



Cooperative Research Centre for Sustainable Rice Production



REPORT ON STUDY TOUR TO JAPAN, ITALY, CANADA AND USA

Dr Graeme Batten

Program 2: Sustainable Production Systems



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Rice CRC Workshop Report P2-09/99



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REPORT ON STUDY TOUR TO JAPAN, ITALY, CANADA AND USA

(7 June - 30 June, 1999)

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ACKNOWLEDGEMENTS

I wish to thank NSW Agriculture for leave to undertake this study tour and NIR Australia for financial assistance, and Mrs Jan Hubatka for helping prepare professional quality posters and making it possible to complete this report.

PURPOSE OF THE TRIP

- To visit Aichi Prefectural Agricultural Institute, Japan to compare rice production techniques.
- To present papers at the 9th International Conference on Near-Infrared Spectroscopy.
- To visit McMaster University, Ontario Canada and the National Small Grains Germplasm Facility, in Aberdeen Idaho to learn methods being used to identify nutrient efficient genotypes of rice, corn and barley.
- To gather germplasm for the Australian Rice Industry

HIGHLIGHTS

1. Observing transplanting of rice into plots for the 75th time at Anjo Research and Experiment Station.
2. Meeting Scientists with the interests, ideas, and enthusiasm needed to undertake joint research of mutual value to the agriculture in both countries.
3. Observing that the Australian rice cultivar Amaroo is clearly more vigorous than Japanese genotypes when grown under experimental conditions at Aichi-ken Agricultural Research Centre.
4. Participating in the 9th International Conference of Near Infrared Spectroscopy via-presenting or being a co-author on 4 posters and 1 oral paper.
 - discussions during and between sessions;
 - subsidiary meetings of:
 - a. the Advisory Board and the Editors of the Journal of NIR Spectroscopy and NIR news; and
 - b. the International Council of NIR Spectroscopy.
5. Inspection of low phytic acid (lpa) mutant barley growing in the field at Aberdeen Idaho.
6. Planning of a collaborative study of the low phytic acid rice mutant involving G Batten, CRC for Sustainable Rice Production, Yanco, Dr Victor Raboy of the USDA-ARS Small Grains Germplasm Research Facility at Aberdeen Idaho, USA and Dr John Lott, Department of Biology, McMaster University, Hamilton, Ontario, Canada.
7. The importation of 5 mutant barley genotypes together with two wild parents (Harrington and naked barley).

8. An invitation to contribute to a book titled: “Near Infrared Spectroscopy in Agriculture” Eds. Dr Craig Roberts, University of Missouri, Dr Bob Windham, USDA-ARS, Athens, GA and Dr Jeromy Workman, Perkin-Elmer Corp, Norwalk, CT.

RECOMMENDATIONS:

1. Arrange to have grain from the 75 year study of Anjo Research and Experiment Station imported so it can be analysed for protein and minerals.
2. Compare the establishment, growth, yield and quality of key Australian and Japanese rice genotypes when grown in both Australia (Yanco NSW) and Japan (Aichi Prefecture).
3. Maintain activities in the field of NIR and maintain the reputation of Australians at the leading edge of this incredibly versatile and cost effective technology.
4. Examine the potential value of low phytate mutants of rice, barley and other species as they become available and gain an update on studies of low phytic acid mutants in rice and other grains.
5. Continue to participate in NIR Conferences and associated activities.

TOUR ITINERARY

DATES: 7 June 1999 - 30 June 1999

<i>DATE</i>	<i>ACTIVITY</i>
June 7	Depart Sydney
June 8 - 11	Mei University Aichi-ken Prefectural Agricultural Institute
June 12 - 13	Travel to Italy
June 14 - 18	9 th International Conference on Near-Infrared Spectroscopy, in Verona Italy.
June 19-20	Travel to Hamilton, Ontario, Canada
June 21-23	Biology Department, McMaster University
June 24	Travel to Aberdeen, Idaho
June 24-26	National Small Grains Research Centre
June 27 - 30	Return to Sydney / Yanco.

JAPAN

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Inspected:

- i. crop rotation study
- ii. Sesame experiment
- iii. Azolla experiment

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Mr Ichiro Shaku – Farmer Agronomist
Mr Yasunori Nakashima – Farmer Agronomist
Mr Yukihiro Hamada – Farmer Agronomist

For the long term rice experiment at Anjo Research and Experiment Station Aichi, the rice is always transplanted into the plots on the 9th of June every year. However, I was greatly honored because in 1999, Mr Yukihiro Hamada, who was a visiting Scientist at Yanco in the CRC for Sustainable Rice Production in 1998-99, arranged for transplanting to occur on 10th June so that I could observe it first hand.

This elegant but simple study of the effects of various combinations of N, P, K, Ca and farm yard manure on rice production offers invaluable opportunities to deduce scenarios/theories/hypotheses for the sustainability of rice production (yield and quality) in Australia. Discussions led to the initiation of possible collaboration between scientists at Aichi and Yanco.

A good level of understanding of rice production by Yanco and Anjo Scientists has been achieved by the 6 month visit by Mr Yukihiro Hamada to Yanco and this short return visit by me. As a result of these visits, we plan to collaborate further by way of comparisons of genotypes available to both centres and exchange of samples and data for analysis.

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Yuko Mizukami, Forage Crop Breeding

Naoki Sugiura, Rice Breeder

Norikuni Saka, Senior Research Scientist

Discussions with the Executive centered on the benefits of past scientific exchanges and possible future exchanges and collaborative research (see highlights). It was clearly stated that further scientific exchanges would be encouraged. Aichi-ken ARC has excellent laboratory facilities and field areas, at both the Main Research Centre site and at the Anjo Research and Extension campus about 40km away.

I observed rice genotypes, including current commercial Australian cultivars, grown with transplanting, and direct seeding techniques with controlled release nitrogen fertilizer (see highlights 2 & 3).

Reasons for lack of root establishment by aerial-sown rice was discussed at length.

A technique, and the equipment used to grade seed (brown rice) was examined to assist us set up an equivalent system at Yanco.

I presented an invited (formal) seminar followed by a discussion session, lasting over 2 hours to Research and Extension staff.

Discussions were held with breeders and genetists on the occurrence, management of and possible role of minerals in the rice disorder known as straighthead.

Inspection of commercial rice crops enabled me to observe the adoption of new seeding techniques on farms.

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Discussions of the role of phytin in seed and possible causes of the production of straighthead. Numerous reprints were obtained.

ITALY

“TOWARDS THE THIRD MILLENIUM”

The 9th International Conference on Near Infra-Red Spectroscopy held in Verona, Italy

This meeting brought together over 400 NIR Scientists from about 30 countries. There were 15 Australian delegates with interests which included barley, dough, fruits, instrument noise, marsupials, paper pulp, pastures, rice, sugar, wheat, wines, wool.

Australian participants presented 5 oral and 13 poster papers during the 5 day meeting.

The total of 83 oral and 184 poster presentation fell into many divergent disciplines but were dominated by articles on food and agriculture as summarised in Table 1. In addition to those listed as being in Food and agriculture there were a further 51 in other sessions.

TABLE 1:

<i>SESSION</i>		<i>ORAL PAPERS</i>	<i>POSTER PAPERS</i>
1.	NIR Theory	2	15
2.	Instrumentation	3	7
3.	Data processing	6	28
4.	Food & Agriculture	6	73
5.	Pharmaceuticals	6	17
6.	Medical Applications	7	4
7.	Emerging fields & environment	7	20
8.	Enlarging horizons	8	20
9.	Workshops	30	-
10.	NIR for Official Methods	8	-
		83	184

The scientific content was clearly dominated by food and agriculture officially with 14 oral and 73 poster presentations out of a total of 83 oral and 171 posters. However, a further 51 food and agriculture papers were classified as chemometric, instrumentation, etc. papers. This is most likely a reflection of secretiveness rather than inactivity on the part of other industries. Given this was the last in this series of conferences in the millenium, the organisers made a policy decision to encourage review papers for the plenary sessions. While these were generally excellent and interesting there was obviously little new knowledge imparted to experienced practitioners. On the other hand, it has to be recognised that the majority of delegates were less experienced. The structure of the Conference involved no parallel sessions and a chaired discussion session on posters associated with each set of oral papers. This was an excellent idea except the same length of time was allocated regardless of the number of posters to be discussed. Specific points of interest are discussed below.

INSTRUMENTATION

Several manufacturers have taken up the challenge of designing low-cost, portable instruments for field use. However, there are few data on the performance of such instruments. Examples on display were Zeltec (USA), Cerea (Zeiss), EsetekJE-270 (Finland) and Esetek AN 800 (Finland). In addition, Bran & Leubbe have introduced a new instrument, the InfraAlyzer T. It is designed for transmission analysis of meat but a grain version is under development. At the other end of the range, Perkin-Elmer are strongly promoting their Identichack FT-NIR System. This is a “Rolls Royce” instrument which is hardly likely to find application for analysis for protein in wheat but it would be a useful research instrument. (See below for discussion of a possible loan).

DATA PROCESSING

Despite a proliferation of data processing methods, to which the NIR Centre itself has contributed, there was for the first time a consensus among experienced chemometricians such as Dardenne and Martens that for precise, linear NIR data no single method gives the best results in all circumstances. The advice was to establish a favourite house method and keep to it. This was very much in line with the philosophy advocated by Shenk on NIR at his training course in Sydney. This conclusion also holds for a network of instruments where they can be optically matched. However, where the instrument differences introduce non-linearities into the data, artificial neural networks (ANN) result in substantial improvement in the prediction accuracy. The point to this is that the spectral database is more important than the regression method.

There are two schools of thought about the size of the database. Essentially, these relate to the choice of calibration method. ANN require a very large database and, conversely, a very large database will require ANN to model it. This is the Infratec philosophy which has resulted in a global network which includes Co-operative Bulk Handling, Perth. This network has developed calibrations for protein and moisture based on 32,000 samples which are claimed to be the most accurate available, applicable to all wheat and barley worldwide and independent of reference laboratory error. Unfortunately, these claims cannot be verified, as the algorithm is confidential. The alternative approach, due to Shenk, is to employ a small database of perhaps 2-3,000 samples and derive a new calibration for each unknown samples.

This section of my report was based on a report due to appear in Issue 12 of “Overtones” – the newsletter of the Australasian NIR Spectroscopy Group later in 1999.

I was involved in the following papers (copies of the posters are attached):

Data Processing (Posters)

Batten, G.D., Ciavarella, S. and Blakeney, A. B. “Choosing the scan number and wavelength range for routine analysis of plant tissues”. (Poster 3.3).

This study is relevant to laboratories where the NIR analysis time is the limitation to sample throughput.

Food and Agriculture

Flinn, P. C., Batten, G.B., and Blakeney, A. B. (1999). “Current and future possibilities for measuring wool quality characteristics using NIR”. (Poster 4.62).

This study reports the accuracy of NIR based fibre diameter determinations of raw and clean wool.

Emerging fields and environment

McGrath, V.B., Blakeney A. B. and Batten G. D. “Analysis of P, S and growth stage in wheat shoots”. (Poster 7.10).

This poster reports recent progress made during GRDC project DAN 324.

Dunn, B.W., Beecher, G. H., Batten, G. D. and Blakeney, A. B. “Estimating the uptake of nitrogen by rice crops using near infrared reflectance analysis of soil”. (Poster 7.12).

This is the first report at an international NIR Conference to show the ability of a soil to provide N to plants. Work from the CRC for Sustainable Rice Production (Poster 2.1). There were reports of several studies on soil but this was most relevant to rice cropping.

Blakeney, A. B., Batten, G. D., Ciavarella, S and Wesley, I.J. “Plant tissue analysis using a diode array instrument”. (Oral 9.2.1).

NIR instruments vary in how samples can be presented to the unit for scanning. This paper compared the accuracy of analyses by wet chemistry, a “slow” NIR unit, and a very fast diode array NIR. Very good agreement was reported.

The following topics were heavily discussed at this NIR Conference.

1. Quality Assurance
 - Instrument standardization
 - Calibration robustness
 - Calibration transferability
 - Low cost (US \$200) hand held NIR units.
 - NIR for soil analysis and farm (fertilizer) management

At this Conference there was further evidence (if it was needed) that:

1. analysis by NIR techniques is usually faster, less costly, more convenient, and less prone to errors than traditional analytical methods; and
2. the NIR region of the spectrum contains information, which is not available to any other analytical techniques.

eg: a paper by Dr I Murray on the composition of meat, illustrated the advantage of NIR to analyse the vitamin content of meat, whereas HPLC determinations on extracts of the same samples was of no use.

New applications include reports that NIR can indicate:

- i. The presence of insecticides (eg Tefluthrin) on seeds at 40g/L.
- ii Mapping of trees, within a forest, which are suitable for native animal species.
- iii. Analysis of the internal quality of fruits using the newly available techniques of “time-resolved” reflectance spectroscopy (0.9.2.2).

A copy of the full program is attached to this report.

A copy of the “List of Delegates” is available upon request from Graeme Batten.

CANADA AND USA

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THE US NATIONAL PLANT GERMPLASM SYSTEM

What the National Plant Germplasm System Is

The National Plant Germplasm System is a network of organisations and people dedicated to preserving the genetic diversity of crop plants. The national system collects plant germplasm from all over the world, including the United States. Curators and other scientists preserve, evaluate, and catalog this germplasm and distribute it to people with a valid use. Coordinating the system is the Agricultural Research Service (ARS).

Support and funding comes from Federal appropriations and State contributions. Private industry underwrites selected projects and develops and transfers germplasm in the form of hybrids and varieties from the public system to farmers and other consumers.

The Nation's only long-term seed storage facility is the National Seed Storage Laboratory at Fort Collins, Colorado. Special collections of particular species still exist outside the national system.

GRIN, The Germplasm Resources Information Network, is the system's computer database. It contains information on all genetic resources preserved by the National Plant Germplasm System. Through GRIN, scientists learn about characteristics of specific germplasm and where its located. ARS maintains the GRIN database at its research centre in Beltsville, Maryland, near Washington, D.C., for scientists and other users cooperating in the national system. All sites in the national system interact with the GRIN database regularly, entering data, conducting searches, recording seed orders, and so on.

What the Collection Contains

The collections include domestic and foreign plants, wild and weedy relatives of crop species, cultivars and inbred parental lines (varieties created through planned breeding programs), elite breeding lines, some rare and endangered species, and genetic stocks.

Genetic stocks include induced and natural mutations, cytological (cellular) stocks of genetic oddities and variations on normal chromosomes, marker genes, polyploids, and pest-resistant stocks.

How Germplasm Is Stored

Seeds are dried to optimum moisture content, evaluated for quality, and sealed in moisture-proof containers. Then they are stored at below freezing (-20°C) for long term preservation. For short term storage, seeds are dried and placed in non-sealed containers at 5°C. But some species have short lived seeds that are difficult to store. For these seeds, other methods are needed.

Germplasm Users

The National Plant Germplasm System is devoted to the free and unrestricted exchange of germplasm with all nations and permits access to U.S. collections by any person with a valid use. Normally, this means plant researchers and breeders. Other users have included medical researchers and educators.

Germplasm users in other countries have the same privileges as those in the United States. About 100,000 requests are made to the Facility each year.

Selected list of Species (by Common Name) stored in the National Plant Germplasm System Collection at Aberdeen, Idaho:

aegilops
barley
barley genetic stocks
oats
rice
rye
triticale
wheat

Low phytic acid mutants (lpa_lines) - Dr Victor Raboy's work

Much of the P in seeds is stored as a mixed salt of phytic acid commonly known as phytate. Poultry and swine lack the phytase enzyme necessary to cleave the P from phytic acid, thereby making the P unavailable to these animals: ruminant animals, however, are believed to fully utilise phytate P because rumen microbes produce phytase. As a result, only about 10 to 20% of the P in corn is available to swine and poultry. Swine and poultry waste account

for about 18% of total animal waste in the USA, yet they account for about one-third of the total P excreted in animal waste. It is estimated that swine excrete approximately 181,000 Mg of P per year and poultry excrete 109,000 Mg.

Phytic acid is a storage form of P in seeds and accounts for approximately 80% of total seed P in many kernels. Phytic acid is involved in several roles in the seed, including maturation, initiation of dormancy, and as a source of P and cations for use during germination. In addition to phytic acid's effect on reduced P availability, phytic acid's chelating potential reduces the bioavailability of several other nutritionally important minerals including Cu, Zn, Mn, Fe and Ca. Because these cations are unavailable for absorption by animals, they pass into the waste and could potentially contribute to metal buildup in soils with heavy animal waste application. Phytic acid also interacts with certain amino acids, forming electrostatic linkages resulting in insoluble complexes and possibly leading to reduced amino acid availability in animals.

There are two approaches being used to deal with the poor availability of P in grains. The most common is for swine and poultry feeders to add inorganic phosphates or animal products (meat and bone meal) to meet animals' P needs. This solves the problem of meeting the P requirement in rations but at a significant cost and it exacerbates the problems of excess P in the waste and phytate's interactions with other minerals and protein. A second approach is to add the phytase enzyme to monogastric feed. Phytase enzymes added to rations increased the availability of P in corn from 15% up to 43% in swine. Kornegay et al. reported the addition of phytase to broiler feed reduced the P excretion by broilers by as much as 24%.

An alternative approach to adding phytase enzyme would be to increase the availability of P in corn grain by genetically manipulating the form of P. It has been observed that the phytic acid content of grain is highly correlated with the total P level. Breeding programs designed to reduce phytic acid in corn would likely reduce total P because of this tight relationship. This is not a desirable outcome from a nutritional perspective. The problem with P in corn is not the total content but the poor availability of this element. Therefore, efforts to improve the nutritional value of P in corn should be directed at increasing the availability of P, not reducing its content. Screenings of existing corn carrying *effective kernel* mutations have shown that embryo mutations have reduced phytic acid P but not total P providing evidence this relationship between the two forms of P could be broken.

The initial goal of Dr Raboy's research was to isolate chemically induced mutants with reduced levels of phytic acid P in corn. Such mutants, referred to as *low phytic acid* or *lpa*, were isolated and were found to have little or no other effect on kernel composition including no effect on total grain P content. The first mutant characterised, *lpa1-1*, contains a 65% reduction in phytic acid and is accompanied by a molar-equivalent increase in inorganic P. This mutant was backcrossed into elite corn inbred lines and resulting hybrids were evaluated for yield and other important agronomic traits. Preliminary field trials indicated germination, stalk strength, grain moisture at harvest, and flowering date were not affected by *lpa1-1*. Some, but not all, *lpa1-1* hybrids had yield reductions. In a preliminary chick feeding trial, the low phytic acid grain resulted in greater P availability and reduced P content in the waste. Altering the phytic acid content genetically in corn is possible and may have the potential to improve feeding efficiencies and reduce P released to the environment.

During my visit with Dr Raboy I saw first hand the simple screening technique which he uses to identify *lpa* mutant lines (see references). To determine the actual amount of phytate in a mutant Dr Raboy uses an Fe precipitation method. This method has some critics and we had discussions about the ion exchange method which I believe gives more reliable P data. While in Aberdeen I saw the very low phytic acid mutant barleys growing in the field and obtained samples of these and their parent lines to bring back to Australia. These are now passing quarantine at Tamworth.

REFERENCES

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2. "Phosphorus in Plant Biology: Regulatory Roles in Molecular, Cellular, Organismic, and Ecosystem Processes" Edited by Jonathan P Lynch & Jill Deikman. Proceedings 12th Annual Penn State Symposium in Plant Physiology, May 28-30, 1998
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4. "A Survey of Maize Kernel Mutants for variation in Phytic Acid", V Raboy, DB Dickinson, MG Neuffer. *Maydica* 35 (1990): 383 – 390.

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The visit with Professor Lott came as we had news that the review paper titled "Phytic acid and phosphorus in crop seeds and fruits: a global estimate" by Lott, Ockenden, Raboy and Batten had been accepted for publication in the *Journal Seed Science Research*. Following the importation into Australia of the *lpa* mutant rice earlier this year, and the positive interaction with Dr Raboy's group in Idaho immediately before this visit with Professor Lott, this visit provided the ideal opportunity to look to the future and plan mutually beneficial projects.

After a seminar by Professor Lott and some general reports on current research activities we planned a study of the *lpa* mutant rices. At Yanco I will grow grains under P treatments using the panicle culture technique being modified for rice panicles (see CRC for Sustainable Rice Production Annual report for 1999...in press). I will examine the phytic acid composition of the grains produced and Professor Lott will examine the structure of aleurone cells and the globoid crystals therein using his Environmental SEM.

The study will broaden the understanding of the P metabolism in *lpa* mutants. Dr Raboy is currently preparing a review paper on the biochemistry of phytate formation in seeds for the *Journal of Plant Physiology*.

Rice CRC ... of growing importance

About the Rice CRC

The Rice CRC is strengthening the rice industry's research and development (R&D) effort through its focus on sustainability.

Its mission is to increase the environmental, economic and social sustainability of the Australian Rice Industry and enhance its international competitiveness through both strategic and tactical research and the implementation of practical, cost-effective programs.

The Centre uses the intellectual resources of some of Australia's peak R&D organisations to target five main program areas:

1. Sustainability of Natural Resources in Rice-Based Cropping Systems
2. Sustainable Production Systems
3. Genetic Improvement for Sustainable Production
4. Product and Process Development
5. Education, Skills Development and Technology Transfer

Rice CRC core participants are Charles Sturt University, NSW Agriculture, CSIRO, Department of Land and Water Conservation, University of Sydney, Ricegrowers' Co-operative Ltd and the Rural Industries Research and Development Corporation.



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