

Use of conserved rice germplasm

G.C. Loresto, E. Guevarra and M.T. Jackson ✉

Genetic Resources Center, International Rice Research Institute, MCPO Box 3127, 1271 Makati City, Philippines.
Tel: (63-2) 845-0563; Fax: (63-2) 845-0606; E-mail: m.jackson@cgiar.org

Summary

Use of conserved rice germplasm

In a 1995 survey of users of germplasm from the International Rice Genebank Collection at IRRI between 1989 and 1994, 48 respondents (from about 200 surveyed) reported evaluation and use of almost 4000 samples. Only a small number were used directly for rice improvement. Most were evaluated for biotic and abiotic stresses. The research generated 59 publications.

Keywords: Conservation, *ex situ*, *Oryza*, rice

Résumé

Utilisation du germoplasme de riz conservé

Dans une étude menée en 1995 auprès des utilisateurs du germoplasme de riz issu de la Collection de la banque de gène internationale de riz à l'IRRI et concernant la période 1989-1994, 48 personnes interrogées sur les 200 sollicitées ont décrit l'évaluation et l'utilisation de presque 400 échantillons. Il apparaît que seul un petit nombre de ces échantillons sert directement à l'amélioration du riz. La plupart sont évalués pour les stress biotiques et abiotiques. La recherche a généré 59 publications.

Resumen

Uso de germoplasma de arroz conservado

En una encuesta realizada en 1995 entre usuarios de germoplasma de la Colección del Banco de Germoplasma Internacional de Arroz del IRRI entre 1989 y 1994, se recibieron 48 respuestas (entre unos 200 encuestados) sobre evaluación y uso de casi 4000 muestras. Sólo unas pocas se utilizaron directamente para la mejora del arroz. En la mayoría se evaluó el estrés biótico y abiótico. La investigación dio lugar a 59 publicaciones.

Introduction

Crop breeders draw on genebanks to raise yield potential, improve nutritional quality and tackle a broad range of challenges to agricultural productivity (Plucknett *et al.* 1987). Genebanks backstop breeding programmes by regularly supplying new materials for specialized breeding pools. When the desired genes are not found in elite breeding lines, scientists turn to conserved germplasm that comprises farmers' or landrace varieties, old cultivars and wild species.

In 1977 the International Rice Research Institute (IRRI) established the International Rice Genebank (IRG) committed to the long term-conservation of rice genetic resources. Today, over 107 000 accessions of *Oryza* are conserved in the genebank. These are duplicate collections of the national programmes from more than 110 countries in Asia, Africa, Oceania/Pacific, and North and South America.

The value of conserving rice genetic resources may be measured in how these genetic resources are used in crop improvement, and eventually in a released variety. The studies by Evenson and Gollin (1994) indicated that the rice germplasm collection at IRRI had a direct impact on the international flow of improved rice varieties, which in turn was directly associated with an increase in varietal releases and yield growth. They concluded that the collection, preservation and cataloguing of rice germplasm directly and unambiguously led to a higher production of rice around the world, and hence played a significant role in feeding millions of people.

The use of rice genetic resources is not limited to plant breeding or crop improvement. Each year, thousands of germplasm samples are sent out for some kind of evaluation and research by users (Chang 1992). Documentation of the reported results, however, is scarce (Jones 1984). Many of the accessions requested from the International Rice Genebank are used in a wide variety of research projects that expand our understanding of the rice gene pool and its value.

Survey of rice germplasm users

In October 1995, we began a survey among scientists in seven countries who received germplasm from 1989 to 1994. The objectives were to document how the requested germplasm was used, what traits were identified and used in breeding or crop improvement, what methodologies were developed, and what other contributions were made to rice science. More than 200 letters were sent to recipients of germplasm in Bangladesh, Indonesia, Japan, Korea, Thailand, UK and USA; 48 users responded to our queries (Table 1).

Use of germplasm

Scientists who received conserved germplasm generated 59 publications. These scientists used 3921 samples of cultivated rice (some 3800 distinct accessions) and 139 samples of wild species (105 distinct accessions) in various research activities. Recipients used about 130 accessions of cultivated rice for crop improvement. The traits transferred were resistance to brown planthopper and bacterial leaf blight, low amylose, seedling vigour, germination ability at low temperature, waxy endosperm, smooth hulls, and milling and cooking quality. Breeders are usually interested in using only a small fraction of the total germplasm at any one time to meet immediate and often pressing objectives (Gill 1989). To use germplasm effectively, breeders need variability available in an agronomically desirable background.

Most of the accessions requested were used in the evaluation for biotic and abiotic stresses (Table 1). For example, in Thailand, plant breeders evaluated 122 accessions for grain quality and found one (Acc. 9032) that could possibly be released to farmers directly without further improvement. Researchers from the University of Wales-Bangor in the UK evaluated more than 2300 accessions for cold tolerance, with the objective of using the varieties identified as parents in a breeding programme in Nepal. The data generated will be stored in a database for other breeders

Table 1. Use of conserved germplasm from the International Rice Genebank Collection at IRRI between 1989 and 1994.

Institution/ country	No. of samples	Purpose	Traits identified [†]	Traits transferred	Varieties released and contributions to rice science [‡]
Bangladesh					
Bangladesh Rice Research Institute (BIRRI)	69	Evaluation, breeding	BPH, WBPH, callus induction & plant regeneration (Acc. 55814)	Acc. 55814 used in crossing programme	None
BIRRI	123	Evaluation	BPH resistance	BPH resistance gene	None
BIRRI	4	Research	Bacterial blight resistance	None	Plan to use as differential varieties in Bangladesh No publication
BIRRI	8	Research (PhD thesis)	Resistance to tungro virus		
University of Chittagong	37	Research	Work on hold		
Indonesia					
Inter University Center for Biotechnology Soegijapranata Catholic University	7	Research	Awaiting sponsorship		
	57	Re-introduced to farmer's field	None	None	
Japan					
Chugoku National Agricultural Experiment Station Gifu University	19	Evaluation & research	RTSV, GLH		(49)
	27	Research	Shade tolerance		Deriphat-SDS page technique for chlorophyll protein No publication
Hokkaido University	20	Research	Cold tolerance at booting stage		
Kagawa University	35	Research	Nitrate reductase Nitrite reductase		(2)
Kobe University	16	Research	Genetics of BPH resistance		No conclusive results No publication
Kyushu University	5	Research	Comparison on floating ability		(1)
Nagoya University	1	Research	Allergenic protein		Physiology and morphology of root system
Nagoya University	7	Research	Salt tolerance		None
National Agricultural Research Center	4	Breeding	Low amylose	Low amylose	
National Institute of Agrobiological Resources	62	Research	Blast resistance		RFLP/RAPD, experiment terminated
University of Tokyo	248	Research	Rooting ability		Used by graduate student
Korea					
Seoul National University	2	Research	Highly resistant to rice blast		Physiological plant pathology, (46)
Yung Nam Crop Experiment Station, RDA	39	Evaluation, breeding, research	Seedling vigour (4 acc.), BPH resistance (1), germination ability at low temperature (1)	Seedling vigour, germination ability	None
Thailand					
Department of Agriculture (DA)	151	Breeding, evaluation	BPH (6 acc.), SB resistance (1 acc.), BLB resistance (2 acc.), grain char. (2 acc.) grain quality (1 acc.)	BPH resistance (Acc. 237), BLB resistance (Acc. 611)	Acc. 9032 may be released to farmers
DA	8	Breeding	BPH resistance		
DA	8	Evaluation	None	None	None
DA	2	Evaluation	Gall midge resistance		Biotyping study of rice gall midge
DA	3	Research	BPH-resistant check		
United Kingdom					
IACR-Rothamsted	147	Research	Root growth in response to mechanical impedance		Root physiology in rice, screening method for root elongation rate

Institution/ country	No. of samples	Purpose	Traits identified [†]	Traits transferred	Varieties released and contributions to rice science [‡]
John Innes Centre	1	Research	Susceptible to isolates of two RTV & all biotypes of <i>Nephotettix virescens</i>	Used for agroinoculation of rice tungro bacilliform virus	Molecular biology & variation of the two tungro viruses For further study
University of Cambridge	13	Research	To confirm work of Yeo & Flowers		
University of Dundee	4	Research	Artificial symbiotic association between rice & cyanobacteria		Research group phased out
University of Nottingham	64	Research			Biotechnology, (3-7,9-16, 20-22,25,26,30-40,47,50-52)
University of Reading	21	Research			Seed storage, seed germination, & seed production; (17, 18, 19) (23, 24, 27, 28, 29)
University of Sussex	116	Research & evaluation	Salinity	Results were used by IRRI breeders	
University of Wales-Aberystwyth	1	Research	Ethylene responsive		Tissue probed for ethylene receptor proteins
University of Wales-Bangor	2,363	Evaluation	Superior cold tolerance	Varieties identified will be used as parents in a future breeding programme in Nepal	Replicable method for screening cold tolerance. Useful as a database for other breeders
University of Wales-Bangor	6	Research	Long, thick roots		Hydroponics root screening
United States					
Private citizen	11	Evaluation, research	Long grain size		
Brigham Young University	4	Research done at IRRI	Observed wax crystal patterns		
California Cooperative Rice Research Foundation, Inc.	96	Breeding	Still being evaluated for stem rot & sheath spot resistance		
California Cooperative Rice Research Foundation, Inc.	6	Breeding	Waxy endosperm (Acc. 76311); early maturity, cooking & milling quality, short grain (Acc. 76312)	Waxy endosperm, smooth hull (Acc.76311); milling & cooking quality (Acc. 76312)	Many advanced lines for cooking quality. Methodology developed: cooking quality screening
Cornell University	167	Research	BLB-R locus, <i>Xa21</i>		Genetic mapping, gene tagging, evaluation of genetic diversity using molecular markers, RFLP. (8,41,45,48,53,54,59)
Michigan State University	5	Research & training	Proteinase inhibitor II, bar gene	Genes transferred were proteinase inhibitor II and bar gene	Varieties were used to train Rockefeller Foundation students in genetic engineering
The Scripps Research Institute, California	13	Research			Characterization of rice tungro virus. Partial desiccation of mature embryo-derived calli to improve indica rice regeneration. Improving frequency of plant regeneration. (43, 44, 53)
University of California	24	Research	Drought-induced proteins (8 accs.)		Extraction of seedling dehydrins (6 accs.)
University of Washington	4	Research			Genetic engineering using <i>Agrobacterium</i>
Virginia Polytechnic Institute & State University	20	Research	To evaluate photosynthesis, leaf growth and water use		Use in physiological studies of a PhD thesis. (42)
Washington State University	5	Research	Explant used to develop embryogenic calli		Experiment not successful
USDA-Maryland	4	Research	Rice blast reaction		Virulence screening methods refined
USDA-Idaho	3	Conservation & distribution to researchers			

[†] BPH = brown planthopper, WBPH = white-backed planthopper, GLH = green leafhopper, BLB = bacterial leaf blight, SB = stem borer, RTSV = rice tungro spherical virus, RTV = rice tungro virus, RFLP – restriction fragment length polymorphism, RAPD = random amplified polymorphic DNA.

[‡] The number in parentheses refers to the publication in the list of publications generated.

to use. The group also developed a methodology for screening cold tolerance, a vital tool in a breeding programme for this trait. Likewise, another group of researchers worked on 24 accessions of cultivated rice and 11 wild species to understand the mechanism of salinity tolerance, and evaluated germplasm accessions and breeding lines. IRRI breeders used the results of these studies in the breeding programme for salinity tolerance. Plant breeders in the USA identified waxy endosperm, smooth hulls, and milling and cooking quality from two accessions. They developed a methodology to screen and identify advanced lines and varieties for cooking quality.

Use of conserved germplasm is not limited to evaluation and crop improvement. Experimental biologists and biotechnologists also use this conserved germplasm to advance rice science. In some cases, germplasm was used solely in research or for teaching purposes, as at Michigan State University, where the five rice varieties requested were used to train students in genetic engineering. At Virginia Polytechnic Institute and State University, a PhD student used the requested accessions to evaluate photosynthesis, leaf growth and water use of rice.

Conclusions

The value of conserved germplasm thus lies not only in how it has contributed to varietal improvement but also in rice science itself by expanding understanding of the physiological, morphological and genetic diversity of the crop and adaptation to its environment. The distributed germplasm has contributed immensely to knowledge on the physiology and morphology of the root system, physiology of plant resistance to rice blast, a biotype study of rice gall midge and other areas. It also has enhanced the use of molecular tools and biotechnology in evaluating crop diversity and improving the crop. The research work of Ellis *et al.* (1993) at the University of Reading, UK, and at IRRI, on seed physiology has contributed to the production of high-quality seeds for *ex situ* conservation, especially for long-term storage. Moreover, screening methods for different biotic stresses have been refined and further developed. These tools are helping rice scientists to understand better the nature of genetic diversity in rice. In the future, they will also help produce new varieties and make the conservation of rice genetic resources more efficient.

References

- Chang, T.T. 1992. The conservation of crop germplasm for future use. *Recent Adv. Breed.* 33:117-122.
- Ellis, R.H., T.D. Hong and M.T. Jackson. 1993. Seed production environment, time of harvest, and the potential longevity of seeds of three cultivars of rice (*Oryza sativa* L.). *Ann. Bot.* 72:583-590.
- Evenson, R.E. and D. Gollin. 1994. Genetic resources, international organizations, and rice varietal improvement. Center Discussion Paper No. 713. Economic Growth Center, Yale University. New Haven, Connecticut, USA.
- Gill, K.S. 1989. Germplasm collections and the public plant breeder. Pp. 3-16 *in* The Use of Plant Genetic Resources (A.H.D. Brown, O.H. Frankel, D.R. Marshall and J.T. Williams, eds.). Cambridge University Press, Cambridge.
- Jones, Q. 1984. A national plant germplasm system. Pp. 27-33 *in* Conservation of Crop Germplasm - An International Perspective (W.L. Brown, T.T. Chang, M.M. Goodman and Q. Jones, eds.). Crop Science Society of America, Madison, Wisconsin, USA.
- Plucknett, D.L., N.J.H. Smith, J.T. Williams and N. Murthi Anishetty. 1987. Gene Banks and the World's Food. Princeton University Press. Princeton, New Jersey, USA.

Publications generated using rice germplasm from the International Rice Genebank Collection at IRRI, requested from 1989 to 1994 (reference numbers are cited in Table 1).

1. Adachi, T., A.M. Alvarez, N. Aoki, R. Nakamura, V.V. Garcia and T. Matsuda. 1995. Screening of rice strains deficient in 16-kDa allergenic protein. *Biosci. Biotech. Biochem.* 59:1377-1378.
2. Barlaan, E.A. and M. Ichii. 1996. Genotypic variability in nitrate assimilation in rice. Pp. 434-440 *in* Rice Genetics III. Proceedings of the Third International Rice Genetics Symposium (G.S. Khush, ed.). International Rice Research Institute, Manila, Philippines.
3. Blackhall, N.W., M.R. Davey and J.B. Power. 1994. Applications of protoplast technology, Section A: Fusion and selection of somatic hybrids. Pp. 41-48 *in* Plant Cell Culture: A Practical Approach. 2nd Edition (R.A. Dixon and R.A. Gonzales, eds.). IRL Press, Oxford.
4. Blackhall, N.W., M.R. Davey and J.B. Power. 1994. Isolation, culture and regeneration of protoplasts. Pp. 27-39 *in* Plant Cell Culture: A Practical Approach. 2nd Edition (R.A. Dixon and R.A. Gonzales, eds.). IRL Press, Oxford.
5. Blackhall, N.W., P.T. Lynch, J.P. Jotham, M.R. Davey and E.C. Cocking. 1993. A general procedure for the initiation of cell suspension cultures of wild rice. P. 194 *in* Proceedings of the Sixth Meeting of the Rockefeller International Program on Rice Biotechnology, Chiang Mai, Thailand, 1-5 February 1993.
6. Blackhall, N.W., R.P. Finch, J.B. Power, E.C. Cocking and M.R. Davey. 1995. Flow cytometric quantification of electroporation-mediated uptake of macro-molecules into plant protoplasts. *Protoplasma* 186:50-56.
7. Blackhall, N.W., R.P. Finch, M.R. Davey and E.C. Cocking. 1990. Flow cytometry to assess the efficiency of electroporation-mediated delivery of macromolecules to plant protoplasts. P. 21 *in* Abstracts, International Conference on Electroporation and Electrofusion. Marine Biological Laboratory, Woods Hole, Massachusetts, USA. October 1990.
8. Causse, M., T.M. Fulton, Y.G. Cho, S.N. Ahn, K. Wu, J. Xiao, J. Chunwongse, Z. Yu, P.C. Ronald, S.B. Harrington, G.A. Second, S.R. McCouch and S.D. Tanksley. 1994. Saturated molecular map of the rice genome based on an interspecific backcross population. *Genetics* 138:1251-1274.
9. Cocking E.C., P.T. Lynch, N.W. Blackhall and M.R. Davey. 1991. Rice protoplasts genetic manipulations. P. 49 *in* Abstracts, Fifth Annual Meeting of the Rockefeller International Program on Rice Biotechnology.
10. Cocking, E.C., N.W. Blackhall, B. de Touchet, N.B. Jelodar, J.B. Power and M.R. Davey. 1993. Studies on rice somatic hybridisation. XV International Botanical Congress, Tokyo, August 28-September 3, 1993.
11. Cocking, E.C., P.T. Lynch, N.W. Blackhall, J. Jones, J.P. Jotham, G.S. Khehra, S.L. Kothari, S-H. Lee, K. Tang, B. de Touchet, P.S. Eyles and M.R. Davey. 1993. Rice genetic manipulations: use of plants regenerated from protoplasts. Pp. 120-121

- in Proceedings of the Sixth Meeting of the Rockefeller International Program on Rice Biotechnology, Chiang Mai, Thailand, 1-5 February 1993.
12. Cocking, E.C., R.K. Jain, B.S. Gosal, R. Marchant, N.W. Blackhall, G.S. Khehra, S-H. Lee, K. Tang, N.B. Jelodar, B. de Touchet, and J.P. Jotham, S.L. Kothari, J. Jones, G. Webster, C.A. Batchelor, S. Jain, J.B. Power and M.R. Davey. 1994. Transgenic rice production from protoplasts and interaction of plants with rhizobia for nitrogen fixation. International Program on Rice Biotechnology 7th Annual Meeting, 16-21 May 1994, Bali, Indonesia.
 13. Cocking, E.C., S.L. Kothari, R.P. Finch, P.T. Lynch and M.R. Davey. 1993. Genetic manipulations using rice protoplasts. Pp. 141-147 in *New Frontiers in Rice Research* (K. Muralidharan and E.A. Siddiq, eds.). Directorate of Rice Research, Hyderabad, India.
 14. D'Utra Vaz F.B., I.H. Slamet, A. Khatun, E.C. Cocking and J.B. Power. 1992. Protoplast culture in high molecular oxygen atmospheres. *Plant Cell Rep.* 11:416-418.
 15. Davey, M.R., H. Zhang, E.L. Rech, S. Kothari, B.J. Mulligan and E.C. Cocking. 1990. Transgenic rice plants: characterization of seed progeny. P. 175 in *Abstracts, VIIth International Congress on Plant Tissue and Cell Culture*, Amsterdam, June 1990.
 16. Davis, A.S., M.R. Davey, R.C. Clothier and E.C. Cocking. 1991. Quantification and comparison of chloramphenicol acetyltransferase activity in transformed plant protoplasts using high-performance liquid chromatography-radioisotope-based assays. *Anal. Biochem.* 201:87-93.
 17. Ellis, R.H. and T.D. Hong. 1994. Desiccation tolerance and potential longevity of developing seeds of rice (*Oryza sativa* L.). *Ann. Bot.* 73:501-506.
 18. Ellis, R.H., T.D. Hong and E. H. Roberts. 1992. The low-moisture-content limit to the negative logarithmic relation between seed longevity and moisture content in three subspecies of rice. *Ann. Bot.* 69:53-58.
 19. Ellis, R.H., T.D. Hong and M.T. Jackson. 1993. Seed production environment, time of harvest, and the potential longevity of seeds of three cultivars of rice (*Oryza sativa* L.). *Ann. Bot.* 72:583-590.
 20. Eyles, P.S., J.A. Thompson and M.R. Davey. 1990. A comparison of direct gene transfer methods for rice protoplast transformation. P. 54 in *Abstracts, VIIth International Congress on Plant Tissue and Cell Culture*. Amsterdam, June 1990.
 21. Finch, R.P., A. Baset, I.H. Slamet and E.C. Cocking. 1992. *In vitro* shoot culture of wild Oryzaeae and other grass species. *Plant Cell Tissue Organ Culture* 30:31-39.
 22. Finch, R.P., I.H. Slamet and E.C. Cocking. 1990. Production of heterokaryons by the fusion of mesophyll protoplasts of *Porteresia coarctata* and cell suspension-derived protoplasts of *Oryza sativa*: a new approach to hybridization in rice. *J. Plant Physiol.* 136:592-598.
 23. Flowers, T.J., S.A. Flowers, M.A. Hajibagheri and A.R. Yeo. 1990. Salt tolerance in the halophytic wild rice *Porteresia coarctata* Tateoka. *New Phytol.* 114:675-684.
 24. Garcia, A., D. Senadhira, T.J. Flowers and A.R. Yeo. 1995. The effects of selection for sodium transport and of selection for agronomic characteristics upon salt resistance in rice (*Oryza sativa* L.). *Theor. Appl. Genet.* 90:1106-1111.
 25. Jain, R.K., G.S. Khehra, S-H. Lee, N.W. Blackhall, R. Marchant, M.R. Davey, J.B. Power, E.C. Cocking and S. S. Gosal. 1995. An improved procedure for plant regeneration from indica and japonica rice protoplasts. *Plant Cell Rep.* 14:515-519.
 26. Jain, R.K., S.S. Gosal, G.S. Khehra, R. Marchant, N.W. Blackhall, M.R. Davey, J.B. Power and E.C. Cocking. 1994. An efficient protoplast-to-plant system for the Indian indica rice varieties, Pusa Basmati 1 and Jaya. International Program on Rice Biotechnology 7th Annual Meeting, 16-21 May 1994, Bali, Indonesia.
 27. Khatun, S and T.J. Flowers. 1995. The estimation of pollen viability in rice. *J. Exp. Bot.* 146:151-154.
 28. Khatun, S. and T.J. Flowers. 1995. Effects of salinity on seed set in rice. *Plant Cell Environ.* 18:61-67.
 29. Khatun, S., C.A. Rizzo and T.J. Flowers. 1995. Genotype variation in the effect of salinity on fertility in rice. *Plant Soil* 173:239-250.
 30. Kothari, S.L., M.R. Davey, P.T. Lynch, R.P. Finch and E. C. Cocking. 1992. Transgenic rice. Pp. 3-20 in *Transgenic Plants. Vol. 2. Present Status and Social and Economic Impacts* (S.D. Kung and R. Wu, eds.). Academic Press.
 31. Kothari, S.L., M.R. Davey, P.T. Lynch, R.P. Finch and E.C. Cocking. 1993. Transgenic rice. Pp. 3-20 in *Transgenic Plants* (S.D. Kung and R. Wu, eds.). Butterworths.
 32. Lee, S-H, T. Michael, F. Leach, J. Martin-Tanguy, S.L. Kothari, P.T. Lynch, N.W. Blackhall, E.C. Cocking, M.R. Davey and D. Tepfer. 1993. Altering morphology in rice with ORFs 10 and 12 (*rol A* and *rol C*) from the Ri TL-DNA of *Agrobacterium rhizogenes*. Pp. 149-150 in *Proceedings of the Sixth Meeting of the Rockefeller International Program on Rice Biotechnology*, Chiang Mai, Thailand, 1-5 February 1993.
 33. Lynch P.T., J. Jones, H. Zhang, E.L. Rech, P.S. Eyles, S.L. Kothari, N.W. Blackhall, E.C. Cocking and M.R. Davey. 1992. Transgenic rice plants: characterization of two generations of seed progeny. *Physiol. Plant.* 85:362-366.
 34. Lynch, P.T., E.E. Benson, J. Jones, E.C. Cocking, J.B. Power and M.R. Davey. 1994. Rice cell cryopreservation: the influence of culture methods and the embryogenic potential of cell suspensions on post-thaw recovery. *Plant Sci.* 98:185-192.
 35. Lynch, P.T., E.E. Benson, J. Jones, E.C. Cocking, J.B. Power and M.R. Davey. 1994. Cryopreservation of rice cell suspensions: the influence of the embryogenic potential of cultures on post-thaw recovery. *International Congress of Plant Tissue and Cell Culture*, 12-17 June 1994. Firenze, Italy.
 36. Lynch, P.T., J. Jones, H. Zhang, E.L. Rech, P.S. Eyles, S.L. Kothari, E.C. Cocking and M.R. Davey. 1991. Transgenic rice plants: characterisation of two generations of seed progeny. *Abstracts, 8th International Protoplast Symposium*, Uppsala, Sweden, June 1991. Poster 168, 130.
 37. Lynch, P.T., J. Jones, M.R. Davey, E.C. Cocking, J.R. Ambler, W. Buchholz, J. Seay and T.C. Hall. 1993. Cryopreservation of embryogenic rice cell suspension cultures. Pp. 211 in *Proceedings of the Sixth Meeting of the Rockefeller International Program on Rice Biotechnology*, Chiang Mai, Thailand, 1-5 February 1993.
 38. Lynch, P. T., N. W. Blackhall, J. Jones, J. P. Jotham, E. C. Cocking, and M.R. Davey. 1991. Use of flow cytometry to deter-

- mine ploidy levels in protoplast-derived rice plants. P. 188 *in* Abstracts, Fifth Annual Meeting of the Rockefeller International Program on Rice Biotechnology.
39. Lynch, P.T., P.S. Eyles, J. Jones, N.W. Blackhall, M.R. Davey, E.C. Cocking, M.R. Nelson, D.M. Bigelow, T.V. Orum, C.E. Orth and W. Schuh. 1993. The inheritance of introduced genes and their influence on the agronomic traits of transgenic rice plants. P. 209 *in* Proceedings of the Sixth Meeting of the Rockefeller International Program on Rice Biotechnology, Chiang Mai, Thailand, 1-5 February 1993.
40. Lynch, P.T., R.P. Finch, M.R. Davey and E.C. Cocking. 1991. Rice tissue culture and its applications. Pp. 135-155 *in* Rice biotechnology. Biotechnology in Agriculture No. 6 (G.S. Khush and G.H. Toenniessen, eds.). CAB International, Wallingford, England in association with the International Rice Research Institute.
41. McCouch, S.R. and S.D. Tanksley. 1991. Development and use of restriction fragment length polymorphism in rice breeding and genetics. Pp. 109-134 *in* Rice biotechnology. Biotechnology in Agriculture No. 6 (G.S. Khush and G.H. Toenniessen, eds.). CAB International, Wallingford, England in association with the International Rice Research Institute.
42. Mervyn Joseph, K.D.S. 1991. Physiological and agronomic aspects of rice varietal responses to low and high nitrogen management. Dissertation submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Crop and Soil Environmental Science. August 1991.
43. Qu, R., M. Bhattacharyya, G.S. Laco, A. de Kochko, B.L. Subba Rao, M.B. Kaniewska, J. Scott Elmer, D.E. Rochester, C.E. Smith and R.N. Beachy. 1991. Characterization of the genome of rice tungro bacilliform virus: comparison with *Commelina* yellow mottle virus and caulimoviruses. *Virology* 185:354-364.
44. Rance, I.M., W. Tian, H. Matthews, A. de Kochko, R. Beachy and C. Fauquet. 1994. Partial desiccation of mature embryo-derived calli, a simple treatment that dramatically enhances the regeneration ability of indica rice. *Plant Cell Rep.* 13:647-651.
45. Ronald, P.C., B. Albano, R. Tabien, L. Abenes, K. Wu, S. McCouch and S.D. Tanksley. 1992. Genetic and physical analysis of the rice bacterial blight disease resistance locus, *Xa21*. *Mol. Gen. Genet.* 236:113-120.
46. Sang Woo Lee and Hoo Sup Chung. 1993. Evaluation of rice-tenuazonic acid interactions related to blast resistance of cultivars to *Pyricularia grisea*. *J. Nat. Acad. Sci. ROK, Nat. Sci.* 32:161-173.
47. Schuh, W., M.R. Nelson, D.M. Bigelow, T.D. Orum, C.E. Orth, P.T. Lynch, P.S. Eyles, N.W. Blackhall, J. Jones, E.C. Cocking and M.R. Davey. 1993. The phenotypic characterization of R2 generation transgenic rice plants under field conditions. *Plant Sci.* 89:69-79.
48. Song, W.Y., G.L. Wang, L.L. Chen, H.S. Kim, L.Y. Pi, T. Holsten, J. Gardener, B. Wang, W.X. Zhai and L.H. Zhu. 1995. A receptor kinase-like protein encoded by the rice disease resistance gene, *Xa21*. *Science* 270:1804-1806.
49. Takahashi, Y., E.R. Tiongco, P.Q. Cabauatan, H. Koganezawa, H. Hibino and T. Omura. 1993. Detection of rice tungro bacilliform virus by polymerase chain reaction for assessing mild infection of plants and viruliferous vector leafhoppers. *Am. Phytopathol. Soc.* 83:635-659.
50. Tang, K., M.R. Davey, N.W. Blackhall, E.C. Cocking, J.B. Power, M. Wang, Y. Shi, H. Edmonds, K. Powell, A. Gatehouse and J. Gatehouse. 1994. Transformation of japonica rice with an insect resistance gene driven by a phloem-specific promoter. International Plant Molecular Biology Meeting. Amsterdam. June 1994.
51. Tang, K., M.R. Davey, P.T. Lynch, N.W. Blackhall, S.L. Kothari and E. C. Cocking. 1993. Transformation of Japonica rice with insect resistance gene constructs. P. 171 *in* Proceedings of the Sixth Meeting of the Rockefeller International Program on Rice Biotechnology, Chiang Mai, Thailand, 1-5 February 1993.
52. Tian, W., I. Rance, E. Sivamani, C. Fauquet and R. N. Beachy. 1994. Improvement of plant regeneration frequency *in vitro* in indica rice. *Chinese J. Genet.* 21:1-9.
53. Wang, Z.W., G. Second and S.D. Tanksley. 1992. Polymorphism and phylogenetic relationships among species in the genus *Oryza* as determined by analysis of nuclear RFLPs. *Theor. Appl. Genet.* 83:565-581.
54. Wu, K.S. and S.D. Tanksley. 1993. Abundance, polymorphism and genetic mapping of microsatellites in rice. *Mol. Gen. Genet.* 241:225-235.
55. Yeo, A.R. 1992. Variation and inheritance of sodium transport in rice. *Plant Soil* 146:109-116.
56. Yeo, A.R., K-S. Lee, P. Iazard, P.J. Boursier and T.J. Flowers. 1991. Short- and long-term effects of salinity on leaf growth in rice (*Oryza sativa* L.). *J. Exp. Bot.* 42:881-889.
57. Yeo, A.R., M.E. Yeo, S.A. Flowers and T.J. Flowers. 1990. Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance and their relationship to overall performance. *Theor. Appl. Genet.* 79:377-384.
58. Yeo, M.E., A.R. Yeo and T. J. Flowers. 1994. Photosynthesis and photorespiration in the genus *Oryza*. *J. Exp. Bot.* 45(274):553-560.
59. Yu, Z.H., S.R. McCouch, T. Kinoshita, S. Sato and S.D. Tanksley. 1995. Association of morphological and RFLP markers in rice (*Oryza sativa* L.). *Genome* 38:566-574.