice clones encoding proteins from MADS-domain were transferred and expressed in tobacco where they altered apical dominance resulting in early flowering and bushy plants. Expression of Os-MADS3 altered floral organ development and homeotic development of petals to stamen. These results establish that the regulatory factors for flowering in dicots and monocots may be similar. Such homeotic genes have been extensively investigated in *Arabidopsis*.

The other significant reports described efforts made towards cloning of blast resistance genes by chromosome walking, presence of transposable elements in rice as well as in the pathogen

for blast disease, encoding of a receptor-like protein kinase by disease resistance gene, molecular analysis of anthocyanin pigmentation pathway, manipulation of starch metabolism during seed development, characterization of mitochondrial genome, silencing of β-glucuronidase gene in transgenic rice.

It has been projected that by the year 2025, the global demand for rice would increase by 70%, requiring production of 810 million tons of unmilled rice compared to the present production of 480 million tons. This will have to be harvested from diminishing cultivated area and availability of water due to competing demands on these resources. It was repeatedly emphasized that to meet this demand the rice yields will have to increase from the present mean of 4.5 tons ha^{-1} to 8.0 tons ha^{-1} under irrigation and from 1.9 tons ha^{-1} to 3.6 tons ha^{-1} in the rainfed areas. This will have to be achieved by increasing the yield ceiling, bridging the gap between the potential and the realized yield and sustaining the present levels. The major role of biotechnology towards this goal would be in the development of transgenic plants with resistance to biotic and abiotic stresses. Therefore there is an urgent need to evolve procedures for field releases of transgenic plants and approval of grain harvested from such plants as safe food similar to those produced using conventional genetic tools. The marker-assisted selection and tissue culture methods would facilitate breeding programmes and reduce the breeding cycle time.

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RESEARCH NEWS

Designer molecules: A chromogenic sensor of creatinine

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Specific recognition of molecules by one another via shape complementarity and specific, weak non-covalent interaction like hydrogen-bonds, van der Waal's interaction, etc. is central to biology. Recognition of a chosen guest by specifically designed host molecules is currently a major area of chemical research.

Molecular and ionic sensors have always been in great demand for the easy detection/assay of a variety of molecules and ions[1]. Such sensors find many applications in medical diagnostics and in detecting environmental pollutants. A variety of chemical sensors, including biosensors, can be designed[2]. However, sensors based on synthetic molecular receptors are becoming more attractive since such molecules are usually much more stable compared to biomolecules. Of sensors, chromogenic sensors are the most valuable ones since the presence of the 'guest' is signalled by a change in colour. A number of chromogenic sensors have recently been designed and synthesized based on the principles of molecular recognition[3].

In a recent communication to *Science* (1995, **269**, 671), Bell and coworkers have designed a receptor which detects creatinine efficiently. Creatinine is one of the end products of nitrogen metabolism, and in healthy human beings it is excreted in the urine. The transport of creatinine from the blood to urine is mediated by the kidneys, hence the blood level of creatinine is a key indicator of renal function. Present methods available for the assay of creatinine are tedious and expensive. Bell's work has produced a simple but sensitive assay of creatinine, in which the formation of a supramolecular complex is signalled by a colour change.

The $H_2N–C=N$ moiety of creatinine has a D-D-A array* for forming hydrogen bonds. The designed receptor however, in its neutral form,* has a D-A-A surface for forming an H-bond. However, a zwitterionic structure is also possible for this receptor, in which the phenolic hydrogen is transferred to the nitrogen atom at the other end of the molecule, thereby converting the D-A-A surface to an A-A-D surface which is now complementary to the creatinine surface. In other words, of the two tautomeric forms only the zwitterionic form (the 'dye'-like form) should bind creatinine. In this example, that is what happens – the receptor exists predominantly in its neutral form, say in dichloromethane solution, which has a yellow colour. However, when this solution is shaken with an aqueous solution of creatinine, the receptor extracts creatinine into the organic solution, forms the complex, and in that process undergoes a change from the neutral to the zwitterionic form. This causes a colour change from yellow to brownish orange (the complex shows an extra absorption band at λ_{max} = 444 nm). This is schematically shown in

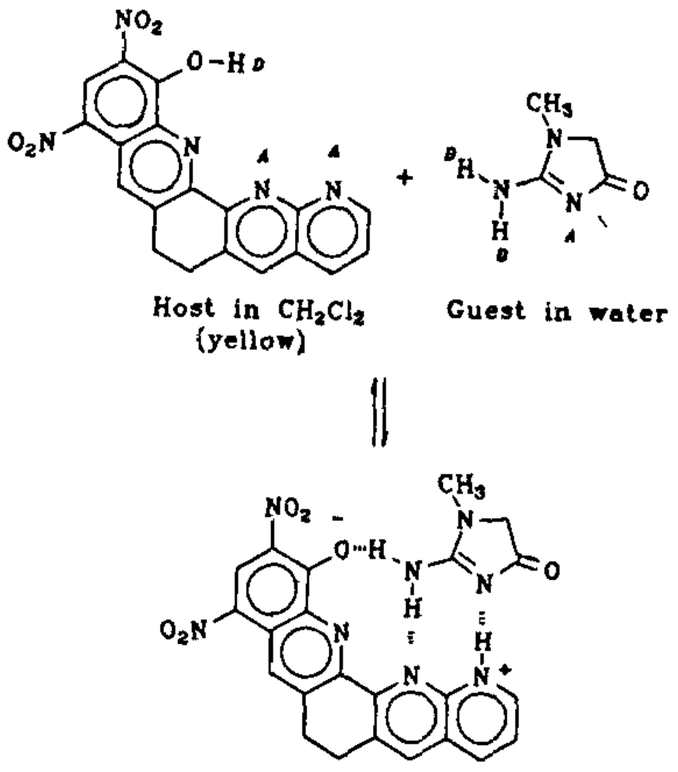

Figure 1

*D and A stand for donor and acceptor groups for forming a hydrogen bond, respectively