

**CRC for Sustainable Rice Production
International Rice Cold Tolerance Workshop
CSIRO Discovery, Canberra, 22-23 July 2004**

Program presented at the Conference and Abstracts are provided herewith.

**Copies of Conference Proceedings (full papers) will be available
on CD from the Rice CRC Office in due course.
Contact: Julie Symes, Rice CRC Tel: 61-2-69512713**

CRC for Sustainable Rice Production
Program of oral presentations at the International Rice Cold Tolerance Workshop
CSIRO Discovery, Canberra, 22-23 July 2004

Thursday 22 July

8:30-8:40 Opening by Dr John Williams, Interim
Chairman, CRC for Value Added Rice-Based
Systems

Session 1 Field Physiology

Chairman: John Angus, CSIRO

8:40-9:00 Water temperature and water height effects on
canopy temperature in a Uruguayan rice field

Alvaro Roel
Instituto Nacional de Investigación
Agropecuaria, Uruguay

9:00-9:40 Effects of panicle temperature, root
temperature and nitrogen status on spikelet
sterility in rice

Thusitha Gunawardena and Shu Fukai
University of Queensland and Rice CRC

9:40-10:20 How to improve reproductive cold tolerance
of rice in Australia

T.C. Farrell, K.M. Fox, R.L. Williams, S.
Fukai and L.G. Lewin
University of Queensland and Rice CRC

10:20-10:50 Morning tea

10:50-11:10 Modelling chilling injury in rice using CERES
Rice ver. 4.1

J. Timsina, U. Singh, D. Godwin and E.
Humphreys
CSIRO Land and Water, Griffith

11:10-11:30 Mechanisms of cold tolerance in rice at
seedling and reproductive stages

B.P. Naidu, **T.A. Gunawardena** and S. Fukai
University of Queensland and Department of
Natural Resources, Mines and Energy

Session 2 Industry Effects

Chairman: Daryl Gibbs (RIRDC Rice R&D
Committee)

11:30-12:10 PREDA: a prototype of a rice cold damage
early warning system at high latitudes

R. Confalonieri, L. Mariani and S. Bocchi
Department of Crop Science, University of
Milano, Italy

12:10-1:15 Lunch

Session 2 (continued)

1:15-1:55 Perceptions of rice cold damage by farmers,
advisers and researchers – The 2004 experience

Laurie Lewin, John Lacy and Russell Ford
Rice CRC, NSW Dept Primary Industries

1:55-2:35 Temporal and spatial analysis of water
temperature impact on California rice
production

Alvaro Roel, **Randall Mutters**, Richard Plant
University of California, USA

2:35-3:15 Forecasting Australian rice yield in relation to
cold damage

John Angus, Tim Farrell, Rob Williams and
Laurie Lewin
CSIRO Plant Industry and Rice CRC

3:15-3:45 Afternoon tea

Session 3 Breeding for cold tolerance

Chairman: Laurie Lewin, Rice CRC

3:45-4:25 Rice breeding for cold tolerance in Southern Brazil

Renata Pereira da Cruz, Antonio Folgiarini de
Rosso, Sérgio Iraçu Gindri Lopes, Mara Cristina
Barbosa Lopes, Paulo Sérgio Carmona, Oneides
Avozani, João Leodato Nunes Maciel, Dieter Kempf
Instituto Rio Grandense del Arroz, Brazil

4:25-4:45 Genetic control of cold tolerance derived from
Eastern European rice cultivars HSC55 and Plovdiv
22

T.C. Farrell, **R.L. Williams**, G.J. Rebetzke, and S.
Fukai
University of Queensland and Rice CRC

4:45-5:05 Screening for rice cold tolerance: low temperature
effects on flowering

K.M. Fox, R. Subasinghe, P.D. Looby, D.L. Wornes
NSW Dept Primary Industries and Rice CRC

7:00 for 7:30 Dinner at the Common Room, University House

Friday 23 July**Session 3 Breeding for cold tolerance (continued)**

Chairman: John Herbert, Rice CRC

8:30-8:50 Varietal improvement utilizing cold tolerance genes of Dongxiang wild rice

Haohua He, Haibo Li, Dali Hu, Yibin Liu
Jiangxi Agricultural University, China

8:50-9:10 Screening for low-temperature tolerance at the reproductive stage in rice: difficulties inducing floret sterility

John Smith, Russell Reinke, Shu Fukai and Ken Fischer
NSW Dept Primary Industries and University of Queensland

9:10-9:30 Evaluation of rice cultivars for cold tolerance in relation to cold stress on spikelet sterility

CANCELLED due to non-arrival of speaker**J.D. Yea**, Y.H. Choi, H.G. Hwang, K.K. Jena and M.H. Lee
National Institute of Crop Science, Suwon, Republic of Korea

9:30-9:50 Economic analysis of developing cold-tolerant varieties of rice in Australia

Rajinder P. Singh, John Brennan, John Mullen, Russell Reinke and Laurie Lewin
NSW Dept Primary Industries and Rice CRC

9:50-10:10 Rice breeding for cold tolerance in Uruguay

P. Blanco, F. Molina, R. Méndez, A. Roel and F. Pérez de Vida,
Instituto Nacional de Investigación Agropecuaria, Uruguay

10:10-10:40 Morning tea

Session 4 Cell biology

Chairman: Liz Dennis

10:40-11:00 Mechanism of chilling induced pollen failure in rice

M.E. Mamun, S. C. Alfred, S. Oliver, L.C. Cantrill, Z Li, R. Dolferus, R.L. Overall, and B.G. Sutton.
University of Sydney

11:00-11:20 Illuminating anthers with electrons and lasers: The cell biology of chilling damage in rice.

L.C. Cantrill, Z. Li, M.E. Mamun, S.C. Alfred, R.L. Overall and B.G. Sutton
*University of Sydney and Rice CRC***Session 5 Molecular genetics**

Chairman: Bruce Sutton, Sydney University

11:20-12:00 Molecular biology of rice cold tolerance 1

Sandra Oliver, X.Zhao, Joost van Dongen, Peter Geigenberger, Hargurdeep Saini, Sussan Fernandes, Christopher Blanchard, Liz Dennis and Rudy Dolferus
CSIRO Plant Industry and Rice CRC

12:00-1:00 Lunch

1:00-1:40 Molecular biology of rice cold tolerance 2

Sandra Oliver, X. Zhao, Joost van Dongen, Peter Geigenberger, Hargurdeep Saini, Sussan Fernandes, Christopher Blanchard, Liz Dennis and **Rudy Dolferus**
CSIRO Plant Industry and Rice CRC

1:40-2:20 Diverse mechanisms of low temperature stress tolerance in rice plants

Kazutoshi Okuno*National Institute of Agrobiological Sciences, Tsukuba, Japan*

2:20-2:40 Low temperature changes proteome of male gametophyte development in rice.

Nijat Imin, Tursun Kerim, Jeremy J. Weinman and Barry G. Rolfe.
Australian National University

2:40-3:00 Chromosomal location of QTLs controlling low temperature induced male sterility in temperate japonica rice.

X. Zhao, R.Verma, R. Dolferus, N. Darvey and E.S. Dennis
Sydney University

3:00-3:30 Afternoon tea

3:30-4:30 General discussion

WATER TEMPERATURE AND WATER HEIGHT EFFECTS ON CANOPY TEMPERATURE IN A URUGUAYAN RICE FIELD

Alvaro Roel

Instituto Nacional de Investigación Agropecuaria (INIA),
Ruta 8 km 281, CP 33000, Treinta y Tres, Uruguay.

In Uruguay, periods of low air temperature during rice growing season can be common and have been identified as one of the reasons for rice yield instability. Although a vast literature exists about the effect of water or air temperature on rice sterility, most of the studies were conducted under chamber or green house controlled conditions and little has been done under actual field scale conditions.

The objectives of this study were to determine the spatial variability in water and canopy temperatures in an Uruguayan rice field, quantify the relationship among them, and determine whether these temperatures patterns can be affected by water height.

During the 2002-03 rice-growing season a 12 hectares field located at Paso de la Laguna INIA's Rice Experimental Unit was instrumented with a network of temperature sensors (Hobo H8 Pro, Onset Computer Corporation, MA) that allowed to measure water and canopy air temperature in an hourly basis.

Results from the study showed that canopy and water temperature daily cycles were quite different and significantly affected by weather conditions. Water temperatures presented smaller daily oscillations than air temperature. Locations in the field situated in areas with deeper water presented very small differences in canopy temperature patterns with respect with the ones located in shallower areas. These results indicate that under Uruguayan rice conditions the possibility of ameliorating cold temperature damages relies on the fact of having the rice panicle below water level. Once the panicle is exposed to ambient air little can be done through water management.

EFFECTS OF PANICLE TEMPERATURE, ROOT TEMPERATURE AND NITROGEN STATUS ON SPIKELET STERILITY IN RICE

T. A. Gunawardena and S. Fukai

School of Land and Food Sciences, The University of Queensland, QLD 4072, Australia.

Low temperature during panicle development in rice increases spikelet sterility. This effect is exacerbated by high rates of nitrogen (N) application in the field.

In temperature-controlled glasshouse experiments, spikelet sterility induced by low temperature and N fertilisation was determined. Experiments also attempted to clarify whether low temperature experienced by the root, panicle or foliage is responsible for increased spikelet sterility. Water depth and, water and air temperatures, were changed independently to investigate the effects of low temperature in the root, panicle and foliage during microspore development on spikelet sterility.

The total number of pollen and number of engorged pollen grains per anther, and the number of intercepted and germinated pollen grains per stigma, were measured. Spikelet sterility was then analysed in relation to the total number of pollen grains per spikelet and the efficiency with which these pollen grains became engorged, were intercepted by the stigma, germinated and were involved in fertilisation.

Although there was no significant effect of low temperature on spikelet sterility in the absence of applied N, low temperature greatly increased spikelet sterility as a result of a reduction in the number of engorged pollen grains per anther in the presence of applied N. The increased tillering due to application of high rates of N, increased both spikelet number per plant and spikelet sterility under low temperature conditions. It is concluded that N increased tillering and spikelet number per plant

and this, in turn, reduced the number of engorged pollen per anther leading into increased spikelet sterility under low temperature condition.

There was a significant combined effect of average minimum panicle and root temperatures on spikelet sterility which accounted for 86% of the variation in spikelet sterility. Total number of pollen grains per anther was reduced by low panicle temperature, but not by low root temperature. While engorgement efficiency (the percentage of pollen grains that were engorged) was determined by both root and panicle temperature, germination efficiency (the percentage of germinated pollen grains relative to the number of engorged pollen grains intercepted by the stigma) was determined only by root temperature. Interception efficiency (i.e. percentage of engorged pollen grains intercepted by the stigma), however, was not affected by either root or panicle temperature. Engorgement efficiency was the dominant factor explaining the variation in spikelet sterility. It is concluded that both panicle and root temperature affect spikelet sterility in rice when the plant encounters low temperatures during the microspore development stage.

HOW TO IMPROVE REPRODUCTIVE COLD TOLERANCE OF RICE IN AUSTRALIA

T.C. Farrell^{1,2}, K.M. Fox^{1,3}, R.L. Williams^{1,4}, S. Fukai^{1,2} and L.G. Lewin¹

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Rice production in temperate Australia is successful due to many factors including full irrigation, high levels of solar radiation and no major pests and diseases. However, low temperature, particularly during the reproductive stage of the development of rice, limits productivity in the Riverina region of New South Wales (NSW). Low temperatures between late January and early February coincide with the development of young microspores and this has led to a reduction of industry yields in Australia by up to 25% in extreme years. Results from experiments in temperature-controlled rooms and a cold-water facility were combined with those from four years of field experiments, which used natural exposure to low temperature to examine the response of over 50 cultivars from diverse origins. Plants were exposed to day/night air temperatures of 27/13°C in temperature-controlled rooms and also exposed to 19°C in the cold-water facility from panicle initiation to 50% heading. In field experiments several techniques were used to increase the likelihood of inducing cold damage such as multiple sowing dates, shallow water depths and high nitrogen rates. Cultivars from many countries have been tested in these three facilities in Australia for cold tolerance during reproductive development. The performance of cultivars was consistent across facilities with the temperature-controlled room and cold-water facility being preferred to field screening because of the improved reliability of exposure to low temperature in both environments. Incorporating cold tolerant genes from cultivars originating from cooler climates is one strategy to increase the cold tolerance of commercial cultivars. Reducing the impact of cold damage in Australia through the development of cold tolerant cultivars and improved cultural techniques can increase productivity and sustainability through increased water use efficiency.

MODELLING CHILLING INJURY IN RICE USING CERES RICE ver. 4.1

J. Timsina¹, U. Singh², D. Godwin³ and E. Humphreys¹

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Rice crops in the Riverina region suffer from cold damage during the reproductive stage, from panicle initiation to flowering. Low night temperature during the early pollen microspore stage is the main cause of cold damage and resulting yield loss. Growth and development of rice during this stage is more affected by floodwater or soil surface temperature than ambient air temperature (measured at the standard height of ~1.2 m) as the developing panicle is below the top of the plant canopy and near the soil or floodwater surface, or under the water, and also the developing root is under the water. Simulation of this effect of low air temperature on rice yield requires firstly, simulating the temperature experience by the developing panicle and root, and secondly, simulating the effect of low temperature on spikelet sterility and yield. Rice crop models with the ability to simulate the effect of low temperature on rice yield have many potential uses, such as (1) yield forecasting, (2) evaluation of the tradeoffs between yield and management to save water (e.g. intermediate irrigation, sowing date, etc.), (3) investigation of the impact of climate change on yield.

Floodwater temperature and chilling injury routines were developed and incorporated into CERES Rice ver. 4.1, and the model was tested against data from southern NSW. The model overestimated the daily minimum floodwater temperature in deep water throughout the 39 days of measurement from 1 January to 8 February in 2000 in an experiment at Yanco, but in shallow water, the simulated minimum temperatures matched the measured data well. Daily maximum temperatures in deep water were simulated well except for some overestimation during the later stage, but in shallow water, the maximum temperatures were consistently overestimated. Simulated daily mean flood water temperatures were 5-10% higher than the measured data. Simulated grain yield response to shallow and deep water was within 10-20% of measured data for early planting (26 September 1991) at Deniliquin but agreement was poor for late planting (31 October). Sensitivity analysis showed greater cold damage with pre-flood N than PI N, confirming with the observed yields from many experiments in southern NSW.

Initial attempts to develop and test the floodwater temperature and chilling injury routines show an optimistic promise for the simulation of yield loss in rice due to cold injury in the Riverina region of southern NSW. Refinements of both floodwater temperature and chilling injury routines and the collation and scrutiny of data for model validation are in progress. More and better data sets, and further refinement and testing of the model, are required to accurately simulate the effects of low temperature, and deep water and N management on chilling injury and grain yield of rice.

MECHANISMS OF COLD TOLERANCE IN RICE AT SEEDLING AND REPRODUCTIVE STAGES

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We investigated if rice (*Oryza sativa* L.) cultivars with higher cold tolerance during seedling growth will also have increased level of cold tolerance during the reproductive stage and the mechanisms associated with such tolerance.

Temperature treatments (18/13 and 28/23 °C 12h day/night) were imposed in temperature controlled glasshouses during seed germination/seedling establishment and reproductive stages of 32 diverse rice cultivars and 30 recombinant inbred lines (RILs) between Millin and HSC 55. At the seedling stage, biomass production which was considered as a measure of seedling cold tolerance was positively correlated with the ability of cultivars to accumulate spermine ($r=0.68$; $p<0.01$) and gibberellic acid (GA) ($r=0.71$; $p<0.01$). Cold tolerant rice cultivars accumulated higher amounts of spermine ($r=-0.89$; $p<0.01$) and produced higher number of engorged pollen grains ($r=-0.75$; $p<0.01$), resulting in lesser sterility. However, the correlation between GA in leaves and spikelet sterility was weak.

Significant negative correlation between seedling biomass and spikelet sterility showed that >50% of the variation in sterility was explained by seedling biomass under low temperature condition. This suggests that screening for cold tolerance could be performed at the seedling stage which serves as an indirect selection for reproductive cold stress tolerance.

PREDA: A PROTOTYPE OF A RICE COLD DAMAGE EARLY WARNING SYSTEM AT HIGH LATITUDES

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Rice, as a tropical crop, at mid or high latitudes is threatened by cold air irruptions occurring during the pre-flowering period which may cause flower sterility and consequent yield losses. In northern Europe, during the last 30 years, this phenomenon verified 5 times, determining significant economic problems to rice farmers. At higher latitudes, the water layer affects the vertical thermal profile by mitigating extreme daily-night temperatures fluctuations, so that rice growers (North Italy, North Japan, North Korea, North China, Southern Australia) traditionally consider water an important tool to be used to mitigate low temperature.

The models framework PREDA was developed to provide early warnings to farmers on cold damage risk and to suggest whether to increase or not water level. Its components are the thermal profile model TRIS (used for the simulation of temperatures at the height of sensitive organs), a crop growth model, the flower sterility model STEFI and the model for the simulation of the height of sensitive organs ALTEA. PREDA uses forecasted meteorological data for evaluating cold damage risk and cold effects in presence of two water levels: low and high. If the difference between the simulated yields obtained under these two management options is significant, it is suggested to increase the water level.

The system is implemented in Turbo Pascal and an object oriented version (free downloadable DLLs) will be soon developed. PREDA is going to be introduced in the MARS crop yield forecasting system of the European Commission for further tests and validations.

PERCEPTIONS OF RICE COLD DAMAGE BY FARMERS, ADVISERS AND RESEARCHERS – THE 2004 EXPERIENCE

L. Lewin¹, J. Lacy¹, R. Ford², R. Subasinghe¹

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There was significant cold damage to commercial rice crops in the 2003/04 rice season. Yield reductions were largely due to low temperatures in the first half of the critical reproductive period. The damage was marked by extreme variability between cultivars, regions, time of sowing and even within rice bays.

Much of the observed variability can be explained by the effects of nitrogen, water depth, time of development and cultivar differences.

Grower perception of damage varied depending on their particular situation. They found it difficult to explain: greater damage around the water inlet; less damage along the edge of each bay; some situations where deep water made little difference; apparently greater damage in thicker crops; less impact of nitrogen topdressed as opposed to pre-flood nitrogen. Most of this variability can be explained from past results.

Growers are still not well-informed on the nature of cold damage, the temperatures that cause it and the importance of the cold by nitrogen interaction. They do generally understand the value of management options that can make a difference, such as water depth and nitrogen management.

TEMPORAL AND SPATIAL ANALYSIS OF WATER TEMPERATURE IMPACT ON CALIFORNIA RICE PRODUCTION

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Water temperature has increasingly become a matter of concern for California rice growers due to a need for public water agencies to improve habitat for fish. There have been few systematic efforts to measure at the field level the effect of low water temperature on rice yield. Neither have there been systematic efforts to measure the spatial distribution of water temperature in a check and associated effects on productivity and quality. The objectives of this study were to determine the spatial variability in water temperature in two California rice fields, to quantify water temperature (T_w) effects on plant development, yield, and to determine whether water temperature can be estimated from thermal infrared images. Two rice fields were instrumented with a network of temperature sensors. In order to reveal the spatial dimension of water temperature and yield reduction an interpolation procedure was used to generate maps that show the spatial distribution of the number of hours that water was below a certain temperature threshold across fields and the levels of yield reduction. The area affected by low T_w effects had an inverse relation with the level of yield reduction. Affected areas were clustered together and not randomly dispersed, which revealed the spatial nature of the problem. A GIS model was capable of accurately estimating the size and location of the areas that were affected by low T_w . The cold-water effects were present over a wide area and detectable using remotely sensed thermal images. Overall, the study showed that the deleterious effects of cold water on rice productivity occur well beyond the visually impacted area immediately adjacent to the water intake. Implications for management at the farm level and at the regional scale are discussed.

FORECASTING AUSTRALIAN RICE YIELD IN RELATION TO COLD DAMAGE

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Yield forecasts are needed by the Australian rice industry to schedule loans, payments to ricegrowers, transport, storage and sales. The main source of yield variation is cold damage during reproductive development, but recent evidence suggests that low temperatures at other stages, particularly vegetative development, also cause yield loss. Other sources of yield variation are solar radiation, new cultivars, increased inputs and the use of deep water to insulate young pollen grains against cold damage. All crops are fully irrigated, so water supply plays no part in yield variation. Since most of the variation is known by the time of flowering, yield can be forecast about 6 weeks before harvest.

Reliable yield and weather data from three regions for periods of up to 49 years are available for model development. During that time, when mean yields increased from 5 to 9.5 t/ha, 3 main cultivars occupied most of the area sown: Caloro from 1955 to 1972, Calrose from 1967 to 1992 and Amaroo from 1987 to 2002. By allowing for the yield increases due to cultivar changes it is possible to model yield for 236 region-year combinations. Two forecasting systems were tested. One was a set of regression models for individual regions, based on monthly temperatures. The other was a dynamic model that simulated daily growth in relation to solar radiation and temperature for all regions. Both systems included variables describing the technological changes and both led to acceptable yield forecasts, with a standard error of prediction of about 0.5 t/ha.

RICE BREEDING FOR COLD TOLERANCE IN SOUTHERN BRAZIL

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Rio Grande do Sul (RS) is the Southern most state of Brazil and the first in rice production, contributing with almost 50% of the total rice produced in this country. Every year about 1 million ha of rice are planted in RS, of which 100% is cultivated under permanent flood. Within the rice growing season, which extends from late September until late March, some cold periods are observed, in which the average temperature goes below 20°C, which affect rice development and the final yield. The intensity and duration of these cold temperatures vary according to the region within the state and to the stage of development of the crop. In general, cold damage during the reproductive stage is more often observed in the Southern part of RS, near the frontier with Uruguay, where most of the cold temperatures come from. But during the initial stages of development, such as germination and establishment, cold temperatures are usual in most rice growing regions, lowering germination rates and extending the time to crop establishment and further development. Based on this, the breeding program for cold tolerance conducted at the RS Rice Institute (IRGA) has a short term goal of developing genotypes with cold tolerance at the germination stage and a long term goal of developing genotypes with cold tolerance at the reproductive stage. In this paper we intend to briefly describe the strategies of the program in order to achieve these goals.

The cold tolerance breeding program conducted in IRGA started in the 1993/94 growing season with the sowing of advanced breeding lines in Santa Vitória do Palmar. This town council is located 20

Km from the frontier with Uruguay, at the 33° S Latitude and is one of the coldest regions of RS. The objective was to do a cold screening under field conditions in order to select cold tolerant rice genotypes. Selection of segregant breeding lines only started in the 1995/96 growing season with the sowing of the F₂ generation of crosses between Indica adapted genotypes and cold tolerant sources from USA. The subsequent generations were field selected at that location and at the 2000/01 growing season the best genotypes entered the Preliminary Yield Trial conducted at Santa Vitória. During the subsequent two years the best lines were further evaluated in an Advanced Yield Trial. This strategy proves to be efficient to select adapted genotypes, but due to the unpredictable occurrence of cold temperatures, there was no progress in selecting cold tolerant genotypes at those conditions. Due to this, since 2001/02 selection of cold tolerant segregant lines is being performed under controlled temperature conditions with the results of these tests being used as an additional criterion for selection. This type of test is applied only for the germination stage, in which a sample of the segregant generations sowed at Santa Vitória are submitted to germination under 13°C for 25-30 days with two controls (a tolerant and a sensitive) and evaluated for percentage of seeds with coleoptile longer than 5 mm. Evaluation of genotypes at the vegetative and at the reproductive stages under controlled temperature conditions are also performed, but only for the choice of parents in order to know more precisely the reaction of the introduced sources. At this point, studies regarding methodology adjustment for cold tolerance evaluation under controlled temperature were performed as well as studies on the inheritance of cold tolerance at the germination stage.

Recently, genotypes of the Preliminary Yield Trial (2003/04) conducted at Cachoeirinha were characterized for cold tolerance at the germination stage under controlled temperature, with many of them presenting a high level of tolerance combined with high yield potential, adaptation and excellent grain quality.

A critical evaluation of the results obtained so far and the further strategies to be adopted by the IRGA breeding program for cold tolerance will also be presented.

GENETIC CONTROL OF COLD TOLERANCE DERIVED FROM EASTERN EUROPEAN RICE CULTIVARS HSC55 AND PLOVDIV 22

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Cold tolerance is an important trait for rice cultivars grown in temperate regions of the world. Previous studies have identified useful genotypic variation for cold tolerance among diverse rice cultivars. However, in Australian studies, many of the cold tolerant cultivars had shorter growth duration compared to commercial cultivars. To determine if there was a relationship between growth duration and cold tolerance, the cultivars HSC55 and Plovdiv 22 were each hybridised with two NSW commercial cultivars Illabong and Millin and the progeny (206 F_{5:7} lines) evaluated for cold tolerance in temperature-controlled rooms. Four populations were exposed to cold air temperature during the reproductive stage. Cold tolerance, measured as number of fertile grains, was independent of early flowering. We conclude that cold tolerance could be improved while selecting for cultivars with a range of growth durations. We further concluded that the cold tolerance of Eastern European varieties was not genetically linked with short crop duration, but is non-causally associated as cultivars grown in Eastern Europe require a short duration and mid-season cold tolerance. The challenge for Australian plant breeders is to capture the cold tolerance in cultivars of appropriate growth durations, while maintaining grain quality. This is a reasonable breeding objective given the high heritability and high frequency of progeny having cold tolerance in this experiment, especially from the Hungarian cultivar HSC55.

SCREENING FOR RICE COLD TOLERANCE: LOW TEMPERATURE EFFECTS ON FLOWERING

K.M. Fox^{1,2}, R. Subasinghe^{1,2}, P.D. Looby^{1,2}, and D.L. Wornes¹

¹NSW Dept Primary Industries, Yanco Agricultural Institute, PMB, Yanco, NSW, 2703;
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Low temperature damage during the early pollen microspore stage of rice (*Oryza sativa*) is a major factor limiting yield in the Australian Rice Industry. A key focus of the research is to identify cold tolerant cultivars by comparing characteristics such as flowering date, engorged pollen number and anther and stigma size at flowering, with estimated and real sterility at harvest. Estimated sterility has previously been found to be highly correlated to real sterility ($r^2 = 0.80$) and is a quick and effective method for determining the cold tolerance of cultivars. This study compared the delay in flowering due to the cold treatment with estimated sterility at harvest. Control and treatment plants, consisting of 103 cultivars from diverse origins (Australian and International) were grown in a glasshouse until a panicle length of approximately 20mm was reached. Treatment plants were then transferred to a cold water (bore) facility and exposed to a constant water temp of 19°C and a water depth of 30cm. Flowering was delayed for most cultivars when exposed to the cold treatment. However, the relationship between this delay in flowering and the changes in estimated sterility was low ($r^2 = 0.17$). This indicates that the effect of low temperature on growth and phenology and sterility are independent phenomena. Furthermore, the results showed that the duration of cold treatment had some effect on delay in flowering ($r^2 = 0.49$) and on sterility ($r^2 = 0.16$). Therefore, further research reducing the time plants are exposed to low temperature is recommended.

VARIETAL IMPROVEMENT UTILIZING COLD TOLERANCE GENES OF DONGXIANG WILD RICE

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Dongxiang wild rice (DXWR) was found in 0.3 hectares of a reserve on Dongxiang County (28°14'N, 116°36'E) in Jiangxi Province in China. It shows pest-resistance and survives cold weather and arid regions. It can stand temperatures of minus 12.8°C, far outstripping other varieties of rice with strong cold tolerance. We screened four chilling-tolerant varieties and a male sterile line surviving in winter from crossbreeding of DXWR and cultivars. The chilling-tolerant varieties of TD-1, TD-2, TD-3, TD-4 were derived from the DXWR×cultivar 88-1. We measured seeding survival rate (SSR), relative conductivity (RC) and chloroplast pigment degradation rate (CPDR) on the 4 chilling-tolerant varieties and four conventional varieties of R120, R128, R195 and Gui 99, when subjected to a temperature of 1±1°C for 3 days during the seeding stage. The results indicated that the average SSR of chilling-tolerant varieties were 71% higher than that of conventional varieties, the average RC and CPDR of chilling-tolerant varieties were respectively 98% and 124% lower than those of conventional varieties. It showed these varieties have strong chilling-tolerant features. The male sterile lines derived from the DXWR×cultivar 88-2 can survive in the winter in Nanchang region where it can flower at a temperature under 15°C.

SCREENING FOR LOW-TEMPERATURE TOLERANCE AT THE REPRODUCTIVE STAGE IN RICE: DIFFICULTIES INDUCING FLORET STERILITY

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An accurate and repeatable screen for cold tolerance is essential for the genetic improvement of rice cultivars. During the development of a screening facility at Deniliquin, initial experiments were conducted using standard varieties of known and varying levels of low temperature tolerance. We identified a number of sources of variation which influenced floret sterility levels. Spatial variability in the glasshouse, effects of pot location within tubs and duration of exposure to low temperature have all influenced floret sterility. Experimental design and methods need to account for these sources of variation to avoid misclassification of genotypes under evaluation.

However, the ability to adequately and regularly induce floret sterility as a measure of low temperature tolerance during the reproductive stage has proven difficult. In a similar screening system using controlled-environment facilities at Yanco Agricultural Institute, a temperature regime of 27°C day and 13°C night effectively identified genotypic variation for low temperature tolerance during the reproductive stage. Adoption of this temperature regime in the Deniliquin facility has failed to produce similar levels of floret sterility.

This paper discusses progress in establishing a reliable and repeatable screen in the Deniliquin glasshouse facility.

EVALUATION OF RICE CULTIVARS FOR COLD TOLERANCE IN RELATION TO COLD STRESS ON SPIKELET STERILITY

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Low temperature stress causes serious cold damage to rice plant during different stages of growth. Cold stress induces low seed germination rate, leaf discoloration, delayed heading and spikelet sterility. The most sensitive stage to cold stress is heading that determines grain yield. We have used three evaluation methods to determine cold stress induced spikelet sterility such as; (1) Irrigating cold tolerance nursery to a water temperature gradient of 17°C with continuous flowing of cold water at 5 cm water depth until maturity; (2) Irrigating cold tolerance nursery under different water depth with 17°C water temperature; (3) Screening for spikelet sterility under 18°/18°C of air and water temperatures for 10 days during heading. Spikelet sterility was positively correlated with heading delay, reduction of culm length and panicle exertion. Rice cultivars were classified into five model groups according to percent spikelet sterility from highly tolerant to sensitive cultivars at different water temperature gradient in the cold tolerance nursery. Tall indica rice cultivars are more sensitive to spikelet sterility under different cold-water depth treatment compared to temperate japonica rice cultivars. The derivatives of indica and japonica crosses, Tongil rice cultivars were also highly susceptible to cold stress. However, the cold tolerant Korean japonica rice varieties, Odaebyeo and Jinbubyeo showed high spikelet fertility at all levels of water depth with cold stress. Other rice cultivars such as Hexi 25 and Stejaree 45 (japonica adapted to high altitude areas) and K39-96-1-1-2 and China 1039 (tropical indica) had higher spikelet fertility under cold stress.

ECONOMIC ANALYSIS OF DEVELOPING COLD TOLERANT VARIETIES OF RICE IN AUSTRALIA

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Rice is the most important crop on irrigated large area farms in the southern NSW. Australian rice yields are among the highest in the world, but because of the occurrence of low night temperatures during reproductive development the yields are not reliable. In 75% of the years, rice farmers suffer yield losses due to cold damage. Research is being undertaken to develop cold tolerant varieties that have equivalent yield and quality to current local commercial varieties. This paper examines measures of losses due to cold and the gains from developing new cold tolerant rice varieties. In addition, the returns on R&D investments in breeding for the development of cold tolerant varieties are estimated, considering total benefits and costs involved, lags in development and adoption of new varieties, rate and extent of adoption of new varieties etc. The results provide a basis for establishing improved priorities for research in rice.

RICE BREEDING FOR COLD TOLERANCE IN URUGUAY

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Uruguay grows 195,000 ha of irrigated rice, all long grain *indica* and tropical *japonica* varieties. Grain yield average in recent years has been 6.3 t/ha. The warmer months of summer, January and February, have an average of 10 days each with minimum temperatures below 15°C and yields are often affected by low temperatures during the reproductive phase, resulting in increased sterility. The local breeding program has released some varieties, such as INIA Tacuarí, that has contributed to stable grain yield and provided more flexibility to seeding dates. In previous field experiments, average minimum temperatures during 10 days pre-flowering (10DPF) ranged from 12 to 22° C. Average minimum temperatures below 17° C for 10DPF, resulted in a sharp increase in grain sterility in susceptible cultivars. For the lowest temperatures of the range (12-13° C) grain sterility was higher than 70% for the susceptible varieties Bluebelle and El Paso 144, and lower than 30% for INIA Tacuarí. All these varieties show an important reduction in growth duration to heading when seeding is delayed from early October to late December. If degree days accumulation is considered, however, all varieties have fairly constant requirements, either in early or late plantings, showing that reduction in days to flowering is not associated with photoperiod response. Some new promising breeding lines, as L2825CA and L3616, have also shown improved cold tolerance compared with INIA Tacuarí. In recent field experiments, grain sterility of L3616 was not significantly associated to minimum temperatures 12 days pre-flowering. Its mean sterility was 15.8% while INIA Tacuarí reached 40% for the lowest temperatures of the range.

MECHANISM OF CHILLING INDUCED POLLEN FAILURE IN RICE

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A cellular approach has been undertaken to understand development and cellular organization of anthers in normal and chilling-induced rice plants. Changes in cellular ultrastructure that have been exposed to mild chilling during microspore development include unusual starch retention and accumulation in the plastids of the endothecium in postmeiotic anthers, abnormal vacuolation and hypertrophy of the tapetum and lack of normal pollen wall formation. Anthers observed during late meiosis and tetrad release also seem to have callose (1,3- β -glucan) wall abnormalities as shown by immunocytochemical labeling.

These ultrastructural lesions might arise from damage to critical biochemical processes including sugar metabolism in the anthers and its use by the microspores. Vacuolation and hypertrophy might be symptoms of osmotic imbalance triggered by the reabsorption of callose breakdown products. Premature callose breakdown could also restrict the basic framework of the future pollen wall. Perturbed carbohydrate metabolism during chilling could mediate unusual starch storage in the endothecium and also enhance other symptoms of vacuolation and poor microspore wall formation.

To understand the processes of carbohydrate metabolism, temporal and spatial gene expression of apoplastic invertase (*OsINV4*) and monosaccharide transporter (*OsMST7*) genes have been studied in normal and chilling-induced rice anthers. mRNA *in situ* hybridization studies of both *OsINV4* and *OsMST7* genes show distinct temporal and spatial expression patterns in the normal anthers and different levels of expression in chilling-induced rice anthers.

ILLUMINATING ANTHERS WITH ELECTRONS AND LASERS: THE CELL BIOLOGY OF CHILLING DAMAGE IN RICE

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Much of the information that we have from the literature concerning normal development and stress-induced cellular changes in anthers has come from static light and electron microscope images from fixed and sectioned material. These images have provided much useful information on the cell biology of anther development and the response of these cells to chilling. However there are always concerns that such ultrastructural changes are merely artefacts of tissue preparation. While continuing to develop high quality electron micrographs, we have also been using confocal laser scanning microscopy to confirm that many of the observed changes are clearly present in living anthers. Although confocal microscopy does not have the resolution to provide fine detail of cellular changes, it does have the ability to reveal structural and physiological changes in the rice anther as they are occurring. With these techniques we have revealed a highly dynamic system ranging from developmental changes in mitochondrial activity and images of highly energetic microspores through to chilling induced tapetal plasmolysis, disruption to the tapetal tubular vacuole system, and vacuolation of microspore nuclei.

MOLECULAR BIOLOGY OF RICE COLD TOLERANCE: I. EFFECT OF COLD ON ANTHHER SUGAR METABOLISM AND ABA BIOSYNTHESIS

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The young microspore stage of rice pollen development is highly sensitive to cold temperature damage. Cold temperatures at the young microspore stage cause abortion of normal pollen development and result in pollen sterility. Australian rice varieties are particularly sensitive to cold-induced pollen sterility. One of the obstacles to successfully breeding cold tolerant rice varieties in Australia is that the molecular mechanism of cold-induced pollen sterility has not yet been defined. Previous studies of drought-induced pollen sterility in wheat and rice have indicated that a disruption in anther sugar metabolism is involved in causing sterility. Our work has shown that sugar metabolism is also disrupted in rice anthers by cold stress. Cold temperature at the young microspore stage causes an accumulation of sucrose and reduction in cell wall invertase activity in anthers, and a failure of starch deposition in mature pollen. With the aim of identifying the genes responsible for causing these changes in sugar metabolism, we have identified four invertase genes and two monosaccharide transporter genes that are expressed in rice anthers. Of these, the cell wall invertase gene *OSINV4* appears to be anther-specific, and is down-regulated by cold in anthers. The monosaccharide transporter gene *OSMST8* is also down-regulated by cold in anthers, suggesting that a sugar transport pathway involving *OSINV4* and *OSMST8* could be blocked by cold. The expression of another monosaccharide transporter gene, *OSMST7*, was induced by cold, which could suggest that cold stress triggers the re-direction of sugars away from the developing pollen. We also investigated the possible role of the abscisic acid (ABA) hormone as a signal for causing the cold-induced changes in sugar metabolism. ABA levels increased in anthers in response to cold treatment at the young microspore stage. ABA application to developing panicles mimicked the effect of cold treatment on grain yield and the expression of the sugar metabolic genes *OSINV4*, *OSMST7*, and *OSMST8*. Our results indicate that an anther-specific synthesis of ABA is associated with pollen sterility, and that ABA could act as a signal for causing sterility, possibly in combination with the changes in sugar metabolism.

MOLECULAR BIOLOGY OF RICE COLD TOLERANCE: II. GENE EXPRESSION PROFILING OF THE COLD RESPONSE IN COLD SENSITIVE AND COLD TOLERANT RICE

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We constructed microarrays containing 18,000 anonymous clones from rice anther cDNA libraries to carry out gene expression profiling of the cold response in rice anthers. We identified a set of genes in the cold sensitive Australian variety *Doongara* that is differentially expressed in response to cold at the cold-sensitive stage of anther development, the young microspore stage. We also compared the cold response at different stages of anther development (mothercell, pre-mothercell, tetrad and early and mid uni-nucleate stage) and found that the cold response changes quickly during anther development. Comparison of the response to cold and water stress demonstrated that there is a significant overlap between the two abiotic stresses. This may suggest that cold and water stress use the same mechanism to induce pollen sterility in rice. The availability of cold-tolerant lines allowed us to use microarray gene expression profiling to compare the cold response in cold tolerant rice to the cold response of *Doongara*. We found that expression of the genes encoding anther cell wall invertase (*OSINV4*) and monosaccharide transporter (*OSMST8*) were not repressed to the same extent by cold in two Chinese cold-tolerant rice varieties. We used microarray screening to compare the cold response of these two Chinese cold-tolerant varieties to *Doongara* and found substantial differences. The possibility to exploit this information for the development of molecular markers for marker assisted breeding of cold-tolerant rice will be discussed.

DIVERSE MECHANISMS OF LOW TEMPERATURE STRESS TOLERANCE IN RICE PLANTS

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Rice (*Oryza sativa* L.) which was originated from the tropics or subtropics, is widely cultivated in diverse environmental conditions. Compared with winter cereal crops, rice is much more sensitive to low temperature during the growth period. Male sterility is the most severe consequence among the chilling-induced agronomic damages in rice production. The developmental stages from pollen formation to fertilization are sensitive to low temperature throughout the life cycle of rice plants. In particular, the young microspore stage in pollen development is the most sensitive to low temperature. Exposure of rice plants at the tetrad stage to moderately low temperature (12°C) for 4 days resulted in male sterility in 80% of spikelets. Microscopic observation of developing anthers of rice suggested that one possible reason for the male sterility after low-temperature treatment was the failure of anther development. Low temperature also affected the physiological status of anthers. Non-reducing sugar content increased rapidly, while the acid phosphatase activity decreased in the moderately temperature-treated rice plants. Phytohormones, gibberellins and auxin, were associated with chilling-induced male sterility. However, it is still largely unknown how chilling temperature induces molecular events that result in male sterility in rice. In this report I will discuss recent advances in genetics and genomics on low temperature stress tolerance of rice plants which have been carried out at National Agricultural Research Center for Hokkaido Region, Sapporo, Japan.

LOW TEMPERATURE CHANGES PROTEOME OF MALE GAMETOPHYTE DEVELOPMENT IN RICE

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We used proteomic analysis to investigate the changing patterns of protein synthesis during pollen development in rice anthers and their responses to low temperature treatment that cause male sterility in rice. An anther proteome database of the proteins separated by two-dimensional gel electrophoresis (2-DE) was established and publicly accessible at <http://semele.anu.edu.au/2d/2d.html>. Over 3,000 protein spots were detected over the pH range of 4-11. Of these, over 500 protein spots were analysed by MALDI-TOF MS (matrix-assisted laser desorption/ionisation time-of flight mass spectrometry), tandem MS and N-terminal sequencing and putative identities were assigned to more than 100 protein spots. Then, we compared the anther proteome maps of different developmental stages and detected approximately 150 protein spots changed consistently during development. Of these, 44 proteins were identified including proteins are closely associated with sugar metabolism, cell elongation and cell expansion. Furthermore, we detected 37 protein spots as differentially displayed after one, two and/or four days of cold temperature treatment in the cold sensitive cultivar Doongara. Of these, 13 proteins were identified including a potential negative regulator of male gametophyte development. We have further analysed specific expression and localisation of some of the most interesting proteins. Most significant findings of these studies will be highlighted.

CHROMOSOMAL LOCATION OF QTLs CONTROLLING LOW TEMPERATURE INDUCED MALE STERILITY IN TEMPERATE *JAPONICA* RICE

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Cold sensitivity at booting stage is considered to be the most yielding-affecting factor of the Australian rice industry. To date, the mechanism and genetics of cold tolerance are not well understood. In this study, we established a double haploid (DH) population of 95 lines derived from a cross between two temperate *japonica* rices, Doongara (cold sensitive Australian variety) and M103 (moderately cold tolerant Californian variety), was used to identify the chromosomal location of QTLs for cold tolerance using microsatellite mapping. Cold tolerance was measured as the degree of floret sterility of plants treated at a 12°C for 3 days in a growth chamber. Floret sterility from plants grown under normal conditions was used as a control to screen for background sterility caused by genetic variation. 42 polymorphic microsatellite markers were identified between the two parent lines for QTL analysis in the double haploid lines. Putative QTLs for cold tolerance were identified on chromosomes 3, 5, 6, 7, 8, 9 and 12. The amount of phenotypic variation that was explained by individual chromosome location ranged from 9% to 37% ($P < 0.05$). The QTLs located on chromosome 3 and 7 showed the strongest linkage ($P < 0.01$) with cold tolerance.

Posters

CHALLENGES FOR RICE PRODUCERS IN MYANMAR

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Myanmar is a country of over 50 million people, 75% of whom live in rural areas. Over 40%, about 6.5 million ha, of the 15.8 million hectares of agricultural land under various crops is used to produce rice but rice shortages are widespread. Average yield are about 3.4 tonnes/hectare. The land area is 671,000 km² (approximately 925 km from east to west and 2090 km from north to south) and is located between 9°32' and 28°31'N latitudes and 92°10' and 101°11'E longitudes. The agriculture sector contributes 45.1% of GDP, 18% of total export earnings and employs 63% of the labour force. Myanmar is rich in water resources. Annual rainfall ranges from 750 mm in the alluvial lowlands of central region to 1500mm in the eastern and western mountains to 4000-5000 mm in the coastal region. Only 6% of the total surface water resources of 1081km³ per annum are being utilised. The total ground water potential is approximately 500 km³ per annum.

So what are the challenges for rice producers in Myanmar? A visit to the more northern area in Shan State in 2004 revealed several aspects of rice production which impact on yield potential.

1. Cold in northern regions, especially between December and April when summer crops are grown.
2. Efficiency of water capture and delivery.
3. Fertilizer inputs and utilization.
4. Pests and diseases.

The paper will consider restrictions to yield due to temperature, water and nutrient interactions in cooler northern highland areas of Myanmar, and suggest breeding and management options. International linkages are urgently needed to harness and apply relevant technologies.

CELL BIOLOGY OF CHILLING DAMAGE IN RICE ANTERS

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Chilling temperatures cause a great reduction of yield in rice during anther development in Australia. To address this problem, an Australian sensitive variety, Doongara, and two Chinese tolerant varieties, R31 and R32, were used to determine their cellular response to chilling. Overall, the chilling-induced ultrastructural changes were similar but the severity of the damage was different between the three varieties. Ultrastructural changes included cell degeneration, death and/or shrinkage, formation of vacuoles or spaces in the tapetum and sporogenous cells, visible starch storage in the endothecium chloroplasts and delay or poor formation of orbicles and the microspore wall. Both ultrastructural and living anther observations indicated that cell degeneration at the pollen mother cell stage was significantly higher than that at other stages. Anthers of R32 had high levels of ultrastructural damage at this stage of development but its seed set, while slightly lower than R31, was significantly higher than Doongara. Sporogenous cell damage in R31 was the lowest of the three varieties. The mechanism of response to chilling temperatures seemed to be different in the three varieties.

MANAGE RICE – CROP-MANAGEMENT SOFTWARE FOR THE AUSTRALIAN RICE INDUSTRY

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MANAGE RICE is a software package used by about 600 of the 2000 ricegrowers and advisers in the Murray and Murrumbidgee valleys. The main function is prediction of yield response to topdressed N fertiliser using a simulation model based on cold damage, tissue-N status, flood-water depth at the microspore stage, cultivar and sowing date. It calculates riskiness of yield response to N in relation to cold damage, profitability in relation to grain price and fertiliser cost, and the probability of achieving a target return.

The software also assists N management of zones of a field, drawn on the computer screen by the ricegrower. The basis for delineating zones is an underlaid image such as a yield or electromagnetic induction map, imported into the software and georeferenced by the user. The optimum N is calculated by the simulation model for each zone.

Since 2001 three new functions have been added, based on automatic downloads of daily temperature, evapotranspiration (ET) and rainfall (<http://www.clw.csiro.au/services/weather>).

(1) Predictions of the developmental stages of panicle initiation, microspore, flowering and physiological maturity, based on cultivar, sowing date, photoperiod and daily downloaded temperature. (2) A water-use calculator based on daily ET, rainfall, percolation below the root-zone and crop duration. The calculator uses downloaded ET from sowing to the current date (from the computer clock) and then long-term average ET from that date until maturity. The information assists ricegrowers in planning future needs of irrigation water. (3) Prediction of optimum harvest date, based on rate of grain-drying, percent whole grain and soil dry enough for harvesting. The rate of grain drying is estimated from an initial measurement of grain moisture, daily ET (actual downloaded or estimated long-term average), and soil moisture status. Percent whole grain is estimated from the moisture content and rate of drying. The soil moisture is estimated from the date of drainage and daily ET.

INVERTASE GENE EXPRESSION IN NORMAL AND CHILLING INDUCED RICE ANTHERS

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Cold induced male sterility is a major problem in Australia and other rice growing areas. Ultra structural studies from our group have shown that, among other symptoms, starch accumulation and poor microspore wall formation are the major consequences of chilling in rice anthers. These problems are likely to be related to sugar metabolism being affected by chilling. Of particular interest is the apoplastic invertase enzyme which plays a key role in cleaving sucrose in the cell wall. Using mRNA *in situ* hybridization technique, we have seen spatial expression of the invertase gene (*OsINV4*) in the anther. Results show differences in the level of expression of invertase, between normal and chilled anthers.

PROTEOME ANALYSIS OF MALE GAMETOPHYTE DEVELOPMENT IN RICE ANTHEERS

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We applied proteomic analysis to investigate the changing patterns of protein expression during the pollen development in rice anthers. Proteins were extracted from the anther samples at different stages of microspore development and separated by two-dimensional gel electrophoresis (2-DE) to produce proteome maps. The anther proteome maps of different developmental stages were compared, and 150 protein spots, which changed consistently during development, were analyzed by MALDI-TOF mass spectrometry to produce peptide mass fingerprinting (PMF) data. Database searches using these PMF data revealed the identities of 40 of the protein spots analyzed. These proteins are closely associated with sugar metabolism, cell elongation and cell expansion; all of which are cell activities that are essential to pollen germination. Eight protein spots that could not be identified by PMF analysis were analyzed by *N*-terminal micro sequencing and database searching. Three of them were identified as novel isoallergens of Ory s 2, the rice homologues of grass group II pollen allergens. Polyclonal antibodies were produced in rabbit using 2-DE purified Ory s 2 proteins and used to investigate the stage and tissue specific expression of Ory s 2 by immunochemical analysis. Interaction of Ory s 2 proteins with other anther and style proteins are currently being investigated using co-immunoprecipitation techniques.