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Progress in Rice Biotechnology

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World population which now stands at over 5 billion is likely to exceed 6 billion by the end of this century and 8 billion by 2020 by which time the number of rice consumers will probably have doubled. All current predictions are that demand for rice will exceed production even by the end of this century. New rice varieties with higher yield potential, resistance to biotic and abiotic stresses and which use inputs more efficiently are therefore required.

Several years ago it was perceived that biotechnology utilising cell and molecular procedures could significantly strengthen rice breeding programmes helping to produce new varieties with higher yield potential and greater yield stability, thereby improving the efficiency of rice production and allowing expansion of rice-growing areas. In less than a decade rice biotechnology has moved from a position of neglect to a position where rice is now regarded as a model plant for cereal research and biotechnological developments.

I – Rice tissue and its application

It was not until the mid 1950s that tissue cultures of rice were initiated with studies on the culture of excised rice roots and immature rice embryos. The regeneration of rice plants was subsequently reported from root-derived callus (1) as was the regeneration of haploid rice cells from cultured anthers (2) and shoots were obtained in 1968 from callus initiated from rice embryos (3). The production of haploid rice through the culture of anthers or microspores had important applications in rice breeding. Doubling the chromosome complement of haploid material was a rapid method of inducing homozygosity, thereby shortening the time required for the development of new rice varieties and enabling recessive genes expressed in haploids to become fixed when the chromosome complement is doubled. In 1976, the first rice varieties from haploid breeding were released for commercial production (4) and currently about 100 new varieties of rice derived from anther culture are grown on 250,000 hectares in China.

It has been known for more than 20 years that phenotypic variation occurs in plants regenerated from cultured cells and thus the culture of plant tissues is in itself the simplest form of genetic engineering. Commonly observed variation in rice tissue culture derived plants includes the number of tillers per plant, plant height, flag-leaf length, heading date, panicle length, fertility and the number of seeds produced. The genetic variation exposed constitutes the basis of somaclonal variation (5). The development of procedures for the regeneration of plants from cultured single cells of rice lacking a cell wall (protoplasts) subsequently led to the assessment of protoclonal variation in protoplast-derived plants (protoclones) and their seed progeny. In an analysis of protoclonal variation seed progeny of protoplast-derived plants of the japonica Taipei 309 were evaluated for 13 phenotypic characters in a field trial (6). Seed progeny exhibited delayed flowering and decreased lengths of flag leaves and panicles. Flag leaf width increased as did the number of branches per panicle. It was inferred that protoclonal variation shifts means away from the direction of the previous selection history for the variety, and that protoclones exhibiting positive variation could be selectively exploited for crop improvement.

Between 1976 and 1986 six publications reported the sustained division of rice protoplasts to form callus, and from 1985 to 1990 there were 13 primary publications from 12 different laboratories concerning the regeneration of rice plants from protoplasts. Initially most successes occurred with japonica varieties, but in recent years there has been increasing success with indica varieties (7). This ability to use

rice cell and tissue culture procedures to regenerate fertile rice plants from protoplasts opened up the possibility of the genetic engineering of rice using both japonica and indica varieties via direct uptake of DNA into protoplasts and subsequent regeneration of transgenic plants from such transformed protoplasts. It also opened up the opportunities for gene flow by the fusion of plant protoplasts and the production of rice somatic hybrids thereby extending the range of hybridisations, both nuclear and cytoplasmic, possible by sexual crossing in rice.

II – Transgenic plant production from protoplasts

Fertile transgenic protoplast-derived rice plants have been produced using varieties from all of the six rice subgroups. These subgroups can now be clearly defined by either their isozyme (8) or their RFLP patterns (9) into six varietal groups (VGs). Japonica and javanica (tropical Japonicas) rices are VG6. Intermediate varieties such a Chinsurah Boro II can be differentiated from the true indicas, such as IR54, IR43 and IR36 which belong to VG1. In general, it is progressively more difficult to produce fertile transgenic protoplast-derived rice plants as one utilises Group 6 to Group 1 varieties. Suspension-cultured cells have been the primary source of protoplasts used for rice plant regeneration and many different media have been used for in vitro culture of rice suspension cultures and protoplasts. The use of the AA medium which provides all nitrogen via amino acids has been found to be highly effective for suspension culture maintenance and release of protoplasts by enzymatic degradation of their cell walls, especially for Japonica rices (10).

DNA transfer to plant protoplasts utilises either a chemical treatment (polyethylene glycol, PEG) or electroporation for transformation, but it is still not clear whether electroporation or PEG mediated DNA uptake is preferable. Moreover, a general problem associated with genetic transformation of rice protoplasts using either of these transformation procedures is that the foreign DNA often integrates in many copies and undergoes re-arrangements during the uptake and integration processes (7). Initially the genes used for transforming protoplasts and selecting transgenic rice plants were mainly from the group of genes encoding the aminoglycoside phosphotransferases including the neo gene encoding for neomycin phosphotransferase (NPTII) which confers to plant cells resistance to the antibiotics, kanamycin and G418. Transformed callus derived from protoplasts was selected using either kanamycin or G418. Reliable systems for the transformation and regeneration of rice protoplasts yielding fertile transgenic plants have now been established for a steadily increasing number of rice varieties including many Japonicas (VG6) (11)(12), several intermediate type Indicas and a few true Indicas (VG1) (13), but there is still considerable room for improvement in the efficiency of these systems, particularly for the true (VG1) Indicas.

Recent discussions (12) have highlighted the fact that we are presently in the early stages of development and testing of transgenic rice plants containing novel genes capable of conferring beneficial agronomic traits. These include the difficulty experienced in combining transformation and regeneration for elite cultures and the relative infrequency with which viable seed is obtained from transgenic seed. Only a few detailed studies have been undertaken of the inheritance of genes in transgenic rice plants highlighting problems likely to be encountered in the control of gene expression in transgenic seed progeny (14). Procedures for direct integration of foreign DNA sequences, including the use of biolostics (15), currently yield unpredictable numbers of gene inserts, frequently bearing many rearrangements and deletions. It has also been emphasised (12) that the biolistic approaches requires special selection against the regeneration of plants consisting of a chimeric array of cells, some of which are transformed with the gene of interest, others not; and that researchers must be particularly careful to develop and report rigorous testing of transgenic plants.

III – Potentially useful genes for rice genetic engineering

The availability of procedures for the production of transgenic rice have focused attention on identifying gene constructs that can instill cultivars with useful new traits of agronomic, nutritional and commercial value. This has led to the identification of research priorities for rice biotechnology for developing country rice regions and for region-specific agro-ecology. Analysis of the potential for a biotechnology solution is rendered difficult because application of biotechnology to solve agricultural problems is still at an early

stage; there are few data reflecting the potential of biotechnology to meet a given challenge. Currently the approach is to use a score based on the judgement of knowledgeable scientists that reflects the potential that biotechnology has to lead to a successful solution to each challenge (16). This has led to the ranking of priorities for biotechnological thrusts for rice diseases (tungro virus, bacterial blight, sheath blight, ragged stunt virus, blast), soil factors (coastal saline/acid, iron deficiency), temperature and water, physiological opportunities (greater lodging resistance, cytoplasmic male sterility, apomixis) and insects (brown plant hopper, yellow stem borer). Successful approaches to these biotechnological objectives must be put in the perspective that many of these objectives are already being attempted (often with significant success) by breeding procedures utilising a range of hybridisations including the use of the genetic diversity of wild rice species in wide hybridisations. For biotechnological applications linked to the production of transgenic plants research strategies are required aimed at identifying and constructing such useful genes. Progress is often slow because either the genes have not been identified and isolated or because many genes are involved making the production of transgenic plants difficult, or with our current knowledge presently impossible. Objectives that have been identified include improvements in cytoplasmic male sterility for improved yields through hybrid rice production and apomictic seed production, since once a productive hybrid is produced it could be multiplied easily and quickly through apomictic seed production of successive generations—there would be no segregation and no loss of hybrid vigour.

More than 100 species of insects attack rice with at least 20 insects regarded as major pests. The insertion into rice of genes coding for protein toxins from entomocidal bacteria, protein inhibitors of insect digestive enzymes, and certain lectins are the prime targets of current research (17).

The principal strategy for controlling rice diseases is breeding for resistance and to use molecular probes for disease diagnosis and monitoring that will allow these existing sources of resistance to be used more effectively. These approaches have been comprehensively discussed and described and it is clear that the availability of suitable probes, such as repetitive DNA elements or cloned avirulence genes to characterise different pathogen populations from broad geographic areas will provide the information needed for the rational use of resistant germplasm among rice-growing countries (18).

Three strategies have been developed for improving stress tolerance in rice all of which are benefiting from biotechnology. These include the use of rice genetic maps and markers to help in identifying the most important genetic components, even though the function of the gene products may be known. Also included is modifying the level of expression of stress-induced rice genes and the transfer of alien genes that enhance a desired response. These strategies relate to improvements in drought and salinity tolerance and temperature tolerance. Improved product quality, relating to protein quantity and vitamin content, require other strategies some of which centre, for instance, in relation to enhanced Vitamin A content, on the production of transgenic rice in which the carotenoid biosynthetic pathway has been turned on in rice endosperm.

IV – Wide crosses

Wide cross hybridisation, including the use of embryo rescue procedures, has allowed the successful introgression of genes from wild Oryza species to elite rice cultivars. The genus Oryza has about 20 species, two of which are cultivated, O. sativa the common cultivated rice which is grown world wide and O. glaberrima, the cultivated African rice which is grown in West africa. The others are wild rice species many of which can be successfully crossed with O. sativa for the introgression of useful genes. Of particular interest is the use of protoplast fusion procedures, coupled with the regeneration of plants from the products of the fusion, for the introgression of both nuclear and cytoplasmic genes (19). Heterokaryons were produced by fusing cell suspension-derived protoplasts of the Japonica variety Taipei 309 and mesophyll protoplasts isolated from the salt-tolerant wild species Porteresia coarctata (20). Recently improvements in culture procedures and section strategies have resulted in the production of somatic hybrid plants, opening up the possiblity of using protoplast fusion technology for the introgression of genes for salinity tolerance into cultivated rice (21). The selection of protoplast fusion products has also led to the transfer of cytoplasmic male sterility (CMS) in rice, including the transfer of CMS from VG2 variety Chinsurah Boro II into the Japonica variety Nipponbare (22). These are important, yet presently

not fully developed, biotechnological procedures because they may enable the transfer of polygenic characters presently impossible using transgenic plant production procedures in the instance of salinity tolerance, and in the instance of CMS may enable the range of CMS lines for hybrid rice production to be significantly increased.

V – Symbiotic biological nitrogen fixation for rice

The introduction of symbiotic biological nitrogen fixation into rice would be one of the most significant contributions biotechnology could make to agriculture. It was suggested that rice has the genes necessary to form nodules but is not producing the required chemical signals (17).

The current renewed interest in developing symbiotic nitrogen fixation in the major non-legume crops of the world has been reflected in recent symposia devoted to discussion of this topic in rice (23) and by the establishment of the Rockefeller Foundation funded International Rice Nodulation Group to co-ordinate work in Australia, China, Mexico and the U.K. (24). Detailed planning is also being undertaken to establish a collaborative project between countries in the Mediterranean climate region for collaborative work on the nodulation of Mediterranean climate rices for nitrogen fixation. This project has been stimulated by the finding that certain naturally occurring strains of rhizobia are able to initiate the development of lateral root nodules by invading emerging rice lateral roots by crack entry (25).

We have previously demonstrated that some naturally occurring rhizobia, such as those isolated from root nodules of non-legume Parasponia species and from stem nodules of tropical Aeschynomene legume species, are able to enter the root systems of maize, rice and wheat by ‘crack entry’. This occurs where lateral roots emerge through the root cortex, resulting in the penetration of rhizobia both between and into cells of the cortex, particularly in the cortex of emerging lateral roots (26).

Recently, we have interacted oxygen tolerant Azorhizobium caulinodans ORS571 isolated from stem nodules of the tropical legume Sesbania rostrata with the root systems of rice and wheat. We have found that intracellular invasion of cells of the cortex of roots of both rice (IR42 and Lemont) results in plants that are active in nitrogen fixation as determined using acetylene reduction assays. Currently, we are quantifying, using both acetylene reduction and 15N dilution assays, the extent to which this nitrogen fixation is due to symbiotic intracellular rhizobia, and whether it will be beneficial to the growth and development of rice plants. Perhaps, only a few legume genes may need to be transferred to non-legume crops such as rice and other cereals such as maize and wheat to produce the necessary signals for effective symbiotic nitrogen fixing interaction with soil rhizobia. The recent evidence for the conservation of genome structure between rice and wheat, indicating that many wheat chromosomes contain homeologous genes and genomic DNA fragments in a similar order to that formed on rice chromosomes (27), may be particularly pertinent in this respect.

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