FINAL TECHNICAL REPORT

Molecular analysis of sexual and somatic hybrids of *Oryza sativa* and *O. granulata* for comparative genome characterisation. R8024

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 ensure that novel plants produced by somatic hybridisation of *O. sativa* and *O. granulata* in previous DFID PSP research are made available for the rice breeding community. It was envisaged that the germplasm could be used to transfer traits to cultivated rice and these may include drought tolerance and adaptability to low levels of solar radiation which occur in the wet season in most areas where rice is grown. Detailed molecular characterisation using microsatellite primers showed that the original population of 55 plants was composed of 33 cybrids and 22 *O. sativa* plants, of which 7 were somaclonal variants. There are no plans to further utilise this germplasm.

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.

Wild species have played a key role in the improvement of cultivated rice. For example, *O. nirvana* contributed to the parentage of IR64, at one time the most widely grown rice genotype in the world. More recently, WARDA has used *O. glaberrima* in its breeding programme to produce a number of widely accepted cultivars for west Africa.

O. granulata could also contribute to the cultivated rice gene pool. Two traits of *O. granulata* are drought tolerance and tolerance to shade. However, the genetic contribution of a wild species cannot be predicted on its phenotypic performance alone and *O. granulata* could contribute many other useful characteristics to rice. Drought and shade tolerance, have been identified by IRRI as key traits for increasing rice yield. The report of the Fourth External Programme and Management Review of IRRI by the Technical Advisory Committee and CGIAR Secretariat made the following key recommendation:
'… that IRRI explore the feasibility of combining with cultivated rice the ability of some wild species to grow under low solar radiation, in order to increase wet season rice productivity.'

Somatic hybridisation potentially offers an alternative and rapid method of transferring polygenic traits from wild germplasms to cultivated species. Unlike sexual hybridisation, wherein fertilization results in seed formation, protoplast fusion produces heterokaryons

which develop into plantlets. Within heterokaryons and their cellular derivatives, mixing of interphase nuclei occurs leading to their interaction during subsequent mitoses. *O. granulata* is particularly suitable for use in such studies since it possesses drought tolerance and the ability to grow well in the shade. These have been identified by IRRI as being key traits for increasing rice yield.

The sexual hybrids (2n=2x=24, Aggarwal *et al*. 1996) are available as monosomatic alien addition lines for chromosomes 9 and 11 for which *O. sativa* was always used as the female parent. A population of 54 somatic hybrids (2n=4x=48) have been produced at Nottingham by protoplast fusion. These have been shown to contain novel nuclear-cytoplasmic combinations and have a range of phenotypes intermediate between the two parents. Preliminary evidence suggests that these hybrids are fertile and can be backcrossed with the aid of embryo rescue and thus are providers of the key traits to be transferred and established in rice.

Somatic hybridisation facilitates novel gene introgression and (chromosome) recombination events which result in different (and often preferable) introgression patterns when compared to sexual hybridisation. For instance, in tomato, Parokonny *et al*. (1997) crossed *L. esculentum* (2n=2x=24) (+) *L. peruvianum* (2n=2x=24) to give somatic hybrids (2n=6x=72) consisting of 24 *L. esculentum* and 48 *L. peruvianum* chromosomes. Importantly, backcrossing of these polyploid hybrid plants to *L. esculentum* produced plants with 2n=2x=24 (12 chromosomes from *L. esculentum* and 12 from *L. peruvianum*) in BC_1 following embryo rescue. However BC_2 plants consisted of 4-10 *L. peruvianum* chromosomes (14-20 chromosomes of *L. esculentum* correspondingly) and BC_3 plants contained 2-4 *L. peruvianum* chromosomes (and 20 –22 *L. esculentum* chromosomes). Pollen tetrad nuclei of the somatic hybrids contained one or two micro-nuclei along with a reduced selection (near haploid) number of chromosomes i.e. gametic rather than zygotic selection was occurring. Similar events would be expected to occur during backcrossing to rice of the *O. sativa* (+) *O. granulata* somatic hybrids, giving rise to a spectrum of plants with 1-12 *O. granulata* chromosomes.

In the Sainsbury Laboratory at the John Innes Centre, the use of map-based fingerprinting has been applied to indica-japonica crosses of rice (Zhu *et al.,* 1999). This technique employed high-throughput techniques (AFLP and microsatellites). Chromosomes were divided into 'DNA fingerprint linkage blocks' defined by specific markers. Using these blocks, the degree of similarity or divergence within specific chromosome regions was calculated for hybrid plants. AFLP markers provide a fast genome scan with good genome coverage whereas microsatellites have the flexibility to allow particular chromosomal segments to be targeted and are easier to transfer between different species. Application of these analytical techniques to the sexual and somatic hybrid rice plants will produce detailed chromosome maps for each plant line which facilitate identification of the origins of individual gene segments.

Aggarwal RK, Brar DS, Huang N, Khush GS (1996) Molecular analysis of introgression into *Oryza sativa/O. brachyantha* and *O. sativa/O. granulata* derivatives. International Rice Research Notes 21:14-15.

Parokonny AS, Marshall JA, Bennett MD, Cocking EC, Davey MR, Power JB (1997) Homoeologous pairing and recombination in backcross derivatives of tomato somatic hybrids [*Lycopersicon esculentum* (+) *L. peruvianum*] Theor. Appl. Genet. 94:713-723.

Zhu JH, Stephenson P, Laurie DA, Li W, Tang D, Gale MD. Towards rice genome scanning by map-based AFLP fingerprinting (1999) Mol. Gen. Genet. 261:184-195.

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 ensure that novel plants produced by somatic hybridisation of *O. sativa* and *O. granulata* in previous DFID PSP research are made available for the rice breeding community. It was envisaged that the germplasm could be used to transfer traits to cultivated rice and these may include drought tolerance and adaptability to low levels of solar radiation which occur in the wet season in most areas where rice is grown.

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.

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 and grown at a temperature of 26°C, 16 hour photoperiod.

Seven days before shipping to IRRI, the plants were sub-cultured into polypropylene test-tubes (10 cm x 1.5 cm with nylon push-fit caps) containing 5mL of micropropagation medium. Following inspection by an official from DEFRA and the issue of a Phytosanitary certificate, the tubes were heat-sealed inside polythene bags. Five tubes were placed inside a 12 cm x 12 cm plastic bag which was sealed between each tube and at the end. The bags of tubes were placed inside a strong cardboard box surrounded by small expanded polystyrene pieces. The next morning, the box was collected by a courier (DHL) for transport to IRRI.

DNA was extracted from leaves of *in vitro* grown plants by freezing 100 mg in liquid nitrogen and grinding to a fine powder using a pestle and mortar. The DNA was isolated using a plant genomic DNA extraction kit (Sigma) in accordance with the manufacturer's instructions. The final DNA pellet was dissolved in 50 µl of sterile water and stored frozen at –80°C. Five extractions were made for each plant.

Microsatellite analysis was performed as described by Panaud *et al.,* 1996 using the 255 primer pairs described in Table 2. Initially, each pair of primers was tested for the ability to distinguish between *O. sativa* and *O. granulata*. The primers which showed polymorphisms were used to analyse the population of putatively hybrid plants.

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: Microsatellite primers and polymorphisms between *O.sativa* and *O. Granulata.*

Marker	Primer forward	Primer reverse	Polymorphism
RM1	gcgaaaacacaatgcaaaaa	gcgttggttggacctgac	Yes
RM2	acgtgtcaccgcttcctc	atgtccgggatctcatcg	Yes
RM3	acactgtagcggccactg	cctccactgctccacatctt	Yes
RM4	ttgacgaggtcagcactgac	agggtgtatccgactcatcg	No
RM5	tgcaacttctagctgctcga	gcatccgatcttgatggg	Yes
RM6	gtcccctccacccaattc	tcgtctactgttggctgcac	No
RM7	ttcgccatgaagtctctcg	cctcccatcatttcgttgtt	No
RM8	cacgtggcgtaaatacacgt	ggccaaaccctaaccctg	Yes
RM9	ggtgccattgtcgtcctc	acggccctcatcaccttc	Yes
RM10	ttgtcaagaggaggcatcg	cagaatgggaaatgggtcc	Yes
RM11	tctcctcttcccccgatc	atagcgggcgaggcttag	Yes
RM13	tccaacatggcaagagagag	ggtggcattcgattccag	No
RM14	ccgaggagaggagttcgac	gtgccaatttcctcgaaaaa	No
RM16	cgctagggcagcatctaaaa	aacacagcaggtacgcgc	Yes
RM17	tgccctgttattttcttctctc	ggtgatcctttcccatttca	Yes
RM18	ttccctctcatgagctccat	gagtgcctggcgctgtac	No
RM19	caaaaacagagcagatgac	ctcaagatggacgccaaga	Yes
RM20	atcttgtccctgcaggtcat	gaaacagaggcacatttcattg	Yes
RM21	acagtattccgtaggcacgg	gctccatgagggtggtagag	Yes
RM22	ggtttgggagcccataatct	ctgggcttctttcactcgtc	Yes
RM23	cattggagtggaggctgg	gtcaggcttctgccattctc	Yes
RM24	gaagtgtgatcactgtaacc	tacagtggacggcgaagtcg	Yes
RM25	ggaaagaatgatcttttcatgg	ctaccatcaaaaccaatgttc	No
RM26	gagtcgacgagcggcaga	ctgcgagcgacggtaaca	Yes
RM27	ttttccttctcacccacttca	tctttgacaagaggaaagaggc	Yes
RM29	cagggacccacctgtcatac	aacgttggtcatatcggtgg	No
RM30	ggttaggcatcgtcacgg	tcacctcaccacacgacacg	No
RM31	gatcacgatccactggagct	aagtccattactctcctccc	Yes
RM32	agtctacgtggtgtacacgtgg	tgcggcctgccgtttgtgag	Yes
RM34	gaaatggcaatgtgtgcg	gccggagaaccctagctc	Yes
RM35	tggttaatcgatcggtcgcc	cgacggcagatatacacgg	No
RM36	caactatgcaccattgtcgc	gtactccacaagaccgtacc	Yes
RM38	acgagctctcgatcagccta	tcggtctccatgtcccac	Yes
RM39	gcctctctcgtctccttcct	aattcaaactgcggtggc	Yes
RM40	tcatcacgtaccgcgttg	cgcatagccggaaaagtaag	Yes
RM41	aagtctagtttgcctccc	aatttctacgtcgtcgggc	Yes
RM42	atcctaccgctgaccatgag	tttggtctacgtggcgtaca	No
RM44	acgggcaatccgaacaacc	tcgggaaaacctaccctacc	No
RM47	actccactccactccccac	gtcagcaggtcggacgtc	Yes
RM48	tgtcccactgctttcaagc	cgagaatgagggacaaataacc	Yes
RM49	ttcggaagttggttactgatca	ttggagcggattcggagg	Yes
RM50	actgtaccggtcgaagacg	aaattccacgtcagcctcc	Yes
RM51	tctcgattcaatgtcctcgg	ctacgtcatcatcgtcttccc	No
RM52	ctactcgcgcgtggagtt	tgtcttactggtgaagctgg	Yes
RM53	acgtctcgacgcatcaatgg	cacaagaacttcctcggtac	Yes
RM54	aacggagggattcgaattc	ggggatggtttatatgggct	No
RM55	ccgtcgccgtagtagagaag	tcccggttattttaaggcg	No

Marker	Primer forward	Primer reverse	Polymorphism
RM60	agtcccatgttccacttccg	atggctactgcctgtactac	No
RM70	gtggacttcatttcaactcg	gatgtataagatagtccc	Yes
RM71	ctagaggcgaaaacgagatg	gggtgggcgaggtaataatg	Yes
RM72	ccggcgataaaacaatgag	gcatcggtcctaactaaggg	No
RM80	ttgaaggcgctgaaggag	catcaacctcgtcttcaccg	No
RM81	gagtgcttgtgcaagatcca	cttcttcactcatgcagttc	No
RM82	tgcttcttgtcaattcgcc	cgactcgtggaggtacgg	No
RM83	actcgatgacaagttgagg	cacctagacacgatcgag	Yes
RM84	taagggtccatccacaagatg	ttgcaaatgcagctagagtac	Yes
RM85	ccaaagatgaaacctggattg	gcacaaggtgagcagtcc	No
RM86	tacacctcatcgatcaatcg	ctttcgaatctgaagatc	Yes
RM87	cctctccgatacaccgtatg	gcgaaggtacgaaaggaaag	No
RM88	actcatcagcatggccttgctc	taatgctccaccttcaccac	No
RM101	gtgaatggtcaagtgacttaggtggc	acacaacatgttccctcccatgc	Yes
RM102	aactttcccaccaccaccgcgg	agcagcagcaagccagcaagcg	No
RM103	cttccaattcaggccggctggc	cgccacagctgaccatgcatgc	No
RM104	ggaagaggagagaaagatgtgtgtcg	tcaacagacacaccgccaccgc	Yes
RM105	gtcgtcgacccatcggagccac	tggtcgaggtggggatcgggtc	Yes
RM106	cgtcttcatcatcgtcgccccg	ggcccatcccgtcgtggatctc	Yes
RM107	agatcgaagcatcgcgcccgag	actgcgtcctctgggttcccgg	Yes
RM108	tctcttgcgcgcacactggcac	cgtgcaccaccaccaccaccac	Yes
RM109	gccgccggagagggagagagag	ccccgacgggatctccatcgtc	Yes
RM110	tcgaagccatccaccaacgaag	tccgtacgccgacgaggtcgag	No
RM111	cacaacctttgagcaccgggtc	acgcctgcagcttgatcaccgg	Yes
RM112	gggaggagaggcaagcggagag	agccggtgcagtggacggtgac	No
RM113	caccattgcccatcagcacaac	tcgccctctgctgcttgatggc	No
RM114	cagggacgaatcgtcgccggag	ttggcccccttgaggttgtcgg	No
RM115	ttgccgcagtggccgttaccac	aggaggcggcggaaatggaagg	Yes
RM117	cgatccattcctgctgctcgcg	cgcccccatgcatgagaagacg	Yes
RM118	ccaatcggagccaccggagagc	cacatcctccagcgacgccgag	No
RM119	catccccctgctgctgctgctg	cgccggatgtgtgggactagcg	No
RM120	cacacaagccctgtctcacgacc	cgctgcgtcatgagtatgta	Yes
RM121	accgtcgccttccactttcccc	ttcggggttgccggtgatgttg	Yes
RM122	gagtcgatgtaatgtcatcagtgc	gaaggaggtatcgctttgttggac	Yes
RM124	atcgtctgcgttgcggctgctg	catggatcaccgagctcccccc	Yes
RM125	atcagcagccatggcagcgacc	aggggatcatgtgccgaaggcc	Yes
RM126	cgcgtccgcgataaacacaggg	tcgcacaggtgaggccatgtcg	No
RM127	gtgggatagctgcgtcgcgtcg	aggccagggtgttggcatgctg	Yes
RM128	agcttgggtgatttcttggaagcg	acgacgaggagtcgccgtgcag	Yes
RM129	tctctccggagccaaggcgagg	cgagccacgacgcgatgtaccc	No
RM130	tgttgcttgccctcacgcgaag	ggtcgcgtgcttggtttggttc	Yes
RM131	tcctccctcccttcgcccactg	cgatgttcgccatggctgctcc	Yes
RM132	atcttgttgtttcggcggcggc	catggcgagaatgcccacgtcc	No
RM133	ttggattgttttgctggctcgc	ggaacacggggtcggaagcgac	Yes
RM134	acaaggccgcgagaggattccg	gctctccggtggctccgattgg	No
RM135	ctctgtctcctcccccgcgtcg	tcagcttctggccggcctcctc	No
RM136	gagagctcagctgctgcctctagc	gaggagcgccacggtgtacgcc	Yes
RM137	gacatcgccaccagcccaccac	cgggtggtccccgaggatcttg	Yes
RM138	agcgcaacaaccaatccatccg	aagaagctgcctttgacgctatgg	No

Marker	Primer forward	Primer reverse	Polymorphism
RM139	gagagggaggaagggaggcggc	ctgccatggcagagaaggggcc	No
RM140	tgcctcttccctggctcccctg	ggcatgccgaatgaaatgcatg	Yes
RM141	caccaccaccaccacgcctctc	tcttggagaggaggaggcgcgg	Yes
RM142	ctcgctatcgccatcgccatcg	tcgagccatcgctggatggagg	No
RM143	gtcccgaaccctagcccgaggg	agaggccctccacatggcgacc	No
RM144	tgccctggcgcaaatttgatcc	gctagaggagatcagatggtagtgcatg	No
RM145	ccggtaggcgccctgcagtttc	caaggaccccatcctcggcgtc	Yes
RM146	ctattattccctaacccccataccctcc	agagccactgcctgcaaggccc	Yes
RM147	tacggcttcggcggctgattcc	cccccgaatcccatcgaaaccc	Yes
RM148	atacaacattagggatgaggctgg	tccttaaaggtggtgcaatgcgag	Yes
RM149	gctgaccaacgaacctaggccg	gttggaagcctttcctcgtaacacg	No
RM150	cacgacgacgacgagcagcagc	gctcgagggagagcgacctgcc	Yes
RM151	ggctgctcatcagctgcatgcg	tcggcagtggtagagtttgatctgc	Yes
RM152	gaaaccaccacacctcaccg	ccgtagaccttcttgaagtag	Yes
RM153	gcctcgagcatcatcatcag	atcaacctgcacttgcctgg	Yes
RM154	accctctccgcctcgcctcctc	ctcctcctcctgcgaccgctcc	Yes
RM155	gagatggccccctccgtgatgg	tgccctcaatcggccacacctc	No
RM156	gccgcaccctcactccctcctc	tcttgccggagcgcttgaggtg	Yes
RM157	cctcctcctcacgaatcccgcc	gggcttcttctccgccggcttc	No
RM158	atggtgagagttgctgccgccg	gatgacgcagaacggcatcgcc	Yes
RM159	ggggcactggcaagggtgaagg	gcttgtgcttctctctctctctctctc	No
RM160	agctagcagctatagcttagctggagatc	tctcatcgccatgcgaggcctc	No
RM161	tgcagatgagaagcggcgcctc	tgtgtcatcagacggcgctccg	Yes
RM162	gccagcaaaaccagggatccgg	caaggtcttgtgcggcttgcgg	No
RM163	atccatgtgcgcctttatgagga	cgctacctccttcacttactagt	No
RM164	tcttgcccgtcactgcagatatcc	gcagccctaatgctacaattcttc	No
RM165	ccgaacgcctagaagcgcgtcc	cggcgaggtttgctaatggcgg	Yes
RM166	ggtcctgggtcaataattgggttacc	ttgctgcatgatcctaaaccgg	Yes
RM167	gatccagcgtgaggaacacgt	agtccgaccacaaggtgcgttgtc	No
RM168	tgctgcttgcctgcttccttt	gaaacgaatcaatccacggc	No
RM169	tggctggctccgtgggtagctg	tcccgttgccgttcatccctcc	Yes
RM170	tcgcgcttcttcctcgtcgacg	cccgcttgcagaggaagcagcc	Yes
RM171	aacgcgaggacacgtacttac	acgagatacgtacgcctttg	Yes
RM172	tgcagctgcgccacagccatag	caaccacgacaccgccgtgttg	No
RM173	cctacctcgcgatcccccccctc	ccatgaggaggaggcggcgatc	No
RM174	agcgacgccaagacaagtcggg	tccacgtcgatcgacacgacgg	No
RM175	cttcggcgccgtcatcaaggtg	cgttgagcagcgcgacgttgac	Yes
RM176	cggctcccgctacgacgtctcc	agcgatgcgctggaagaggtgc	Yes
RM177	ccctcttagacagaggccagaggg	gtagccgaagatgaggccgccg	Yes
RM178	tcgcgtgaaagataagcggcgc	gatcaccgttccctccgcctgc	Yes
RM179	ccccattagtccactccaccacc	ccaatcagcctcatgcctcccc	No
RM180	ctacatcggcttaggtgtagcaacacg	acttgctctacttgtggtgagggactg	No
RM181	acgggagcttctccgacagcgc	tatgcttttgccgtgtgccgcg	Yes
RM182	tgggatgcagagtgcagttggc	cgcaggcacggtgccttgtaag	No
RM183	ggagcgggagagagagcccacg	tgccgatgaaggactgcgacgc	Yes
RM184	atcccattcgccaaaaccggcc	tgacacttggagagcggtgtgg	No
RM185	agttgttgggagggagaaaggcc	aggaggcgacggcgatgtcctc	No
RM186	tcctccatctcctccgctcccg	gggcgtggtggccttcttcgtc	No
RM187	ccaagggaaagatgcgacaattg	gtggacgctttatattatggg	No

Marker	Primer forward	Primer reverse	Polymorphism
RM188	tccgcctctcctctcgcttccc	gcaacgcacaaccgaaccgagc	Yes
RM189	cgtcttccccaacgctaaaa	cgccgggcttcgcttc	Yes
RM190	ctttgtctatctcaagacac	ttgcagatgttcttcctgatg	No
RM191	cccatcctcaccgatctctctaaac	gtgcgcacggaggaggaaaggg	Yes
RM192	gcggcggatcatgaattgcgag	cttgttccccggcgtcgagtcc	Yes
RM193	cgcctcttcttcctcgcctccg	cgggtccatccccctctcctc	Yes
RM194	gccctgcttcttgcccaccacc	tccagggagggcaaggctgagc	Yes
RM195	agaaagagaggccgtcggcggc	gggctcacccccaaacctgcag	Yes
RM197	gatccgtttttgctgtgccc	cctcctctccgccgatcctg	No
RM200	cgctagggaatttggattga	cgatgagcaggtatcgatgagaag	Yes
RM201	ctcgtttattacctacagtacc	ctacctcctttctagaccgata	Yes
RM202	cagattggagatgaagtcctcc	ccagcaagcatgtcaatgta	No
RM203	cctatcccattagccaaacattgc	gacgccaacctggagttaattacc	Yes
RM204	gtgactgacttggtcataggg	gctagccatgctctcgtacc	No
RM205	ctggttctgtatgggagcag	ctggcccttcacgtttcagtg	Yes
RM206	cccatgcgtttaactattct	cgttccatcgatccgtatgg	Yes
RM207	ccattcgtgagaagatctga	cacctcatcctcgtaacgcc	Yes
RM208	tctgcaagccttgtctgatg	taagtcgatcattgtgtggacc	Yes
RM209	atatgagttgctgtcgtgcg	caacttgcatcctcccctcc	No
RM210	tcacattcggtggcattg	cgaggatggttgttcacttg	No
RM211	ccgatctcatcaaccaactg	cttcacgaggatctcaaagg	Yes
RM212	ccactttcagctactaccag	cacccatttgtctctcattatg	No
RM213	atctgtttgcaggggacaag	aggtctagacgatgtcgtga	No
RM214	ctgatgatagaaacctcttctc	aagaacagctgacttcacaa	Yes
RM215	caaaatggagcagcaagagc	tgagcacctccttctctgtag	No
RM216	gcatggccgatggtaaag	tgtataaaaccacacggcca	No
RM217	atcgcagcaatgcctcgt	gggtgtgaacaaagacac	No
RM218	tggtcaaaccaaggtccttc	gacatacattctacccccgg	Yes
RM219	cgtcggatgatgtaaagcct	catatcggcattcgcctg	Yes
RM220	ggaaggtaactgtttccaac	gaaatgcttcccacatgtct	Yes
RM221	acatgtcagcatgccacatc	tgcaagaatctgacccgg	Yes
RM222	cttaaatgggccacatgcg	caaagcttccggccaaaag	No
RM223	gagtgagcttgggctgaaac	gaaggcaagtcttggcactg	No
RM224	atcgatcgatcttcacgagg	tgctataaaaggcattcggg	No
RM225	tgcccatatggtctggatg	gaaagtggatcaggaaggc	Yes
RM226	agctaaggtctgggagaaacc	aagtaggatggggcacaagctc	Yes
RM227	acctttcgtcataaagacgag	gattggagagaaaagaagcc	Yes
RM228	ctggccattagtccttgg	gcttgcggctctgcttac	No
RM229	cactcacacgaacgactgac	cgcaggttcttgtgaaatgt	No
RM230	gccagaccgtggatgttc	caccgcagtcacttttcaag	Yes
RM231	ccagattatttcctgaggtc	cacttgcatagttctgcattg	Yes
RM232	ccggtatccttcgatattgc	ccgacttttcctcctgacg	No
RM233	ccaaatgaacctacatgttg	gcattgcagacagctattga	No
RM234	acagtatccaaggccctgg	cacgtgagacaaagacggag	Yes
RM235	agaagctagggctaacgaac	tcacctggtcagcctctttc	Yes
RM236	gcgctggtggaaaatgag	ggcatccctctttgattcctc	No
RM237	caaatcccgactgctgtcc	tgggaagagagcactacagc	No
RM238	gatggaaagcacgtgcacta	acaggcaatccgtagactcg	No
RM239	tacaaaatgctgggtacccc	acatatgggacccacctgtc	No

Marker	Primer forward	Primer reverse	Polymorphism
RM240	ccttaatgggtagtgtgcac	tgtaaccattccttccatcc	No
RM241	gagccaaataagatcgctga	tgcaagcagcagatttagtg	Yes
RM242	ggccaacgtgtgtatgtctc	tatatgccaagacggatggg	Yes
RM243	gatctgcagactgcagttgc	agctgcaacgatgttgtcc	No
RM244	ccgactgttcgtccttatca	ctgctctcgggtgaacgt	Yes
RM245	atgccgccagtgaatagc	ctgagaatccaattatctgggg	No
RM246	gagctccatcagccattcag	ctgagtgctgctgcgact	No
RM247	tagtgccgatcgatgtaacg	catatggttttgacaaagcg	Yes
RM248	tccttgtgaaatctggtccc	gtagcctagcatggtgcatg	No
RM249	ggcgtaaaggttttgcatgt	atgatgccatgaaggtcagc	No
RM250	ggttcaaaccaagctgatca	gatgaaggccttccacgcag	Yes
RM251	gaatggcaatggcgctag	atgcggttcaagattcgatc	Yes
RM252	ttcgctgacgtgataggttg	atgacttgatcccgagaacg	Yes
RM253	tccttcaagagtgcaaaacc	gcattgtcatgtcgaagcc	No
RM254	agccccgaataaatccacct	ctggaggagcatttggtagc	Yes
RM255	tgttgcgtgtggagatgtg	cgaaaccgctcagttcaac	Yes
RM256	gacagggagtgattgaaggc	gttgatttcgccaagggc	No
RM257	cagttccgagcaagagtactc	ggatcggacgtggcatatg	Yes
RM258	tgctgtatgtagctcgcacc	tggcctttaaagctgtcgc	Yes
RM259	tggagtttgagaggaggg	cttgttgcatggtgccatgt	Yes
RM260	actccactatgacccagag	gaacaatcccttctacgatcg	No
RM261	ctacttctccccttgtgtcg	tgtaccatcgccaaatctcc	Yes
RM262	cattccgtctcggctcaact	cagagcaaggtggcttgc	Yes
RM263	cccaggctagctcatgaacc	gctacgtttgagctaccacg	Yes
RM264	gttgcgtcctactgctacttc	gatccgtgtcgatgattagc	No
RM265	cgagttcgtccaagtgagc	catccaccattccaccaatc	No
RM266	tagtttaaccaagactctc	ggttgaacccaaatctgca	Yes
RM267	tgcagacatagagaaggaagtg	agcaacagcacaacttgatg	Yes
RM268	gtgctatgcacgatccatagca	cgtttctttggaagcggaggga	Yes
RM269	gaaagcgatcgaaccagc	gcaaatgcgcctcgtgtc	Yes
RM270	ggccgttggttctaaaatc	tgcgcagtatcatcggcgag	No
RM271	tcagatctacaattccatcc	tcggtgagacctagagagcc	Yes
RM272	aattggtagagaggggagag	acatgccattagagtcaggc	Yes
RM273	gaagccgtcgtgaagttacc	gtttcctacctgatcgcgac	Yes
RM274	cctcgcttatgagagcttcg	cttctccatcactcccatgg	Yes
RM275	gcattgatgtgccaatcg	cattgcaacatcttcaacatcc	Yes
RM276	ctcaacgttgacacctcgtg	tcctccatcgagcagtatca	No
RM277	cggtcaaatcatcacctgac	caaggcttgcaagggaag	No
RM278	gtagtgagcctaacaataatc	tcaactcagcatctctgtcc	Yes
RM279	gcgggagagggatctcct	ggctaggagttaacctcgcg	No
RM280	acacgatccactttgcgc	tgtgtcttgagcagccagg	No
RM281	accaagcatccagtgaccag	gttcttcatacagtccacatg	Yes
RM282	ctgtgtcgaaaggctgcac	cagtcctgtgttgcagcaag	No
RM283	gtctacatgtacccttgttggg	cggcatgagagtctgtgatg	Yes
RM284	atctctgatactccatccatcc	cctgtacgttgatccgaagc	Yes
RM285	ctgtgggcccaatatgtcac	ggcggtgacatggagaaag	Yes
RM286	ggcttcatctttggcgac	ccggattcacgagataaactc	No
RM287	ttccctgttaagagagaaatc	gtgtatttggtgaaagcaac	No
RM288	ccggtcagttcaagctctg	acgtacggacgtgacgac	Yes

Marker	Primer forward	Primer reverse	Polymorphism
RM289	ttccatggcacacaagcc	ctgtgcacgaacttccaaag	No
RM290	acccttattcctgctctcctc	gtgctgtagatggaagggag	No

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

Panaud, O., Chen, X., and McCouch S.R. (1996) Development of microsatellite markers and characterisation of simple sequence length polymorphism (SSLP) in rice (*Oryza sativa* L.). Mol. Gen. Genet. 252:597-607.

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.

Plants were transported to IRRI 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 plants were unable to withstand the rigours of international air transport. In project R7417 a similar problem was encountered while trying to transposrt the same plants to India. When considered together with the previously observed 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 considerably more fragile than seed-derived plants.

When the microsatellite primers were used to analyse *O. sativa* and *O. granulata* plants, 60% were able to distinguish between the 2 species. Analysis of the putative hybrid plants revealed complex profiles for 40 of the plants, suggesting that these plants contained chromatin from both rice species. The remaining 15 plants all possessed the same profile as seed-derived *O. sativa* plants. This finding was reported in the 2001 Annual Report. However, more detailed examination showed that for the 40 putative hybrid plants, the presence of *O. granulata* could not be confirmed but novel profiles were observed. Subsequent analysis of a population of *O. sativa* plants which had been produced by the same protoplast-to-plant culture system revealed similar DNA profiles. This indicates that the 40 putative hybrid plants were in fact *O. sativa* plants which were demonstrating somaclonal variation. Of these 40 plants, it had previously been shown that 33 possessed chloroplasts derived from *O. granulata* while the remaining 7 possessed chloroplasts derived from *O. sativa.* Thus, the original population of 55 plants was composed of 33 cybrids and 22 *O. sativa* plants, of which 7 were somaclonal variants.

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 finding that the population of 55 plants contained no somatic hybrids made an important change on the output of the project. There are no plans to further utilise this germplasm, especially as they could not be transported to either India or the Philippines.

N.W. Blackhall