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Australia–China linkage for enhanced rice cold-tolerance

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1 Acknowledgments

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2 Executive summary

This project aimed at enhancing linkages between Australian and Chinese rice research programs with a specific focus on improving rice cold tolerance, a major constraint to production in temperate rice-producing areas of the world. This was achieved through convening an International Rice Cold Tolerance Workshop, which was held at Yanco Agricultural Institute in December 2006. The workshop reviewed rice production and research related to cold tolerance in Yunnan Province south-west China, Guangxi Province in southern China, and Beijing, Liaoning and Heilongjiang Provinces in the northern and north-eastern regions of China. The value of the workshop was further leveraged by the addition of funds from the Rural Industries Research and Development Corporation to facilitate the attendance of scientists from Japan and South Korea, two countries for which cold tolerance is also a high priority.

The workshop highlighted a range of issues and opportunities for further interaction on rice cold-tolerance research. Foremost amongst these was that Yunnan Province of China was highlighted as part of the centre of diversity of the *japonica* sub-species of rice (Xiong *et al.*, 2010), its unique geography resulting in development of cold-tolerant landraces across the significant altitude range under which rice evolution and domestication has taken place within the province. Recent genetic conservation efforts and diversity studies have significant potential to identify new sources of cold tolerance in addition to the existing genes for cold tolerance which have become relatively widespread in temperate rice breeding programs. Further, Yunnan offers high altitude sites with naturally occurring low temperatures for broad scale phenotyping for cold tolerance, allowing benchmarking studies of existing cultivars and selection within segregating populations. As a result of this linkage project such collaborative evaluations have already occurred. Further opportunities for exchange of germplasm and sharing of information on selection techniques were apparent from provinces in north-eastern China, Liaoning and Heilongjiang. Here selection for cold tolerance is carried out using managed environments, specifically using low temperature groundwater to irrigate the rice during critical sensitive stages, similar to the methodology used for rice improvement programs in South Korea and Japan.

The workshop also explored the ascendancy of aerobic rices (grown without standing water) under development in northern China in response to increasing competition for water resources and the need to grow rice using less water. In temperate environments cold tolerance is a critical adaptive trait for any production system in which there is no standing water of any depth on the field. This is because the thermal mass of the water buffers the temperature of the base of the rice plant preventing temperature excursions to the ambient maximum and minimum. This diurnal range is often more than 10°C in temperate environments and minimum ambient temperatures regularly fall below the threshold for damage. Hence the need for cold tolerance as one of the suite of adaptive traits required for successful aerobic rice production.

The second part of the project allowed a small group of Australian rice researchers to visit a range of locations in China to continue the process of maintaining and extending the scientific links, exploring research activities at a provincial and national level. Specific outcomes from the visit include firstly the exchange of germplasm between breeding programs and secondly the development of two research concept notes to focus on elucidating further genes and/or mechanisms for cold tolerance from within the germplasm resources in Yunnan, and collaborating in testing of segregating population under naturally occurring cold conditions. A further concept note was developed around the development of varieties adapted to aerobic conditions, building on the strengths of each of the research groups, with Chinese research continuing on root traits including vigour and exploration and the Australian component on above-ground traits including carbon

and oxygen isotope discrimination, stomatal aperture and performance under high vapour pressure deficit conditions.

A final legacy of this linkage project will be the ongoing involvement in the Temperate Rice Research Consortium, an affiliation between research programs in temperate rice-producing countries.

The original proposal for a Temperate Rice Research Consortium (TRRC) was tendered by Dr KK Jena (IRRI-Korea Liaison Scientist) to the group present at the Cold Tolerance Workshop in December 2006, and received broad support for the concept. The first planning meeting was held in mid-2007 and a further meeting of the TRRC Steering Committee held in South Korea in April 2008.

At the planning meeting four major constraints were identified for temperate rice: (1) yield potential and grain quality, (2) blast resistance, (3) cold tolerance, and (4) nitrogen and water use efficiency. Working groups were formed among member countries to address each of these issues. Working group 3, focused on cold tolerance, is most relevant to this project. The group aims to evaluate a selected number of cold-tolerant lines provided by partner countries for cold tolerance at seedling and reproductive stages at key sites, and identify promising cold-tolerant cultivars or breeding lines. Further aims are to combine genes from cold-tolerant genotypes to develop suitable cold-tolerant germplasm adapted to different countries, to use potential DNA markers linked to cold tolerance traits (if available) for marker-assisted breeding for cold tolerance, and to develop a common set of cold-tolerant lines for use in breeding in collaboration with partner countries.

2.1 Recommendations

- That projects be developed to maintain and extend collaboration between the I&I DPI Rice Breeding Programme and Yunnan Agricultural University for the purposes of:
 - identifying further promising sources of cold tolerance among the landraces available from high altitude regions of Yunnan,
 - benchmarking existing modern cultivars against traditional cold tolerant varieties using screening facilities located at high altitude,
 - developing populations suitable for screening and selection at both sites, at Yanco using divergent sowing dates and random cold events and at Yunnan Agricultural University's high altitude screening location,
 - discovering genes/QTLs associated with cold tolerance in more recent collections of cold-tolerant lines and comparing those to known sources of cold tolerance to assess their novelty,
- That projects be developed in conjunction with China Agricultural University, Beijing, to complement their research on root traits for aerobic lines by focusing on the above-ground physiological traits associated with adaptation to aerobic conditions, to allow the simultaneous development of cold-tolerant and aerobically-adapted lines,
- That continuing scientific and germplasm exchange take place between Yanco and the rice breeding programs in northern China, specifically Liaoning and Heilongjiang Academies of Agricultural Sciences,
- Maintain involvement in the Temperate Rice Research Consortium activities through active participation in working group 3 focused on cold tolerance, and in working group 1 focused on yield potential improvement in combination with enhanced grain quality.

3 Background

The Australian Rice Industry is located in the Riverina Region of southern NSW (146°E, 34°S) and is confined to a relatively small geographic area. Since rice production ceased in north Queensland in the late 1980's, production has been confined to southern NSW with annual average production of around 1 million tonnes of paddy rice, until drought impacted production from 2002 until the present. Since 2002 production has varied significantly, and 2006 was the only year in which production reached 1 million tonnes.

While earning significant export income for Australia, the industry is small in world terms and isolated for other rice growing areas. The geographic isolation and a rigorous adherence to strict quarantine procedures has kept the industry free of major rice pests and diseases, and the adoption of advanced management and agronomic packages (particularly for the measurement and management of nitrogen supply during crop growth) coupled with high-yielding varieties has resulted in industry-wide average yields across all farms and varieties in excess of 9 tonnes/hectare (t/ha).

Further, meticulous adherence to grain quality traits specific to a range of markets world-wide, coupled with the development of quantitative measures of grain quality throughout the breeding and selection process, has led to the diversification of products from two quality types in the early 1980's to the current range of 7 grain quality classes under current production.

3.1 The importance of scientific linkage

The relative isolation from other rice producing areas has limited the opportunities for interaction between Australian rice breeders, physiologists, agronomists and cereal chemists and their counterparts in other countries. Although there has been sporadic interaction with publicly-funded organisations such as the International Rice Research Institute (IRRI), their focus has necessarily been on tropically-adapted *indica* rice and the need to bolster production to ensure food supply to an ever-increasing population base. Hence, yield potential and protection against yield loss from biotic and abiotic constraints such as pests and diseases, flooding, salinity and drought stress have received much attention.

The focus for the Australian rice industry has similarly been on yield improvement, but within a germplasm pool adapted to temperate growing conditions (*japonica* sub-species) and with a simultaneous focus on grain quality for discerning export markets. There has also been a focus on tolerance to abiotic stresses such as tolerance to low temperatures during the critical reproductive growth stage of the crop.

The opportunity to extend scientific interaction through projects such as this are particularly important for the Australian rice industry, facilitating communication between Australian rice scientists and those in the major rice producing regions such as China, and especially temperate areas where scientists are working largely within a similar gene pool to the Australian rice breeding program.

3.2 Cold tolerance - the foundation for future gains in water productivity

3.2.1 The impact of low temperatures

This project had a very specific focus on cold tolerance. Yield reduction due to cold at critical growth stages is a significant economic, social and natural resource issue facing affecting both Australian and Chinese rice production. Tolerance to low temperature is one of the major unresolved production constraints affecting the rice industry in Australia.

Cold damage incurred during the reproductive stage costs on average 0.8 t/ha in grain yield per year, with more than 1 t/ha yield penalty one year in three, and more than 2 t/ha reduction one year in ten. In the worst years the cost of cold damage at the farm gate is over \$50 million, and with flow-on effects, the costs to the regional communities and to Australia is significantly greater.

Low temperatures during vegetative growth and, more importantly during the critical reproductive stage are the primary driver of year-to-year variability in rice yields in the Riverina region. Significant cold stress in crops harvested in 1996 and in 2005 led to average industry yields of 6.5 and 6.8 t/ha respectively. In contrast, the average yield of commercial crops harvested in 2006 (a year with minimal cold stress during growth) was 9.82 t/ha.

3.2.2 Managing to minimise cold damage

Deep water (20-25 cm above the soil surface) during the most sensitive stage of crop growth is the main management technique used to minimise the impact of cold. The thermal mass of the water acts to reduce the amplitude of temperature variation around the base of the rice plant and the developing panicle as it moves up the stem. Minimum water temperatures are commonly around 7°C higher than the minimum ambient temperature or the first 3-5 days immediately following the passage of a cold front. However, prolonged low temperatures result in a steady decline in water temperature and after a week to 10 days there is little differential between minimum ambient temperatures and water temperatures. Nonetheless, using 20-25cm water depths during the critical reproductive phase of the rice crop has been an effective tool in minimising yield loss due to low temperature damage.

Research conducted by the Cooperative Research Centre for Sustainable Rice Production estimated that improved reproductive stage cold tolerance of 4°C could minimise the impact of cold with existing management, but that a further 7°C would be needed to completely eliminate the need for standing water to protect the developing panicle. This could have a significant impact on rice management and water use, and thus the long-term sustainability of the industry.

The importance of cold damage at the seedling stage has not been quantified for NSW, but contributes to poor establishment, re-sowing and loss of yield uniformity. In years with cold conditions at establishment, more than 30% of crops are likely to have areas of inadequate plant density to sustain high yields.

3.2.3 Increasing water productivity of rice

A long-running drought in southern New South Wales since 2002 has further underscored the pressing need for continual improvement in water productivity of rice-based farming systems. During the years of normal production until 2002, significant improvements in water productivity were made as a result of the removal of more permeable soils from production, use of shorter-duration cultivars, and improved fertilizer management, leading to improved yield potential. Over the 10 year period prior to 2002, the water productivity of Australian rice production increased by around 60%, from 500 kg to 800 - 900 kg of paddy rice per megalitre (ML) of water used.

Continual improvement in water productivity is necessary to secure the future for the rice industry. Initially, these improvements are likely to come from changed production systems: such as changing from broadcasting pre-germinated seed into standing water to direct-seeding into dry soil, followed by intermittent irrigation until the mid-late vegetative stage. This will minimize evaporative losses prior to canopy closure as the application of standing water is delayed until the mid-late vegetative stage. This system has the effect of avoiding an extended period of standing water when the crop canopy is sparse and during which there is significant evaporation from the water surface. Cumulative evapotranspiration for the period from sowing to the mid-vegetative stage (early October to mid-

November) is commonly 80-120mm and much of this loss can be avoided by establishing the crop under intermittent irrigation. Cold tolerance at the seedling stage is essential under this system for adequate and timely germination and establishment of the crop, thereby laying the foundations for future high yield potential.

Further savings in total water use may also be possible by using intermittent irrigation or maintaining aerobic conditions for the entire crop growth cycle, however water productivity may not increase and the plants will be more directly exposed to temperature extremes.

A number of adaptive traits will be required to secure the future for the Australian rice industry with limited water, including tolerance to low temperatures, drought, and high temperatures.

Foremost among these is cold tolerance, particularly at the reproductive stage. The thermal mass of standing water in the current production system minimizes exposure of the developing panicle to damaging low temperatures. In a completely aerobic system, this protective effect is lost.

Secondly, tolerance to transient periods of drought stress will also be required to ensure survival between irrigation applications. Finally, as temperatures become more variable, tolerance to periods of intense heat will also be required, particularly during periods of reduced water supply, (i.e. between irrigations), when the evaporative cooling effect associated with transpiration flow is reduced. Understanding and improvement of these adaptive traits, particularly cold tolerance, is critical for the continued success of rice breeding under a production environment with increasingly limited water.

4 Objectives

- To compare the aims and methodologies of rice cold tolerance research underway in China and Australia
- To explore developments in cold tolerance research and its application in the development of new varieties which combine cold tolerance and superior grain quality (including new techniques such as: wide hybridisation, micro-arrays, new genetic technologies and genome-profiling techniques)
- To cement long term relationships for cold tolerance research and facilitate exchange of technologies and plant material.

5 Methodology

The project comprised two parts. The first part involved convening a workshop to bring together researchers from Australia and China to compare past and current research and to establish key areas for new collaborative projects to be funded by each country independently. It was proposed that the subsequent research be funded through resources supplied in-country.

The second part involved an exchange visit in order to examine and evaluate the progress and impact of each project by a small group in China and in Australia at the optimum timing for each location. The workshop and subsequent evaluations capitalized on the research priorities established through RIRDC's Rice Research and Development Committee. This concept was developed from a specific research committee meeting and discussion to develop a framework for cold tolerance research. Exchange visits such as this were seen as the best way to build on the relationships already developed between Australian and Chinese scientists and maintain effective collaboration.

For example, Rice CRC funding from 1997-2005 was instrumental in establishing links between Yunnan Agricultural University and Australia, and this project has maintained and further extended that relationship. Facilitating research on cold tolerance throughout the crop cycle (but particularly at the critical reproductive stage) and combining this with superior grain quality will accrue important benefits to both Australia and China.

5.1 International workshop on rice cold tolerance

A workshop, held in late 2006, brought together researchers from Australia and China to compare past research and to build new collaborative arrangements.

The concept of a workshop was developed in a facilitation session funded by RIRDC to determine the way forward for cold tolerance research. Part of this session examined the most appropriate way of building on the relationships already developed between Australian scientists and those from China to ensure that the flow of information, germplasm and cooperation between Australia and China is maintained.

In ensuring that the continuing relationship is of benefit to both parties, the ACIAR-sponsored workshop focused on combining cold tolerance at all growth stages with development of high quality varieties and use of new genetic technologies including marker development from the use of genome profiling and association mapping for gene discovery.

We conducted an international workshop of Australian and Chinese scientists to review the current status of rice cold tolerance research and to discuss the potential for cooperative projects on cold tolerance. The workshop was to provide the basis for discussion with the objective of initiating joint activities on cold tolerance research.

The workshop was held from December 3rd to 8th, 2006, and the international participants are listed in Table 3 on page 16. The workshop comprised both field and laboratory visits and workshop sessions. International participants were met in Sydney on December 3, and visited the University of Sydney, Cobbitty on Monday 4th December. After travelling to Canberra, the participants inspected the work at CSIRO Plant Industry, particularly focusing on the biotechnology work with rice. A visit to Diversity Arrays Pty Ltd (DArT) on Tuesday 5th December promoted important discussion on the use of various micro-array technologies to assist selection for cold tolerance. This was followed by a visit to CAMBIA to meet with Dr Richard Jefferson. The main topic of discussion was intellectual property management and the potential for 'open source' management.

A field tour was arranged on Thursday 7th. This covered SunRice facilities in Leeton, a rice farm in Coleambally and the facilities at Rice Research Australia Pty Ltd (RRAPL) in Jerilderie.

5.2 Evaluation visit

This study tour encompassed visits to five locations in China, all of which were related to rice cold tolerance research and development. Participants in the study tour included Dr Laurie Lewin, rice industry consultant, Dr Peter Snell, Rice Breeder, Mr Russell Ford, Manager, Rice Research Australia and delegate to the RIRDC Rice Research Committee, and Mr Leigh Vial, rice grower and delegate to the RIRDC Rice Research Committee.

The five locations in China, included Yunnan Agricultural University, Kunming, Guangxi Academy of Agricultural Sciences, Nanning, China Agricultural University, Beijing, Liaoning Academy of Agricultural Sciences, Shenyang and Heilongjiang Academy of Agricultural Sciences, Harbin. The itinerary for the visit to research institutions throughout China is shown in Table 1 below.

Table 1. Itinerary for China Linkage visit

Sector	Travel/Visit	Date	Depart	Arrive
Yanco-Sydney	Transit	September 8th, 2007	10.00am	5.00pm
Sydney-Hong Kong	Transit	September 9th, 2007	7.35am	3.00pm
Hong Kong-Kunming	Transit	September 9th, 2007	5.55pm	8.15pm
Kunming	Prof Tan Xuelin, Yunnan Agricultural University	September 10th, 2007		
Kunming-Nanning	Transit	September 12th, 2007	8.10am	9.15am
Nanning	Dr Li Rongbai, Guangxi Academy of Agricultural Sciences	September 12th, 2007	11.00am	4.30pm
Nanning-Beijing	Transit	September 12th, 2007	6.15pm	9.20pm
Beijing	Prof Wang Huaqi, China Agricultural University	September 13-14, 2007		
Beijing-Shenyang	Transit	September 14th, 2007	6.10pm	7.10pm
Shenyang	Dr Wang Hui, Liaoning Academy of Agricultural Sciences	September 15-16, 2007		
Shenyang-Harbin	Transit	September 17th, 2007	2:00pm	4:00pm
Harbin	Prof Zhang Fengming, Heilongjiang Academy of Agricultural Sciences	September 18th, 2007		
	Return	September 19th, 2007	8:00am	

6 Achievements against activities and outputs/milestones

Objective 1: To compare the aims and methodologies of rice cold tolerance research underway in the Australia and China.

no.	activity	outputs/ milestones	completion date	Comments
1.1	International Rice Cold Tolerance Workshop	Maintain and extend the scientific connections between Chinese and Australian rice research programs, particularly those focusing on tolerance to low temperatures.	December 2006	<p>Linkage to rice cold tolerance research in Yunnan province is essential for: a) genetic diversity, and b) reliable cold tolerance screening facilities to test large populations</p> <p>Linkage to the Upland Rice Research Centre at China Agricultural University is warranted to interact in the development of aerobic rice. Germplasm exchange with research programs in the far north of China, Heilongjiang and Liaoning Provinces Participate in new Temperate Rice Research Consortium. Dr Jena (IRRI representative, Korea) proposed that a Temperate Rice Research Consortium (TRRC) be developed to continue and improve the liaison between producers of japonica rice. Seed funding for this initiative is to be provided by the Rural Development Administration of South Korea. All participants at the workshop supported the concept, and cold tolerance was seen to be a significant focus of the TRRC.</p>
1.2	Study tour to five research institutions in China	Reinforced links to Yunnan Agricultural University, China Agricultural University and Liaoning and Heilongjiang Academies of Agricultural Science	September 2007	<p>The visit to Yunnan Agricultural University underscored the value of Yunnan province as the centre of origin of temperate, cold tolerant rice. May provide new sources of cold tolerance genes and alternative mechanisms of tolerance, which may be defined then pyramided into newer high yielding varieties.</p> <p>The visit to Liaoning and Heilongjiang provinces highlighted the importance of comparative screening methods for selecting cold tolerant lines.</p> <p>Benchmarking of Australian varieties against Chinese varieties will take place within the cold tolerance project funded by the Temperate Rice Research Consortium.</p> <p>Links to the rice breeding program for aerobic rice based at China Agricultural University is warranted</p>

PC = partner country, A = Australia

Objective 2: To explore synergies between cold tolerance research and the development of new varieties which combine cold tolerance and superior grain quality.

no.	activity	outputs/ milestones	completion date	Comments
2.1	Participate in the Temperate Rice Research Consortium (TRRC) steering committee meeting	Cold tolerance project funded by TRRC steering committee to benchmark cold tolerance of temperate varieties in key locations in northern China and South Korea. Australian varieties provided.	April 18-19, 2008, September 1-3 2009	At the conclusion of the cold tolerance workshop in November 2006, Dr KK Jena (IRRI Korea rep) mooted the concept of a Temperate Rice Research Consortium. The Rural Development Association of Korea has provided seed funding for this initiative and since the workshop a steering committee has been formed and a meeting of rice scientists from temperate countries was held in Korea in May 2007 to determine the key research areas for the consortium. Cold tolerance is a key focus of the consortium, together with disease, resource use efficiency, and yield and grain quality. Participation in the consortium will provide valuable linkages, not only to China, but to all countries producing temperate rice.
2.2	Development of joint proposal with China Agricultural University under the Australia-China special fund administered by the Dept of Innovation, Industry, Science and Research	A proposal has been developed by Dr Peter Snell, however the matching application from China Agricultural University was not submitted in time for the 2008 round of funding.	March 2008	This is a joint Australia-China fund for collaboration with a Chinese partner, and both the Australian and Chinese partners must submit an application to their respective Government. The primary function of this support is to facilitate interaction, not to directly fund research. The focus of this project is to evaluate Chinese aerobic rices under Australian conditions, to find the traits necessary for aerobic rice production across a range of environments. Discussions at China Agricultural University resulted in agreement that China would focus on root traits such as density and length conditioning adaptation in aerobic rice, while Australia would focus on the physiology of above ground plant parts with traits such as osmotic adjustment and carbon and oxygen isotope discrimination, and stomatal conductance.
2.3	Development of Sydney University /NSW DPI joint proposal for extending the links to Yunnan Agricultural University for uncovering new cold tolerance genes and screening at high altitude sites.	An initial proposal has been developed at Sydney University, however the source of funds is still unclear.	May 2008	Key projects for future development will be linkage to Yunnan Agricultural University by building on the existing links between Dr Zhao Xiaochun, Sydney University Cobbitty, and Dr Tan Xuelin, Yunnan Agricultural University. Such a project will be directed at further investigation of cold tolerance mechanisms in landraces from high altitude areas in Yunnan and transfer into adapted genetic backgrounds.

7 Key results and discussion

7.1 The importance of cold tolerance for rice production in China

Rice is the largest crop in China and has been cultivated for over 7,000 years. In the year 2000, the total rice sown area was 29.962 million hectares, accounting for 27.6% of total food crop sown area. The total production in that year was 187.91 million tonnes, which accounted for more than 40% of total food crop production. Rice production in China is classified into 6 cultivation regions and 16 sub-regions described below in Table 2.

Table 2. Description of the 6 major regional classifications of rice production throughout China.

Region	Description	Extent
Region I.	South double crop region	Including Fujian, Guangdong, Guangxi, south of Yunnan and Hainan provinces, the majority of which is <i>indica</i> rice. Region I comprises 17.6% of total rice sown area.
Region II.	Central double and single crop region	Including Jiangsu, Shaihai, Zheijian, Anhui, Jiangxi, Hunan, Hubai, Shichuan and part of south Shanxi and Henan provinces. This region accounts for 67% of total rice sown area. About 70% of the region is double cropped, and the proportion of <i>japonica</i> rice is about 40%.
Region III.	South-western single and double crop region	This region includes the major part of Yunnan-Guizhou plateau and part of Qinghai-Tibet Plateau. Region III represents 8% of the total rice sown area, and the majority is single crop <i>japonica</i> rice. There is also a small area of upland rice.
Region IV.	North single crop region	Includes Beijing, Tianjiang, Shandong, a major portion of Hebei, Henan and some part of Shanxi, Shanxii, Anhui and Jiangsu provinces. Accounts for 3% of total area, and is primarily <i>japonica</i> rice.
Region V.	North-eastern single crop region	This region includes Heilongjiang, Jieling, most part of Liangling, and the northern part of Inner Mongolia. Accounts for 3% of total region. This is a <i>japonica</i> rice only region.
Region VI.	North-western single crop region	This region is subject to drought. It includes Xinjiang, part of Ganshu, Ningxa, Shanxi and Inner Mongolia, and accounts for 0.5% of total area. Single crop <i>japonica</i> rice only.

Low temperature damage is one of the important problems for rice production in China. On average it causes 2% of total rice production loss, representing more than 3.7 million tonnes annually. According to many of the scientists to whom we spoke, losses due to cold damage are not as significant as they were 20 – 30 years ago and one possible reason for this mitigation is that breeding programs have made steady improvement in levels of cold tolerance.

The most critical stages are seedling stage and booting stage, and cold tolerance remains an important selection criterion in breeding programs. Research on the identification of cold tolerance in a range of germplasm sources, methods of effective selection for enhanced cold tolerance, understanding the underlying genetics of cold tolerance, mapping the genes for cold tolerance and the improvement of cultivation and management techniques is being conducted in many research organisations across China (Table 3).

The main incidence of low temperature damage to rice in Yunnan province (in the south-western part of China) occurs at the reproductive stage. The frequency of damage is approximately once every 3 - 4 years. The north-eastern and eastern regions of Yunnan province are most prone to cold damage, with cold damage occurring with less severity and less frequently in the north-western and central regions. In contrast, very little cold damage occurs in the lower altitude western and southern regions.

The temperature threshold for cold damage ranges from 14.8°C to 20.2°C, where each occurs for three consecutive days, from the north-eastern regions to the south-western regions respectively, and the variation in threshold relates to the change in proportion of sown area from *japonica* to *indica* rice varieties across the regions. Generally, for every degree below the threshold there is a yield reduction of 150 – 300 kg/ha.

Nation-wide, damaging low temperature events occur every 3 - 5 years from region to region, with yield losses of 30 - 50% if there is a significant low temperature event. Low temperature induced delay in vegetative growth and seedling mortality occurs about 4 years once in most regions, principally to early-sown crops and in single rice crop regions.

A severe cold event may result in crop failure due to seedling mortality, or induce significant delay in development, increasing the likelihood of exposure to critical low temperatures at the highly sensitive pollen development/booting stage. In areas where rice is double cropped, delay in the first crop has a flow-on effect, delaying the second crop as well.

Management options used to reduce the effect of low temperatures include covering nursery seedlings with plastic film to prevent cold damage, and the use of deep irrigation water during the critical reproductive stage to protect developing panicles from transient low minimum temperatures.

7.1.1 Examples of low temperature damage

In 1970 a severe low temperature event caused widespread seedling mortality in the mid to lower reaches of the Yangtze River rice region, in Region II (Table 2).

Low temperature damage during the reproductive stage also occurs every 3 - 4 years, mostly in regions I, II and III, and particularly in the double crop region. Floret sterility ranges from 20 – 30%, and is as high as 40 – 70% in seasons of severe damage, during which temperatures can be more than 10°C lower than normal temperatures. The level of damage is exacerbated due to the greater cold sensitivity of the *indica* rice varieties which dominate the double crop regions.

As another example, in September of 1971, a low temperature event damaged the late-sown rice crop throughout most of Region II. The average temperature dropped down to 16°C and yield loss averaged 30 –50%, reaching 70% in some localised regions.

7.1.2 Research to improve cold tolerance

Research to improve cold tolerance is mostly carried out through breeding programs in different research organisations such as the Academy of Agricultural Sciences within each province and agricultural universities.

Yunnan province is one of the most important sources of cold tolerant rice germplasm, owing to the wide range of altitudes at which rice is grown. Yunnan Province is the most extensive centre of ecological and genetic diversity in China. It constitutes part of the centre of genetic diversity of cultivated rice (*Oryza sativa* L.), an area that includes East Nepal, Bhutan, Assam, Myanmar, Laos and northern Thailand (Chang 1976; Wang and Sun 1996; Zeng et al. 1998).

Extensive research has been carried out to identify and classify the cold tolerant germplasm native to Yunnan, including research into the underlying genetics of cold tolerance by the Biotechnology and Genetic Resources Institute, Yunnan Academy of Agricultural Sciences. Further, the Rice Research Institute of Yunnan Agricultural University has over 30 years history in rice breeding, and cold tolerance is one of the key selection criteria for rice improvement. Average rice yields are significantly lower (3 to 4.5 t/ha) in the high altitude region of Yunnan (above 2100m, which accounts for around 20% of total rice sown area), improved cold tolerance for both seedling and reproductive stages is an essential foundation for future varieties.

Dr. Tan Xuelin, Director of the Rice Research Institute, has collected more than 70 landraces of cold tolerant rice from high altitude rice regions and has initiated research on development of high yielding, cold tolerant varieties. An experimental station has been established at an elevation of 2200m, where the irrigation water temperature used for screening is only 17°C. Some breeding and selection work has been conducted and some promising results have been achieved. Interestingly, a number of hybrid rice lines have been found to have better cold tolerance than conventional inbred rice varieties. Research to develop a microsatellite marker for cold tolerance is also underway, for eventual use in breeding programs (Dazhou et. al., 2002).

Cold tolerance has been an important objective in many breeding programs nation wide. Cold tolerant germplasm has been identified from several other regions too. One relatively recent discovery has been the ‘Dongxian wild rice’ in Jiangxi province. This *Oryza rufipogon* appears to have cold tolerance in vegetative and reproductive stages, and a degree of perenniality and frost resistance to enable the plant to overwinter and shoot again the following spring. Genetics research for cold tolerance such as molecular mapping, and identification of cold tolerance genes have been conducted in several research organisations as well. Table 3 lists a range of institutions and their research focus.

It is difficult to estimate the extent of funding allocations specifically for rice cold tolerance research. It varies among the many research institutions working on cold tolerance. An estimate from the range of scientists we spoke to would be 1-2% of the total budget on rice research.

7.1.3 Aerobic rice production systems and the associated need for increased cold tolerance

Aerobic rice is becoming an increasing topic in China and other countries, since availability of water for irrigation of rice crops is limited, particularly in the northern and western regions of China.

Cold tolerance is not a major limiting factor in most of traditional upland rice growing regions, which are in the tropical and sub-tropical zones. However, aerobic rice production systems may replace traditional lowland rice in water-limited areas, and may be extended to upland rice production regions depending on rainfall and irrigation conditions. Some of these regions will require cold tolerance for aerobic rice, especially those regions in which low temperature events limit rice production under traditional lowland systems (with standing water throughout growth).

Table 3. Institutions working on cold tolerance research, their specific focus and their key researchers.

Institution	Research focus	Key researchers
China Agricultural University	Mapping and gene identification	Li Zichao, Wang Xiangkun and Sun Chaunqing
China Rice Research Institute	Mapping and gene identification	Qian Qia, Yu Shouwu, Zhuang Jieyun and Zheng Kangle.
Institute of Crop Germplasm Resources, Chinese Academy of Agricultural Sciences	Germplasm management	Han Longzhi
Institute of Botany, Chinese Academy of Sciences	Mapping	Xu Yunyan
Institute of Genetics, Chinese Academy of Sciences	Mapping and gene identification	Zhu Lihuang
Wuhan University	Mapping	Zhang Zhihong
Rice Research Institute, Jiangxi Academy of Agricultural Sciences	Cold tolerance breeding	Chen Dazhou, Xie Jiankun, Xiao Yeqing, Yin Jianhua, Shen Xianhua, Wan Jianlin and Yu Chuanyuan

Jiangxi Agricultural University	Cold tolerance breeding	He Haohua, Huang Yinjin, ZHu Changlan and LIU Yibai
Rice Research Institute, Guizhou Agricultural University	Cold tolerance breeding	Yu Xianquan
Rice Research Institute, Hunan Academy of Agricultural Sciences	Cold tolerance breeding	Zhan Qingcai
Shenyong Agricultural University	Cold tolerance breeding	Chen Wenfu
College of Agronomy, Northwest Sci-Tech University of Agriculture and Forestry	Mapping	Qiao Yong-li

7.2 International Rice Cold Tolerance Workshop at Yanco

The first activity within this project was to convene a cold tolerance workshop at Yanco in early December 2006. The workshop, entitled, "An International Rice Cold Tolerance Workshop" was held in Australia from December 4 – 8, 2006. Additional funding (approximately \$15,000) was obtained from the Rural Industries Research and Development Corporation to support the participation of three rice cold-tolerance researchers from Japan and South Korea. Attendees to the workshop and their affiliations are shown in Table 4 below.

The workshop comprised both field and laboratory visits, and workshop sessions. International participants were met in Sydney, and together with Mr Russell Ford, Dr Laurie Lewin and Dr Russell Reinke, visiting Sydney University, Cobbitty, CSIRO Plant Industry, Canberra, Diversity Array Pty Ltd (DARt) Canberra, and Centre for the Application of Molecular Biology in International Agriculture (CAMBIA), Canberra, before travelling to Yanco for the workshop and field visits to Rice Research Australia Pty Ltd, Jerilderie.

7.2.1 Workshop Sessions - Field Sessions

International participants for the workshop visited the University of Sydney, Cobbitty on Monday 4th December to discuss the work of Dr Xiaochun Zhao at that location. Discussion centred on screening for cold tolerance in controlled temperature conditions and the contributions from cold tolerant cultivars from Yunnan, China. The lively discussion at this location set the stage for a successful workshop.

After travelling to Canberra, the participants inspected the work at CSIRO, particularly focusing on the biotechnology work with rice. Dr Rudy Dolferus explained his research on tracking specific sugar transport enzymes (invertase) associated with increased levels of cold tolerance and its hormonal regulation by abscisic acid (ABA).

A visit to Diversity Arrays Pty Ltd (DARt) on Tuesday 5th December promoted important discussion on the use of various micro-array technologies to assist selection for cold tolerance. Data presented from Dr Li Rongbai showing results from screening more than 1,000 *Oryza rufipogon* accession for cold tolerance and initiating a backcrossing program with the 5 tolerant accessions identified using the Australian rice variety Millin as the recurrent parent

This was followed by a visit to CAMBIA to meet with Dr Richard Jefferson. The main topic of discussion was intellectual property management and the potential for 'open source' management.

A field tour was arranged on Thursday 7th. This covered SunRice facilities in Leeton, a rice farm in Coleambally and the facilities at RRAPL in Jerilderie. At this latter site there was discussion and inspection of the cold tolerance and aerobic rice work of Dr Peter Snell and inspection of the seed cleaning and grading facilities along with a discussion of the pure seed scheme.

Table 4. Delegates to the International Rice Cold Tolerance Workshop and their affiliations.

Attendee	Affiliation
Mrs Kathryn Bechaz	NSW Department of Primary Industries, Yanco Agricultural Institute, PMB Yanco NSW 2703
Dr Ye Changrong	Yunnan Academy of Agricultural Sciences, and The University of Queensland, St Lucia QLD 4067
Dr Rudy Dolferus	CSIRO Plant Industry, Po Box 1600, Black Mountain ACT 2600
Dr Ken Fischer	RIRDC Rice Research and Development Committee, and The University of Queensland, St Lucia QLD 4067
Mr Russell Ford	RIRDC Rice Research and Development Committee, and Rice Research Australia Pty Ltd, Jerilderie NSW 2700
Dr Thusitha Gunawardena	The University of Queensland, St Lucia QLD 4067
Dr KK Jena	IRRI Korea office, National Institute of Crop Science, Rural Development Administration, Suwon
Dr Andrzej Kilian	Diversity Arrays, Pty Ltd, Canberra. ACT
Mr John Lacy	NSW Department of Primary Industries, Yanco Agricultural Institute, PMB Yanco NSW 2703
Dr Laurie Lewin	NSW Rice Industry Consultant, Farm 1832 Colando Mail, Leeton NSW 2705
Dr Zhikang Li	Chinese Academy of Agricultural Sciences, Beijing, China
Prof Don Marshall	RIRDC Rice Research and Development Committee
Dr Kuniaki Nagano	Miyagi Prefectural Furukawa Agricultural Experiment Station, Furukawa, Miyagi Prefecture, Japan
Mr Ben Ovendon	RIRDC Rice Research and Development Committee
Dr Russell Reinke	NSW Department of Primary Industries, Yanco Agricultural Institute, PMB Yanco NSW 2703
Dr Yutaka Sato	Crop Cold Tolerance Research Team, National Agricultural Research Center for Hokkaido Region, Japan
Dr Peter Snell	NSW Department of Primary Industries, Yanco Agricultural Institute, PMB Yanco NSW 2703
Dr Xuelin Tan	Yunnan Agricultural University, Kunming, Yunnan Province, China
Dr Huaqi Wang	China Agricultural University, Beijing, China
Dr Dan Waters	Southern Cross University, Lismore, NSW
Dr Fengming Zhang	Heilongjiang Academy of Agricultural Sciences, Harbin, Heilongjiang Province, China
Dr Xiaochun Zhao	Sydney University, Cobbitty NSW

7.2.2 Workshop Sessions - Regional reports

Wednesday 6th December was the first formal workshop day and its program focused on country reports with presentation on rice production in Australia, Yunnan, Heilongjiang, China generally, the potential (and need) for aerobic rice in China, Japan, Hokkaido and Korea.

Generally, the area of rice production is declining in all countries discussed (although not all provinces). This was due to different factors including increased yields (all countries), competition for water (Australia and China), declining demand (Korea and Japan) and competition from more profitable enterprises. In South Korea area has declined by around 20% since 1990, to around 1 million ha, although production has remained relatively constant at approximately 5M tonnes.

A notable exception was in Heilongjiang Province in China where production had increased dramatically from around 100,000 ha in 1949 to 2 million ha in 2006 with a predicted area of 3 million ha within the next decade. This is all japonica rice and is of potential interest to Australia.

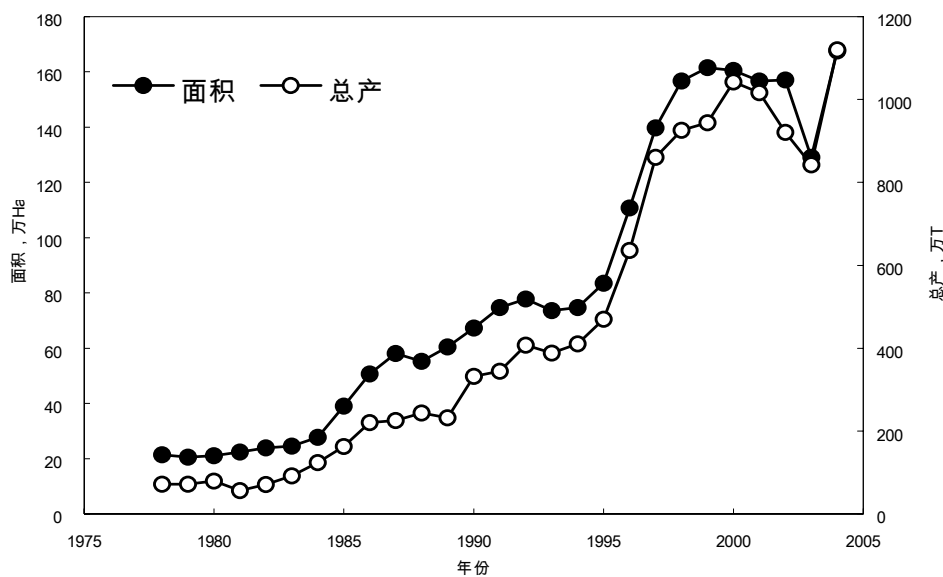


Figure 1. Increase in rice production area and yield in Heilongjiang Province from 1975 to 2005. Closed symbols represent area ($\times 10,000$ ha, left hand axis) and open symbols represent total production ($\times 10,000$ t, right hand axis).

All areas discussed were producers of japonica rice. Hybrid varieties were not important in any region and the *indica/japonica* (Tongil type) varieties were of declining importance in Korea.

Cold damage was a major issue in all areas with the magnitude of the problem varying. Most areas, however, reported serious damage about one year in three with very serious damage about one year in ten (similar to Australia). Around 80% of the rice area was affected by cold in South Korea in 1980. Similarly in northern China, cold damage causing floret sterility of rice in Heilongjiang province was widespread in the paddy fields around Changbai Mountain, and the Zhangguangcailing and Xiaoxing'anling ranges. The chilling injury occurs every 2-3 years and yield reductions of 20%-40% are common. Due to the effect of cold current from the sea of Okhotsk, severe sterile-type cold damage has occurred on average every 5 years in Changbai mountain ranges, especially in recent years.

In the northern rice-growing region of Hokkaido in Japan significant cold damage occurs on average once every four years. In the past 120 years, cold damage has occurred 30 times. The difference in yields between normal years and cool-weather years was about 1 t/ha, and this has been unchanged in the past 120 years. This region of Japan suffered very severe cold damage in 1993, with the shortfall in production leading to importation of rice from Thailand, China, USA and Australia.

In South Korea, for example, low temperature events in 1971, 1980, and 2003 resulted in yield reductions of 17%, 80%, and 20%, respectively. In 1980, the yield reduction of milled rice was as high as 3.9 tons per hectare. Also in China, the recorded yield loss per year due to low temperature damage is 3–5 million tons. In Vietnam, a 30-day cold spell hit in February 2008 and reportedly destroyed more than 53,000 hectares of rice.

Disease, particularly blast was important in all areas other than Australia.

Selection and Mechanisms for Cold Tolerance

Reports on mechanisms of cold tolerance, selecting for tolerance and specific characters commenced late on 6th December and continued on Friday 8th, with discussions centred on a number of key areas.

Sources of tolerance.

Most programs used a mixture of local cultivars and introductions – principally cold tolerant sources from Yunnan, Indonesia and Japan. The need for extensive backcrossing to local parents to incorporate desirable traits was emphasized. Genetic resources have been collected throughout the high elevation regions of Yunnan to broaden the genetic base of cold tolerant varieties. Dr Tan Xuelin reported on a recent collection which included 197 cultivars, most of which were not pure lines, with smooth leaves and glumes, high floret fertility, and were relatively tall plants with low number of panicles. Recent research conducted at Yunnan Agricultural University uncovering additional germplasm resources for cold tolerance genes is described briefly in section 7.3.1 on page 22.

Methods for selection.

Most programs used a mixture of natural selection using multiple sites or cold water screening. For example the Heilongjiang Academy of Agricultural Sciences uses both cold water irrigation for the critical growth stage of the crop and controlled environment testing. The selection criteria for cold tolerance is a percentage fertility of 80% or greater after low temperature treatment of 16°C continuously for 7 days, or after exposure to irrigated cold water in field at the critical meiosis stage, and that the developmental delay of less than four days in comparison to the cold tolerant check lines. Similarly the South Korean Rural Development Administration uses cold water pumped from the bottom of a dam at the Chuncheon sub-station.

In contrast, in Yunnan Province there are cool, high altitude sites at elevations up to 2400m which provide ideal screening conditions for tolerance to low temperatures at all growth stages.

The Australian programs were the only ones to report on use of cold air screening or multiple sowing times.

Characters for cold tolerance.

Many important characters were reported, including maturity, invertase, anti-oxidant enzymes, proline accumulation and anther size. Dr Sato from Japan presented evidence of anti-oxidant enzyme activity such as ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD), heat shock proteins conditioning cold tolerance. To test the hypothesis that plants endowed with higher levels of APX enzyme are better protected against chilling stress, transgenic rice plants were constructed via *Agrobacterium tumefaciens* mediated transformation. Transgenic rice plants over-expressing APXa were generated. Relative to non-transformed control plants, transgenic rice showed significantly less injury from the chilling stress. Chilling tolerance of transgenic and non-transformed plants were tested by exposing rice seedlings to a chilling temperature of 5 °C for 10 days and were transferred to the greenhouse and grown for 7 days. This result was relevant for exposure to cold at the seedling stage, but the beneficial effect was less evident at the critical microspore development (booting) stage.

Several reports focused on identifying QTL's (Quantitative trait loci) for cold tolerance.

Cold tolerant varieties.

Most participants reported on recent releases of tolerant varieties. Most interesting to Australia were the cold tolerant landraces from high altitude regions of Yunnan, Jyoudeki (from Miyagi prefecture) and the series of releases from Heilongjiang (see Table 5). The Longdao series from Heilongjiang had increased cold tolerance and diverse morphological characteristics, with some appearing similar to Australian rice varieties in panicle size and

architecture, while others such as Longdao 5 had erect panicles with short panicle branches (see Figure 3).

Table 5. Cold tolerant rice varieties released in Heilongjiang Province

Varieties	Longdao 2	Longdao 3	Longdao 4	Longdao 5	Longdao 2
Year of release	2002	2004	2005	2006	2002
Germination at 10°C (%)	88.8	91.2	94.3	90.4	88.8
Developmental delay (days) after cool water irrigation for the entire crop duration	2	1	0	1	2
Seed set (% fertility) after exposure to cold water irrigation at booting stage	86.7	92.3	90.2	85.9	86.7

The advantage of Jyoudeki over Hitomebore, the most common variety in Miyagi Prefecture, was reported as a reduction in cold-induced spikelet sterility from 18% to 7%, coupled with a slight reduction in plant height and maturity and high yield potential. Varieties such as these are likely to provide useful genetic resources for the Australian rice breeding program when combined with adaptation to Australian rice production environment.



Figure 2. Field plot of the cold tolerant variety Longdao 4 developed in Heilongjiang Province.

Aerobic rice

This was not the principal focus of the workshop but nonetheless it is important for areas of China and likely to be increasing importance in Australia as increasing levels of water-savings are sought. Aerobic rice production in Australia would require greater cold tolerance than for existing cultivars because there is no protection from standing water. Aerobic rice suffers a relatively small yield penalty in China if adapted cultivars are used. The penalty is likely to be much greater in Australia, however, because humidity is so low.

It is unlikely that cultivars could be directly transferred from successful programs in China or Brazil.

7.2.3 Conclusion.

The workshop was very successful in providing an exclusive focus on the complex trait of cold tolerance and it provided a unique opportunity to bring important researchers in the field of cold tolerance together. Undoubtedly the success was enhanced by bringing the researchers from Japan and Korea.

All researchers agreed to keep others informed of their work with follow-up liaison between Australian and Chinese researchers to occur in 2007.



Figure 3 Cold tolerant variety Longdao 5 developed in Heilongjiang Province. Note the erect panicle display.

Temperate Rice Research Consortium

At the conclusion of the workshop Dr Jena proposed that a rice research consortium be developed to continue the liaison between research programs in temperate rice-producing countries. This was generally supported by all participants at the workshop. While the consortium will fall under the auspices of IRRI, the Rural Development Association of Korea has provided seed funding for this initiative. Since the workshop a steering committee has been formed and a meeting of rice scientists from temperate countries was held in Korea in May 2007 to determine the key research areas for the consortium.

Cold tolerance is a key focus of the consortium, together with disease, resource use efficiency, and yield and grain quality. Participation in the consortium will provide valuable linkages, not only to China, but to all countries producing temperate rice.

7.3 Research linkages with rice research programs in China

The second activity of this project was the completion of a study tour to five locations in China in September 2007. Participants in the study tour included Dr Laurie Lewin, rice industry consultant, Dr Peter Snell, Rice Breeder, Mr Russell Ford, Manager, Rice Research Australia and delegate to the RIRDC Rice Research Committee, and Mr Leigh Vial, rice grower and delegate to the RIRDC Rice Research Committee.

The visit encompassed five locations in China, including Yunnan Agricultural University, Kunming, Guangxi Academy of Agricultural Sciences, Nanning, China Agricultural University, Beijing, Liaoning Academy of Agricultural Sciences, Shenyang and Heilongjiang Academy of Agricultural Sciences, Harbin.

7.3.1 Yunnan Agricultural University

The study tour highlighted the importance of Yunnan as the centre of origin of *japonica* cold-tolerant rice, and the novel genes for cold tolerance which exist in the genetic background of traditional varieties. Following the visit the strategy for the future is to develop collaborative projects which seek to identify new genes for adaptation to low temperature and their mechanism of action such that new varieties can have multiple sources of cold tolerance.

Much work has been carried out on developing cold-tolerant varieties in a number of improvement programs world-wide, however pedigree analysis reveals that many varieties are built on the same sources of cold tolerance, thus combining varieties may be fruitless if the original gene/s for tolerance are present in each of the parents.

A shift in emphasis to the identification of cold tolerance genes in landraces gathered from high altitude areas in Yunnan is warranted. Although ambitious, new research should be brought to bear on uncovering the genetic basis for cold tolerance in landraces and traditional varieties from cold, high altitude areas, so that these genomic regions can be transferred into other genetic backgrounds to provide robust tolerance at all growth stages.

Technology to firstly identify genomic regions identified as contributing to cold tolerance and then to facilitate the efficient transfer of such regions will be increasingly important. Diversity Array Technology (DART) is one means of obtaining an immediate representation of the genome, and by associating genotype with phenotype, regions associated with cold tolerance can be identified. Sequencing the DART clones identified with specific positive regions allows identification of the location within the genome and suggests candidate genes for additional study. Further, these areas can be tracked with DART analysis throughout subsequent crosses and back-crosses made to transfer the traits into varieties with appropriate adaptation and grain quality attributes. Elements of this work are being carried out with segregating populations varying for cold tolerance already developed at

Sydney University, however additional funding is necessary to advance the work and enhance the linkages with Yunnan Agricultural University.

Recent research conducted in Yunnan involved the genetic analysis of cold tolerance characteristics of 15 rice crosses (including near-isogenic lines) developed from different cold tolerant cultivars, together with the parental lines. Cultivar Kunmingxiaobaigu had one major gene controlling grain fertility, while Lijing No. 2 had one or two loci of the major gene controlling fertility, and the gene expression of their cold tolerance was related to the cytoplasm effect. There were different cold tolerance genes in cultivars Kunmingxiaobaigu, Lijing 2 and Hexi 4. A set of standard near-isogenic rice lines with major genes for cold tolerance were developed, with agronomic characters similar to the recurrent parent (Towada) but with significant differences in cold tolerance, highlighting the underlying diversity in rices derived from Yunnan.

The visit to Yunnan further cemented our relationship through Dr Zhao Xiaochun (Sydney University, Cobbitty) and Dr Tan Xuelin (Yunnan Agricultural University), and extended the relationship through the work of Dr Ye Changrong at UQ.

Following the visit it was agreed that Dr Zhao would test a population of recombinant inbred lines under high altitude cold conditions in Yunnan. The lines were developed from a cross between the cold-sensitive Australian cultivar Doongara and the cold-tolerant landrace from Yunnan Lijiangheigu. Through collaboration between Dr Ye Changrong at the University of Queensland, this population has now been tested under three cold tolerance screening regimes. The high altitude screening wrought the most damage with a high proportion of lines having low floret fertility (top of Figure 4) while the

Queensland University system was less severe (15/23°C for 3 weeks) and the Cobbitty system (12°C for 4 days) producing a wider distribution of floret fertility. Work carried out at Queensland University by Dr Ye Changrong through the life of this project has led to the development of glasshouse based screening systems a putative QTL for cold in a similar population, developed from a cross between the Australian medium grain variety Reiziq and the Yunnan landrace Baijiemang (Ye *et al.*, 2009).

7.3.2 Nanning Academy of Agricultural Science

A total of 740 varieties from the *Oryza rufipogon* collection in Nanning, Guangxi Province, have been screened for cold tolerance at both the seedling stage and at the reproductive stage, resulting in 4 varieties sowing seedling stage tolerance and 2 varieties with reproductive stage tolerance. A backcrossing program has been initiated to transfer

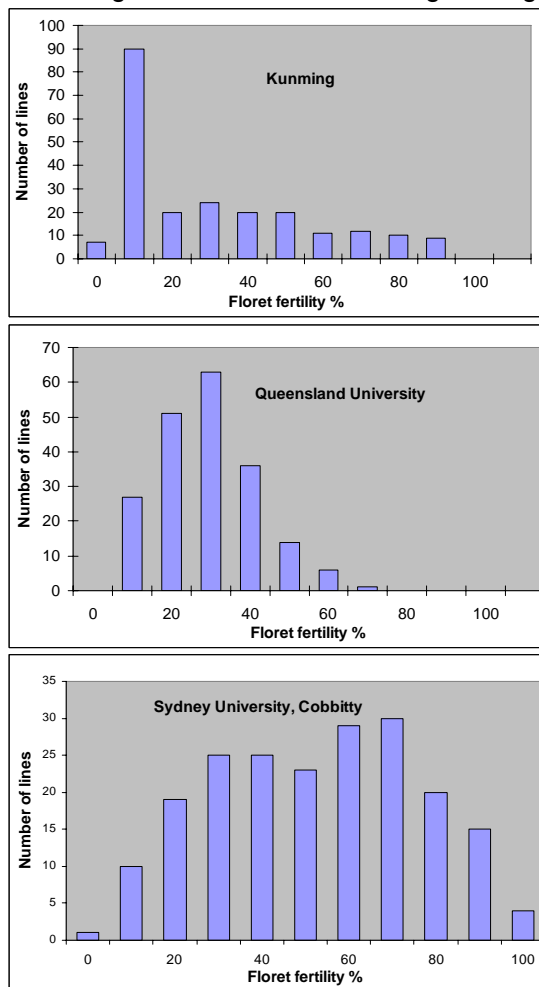


Figure 4. Frequency distribution of floret fertility (% filled grains) for a population of recombinant inbred lines from a cold-tolerant x susceptible cross across three screening methods.

tolerance from the wild background into the Australian variety Millin, with regular screening throughout development.

On visiting Dr Rongbai Li of the Guangxi Academy of Agricultural Sciences it was evident that this project has not received direct funding and thus is of lower priority with efforts proceeding in the background. Given the location of the institute in the southern part of China where production of *indica* types predominates, cold tolerance naturally accords lower research priority. Ideally future research should be directed at elucidating the mechanism and genomic location of *O. rufipogon* cold tolerance genes in comparison with those of Yunnan *O. sativa* landraces, with a view to development of suitable molecular markers to facilitate transfer of the traits with minimal linkage drag.

7.3.3 China Agricultural University - Beijing

Dr Wang Huaqi of the Upland Rice Research Centre at China Agricultural University reported that approximately 25% of the rice production area *i.e.* 6.6 million ha, is threatened by water scarcity and drought in China every year. Hence there is a pressing need to develop rice with a degree of drought tolerance to secure rice production in the face of water limitations, especially in the northern part of China in the Beijing area. Most cultivars of rice are highly susceptible to drought except upland rice limited germplasm

Dr Wang's strategy is to use upland rice germplasm for increasing drought tolerance in lowland rice genetic improvement, seeking to combine drought tolerance with high yield potential. Key traits include the capacity to break through topsoil following germination, high levels of vegetative vigour, strong root growth and exploration

The use of upland rice germplasm in breeding for drought tolerance is aimed at combining this trait with the short stature, early maturity and erect canopy of lowland rice (conferring high yield potential). The varieties developed from this program are known as the Han Dao series, and their utilisation in northern China is shown in Table 6.

Table 6. Elite Han Dao rice varieties released from China Agricultural University

Variety	Adapt region	Growth period (days)	Irrigation (m ³ /ha)	Yield (t/ha)	Total area (ha)
Han Dao 277	Huang-Huai-Hai Region	105-115	1500~2250	5.2~6.0	80,000
Han Dao 297	The North and Southeast	130~140	2250~3750	5.2~6.7	10,000
Han Dao 502	Huai River and Changjiang River Valley	115~130	1500~2250	6.0~7.5	10,000
Han Dao 65	North and Northeast	105~130	2250~3000	4.5~6.0	20,000

Interestingly, Dr Wang noted that an important source of genetic diversity for his breeding program comes from Yunnan and its surrounding areas, known as a centre of origin of upland rice cultivars, with many traditional varieties and landraces still used in rice production.

This germplasm is being used by Dr Wang's program to make lowland × upland crosses in order to combine drought tolerance from the upland cultivars with high yield potential and good grain quality of the lowland types.

Further collaborative linkages are warranted with the comprehensive rice breeding program of China Agricultural University aimed developing varieties better adapted to aerobic growing conditions. This research focuses on the development of varieties tolerant of water-limited conditions at all growth stages. Although not specifically aimed at

cold tolerance, strategically these are high priority traits to build into future varieties as the Australian Rice Industry faces the prospect of generally lower and less reliable rainfall.

Following the visit to China, a joint project proposal was initiated with Dr Wang Huaqi, to begin the process of developing rice adapted to limited water and modified irrigation practices for the Australian rice industry. Funding was sought by Dr Peter Snell, in conjunction with Dr Wang Huaqi, through a joint Australia-China Agricultural Cooperation Program for scientific collaboration administered by the Commonwealth Department of Agriculture, Fisheries and Forestry. A brief justification for the project is shown below.

The production of extremely high-yielding rice crops under flooded culture (lowland) is a practice most at risk from high temperatures and increasing demand for water resources under models of predicted climate change and population growth. Understandably China has been the first to experience the impact of increased competition between conventional lowland production systems and other uses for scarce water resources due to a rapidly growing population and fast-encroaching urban and industrial enterprise. Upland rice cultivation systems are historically more resilient to fluctuation to water availability and temperature, but these systems struggle to provide significant productivity much past the level of subsistence. It is not surprising therefore that China has led the way in developing aerobic rice varieties that represent a viable middle ground between high yielding lowland production and sturdiness of upland production into a package that could overcome the global need for more rice while using less water and land. Over the last 30 years Australian rice researchers have focused on alternative farming systems such as saturated soil culture and raised beds as a means to both reduce water consumption of the rice crop and allow crops less tolerant of waterlogging (wheat, soybeans, maize) to be grown in rotation. Although these systems are economic for crops other than rice, they were not able to provide a viable rice grain yield due to combination of dry days with high evaporative demand and cold nights, limiting yield through low temperature damage at critical growth stages and delaying crop development.

The work in China is centred on root traits with extensive efforts and resources available for measuring root length and exploration under field conditions. In a joint project Australian work could concentrate on above-ground plant parts using isotopic discrimination measures of carbon and oxygen, osmotic adjustment and direct measures of stomatal aperture, as well as assessing performance on different soil types with less permeable layers at depth, and under different environmental conditions.

This project aims to link to the aerobic breeding program at China Agricultural University to incorporate the necessary traits for adaptation of rice to alternative irrigation strategies using less water, and to further research additional traits which may prove beneficial.

The project required applications to be submitted from both Australia and China through their respective government departments and unfortunately was not successful in attracting support.

7.3.4 Liaoning Academy of Agricultural Sciences

There is a need to further explore the origins and mechanisms of cold tolerance in varieties utilised throughout the Liaoning and Heilongjiang provinces and develop the linkages between research institutions.

Of particular interest in Liaoning Province is the dual breeding programs for inbred japonica rice and hybrid japonica rice. During the visit we saw a number of field plots at the Liaoning Rice Research Institute of both inbred *japonica* lines and hybrid *japonica* types. We visited a rice seed producer to the north of Shenyang to see seed increase of hybrids and inbreds (Figure 5).



Figure 5. Seed production crop of new inbred rice variety Liaoning Province, China.

cross section.

Physiological analysis (Zheng *et al.*, (2009) has shown that "super" hybrids have increased yield potential of around 10% and this was attributed to their capacity to accumulate more biomass (8%), and through their larger sink size, apportion slightly more to the grain (harvest index) thereby improving yield. The challenge facing *japonica*-based improvement programs seeking to improve yield potential through hybrid breeding is the lack of clear yield advantage in *japonica x japonica* hybrids, and the strong genetic barriers to floret fertility in *japonica x indica* hybrids. The yield advantage of *japonica x japonica* hybrids is not readily apparent, and there would need to be significant heterosis to warrant the added investment in the long-term process of developing two and three-line hybrid systems, and the added costs of hybrid seed production.

Key factors for future interaction between will include the evaluation of Liaoning rice varieties for cold tolerance and adaptation under Australian rice production environments, and the direct comparison of inbred *japonica* lines and their hybrid counterparts.

7.3.5 Heilongjiang Academy of Agricultural Sciences

Heilongjiang province is located in the north eastern part of China, at a latitude of more than 40°, ranging between 43 and 53°N. At this latitude daylength during summer reaches 16 hours and total solar radiation levels are correspondingly high. There is a pronounced summer peak in rainfall distribution with most rain occurring in July. The winters are harsh, with the soil below freezing from October to April each year. Consequently many pests and diseases do not over-winter, limiting their prevalence and spread in subsequent seasons. There is also a relatively large diurnal variation in temperature (approximately 12°C) in comparison with other rice production areas in China, thus limiting respiration losses at night, and contributing to high yield.

There are four major river systems and three large lakes, facilitating the use of irrigation for rice production. Most rice production occurs on the river plains, and there has been significant land reclamation in recent years, leading to large increases in rice production area (see Figure 10 on page 19). In the 10 years between 1997 and 2006 production area rose to approximately 1.6 m ha, with average yields of 6 t/ha, and since then both area and yield has risen to 2 m ha and 6.5 t/ha respectively, resulting in a total production of more than 13 m t. According to Professor Zhang Fengming the principal reasons for this improvement include changing cultivation methods, from sowing directly into paddies to transplanting rice seedlings, methods of raising rice seedling: from sowing in water bed to sowing in drought bed, and a reduction in transplanting density thereby encouraging tiller formation.

At this latitude there are only 100-140 frost-free days in the summer, and low temperature damage occurs every three to five years. It mostly occurs early in the season, thus

delaying rice growth and establishment, however low temperatures during the reproductive stage also occur with similar frequency, impacting at both the microspore development stage and anthesis. A yield reduction of 20-40% is common, and mountainous areas may suffer complete yield loss.

The Heilongjiang Academy of Agricultural Sciences has a cold water screening facility at Harbin in which groundwater at 7°C is mixed with surface irrigation water to obtain the desired temperature for screening segregating material. This is undoubtedly an excellent resource for the screening of segregating populations.

The key elements of ongoing interaction with Heilongjiang will be current and ongoing exchange of germplasm and ongoing evaluation of representatives of the Long Dao series of rice cultivars under Australian conditions.

7.4 International Temperate Rice Research Consortium

A final outcome from the Cold Tolerance Workshop held at Yanco Agricultural Institute was the proposal for a Temperate Rice Research Consortium, tendered by KK Jena to the group.

To further strengthen international efforts focused on rice research in temperate regions, IRRI established the Temperate Rice Research Consortium (TRRC) in 2007. The consortium has, as part of its charter, a specific focus on the development of cold-tolerant varieties. Its country members are Australia, Bhutan, Chile, China, Egypt, Japan, Kazakhstan, Nepal, the Philippines, Russia, South Korea, Spain, Tanzania, Uruguay, Uzbekistan, and the U.S.

Dr. Seong-Hee Lee, Korean member of the IRRI Board of Trustees, was instrumental in the formation of this Consortium, bringing along with him the full financial support of the RDA. A steering committee and four working groups were formed. Each working group focused on one of the four major constraints identified for temperate rice: (1) yield potential and grain quality, (2) blast resistance, (3) cold tolerance, and (4) nitrogen and water use efficiency. The third group in particular, aimed to evaluate a selected number of cold-tolerant germplasm accessions provided by working group partners at seedling and reproductive stages

Impacts
The first planning meeting was held in mid-2007 and a further meeting of the Steering Committee held in South Korea in April 2008. At the latter meeting the workgroups were formally organised and funds were allocated to each. Working group 1 is focused on yield potential and grain quality (led by Dr. Hua Zetian, Liaoning Academy of Agricultural Sciences, Shenyang). The justification for this project is that the improvement of yield potential has slowed for the past decade due to lack of sufficient genetic diversity, inadequate application of appropriate agronomic management practices, and lack of heterotic gene combinations to further improve yield. The demand for rice is also associated with demand for grain quality characteristics. In this workgroup donor lines provided by each member country will be used to backcross with indigenous elite varieties (recurrent parents). The F₁ hybrid will be backcrossed 1-3 times with recurrent parents to develop breeding populations. The developed breeding populations will be distributed to members to select target varieties.

The activities of working Group 2 are focused on breeding for rice blast resistance (led by Dr. Hei Leung, IRRI) and more specifically, aimed at development of pre-breeding genetic resources for blast resistance breeding in temperate rice.

Temperate rice is particularly vulnerable to blast epidemics the temperate environment is conducive to its development and spread. Hence, there is an urgent need to achieve and maintain stability of blast resistance in the production system. In recent years, there has been increased severity of blast in both temperate and tropical environments. This suggests either an erosion of resistance due to pathogen evolution or a lessening of

screening efforts in breeding programs. Dr Leung's preproject aims to develop cross-cutting genetic materials for rice blast research in the temperate rice production system by: 1) developing a set of breeding-ready NILs carrying key Pi and QTLs useful for TRRC participating members, 2) documenting of the effectiveness of Pi genes and QTL in at least three representative regions for temperate rice breeding, 3) cloning and characterising the new resistance gene, Pi40, that confers broad-spectrum resistance to blast, and 4) developing a panel of pathogen markers for functional diagnostics of the pathogen populations.

Working group 3 is most relevant to this project, and is specifically aimed at breeding for cold tolerance (led by Dr. Sae-Jun Yang, Rural Development Administration, South Korea). Rice is a tropical crop, and when grown at mid or high altitudes is threatened by cold events during the vegetative and pre-flowering period, causing seedling mortality and spikelet sterility, and significant yield losses. Identification of new genetic resources for cold tolerance is of primary importance to rice breeders to develop and grow cold-tolerant rice cultivars in temperate regions. There is a need to develop research activities on germplasm evaluation at key sites for various stages of cold tolerance in temperate rice breeding programs, in collaboration with partners of the working group, and to develop improved breeding lines for cold tolerance. Activities of the group include: 1) evaluate at key sites a selected number of cold-tolerant germplasm provided by WG partners for cold tolerance at seedling and reproductive stages and identify promising cold-tolerant cultivars or breeding lines, 2) to combine genes from cold-tolerant genotypes to develop suitable cold-tolerant germplasm adapted to different countries, 3) to use potential DNA markers linked to cold tolerance traits (if available) for marker-assisted breeding for cold tolerance, 4) to develop a common set of cold-tolerant lines for use in breeding in collaboration with WG partners

The key sites for benchmarking cold-tolerant varieties were the Jilin Academy of Agricultural Sciences in Jilin, China, and an artificial environment for cold stress at vegetative and reproductive stages in field condition at Chuncheon substation, National Institute of Crop Science, RDA, Republic of Korea, which has modern cold water irrigation facilities.

Finally working group 4 is aimed at improving fertilizer N use efficiency (NUE) of temperate rice. Fertilizer N use efficiency in rice production has received great attention in recent years due to concerns about environmental pollution and global warming. A large variation in NUE has been observed in *japonica* rice across temperate rice-growing areas. These differences may be attributed to variety, climate, and N management. The specific aims are to 1) compare varietal (*japonica* vs. *indica*, Korean vs Chinese *japonica*, hybrid vs inbred) differences in NUE component traits, 2) to determine the effects of N management practices and climate on NUE component traits, and 3) to improve N management practice for high grain yield and NUE. The focus of this working group is highly relevant to production practises in China where N application can often reach 300 kg N/ha and significant losses of N can occur. This is less relevant to Australian rice growing, as growers have ready access to NIR technology to determine N content of crops at critical growth stages and can adjust application rates accordingly.

As a result of this project the ongoing linkage with China will be via the Temperate Rice Research Consortium, which will meet annually to review progress on its four project areas, namely yield and quality, cold tolerance, blast resistance and resource-use efficiency.

Involvement in the TRRC is a significant legacy of this China Linkage Project, which gives the Australian Rice Industry and research effort a direct and ongoing link, not only to China but to the wider world of rice production in all temperate areas.

7.5 Scientific impacts – now and in 5 years

Better definition and understanding of the sources and mechanisms of cold tolerance is an essential basis for breeding so that efforts can be more effectively directed at assembling multiple sources and types of cold tolerance. Analysis of the sources of cold tolerance in temperate rice varieties from around the world shows that in many cases the original source of cold tolerance was a single variety. For example, the variety *Silewah* was the original source of cold tolerance in many of the varieties developed in northern Japan and cold tolerance genes from the same source have featured extensively in Californian pedigrees. Therefore, continually introducing lines from these programs does not necessarily broaden the genetic base for cold tolerance, unless those programs in turn are finding new and unique sources of cold tolerance.

The real value of this project lies in the development of direct linkages to scientists located in the centre of diversity of japonica rice, and the prospects for finding new sources and mechanisms of cold tolerance. The key impacts of this project are a better definition of the current and future sources of cold tolerance in temperate rice, the identification of a number of collaborative opportunities for screening and evaluation of breeding material, and the exchange of cold tolerant rice varieties which will feed into rice breeding programs in both Australia and China.

Clearly the broadening of the genetic base for cold tolerance through variety exchange will take a minimum of five years to filter through the breeding, screening and selection process, but it offers real prospects for improving the base level of cold tolerance, which in turn will have significant flow-on effects in the development of new varieties with the suite of traits required to maintain productivity in the water-limited production systems of the future.

7.6 Capacity impacts – now and in 5 years

The project has assisted in maintaining and extending scientific linkages to research programs in China through two activities directly supported by the project - the International Rice Cold Tolerance Workshop held at Yanco and the study tour of rice research programs throughout China, with a focus on cold tolerance.

Further, the project has indirectly supported the visit of a Chinese rice breeder, Dr Wang Hui from Liaoning Rice Research Institute of the Liaoning Academy of Agricultural Sciences (LAAS), who spent 12 months in Australia from March 2007 to March 2008, and who ably assisted our visit to Shenyang, Liaoning Province, in September 2007.

Dr Wang Hui is now Vice Director of the LAAS Rice Research Institute, and recently appointed leader of Working Group 1 of the Temperate Rice Research Consortium, which is focused on yield potential and grain quality of temperate *japonica* rice.

7.7 Community impacts – now and in 5 years

Community impacts will be mediated by the development of new rice varieties with enhanced levels of cold tolerance. Variety improvement is necessarily a long-term process, commonly taking at least 10 years from initial cross to the point of release to rice farmers. The rice improvement program at Yanco will be releasing a new variety in 2010 with enhanced levels of cold tolerance, and while findings from this project did not directly contribute to the development of this variety, the knowledge of screening systems, genetic diversity and variety exchange which occurred within this project will undoubtedly contribute to future variety development of types with enhanced cold tolerance and better adaptation to modified irrigation practices to increase water productivity.

7.7.1 Economic impacts

The economic impact of enhanced rice cold tolerance per se will be decreased year-to-year variation in average yield which is principally determined by the occurrence of low temperatures during critical growth stages. This has the net effect of stabilising farm incomes and improving overall levels of water productivity.

7.7.2 Social impacts

Rice production is a key enterprise in the Riverina region of NSW, providing direct employment for more than 5,000 people throughout the region for receipt, storage, transport, processing and marketing of the final suite of products. Flow-on effects from the operation of this industry indirectly support more than 60 towns and communities within the Riverina region. Securing the future of the rice industry through more resilient rice varieties with better tolerance to the likely impacts of climate change (less water, more variable temperatures) will have long-term social impacts.

7.7.3 Environmental impacts

The key environmental impact of improving rice cold tolerance will be through the more effective use of the available water resources. Cold tolerance is a key driver of producing rice with less water, opening prospects for alternative irrigation strategies such as delayed permanent water, alternate wetting and drying for all or part of the crop growth cycle, or production under completely aerobic conditions, obviating the need for standing water at any stage.

7.8 Communication and dissemination activities

There were no additional communication and dissemination activities undertaken by this project, other than the rice cold tolerance workshop which was one of the key activities of the project.

8 Conclusions and recommendations

This policy linkage and impact assessment project has provided important opportunities for maintaining and extending links with rice research in the temperate rice producing regions of China, underpinning future development of cold tolerant varieties. Opportunities have arisen in direct response to this project for the exchange of germplasm, and the collaborative testing of varieties, lines and populations under alternative screening systems and environments. An important legacy of this project is the contribution to the development of the Temperate Rice Research Consortium and more importantly, the ongoing opportunities for linkage and interaction with research programs, not only in China but also across the broader spectrum of rice producing countries in temperate areas throughout the world.

8.1 Recommendations

- That projects be developed to maintain and extend collaboration between the NSW DPI Rice Breeding Programme and Yunnan Agricultural University for the purposes of:
 - identifying further promising sources of cold tolerance among the landraces available from high altitude regions of Yunnan
 - benchmarking existing modern cultivars against traditional cold tolerant varieties using screening facilities located at high altitude
 - developing populations suitable for screening and selection at both sites, at Yanco using divergent sowing dates and random cold events and at Yunnan Agricultural University's high altitude screening location
 - discovering genes/QTLs associated with cold tolerance in more recent collections of cold-tolerant lines and comparing those to known sources of cold tolerance to assess their novelty
- That a watching brief be maintained on the research associated with cold tolerance in *O. rufipogon* at Nanning, although there is little current research focused on cold tolerance traits
- That projects be developed in conjunction with China Agricultural University, Beijing, to complement their research on root traits for aerobic lines by focusing on the above-ground physiological traits associated with adaptation to aerobic conditions, to allow the simultaneous development of cold-tolerant and aerobically-adapted lines
- That continuing exchange of germplasm take place between Yanco and the rice breeding programs in northern China, specifically Liaoning and Heilongjiang Academies of Agricultural Sciences, in association with two of the Temperate Rice Research Consortium project areas, focusing on yield potential and grain quality for temperate rice production areas and improvement in cold-tolerance.
- Maintain involvement in the Temperate Rice Research Consortium activities through active participation in working group 3 focused on cold tolerance, and in working group 1 focused on yield potential improvement in combination with enhanced grain quality.

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9.2 List of publications produced by project

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10 Appendixes

10.1 Appendix 1:

There are no appendices to this report.