FINAL TECHNICAL REPORT

Cytoplasmic diversification for hybrid rice improvement: phase 3.
R7417

Executive Summary

A very brief summary of the purpose of the project, the research activities, the outputs of the project, and the contribution of the project towards DFID's development goals. (Up to 500 words).

The purpose of the project was to identify novel somatic hybrid rice plants for use in breeding programmes in India. In previous DFID funded work at the University of Nottingham, a population of somatic hybrid plants has been produced between cultivated rice (*Oryza sativa*) and the wild relative (*O. granulata*). In the current project the cytoplasmic composition of these plants has been characterised and it has been shown that 60% contained chloroplasts derived from *O. granulata* while the remaining 40% contained chloroplasts derived from *O. sativa*. Plants with novel nuclear-cytoplasmic genomes are required for the development of new sources of male sterility for improvements of hybrid rice programmes. This germplasm will be of great importance to Indian rice breeders.

Background

Information should include a description of the importance of the researchable constraint(s) that the project sought to address and a summary of any significant research previously carried out. Also, some reference to how the demand for the project was identified.

Spectacular gains in rice productivity have been achieved in China through the adoption of hybrid rice technology. There is presently a requirement to utilise this technology in India. This necessitates the transfer of cytoplasmic male sterility genes into Indian rice cultivars. Male-sterile lines, which are unable to self-pollinate, are essential for a successful hybrid rice programme**.** The most commonly used system, cytoplasmic male sterility, utilises three lines, namely a cytoplasmic male sterile (cms) line with corresponding maintainer and fertility restorer lines. The wild abortive (WA) cytoplasm, by far the most commonly used source of cms, was developed by wide hybridisation between rice cultivars and a wild rice plant with aborted pollen. However, rice growers in the Eastern lowland region of India are unable to use WA lines since the maintainer lines suitable for this area partially restore fertility in the locally adapted varieties. For the rest of India, it is important to diversify the current narrow genetic background of hybrid rice which renders the crop vulnerable to disease and pest outbreaks and adverse environmental conditions. The experience of the devastation of the hybrid maize crop carrying the T-cytoplasm is an important reminder of the importance of this work. Considerable effort is now being directed, both in India and world-wide, towards establishing new sources of male sterility.

Wide hybridisation, involving sexual crosses and back-crossing, is currently being used at CRRI, Cuttack and IRRI Philippines as well as other rice research centres around the world, to develop alloplasmic lines between cultivated rices and other members of the genus *Oryza* in order to develop new sources of male sterility. Ideally, the new lines should be composed of the *O. sativa* nucleus, together with cytoplasm from the wild species. In practice, sexual crossing results in the inheritance of the mitochondrial and chloroplast genomes of the wild species together with the nuclear genomes of both the cultivated and wild rice species. Repeated backcrossing of the hybrid with rice breeding lines is employed to eliminate chromosomes of the wild species, To date, it has been possible to transfer only those cytoplasms from other closely related species ('A' genome) into *O. sativa*.

In phase 2 of the programme somatic hybridisation was exploited to circumvent the sexual barriers to cytoplasmic diversification. Populations of cybrid and somatic hybrid plants were produced at Nottingham from protoplast fusions involving a range of cultivated rices and the distantly related rices *O. australiensis* (F genome), O. *granulata* (C genome) and *O. latifolia* (CD genome). These plants have been sent to collaborating institutions in India for assessment of the extent of male sterility. Production of' these plants represents completion of technology generation step TG5 of Activity 2.2 in the revised PSP Logical Framework (October 1998).

This project was formulated after close collaboration between the Directorate of Rice research, Hyderabad, the Central Rice Research Institute, Cuttack and the University of Nottingham. During April 1999,N.W. Blackhall travelled to India and visited the Directorate of Rice Research Hyderabad and the Central Rice Research Institute, Cuttack. This visit presented the opportunity to converse directly with researchers in both wide hybridisation and hybrid rice research. The progress achieved during Phase 2 was discussed and a well defined course of action was identified in order to build upon the work already completed.

Project Purpose

The purpose of the project and how it addressed the identified development opportunity or identified constraint to development.
The purpose of the project was to identify plants containing diverse cytoplasms derived from wild relatives of cultivated rice suitable for use in hybrid rice breeding programmes in India. The project was targeted towards the needs of rice farmers in high potential production systems in India. In the eastern lowland region, it is difficult for breeders to produce male-sterile lines for distribution to farmers because all of the rice varieties adapted to this area restore fertility when crossed with conventional Wild Abortive hybrid rice. For the rest of India, there is an urgent requirement to diversify the limited genetic background of hybrid rice which renders the crop vulnerable to disease and pest outbreaks and adverse environmental conditions.

Research Activities

This section should include detailed descriptions of all the research activities (research studies, surveys, experiments etc) conducted to achieve the outputs of the project. Information on any facilities, expertise and special resources used to implement the project should also be included.
Indicate any modification to the proposed research activities, and whether planned inputs were achieved.

During phase 2 of the programme, somatic hybrid and cybrid plants were produced between the cultivated rices Taipei 309 and Pusa Basmati 1 and the wild rice *Oryza granulata*. This valuable germplasm was crucial to implementation of the project. Glasshouse facilities at the University of Nottingham were used to grow these plants to maturity.

The plants were maintained *in vitro* by growth in 175 ml capacity screw-capped glass jars ("Powder-Round", Beatson Clark and Co. Ltd., Rotherham, UK) each containing 50 mL aliquots of micropropagation medium [MS basal medium based on the formulation of Murashige and Skoog, 1962 (Sigma, Poole, UK), to which 50 g/L sucrose was added and semi-solidified by the addition of 8 g/L SeaKem LE agarose (FMC BioProducts, Vallensbaek Strand, Denmark), pH 5.8 (see Table 1) supplemented with 2.0 mg/L 6-benzylaminopurine]. The bases of the plants were immersed 5 mm below the surface of the medium and sub-cultured every 28 days.

Prior to transfer to the glasshouse, rooting was induced on the plants by growth for 28 days in rooting medium (MS basal medium as above supplemented with 1.5 mg/L α-naphthaleneacetic acid). Rooted plants were transferred to the glasshouse in initiation compost [a 12:1 (v:v) mixture of M3 soil-less compost (Fisons plc., Ipswich, UK) and Perlite (Silvaperl Ltd., Gainsborough, UK)] in 7.5 cm diameter plastic plant pots and covered with 20 cm x 20 cm clear polythene bag. The plants were maintained under natural daylight with maximum day and night temperatures of 28 ± 2°C and 24 ± 2°C, respectively. The plants were acclimatised by gradually reducing the humidity inside the bags using the following procedure. After 3 days, 5 incisions were made with a pin into the top of the bags. Four days later, one corner of each bag was removed with scissors. After a further 4 days, the other corner of each bag was removed. Subsequently, every 2 days, the top 1 cm of each bag was cut off, until the top-most leaves of the potted plants were exposed. Finally, each bag was removed. Each day the plants were sprayed with a 0.1% (v/v) aqueous solution of Maxicrop Plus Sequestered Iron (Maxicrop Garden Products, Gr. Shelford, Cambridge, UK). Plants producing tillers and roots and which showed healthy, vigorous growth were transferred to 15 cm diameter pots containing growth compost [6:1:1 (v:v) mixture of M3 soil-less compost, John Innes No. 3 compost (J. Bentley Ltd., Barton-on-Humber, UK) and Perlite].

Total genomic DNA was extracted from leaf samples of the plants using the DNeasy Plant mini kit (Qiagen Ltd., Crawley, West Sussex, UK). Analysis of the cytoplasmic genome composition of the plants was carried out using the procedures described by Provan *et al.*, (1997) with the primers listed in Table 2.

Table 1: Formulation of Media - Macronutrients, Micronutrients, Vitamins and other Supplements

Component	Concentration, mg/L MS basal medium
Macronutrients	
$CaCl_2$	332.2
KH_2PO_4	170.0
$MgSO_4$	180.7
KNO_3	1900.0
Micronutrients	
KI	0.83
H_3BO_3	6.20
$MnSO_4$	16.90
$NaMoO_4.2H_2O$	0.25
$ZnSO_4.7H_2O$	8.60
$CuSO_4.5H_2O$	0.025
$CoCl_2.6H_2O$	0.025
$FeSO_4.7H_2O$	27.85
Na_2EDTA	37.25
Vitamins	
Myo-inositol	100.0
Nicotinic acid	0.5
Pyridoxine HCl	0.5
Thiamine HCl	0.1
Glycine	2.0
Sucrose	50000
pH	5.8
Sterilisation	Autoclave

Table 2: Chloroplast Microsatellite primers

Designation	Forward sequence	Reverse sequence
OSCPA	GGATCTAGGCATAATTCCCAA	GAGCATAGGGATCGATTTGAT
OSCPB	GGAATTTGGACATTTTCGC	AAGACAGGGGTAATCTTTCGA
OSCPC	GGAAAAAATAAGTCTCTTTGTTTGA	AGACTCGAAGGATACCGAAGA
OSCPD	GGAAAGGTTAGGGTTTTTAATATTG	CCGCGATGCAATAAGAGTAAA

Murashige, T., and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15**:**473-497.

Provan J, Corbett G, McNicol JW, Powell W. (1997) Chloroplast DNA variability in wild and cultivated rice (*Oryza* spp.) revealed by polymorphic chloroplast simple sequence repeats. Genome 40:104-110.

Outputs

The research results and products achieved by the project. Were all the anticipated outputs achieved and if not what were the reasons? Research results should be presented as tables, graphs or sketches rather than lengthy writing, and provided in as quantitative a form as far as is possible.

The plants were grown under glasshouse conditions at the University of Nottingham and photographed after 120 –180 days. Approximately half of the plants, 55%, failed to reach maturity and remained less than 300 mm tall. The remainder were assessed for pollen fertility. Seed derived Pusa Basmati 1 plants produced viable pollen (mean 86%, std. dev. 4.8%) whereas the somatic hybrid/cybrid plants which did reach maturity produced pollen with reduced viability (mean 62%, std. dev. 33.6%). Seed-derived cms plants (IR58024A mean 27%, std. dev. 8.3%) showed a very different pattern of pollen viability. These unusual results are most probably due to inappropriate light intensities and day lengths. Although rice plants are frequently grown successfully in glasshouses at the University of Nottingham during the period February – July, the conditions will never be optimal.

Plants were transported to DRR, Hyderabad, India on two occasions (six shipments, , 450 plants in total). On each attempt, all of the plants failed to survive *in vitro*. It is suspected that the somatic hybrid/cybrid plants were unable to withstand the rigours of international air transport. When considered together with the inability of many of these plants to reach maturity when grown under glasshouse conditions in the UK, it is apparent that this population of plants is more fragile than seed-derived plants.

Analysis of the cytoplasmic genome of the plants revealed that 65% contained chloroplasts derived from *O. granulata* and the remainder contained chloroplasts derived from *O. sativa*.

Contribution of Outputs

Include how the outputs will contribute towards DFID's development goals. The identified promotion pathways to target institutions and beneficiaries. what follow up action / research is necessary to promote the findings of the work to achieve their development benefit? This should include a list of publications, plans for further dissemination, as appropriate. For projects aimed at developing a device, material or process specify:

a. What further market studies need to be done?
b. How the outputs will be made available to intended users?
c. What further stages will be needed to develop, test and establish manufacture of a product?
d. How and by whom, will the further stages be carried out and paid for.

The results obtained in this project have clearly shown that 65% (from a total of 55) of the plants contained cytoplasms derived from *O. granulata*. These plants were obtained as the result of protoplasts fusion experiments utilising *O. sativa and O. granulata*. A feature of these experiments was the inability to regenerate plants of *O. granulata.* This population of plants represents an important germplasm resource which is being maintained at the University of Nottingham.

In spite of the difficulties experienced in transporting these plants to India, Dr Brar at IRRI, Philippines has shown an interest in this material. These plants will now be used in a further DFID funded project, R8024, Molecular analysis of sexual and somatic hybrids of *O. sativa* and *O. granulata* for comparative genome characterisation. In this project, the plants will be grown at IRRI and seed collected where possible. This seed will then be distributed to the rice breeding community as appropriate via IRRI. This part of the project is being funded by IRRI.

In project R8024, further molecular analyses will be carried out to determine the nuclear composition of these plants. This work will be in collaboration with JIC, Norwich. The data collected during project R7417 will be combined with the results of project R8024 to produce articles for publication in internationally reviewed journals.