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MEETING REPORT

Transformation of rice genetics

Even as late as 1985, a uniform chromosome nomenclature system for rice had not been established and the field of rice genetics was lagging behind genetics of other cereals like barley and wheat. A decade after, the picture is altogether different. The small size of the rice genome and synteny with the genomes of other cereals, coupled with recent advances in rice genetics, have propelled rice into the centrestage of cereal genetics. This was apparent at the recent Third International Rice Genetics Symposium in Manila (15–20 October 1995).

High points at the meeting were reports of establishment of a high-density linkage map of rice with 1904 molecular markers; complete integration of the classical, cytological and molecular maps; a first-generation physical map using YAC contigs; genetic transformation by different methods (including the hitherto elusive *Agrobacterium tumaefaciens* method) for japonica and indica rices; mapping of genes for agronomically important traits using molecular markers; and development of transgenic rice engineered for useful traits.

Genome analysis

Establishment of a nearly saturated linkage map using 1904 molecular markers was described (B. A. Antonio, Tsukuba). An interesting outcome of this work was the detection of a genetic duplication covering a large part of chromosomes 11 and 12. A unique feature of the map is that most of these markers are expressed sequence tags. A first-generation physical map, covering 85% of the genome, using yeast artificial chromosomes was also described. (I. Ashikawa, Tsukuba). The sequencing of over 15,000 rice cDNA clones and strategies to identify genes that have not been sequenced before (new 'hit' genes) give an idea of the magnitude of the effort in the Japanese Rice Genome Program. O. Ideta (Fukuoka) reported integration of the classical and RFLP maps of rice and Gurdev Khush (Los Baños) the orientation of the centromeres of all 12 rice chromosomes with respect to these two maps.

A presentation on the observed synteny (conservation of gene order) in the genetic maps of the various cereals was made by M. D. Gale (Norwich). RFLP crossmapping has revealed that the rice genome is syntenic not only with the three wheat genomes, but also with the genomes of barley, sorghum, maize, oats, pearl millet, foxtail millet, sugarcane, forage grasses, and even bamboo and other members of the grass family. Since the rice genome is one of the smallest in the grass family ($5{\cdot}8 \times 10^5$ kilobase pairs), it is being considered as the species of choice for cloning homologous genes from many other grasses. In the future it is likely that a wheat or barley geneticist will be looking first to map and clone a rice gene before isolating the homologous gene from wheat or barley using the rice gene as a probe.

Genetic transformation of rice

Y. Hiei (Shizuoka) presented pioneering work on rice transformation using *A. tumaefaciens*. He showed, for the first time, that the indica subspecies of rice can also be transformed by this technique. Other groups (T. Hall, College Station; and T. Hodges, West Lafayette) confirmed this work. It is clear that application of this simple and economical method to rice improvement is on the anvil.

Considerable progress was also reported in increasing efficiency of rice transformation by the particle-bombardment method (C. Fauquet, La Jolla; P. Christou, Norwich; and others). The intriguing, and as yet unexplained, observation was that cotransformation with two, three or four different plasmids (with only one of them carrying a selectable marker) can lead to cointegration of all of them at the same chromosomal locus and cosegregation in subsequent generations. This raises the possibility that the rice plant can now be engineered to carry batteries of genes that control entire biochemical pathways. Cointegration ensures that the genes will not be segregated away from each other in subsequent meiotic divisions. It also greatly simplifies the task of making the required constructs because each gene in the pathway can be inserted in a separate plasmid. Genetic engineering of vitamin A synthesis in rice endosperms, as a way of preventing vitamin A deficiency in children, is being attempted by the groups of E. Wurtzel (New York) and I. Potrykus (Zurich). This would require the expression of four new genes in the rice endosperm (P. Burkhardt, Zurich). If these attempts succeed, we may have our proverbial yellow rice directly from the plant. However, it is not clear whether all these newly introduced genes will be expressed efficiently in rice plants. A. Kohli, J. Bennet and colleagues (Los Baños) showed that transgenes, as well as allelic copies of the transgenes, can be silenced in rice, possibly by cosuppression.

Rice plants carrying determinants of antibiotic resistance, introduced as selectable markers during transformation, might never make it to farmer's fields owing to environmental concerns. A method of circumventing this problem by specific excision of the antibiotic-resistance determinant from the genome of transformed rice plants was described (T. Hodges). The strategy involves inserting direct repeats of a sequence that is the substrate for recombination by the *Saccharomyces cerevisiae* FLP recombinase on either side of the antibiotic-resistance determinant on the transforming DNA. Introduction of FLP recombinase, by crossing with rice plants that express the recombinase, was shown to cause excision of the antibiotic-resistance determinant.

Molecular tagging and cloning of rice genes

A number of genes that control various aspects of rice morphology, development, physiology, pathology and agronomy have been tagged with molecular markers. Many of these genes are likely to be cloned in the next few years by targeting these closely linked markers. The utility of obtaining closely linked molecular markers for a gene of interest in rice was demonstrated by the positional cloning of the *Xa21* gene which confers resistance against certain races of the bacterial pathogen *Xanthomonas oryzae* pv. oryzae (P. Ronald, Davis).

The *Xa21* gene belongs to a rice gene family whose other members have also been cloned (they are on the same genomic cosmid clone). This gene encodes a putative receptor kinase with an extracellular domain that contains numerous leucine-rich repeats. The presence of these repeats in other proteins has been implicated in protein–protein interactions. The structure of the *Xa21* gene suggests that the protein has an extracellular domain which may interact with a ligand (possibly a peptide) released by the pathogen. It is likely that the signal from this interaction is transmitted to the interior of the cell by the kinase domain. This triggers a signal transduction cascade that ultimately results in resistance. A notable feature of the *Xa21* gene is that it has striking homology to the handful of disease-resistance genes cloned from other plants. The leucine-rich repeats, a membrane anchor and the serine threonine kinase domain have all been identified in other disease-resistance genes. However, the *Xa21* gene is the only member of this class of genes that has all three domains; the other members have only one or at most two. This feature makes the *Xa21* gene very attractive as a candidate in the receptor kinase model for resistance gene action in plants.

Genetic engineering of stress resistance

Transgenic rice plants that are resistant to various biotic and abiotic stresses were reported at the meeting. Genetically engineered rice plants that overexpress rice chitinase exhibit enhanced tolerance to sheath blight, a fungal disease against which there are no known sources of resistance in the rice germplasm (S. Datta, Los Baños). However, in the absence of disease pressure, the chitinase-overproducing rice plants are not as vigorous as control plants. Attempts are under way to overcome the adverse effect of chitinase overproduction; one way is by making it inducible by pathogen infection. Transgenic plants that exhibit resistance against the yellow stunt viral disease have been produced (R. Fang, Beijing). Transgenic plants produced by other groups are also being tested in greenhouse trials for resistance against other viruses, fungi and insects. The transgenic route is also being pursued to engineer tolerance against abiotic stresses like salinity, submergence and cold.

Quantitative trait loci

The use of molecular markers in rice genetics is likely to have greatest impact in unravelling the genetic basis of quantitative traits. A number of loci (QTL) controlling a diverse range of characters, such as seedling vigour, root thickness and penetration ability, plant height, time required for flowering, seed shattering and yield, have been tagged and mapped using molecular markers. This should facilitate exploitation of QTL in rice breeding. An interesting observation was reported by N. Huang (Los Baños). He and his colleagues mapped a number of QTL that determine plant height and found that all of them, without exception, map to the chromosomal locations of the 12 major genes that control plant height. This supports the hypothesis that QTL are none other than alleles of major genes. In this context it is interesting that the synteny between rice and wheat also extends to QTL (H. Nguyen, Lubbock).

Genetics of rice pathogens

Identification of virulence mutants of pathogens and the cloning of several genes involved in the interaction with rice were described. A number of papers dealt with use of molecular probes to understand the population structure of various rice pathogens in different parts of the world. R. Nelson (Los Baños) described strategies for effective deployment of rice cultivars resistant to *X. oryzae* pv. oryzae based on detailed studies of pathogen population diversity. It appears that the idea of using DNA-based markers to obtain detailed information on plant-pathogen populations is moving from the concept stage to being considered as an integral part of an effective resistance breeding programme.

Future trends

In the light of these wideranging and rapid advances, is it possible to speculate where rice genetics will be heading by the time of the fourth rice genetics symposium in the year 2000? Although it is difficult to predict the exact course of progress, the trends are clear. A second-generation or third-generation physical map encompassing the entire rice genome, generated by use of YAC or BAC (bacterial artificial chromosome) libraries, is likely to be available. Cloning rice genes in the future might be a matter of mapping the gene of interest using molecular markers and then taking the desired clone out of a freezer that contains an ordered library. Many genes controlling the morphology, development, pathology and agronomy of the rice plant will be cloned and characterized. Suitably engineered maize transposons are likely to be deployed as promoter probes for identifying interesting rice genes (as is being done in *Arabidopsis thaliana*). The yeast FLP recombinase (or other site-specific recombinases) might be applied for creating specific chromosomal rearrangements in the rice genome.

On the applied side, the use of molecular markers for bringing together disease-resistance genes and other examples of marker-assisted selection are going to be routine in rice breeding. QTL will be extensively used in this regard. Precise knowledge of the structure of rice pathogen populations is also going to be increasingly used in breeding and deployment of resistant cultivars. Transgenic rice plants that provide enhanced protection against biotic as well as abiotic stresses and new male-sterile systems for hybrid seed production will be available for possible commercial cultivation.

The major credit for the quantum leap in rice genetics that has occurred in recent years must go to the International Rice Biotechnology Program of the Rockefeller Foundation as well as to the Japanese Rice Genome Program in Tsukuba. Whether the pace of these advances can be sustained will depend on continued support from the funding agencies.

A look at the composition of papers presented at successive rice genetics symposia is revealing. George Rothschild, Director-General of the International Rice Research Institute, indicated that at the first meeting in 1985 about 90% of the papers were on classical genetics, about a half of the papers dealt with classical genetics at the second meeting in 1990, and only 20% at the third meeting. The focal point (or at least the glamour and associated support) appears to be moving away from the traditional rice breeder to the rice 'biotechnologist' or 'molecular biologist'.

Some of this is attributable to a natural tendency among established researchers as well as graduate students (especially) to move into frontier areas. In the long term, if taken to an extreme, this could prove to be even detrimental to the crop because the plant breeder must remain the focal point of any crop improvement programme.

In the end, a note of thanks to Gurdev Khush, who was organizing secretary of this meeting as well as the previous one.

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