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Summary

Low night temperatures during late January and early February coinciding with early pollen microspore (EPM) development of rice (*Oryza sativa*) is a major factor limiting productivity in the Riverina region of New South Wales (NSW). This project primarily examined genotypic differences in cold damage that are associated with low temperature during reproductive development. The objectives were to: (1) investigate the effects of low temperature on physio-morphological traits of rice plants, with particular emphasis on reproductive traits; (2) examine the consistency of expression of cold tolerance in different screening environments; and (3) quantify the effects of temperature and daylength on the phonological development among cultivars.

Results from three screening environments including temperature-controlled rooms, a cold water facility and field experiments are reported. Over 50 cultivars from diverse origins, including cold tolerant cultivars from Eastern Europe, Japan and California were screened. Cultivars were exposed to day/night air temperatures of 27°/13°C in temperature-controlled rooms and a constant temperature of 19°C in the cold water facility. Exposure time for plants was from panicle initiation (PI) to 50% heading. To increase the likelihood of inducing cold damage in field experiments, several techniques such as multiple sowing dates, shallow water depths (5cm) and high nitrogen rates (300kgN ha^{-1}) were used.

The three screening methods induced sufficient levels of spikelet sterility to identify genotypic differences and consistently categorise cold tolerant cultivars. Among the common cultivars there was a highly significant relationship for spikelet sterility between temperature-controlled rooms and field experiments (r^2=0.52, p<0.01, n=31), temperature-controlled rooms and the cold water facility (r^2=0.63, p<0.01, n=21) and the cold water facility and field experiments (r^2=0.53, p<0.01, n=21). Screening for cold tolerance in temperature-controlled rooms or the cold water facility was preferred to field screening because of the reliability of exposure to low temperature in both environments. However, it is still important to combine a controlled environment screen with field observations since some cultivars varied in their response under different screening methods.

Several flowering traits such as the number of engorged pollen grains per anther, anther length and anther area produced significant genotypic variation and were negatively related to spikelet sterility at maturity. When low temperature coincided with reproductive development Australian and Californian cultivars were inefficient at producing filled grains, despite them having a similar number of engorged pollen grains and similar sized anthers to cultivars from other origins. This inefficiency may be partly related to a small stigma area.

Several cold tolerant cultivars (M103, HSC55, Plovdiv 22, M104 and Jyoudeki) and cold susceptible cultivars (Sasanishiki, Doongara, Nippon Bare, Sprint and Reiziq) were identified. However, many of the cold tolerant cultivars had a shorter growth duration leading to lower yield potential compared to commercial cultivars. Therefore, two shorter duration cold tolerant cultivars, HSC55 and Plovdiv 22, were hybridised with two NSW commercial cultivars, Illabong and Millin, to determine if cold tolerance could be improved. The progeny were evaluated for cold tolerance in temperature-controlled rooms and there was found to be no relationship between

growth duration and spikelet sterility. Although, it should still be possible to produce cold tolerant cultivars with appropriate growth duration for Australian conditions.

Phenological development was examined in sequentially sown field experiments by exposing plants to low temperatures and providing several different temperature and daylength conditions. Amaroo and Millin were identified as mildly photoperiod sensitive, whilst M103 and HSC55 were found to be photoperiod insensitive. A crop phenology model was developed for Amaroo and used to predict an optimum sowing date based on historical weather data from 1955 to 2002. The model minimizes the possibility of exposure to low temperatures during the young microspore and flowering stages. The analysis indicated that the 15th October was the optimal sowing date for Amaroo. Nevertheless, sowing up to November 1st when seasonal temperatures are average also minimizes the risk of encountering low temperatures. Increasing the photoperiod sensitivity of cultivars above that of Amaroo may further reduce the risk of encountering low temperatures and at the same time increase sowing flexibility.

Results from this project have improved our understanding of the mechanisms of genotypic response to low temperature during reproductive development and provided methods to develop cold tolerant cultivars.

1. Background

Cold damage during the reproductive phase of rice development limits NSW rice yields in most years. Low temperatures at the critical stage of pollen development (between panicle initiation and flowering) have reduced yields by approximately 0.68t per hectare annually, equalling $20 million/year.

Rice crops are typically sown from early October until mid November, depending on the duration of cultivar and the availability of irrigation water. An optimum sowing date for each cultivar ensures the young microspore stage occurs during late January to early February when the minimum temperature is historically high (~17^{o}C). However, low night temperatures of 12^{o}C can occur during mid-summer (Boerema 1974) and reduce the yield substantially due to the cold effect on pollen development and subsequent reproductive stages. For example, extended low temperature during the period of reproductive development in the 1995/96 season induced spikelet sterility, which reduced the industry yields by 25% to below 7t ha^{-1}. In Australia, yield loss due to low temperature damage at the reproductive stage is estimated to be 1t ha^{-1} every four years and greater than 2t ha^{-1} every 10 years (Farrell *et al.* 2001).

Increasing the depth of paddock water level, sowing on recommended dates to reduce the likelihood of encountering low temperatures during reproductive development and the use of short duration cultivars are the main crop management strategies presently practiced to limit this damage. However, increasing the depth of paddock water level is not an environmentally friendly strategy for the rice industry which is already facing the impact of water scarcity. On the other hand, use of short duration cultivars does not avoid low temperature damage during panicle development, in particular during microspore development, because there is a high probability of low temperature during January in southern NSW.

In this context, the use of cold tolerant cultivars is a key to improving water use efficiency and maintaining productivity in the variable climate in the Australian rice area. It was estimated that a degree improvement in the cold tolerance of commercial Australian cultivars would reduce the yield losses by half. Improvement in cold tolerance is therefore essential if the sustainability of the rice industry is to be assured. A vast collection of genetic material which has been selected in cool climates such as China, Nepal and Japan could provide a source of cold tolerant genes needed to develop a cold tolerant cultivar in Australia.

However, screening for cold tolerance has shown to be particularly difficult in both the field and controlled environments. The main problem associated with field screening is the uncertainty of cold stress. Moreover, there is no control over timing or severity of a cold event. This means that cultivars or lines of different phenology will experience very different environments making selections across phenologically different germplasm impossible.

Use of controlled environments appears to be the solution for this since it has the potential to control both timing and severity of the cold damage. Thus it is possible to synchronize the cold treatment to cold sensitive stages of the crop. However, this technique has not been as useful as first thought due to unknown causes. The main observed problem is that different controlled experiments resulted in a different rank order in cold tolerance for the same genotype. This indicates that there is a significant

test **X** genotype interaction and hence no useful selection can be achieved. Therefore, screening genetic material for cold tolerance currently requires an efficient and suitable method to overcome such issues.

Research and screening to improve cold tolerance in rice has previously been studied in other countries, although uncertainties exist as to whether their findings and methods are applicable to rice growing conditions in Australia. According to the findings of many Japanese research institutes, outdoor screening using a deep cold water system could overcome such issues. Therefore, outdoor screening using a deep cold water system backed up with field and glasshouse evaluation will result in an accurate measure of the cold tolerance of advanced lines with quantitative data on the level of cold tolerance. This information in turn allows cultivars to be ranked for cold tolerance. Evaluation of the relationship of cold tolerance to associated characters such as flowering will also help to screen cultivars using indirect selection methods.

2. Objectives

This project aimed to identify cold tolerant cultivars by understanding field and glasshouse response to cold with the following objectives:

- To screen new introductions and cultivars under development for cold tolerance using field, glasshouse and cold water techniques;
- To explore the relationship between engorged pollen number per anther and cold tolerance; and
- To evaluate segregating populations for cold tolerance and develop genetic parameters based on various screening techniques.

3. Introductory technical information concerning the problem or research need

Rice (*Oryza sativa*) is grown in temperate and tropical environments ranging in latitude from 49°N to 35°S and therefore seasonal temperatures and daylength vary widely. The development of cold tolerant cultivars is important for many rice growing regions including temperate and high altitude areas in tropical environments. During any stage of crop growth cool weather and cold irrigation water can damage the rice plant (Majumder *et al.* 1989). The young microspore stage, in the middle of reproductive development, has been identified as the stage most sensitive to cold (Satake and Hayase 1970).

Countermeasures exist for farmers to minimise the current levels of cold damage. In Australia, these include sowing at a time when temperature conditions are favourable for crop development, using conservative applications of nitrogen (N) since cold damage is exacerbated at high N and increasing water depth during reproductive development to protect the developing panicle from low night temperatures. Improving the level of cold tolerance of cultivars not only reduces yield losses but also leads to benefits such as improved food security, greater economic growth, and increased water use efficiency.

Rice growing in Australia began in 1922 and is now confined to the Riverina region of NSW, centred at 35°S and 146°E (Lewin and Heenan 1987). The altitude throughout the region is approximately 120m. Only one rice crop can be grown in Australia each year due to the seasonal temperatures. The Riverina has a large diurnal temperature variation of 12° to 15°C and low humidity.

Low temperature during reproductive development is the primary environmental factor that limits rice productivity in Australia (Lewin and Heenan 1987). Low night temperature during late January and early February induces spikelet sterility, causing a reduction in grain yield (Farrell *et al.* 2001b). Historical weather data illustrates that the average minimum temperature during this period is 17°C (Heenan 1984; Lewin and Heenan 1987). However, temperature variability, which can include extreme periods of low temperature, is a major problem that is shared by Australia and other temperate rice producing countries. For example, yields were reduced by 25% in 1996 when night temperatures were 4°C below average for 30 days during reproductive development (Williams 1997). The frequency of yield loss due to low temperature damage at the reproductive stage in the Australian rice industry equates to 1t ha^{-1} every four years and greater than 2t ha^{-1} every 10 years (Farrell *et al.* 2001b). The average loss due to cold damage each year is approximately 0.68t ha^{-1}, which is equivalent to $A20M (Farrell *et al.* 2001b).

3.1 *Developmental stages and cold damage*

Low temperature has a negative impact on all three stages of development; the vegetative stage (germination to PI), the reproductive stage (PI to flowering), and the grain filling stage (flowering to maturity). The degree of cold injury in rice depends on many factors and one of them is the development stage at the time of exposure to cold (Li 1981). Low temperatures at the vegetative stage can affect the rice plant's developmental processes by reducing leaf area development, which reduces canopy photosynthesis. The nitrogen uptake during the vegetative phase is also reduced. Low temperatures during the vegetative phase significantly reduce the yield as there is a strong positive relationship (r^2=0.73, $p<0.01$) between average temperature during the vegetative period and grain yield in Australia. Low temperatures during this stage also prolong the vegetative phase causing extra water usage resulting in low water use efficiencies. Genotypic variation for cold tolerance during the vegetative stage has been identified (Ayotade and Akinremi 1984; Reinke 2000). The young microspore stage during reproduction is the most susceptible stage to cold injury in rice plants (Satake and Hayase 1970). The young microspore stage occurs approximately 10 to 12 days prior to heading (Satake and Hayase 1970; Heenan 1984). Low temperature during the reproductive stage can have two main effects on rice plants. Firstly, low temperature can delay development, increasing the chance of encountering low temperature at the young microspore and flowering stage. Secondly, low temperature can damage physiological components required for fertilisation, such as pollen grains. The threshold temperature that induces spikelet sterility at the young microspore stage differs between cultivars and depends on factors such as the duration of low temperature exposure and growing conditions (Satake 1969). Satake (1976) found that the critical air temperature that induces cold damage ranges between 15°C and 20°C, depending on the

cultivar. The duration of low temperature is also an essential element determining the extent of cold damage. Spikelet sterility increases with the increase in duration of low temperature during the young microspore stage (Hayase *et al.* 1969; Ito 1976; Ito 1978; Nishiyama 1978; Heenan 1984). Low night temperature has been shown to induce spikelet sterility and high day temperature has been shown to alleviate the effect (Shibata *et al.* 1970; Yoshida 1981).

The reduction in the number of engorged pollen grains per anther has been identified as the main cause for sterility resulting from low temperatures during the young microspore stage (Ito 1971; Uchijima 1976; Nishiyama 1983; Heenan 1984; Matsuo *et al.* 1995). The reduction in the number of engorged pollen grains per anther can be attributed to a decrease in the number of differentiated microspores (Hayase *et al.* 1969; Satake 1969; Satake 1991), a reduction in the number of dehisced anthers (Sawada 1978), the cessation of anther development, unripe pollen, poor extrusion of anthers, partial or no dehiscence of anthers and pollen grains remaining in the anther loculi (Nishiyama 1984). This reduced pollen grain number results in a decrease in the number of pollen grains on the stigma and failure of pollen germination (Sawada 1978; Nishiyama 1984). Cytological abnormalities, distorted carbohydrate metabolism, reduced nutrient supply, malfunctioning of certain enzymes and reduced anther respiration are the possible causes for these abnormalities in anthers when exposed to low temperatures.

The threshold value to achieve less than 10% spikelet sterility was found to be approximately 600 to 800 engorged pollen grains per anther (Nishiyama 1983; Kim *et al.* 1989a; Satake 1991; Nishiyama 1996;). Recent studies by Gunawardena *et al.* (2003b) have indicated that cold treatment not only increases the total number of damaged pollen and microspores, but also decreases the engorgement, interception and germination efficiencies of those pollen grains.

The flowering stage is the second most sensitive stage to low temperatures following the young microspore stage (Matsuo *et al.* 1995). Genotypic variation for cold tolerance at the flowering stage has also been identified (Tanno *et al.* 2000). Spikelet sterility due to low temperatures at the flowering stage is related to the inability of pollen grains to germinate on the stigma (Satake and Koike 1983). Khan *et al.* (1986) found that increased spikelet sterility was associated with a reduction in the number of pollen grains intercepted on the stigma. Additionally, duration of exposure to low temperature at flowering and solar radiation levels influence the amount of spikelet sterility (Matsuo *et al.* 1995). Optimum daily air temperature for flowering is 31 to 32°C (Tanaka, 1962 cited in Matsuo *et al.* 1995).

Duration of grain filling is largely affected by temperature, and typically ranges from 30 days in tropical regions to 43 days in temperate regions such as Australia (Kropff *et al.* 1994). The duration of grain filling is extended in cool

climates because low temperature causes a delay in the translocation of sugars that are converted to starch and stored in the grain. In temperate rice growing regions, grain ripening occurs as air temperature decreases, which can affect grain yield. For example, a late sown December crop in Australia has a reduced yield as a result of truncated grain growth combined with low temperature during the grain filling stage (Reinke 1993). Additionally, low temperature during grain filling in Australia causes increased accumulation of starch, slow grain filling and production of larger grains.

3.2 *Plant characters related to cold tolerance*

Most research on cold tolerance during reproductive development has investigated a wide range of characters other than spikelet sterility. In particular, anthers and pollen grains have been extensively studied. Genotypic differences for cold tolerance in rice are closely related to anther length (Hashimoto 1961 cited in Satake 1989; Suzuki 1982; Nishiyama 1983; Nakamura *et al.* 2000) and the number of engorged pollen grains per anther (Suzuki 1982; Nishiyama 1983; Kim *et al.* 1989b; Satake 1989; Lee 1990; Nishiyama 1996; Nakamura *et al.* 2000). The results of these investigations highlighted the importance of high pollen number in increasing cold tolerance.

Early observations revealed that there is little damage done to pistils (female organs) by cooling at the meiotic stage (Hayase *et al.* 1969). However, stigma length has been shown to be negatively correlated with spikelet sterility (Suzuki 1985) as a long stigma may intercept a higher number of pollen grains at anthesis due to a wider surface area. Measurements of stigma length could therefore be used to give an estimate of the level of spikelet sterility caused by low temperature.

Research has also shown that root activity is related to the ability of rice plants to withstand low temperature stress (Yamamoto and Nishimura 1986; Xiong *et al.* 1993; Nishiyama 1996). Bleeding water from the stem, which depends on root pressure and hence the size and activity of the root system, could be an index for the evaluation of the degree of cold tolerance during reproductive development (Yamamoto and Nishimura 1986). The amount of water that bled under low temperature conditions was highly correlated with spikelet sterility (Yamamoto and Nishimura 1986).

3.3 *Genotypic variation of cold tolerance*

Many investigations have reported that several major genes may be involved in the cold tolerance of cultivars released in northern Japan (Sawada 1978; Moon 1984; Matsuo *et al.* 1995; Nakamura *et al.* 2000). A large variation in the number of engorged pollen grains per anther among cultivars in the cold tolerant group (1200 to 1900) suggests that several genetic factors are involved in cold tolerance of rice (Nakamura *et al.* 2000). These major genes may correspond with the four key fertilisation components including number of differentiated microspores, percentage of developed pollen grains,

percentage of shed pollen grains and fertilisation efficiency of shed pollen grains (Satake and Shibata 1992; Gunawardena *et al.* 2003b).

There are wide genotypic differences for cold tolerance during reproductive development in rice (Board and Peterson 1980; Matsuo *et al.* 1995; Nakamura *et al.* 2000). One of the major differences between the two types of rice, indica and japonica, is how effectively they adapt to low temperature. Japonica cultivars are generally more cold tolerant than the tropical indica cultivars (Kaneda and Beachell 1972; Mackill and Lei 1997). However, despite the japonica type being more adapted to temperate environments than the indica type, the japonica type remains sensitive to low temperature, particularly during reproductive development.

Large genotypic variation for cold tolerance exists within more than 100,000 rice cultivars worldwide. Some cultivars that originated from cooler climates contain cold tolerance genes. The International Cold Tolerance Nursery (ICTN) carries a selection of cultivars from different origins, which have been evaluated in a range of countries for their level of cold tolerance.

Despite Calrose, a japonica from California, being in the genetic background of most Australian cultivars (Lewin and Heenan 1987), these cultivars are cold susceptible. No screening facility has been developed for testing cold tolerant cultivars in Australia, so little is known about the levels of cold tolerance among Australia's cultivars.

3.4 Cold screening methods

Most of the physiological studies have been conducted in temperature-controlled environments because low temperature can be reliably maintained. However, the spatial limitations of these environments have restricted their potential to screen large quantities of genetic material for cold tolerance. Screening for cold tolerance under natural conditions in the field is difficult because of the unpredictability of low temperature conditions combined with differences in phenological development among cultivars. In temperate rice growing areas, five methods are available to increase the likelihood of cold damage during reproductive development in the field. The first option is to stagger sowing dates of cultivars to increase the probability of encountering low temperature events. Secondly, maintaining shallow water levels will expose the developing panicle to cooler air temperature. The third method involves applying high N (200 t0 300kgN ha^{-1}) rates to the plant to increase its susceptibility to low temperatures. The fourth is to establish a field trial situated in cooler climates. Finally, cold water sourced from deep aquifers, chillers, or from the base of dams has been successful in selecting for cold tolerance in the field. Many experimental stations in Japan (Nishiyama 1996; Nagano 1998) and Korea (Lee 2001) have successfully used cold water to screen material for cold tolerance.

Currently in Australia, plant breeders identify cold tolerant rice by growing lines in the Murray Valley, the southern part of the NSW rice industry. However, it is difficult to expose a large range of genetic material to low temperature during the young microspore stage. The challenge therefore exists, to develop screening facilities and protocols to screen for cold tolerance. Successful incorporation of cold tolerance in high yielding cultivars depends on the identification of appropriate genetic donors and on the development of suitable screening methods and breeding strategies (Majumder *et al.* 1989). Screening for cold tolerance can either be done directly at harvest using characteristics such as spikelet sterility and yield, or indirectly with flowering characteristics such as engorged pollen number per anther and anther length (Nakamura *et al.* 2000).

3.5 Sowing time and cold damage

Australian rice crops are sown from late September until mid November, depending on factors such as land preparation, growth duration of a cultivar and seasonal conditions. The sowing time of Australia's rice crop is important for three major reasons. Firstly, to ensure that vegetative growth occurs during a period of suitable temperatures and high levels of solar radiation. Sowing full duration cultivars during early October is a common practice as the temperature is suitable for crop establishment, ensuring plants use the entire season. Secondly, the optimum sowing date for each cultivar ensures the stage susceptible to low temperatures occurs when the minimum night temperatures are historically the warmest during late January to early February. The long term average minimum temperature for this period is around 17°C but the 10 day average temperature can fall below 12.5°C. On average a minimum temperature of less than 12.5°C occurs in one year out of 10. Thirdly, sowing on time ensures that grain filling occurs when milder autumn temperatures are more likely, allowing for good grain quality.

Highest yields are observed when crops flower before late February and low temperature damage is minimal during the microspore stage. This requires long and short-season cultivars sown in late September and late October respectively (Williams and Angus 1994). However, low temperature during the young microspore stage is not the only factor limiting Australian rice yields (Reinke 1993). Other factors, such as temperature conditions during grain filling, contribute to low yields.

3.6 *Management of nutrients*

Nitrogen is one of the major nutrients applied to rice crops, and N uptake by the plant is considered a key determinant for achieving high yields (Williams and Angus 1994). High N content in the rice plant increases the risk of yield reductions due to low temperatures during reproductive development (Sasaki and Wada 1975; Yoshida 1981; Haque 1988). High N reduces the number of engorged pollen grains per anther, causing increased spikelet sterility, when low temperatures occur during reproductive development (Gunawardena *et al.*

2003a). High N conditions can also delay panicle development (Gunawardena 2002), thereby increasing the risk of encountering low temperatures during the young microspore and flowering stages.

Research has shown that phosphate can reduce N induced spikelet sterility (Sasaki and Wada 1975; Choi 1987; Matsuo *et al.* 1995). Potassium may also play an important role in altering the physiological constitution of the rice plant to counteract the combined adverse effect of high N and critically low temperatures at the young microspore stage (Haque 1988). Additionally, application of farmyard manure is believed to be effective in avoiding spikelet sterility due to low temperature at the young microspore stage (Nishiyama 1984).

3.7 *Deep water*

Deep water irrigation is one of the most effective countermeasures against spikelet sterility induced by low temperatures during reproductive development (Sakai 1949 cited in Satake *et al.* 1987; Kobayashi and Satake 1979). Since the specific heat capacity of water is higher than that of air, the flood water in a rice crop cools more slowly at night than air temperature (Godwin *et al.* 1994). The difference between night temperature in the flood water and air will depend on day temperatures, depth of water and its turbidity (Uchijima 1976). Deep water irrigation during the young microspore stage provides protection to the developing panicles as the daily average water temperature is 3 to 4°C higher than that of air (Satake *et al.* 1988). The benefit of deep warm water during low night temperature events is about 2 to 5°C in Japan (Satake *et al.* 1987), 5 to 6°C in California (Board *et al.* 1980) and 3.5 to 7°C in Australia (Williams and Angus 1994; Gunawardena *et al.* 2001).

Many factors affect water temperature such as water depth, amount of canopy cover, diurnal temperature variation (Watanabe and Takeichi 1991; Ichimura *et al.* cited in Collinson *et al.* 1995), incident solar radiation, wind speed and humidity (Collinson *et al.* 1995).

Maintaining deep water to insulate the developing panicle from low air temperature is still one of the most effective ways to counteract spikelet sterility. A water depth of 17 to 20cm covers approximately 80% of the cool susceptible spikelets (Kobayashi and Satake 1979). More recently, it has been shown that more than 25cm is required for complete submergence of panicles during microspore development and more than 30cm is required when a high rate of N is applied (Gunawardena *et al.* 2001). A steady increase in water depth was important for complete submergence (Gunawardena *et al.* 2001).

4. The Methodology

Following paragraphs give a summary of methodologies used in a series of glasshouse and field experiments. The detailed methodology is described in the thesis submitted to the Rice CRC by Dr. T.C. Farrell (2004).

4.1 *Genotypic variation in response to low temperature during reproductive development.*

Two pot experiments were conducted using temperature-controlled environments at Yanco Agricultural Institute (YAI) (34°37'S, 146°25'E, alt. 140m) to evaluate the genotypic variation in spikelet sterility and flowering characteristics in response to low temperature during reproductive development.

The first experiment (experiment 1) was conducted from 16 August 1998 to 10 January 1999, and the second experiment (experiment 2) was conducted from 4 February to 18 May 1999.

Thirty four cultivars from diverse origins were tested in experiment 1 to examine the level of cold tolerance during reproductive development. These cultivars are listed in Table 3-1 of the thesis. The cultivars were mostly of the *japonica* subspecies and had different origins and cold tolerance levels. However, cold tolerance levels of some of the cultivars included were unknown. A subset of 18 cultivars from experiment 1 was used in experiment 2, which included six cultivars from Australia, Japan and other origins with varying degrees of cold tolerance.

Rice plants were established in plastic pots (17cm diameter and 20cm height) containing topsoil of a transitional red/brown earth (Birganbigil clay loam) (Van Dijk 1961) from the rice growing area at YAI. Fifteen seeds were planted in each pot at a depth of 5mm. The sowing date of each cultivar was staggered according to its expected flowering date to synchronise panicle initiation (PI) with other cultivars. Experiment 1 and 2 had 12 and 25 pots of each cultivar respectively.

Pots were kept in 120 x 120cm tubs and were initially soaked to a depth of 7cm from the bottom to facilitate germination. The water in the tubs was then drained to a depth of 2cm until the three leaf stage, when pots were thinned to 6 uniform plants per pot. Water was then completely drained from tubs and N fertiliser as urea was added onto the dry soil surface of pots at a rate equivalent to 75kgN ha^{-1} in experiment 1 and 75 or 150kgN ha^{-1} in experiment 2. Permanent water to a depth of 2cm above the soil surface was maintained until physiological maturity.

Plants were initially grown in a polyhouse with natural daylight at a day/night temperature of 30/24^{o}C. Following panicle initiation (panicle length 10 to 20mm), pots were transferred to temperature-controlled rooms until head emergence. The temperature-controlled room was naturally lit and provided 3 temperature regimes of low, medium and high, which was based on night temperatures as shown in Table 3-2 of the thesis. Night temperatures in experiment 1 for low, medium and high treatments were 13.0^{o}C, 14.4^{o}C and 24.5^{o}C respectively. Corresponding day temperatures in experiment 1 were 26.7^{o}C, 24.8^{o}C and 31.7^{o}C. Similar day/night temperature regimes were maintained in experiment 2 (Table 3-2 of the thesis). The duration of the day and night temperature was set for 12 hours and the change in temperature took approximately 20 minutes. Duration of each cultivar under the cold treatment varied from 11to 43 days depending on the panicle growth rate during the period from PI to head emergence. At 50% heading pots were individually returned to the original tubs in the polyhouse.

In experiment 1, there were 4 replications for the low and medium temperature treatments and 3 replications for the high temperature treatment. In experiment 2, there were 4 replications for each temperature treatment.

Measurements such as number of engorged pollen grains per anther and anther length width and area were measured at flowering using an image analysis system. Detailed procedures are described in Chapter 3 of the thesis. The delay in heading in response to low temperature was also calculated. The plants were harvested at physiological maturity and harvest index and percent sterility were determined as described in the thesis. Spikelet sterility and harvest index were used to determine the levels of cold tolerance of the cultivars. Moreover, the relationship between flowering characteristics and the level of cold tolerance was evaluated.

A linear mixed model was used to analyse the effect of temperature treatment on spikelet sterility, harvest index, anther length, width and area and engorged pollen grains per anther.

4.2 *Genetic control of cold tolerance*

Six populations were developed from a series of reciprocal and single crosses of the cultivars HSC55 and Plovdiv 22 with Millin and Illabong (Table 4-2 of the thesis) to investigate the possibility of incorporating the cold tolerance of foreign cultivars into locally adapted cultivars. The resulting F_1 seeds were sown under glasshouse conditions at YAI. Lines were randomly sampled from more than 2000 F_2 plants from each population. The lines were then advanced three generations (F_3 to F_5) by single-seed descent (SSD). Plants from SSD were grown in the glasshouse, and single generation (F_6) panicle rows were sown in the field. Therefore, the resulting F_7 bulks were derived from a single F_5 plant ($F_{5:7}$). The $F_{5:7}$ plants evaluated in this experiment were derived from seeds from field grown plants at Leeton farm.

A subset of 30 to 46 recombinant inbred lines (RIL) was randomly selected from $F_{5:7}$ populations to estimate their cold tolerance level. The selection included 30 lines from each reciprocal cross between Millin and HSC55 and between Illabong and HSC55; 40 lines from a single direction cross between Millin and Plovdiv22; and 46 lines from a single direction cross between Illabong and Plovdiv 22. $F_{5:7}$ lines were used because they were homogeneous and could be replicated.

All plants were initially grown in pots (13.5cm diameter and height) in a polyhouse as described in section 4.1. Sowing was staggered to obtain synchrony of plants reaching PI. The cultural operations and conditions given were similar to experiments in section 4.1. At the three leaf stage, pots were thinned to four uniform plants and fertilised with nitrogen at 150kgN ha^{-1} rate. There were 11 pots per each parent cultivar and 7 pots for each RIL.

Following panicle initiation (panicle 10 to 20mm long), pots were transferred to temperature-controlled rooms until head emergence as described in section 4.1. In the temperature-controlled rooms, plants were exposed to day temperatures ranging from 23.5^{o}C to 25.1^{o}C and night temperatures ranging from 11.5^{o}C to 14^{o}C (Table 4-3 of the thesis). At 50% heading pots were individually returned to the original tubs in the glasshouse. Flowering dates of control and cold treated plants were recorded. When physiological maturity was reached, harvest index and spikelet sterility were calculated and a comparison of harvest index and spikelet sterility was made between cold treated parents and their progeny.

The experiment was analysed as a completely randomised design within control and cold treatment. Three statistical models were used to analyse data from this trial and are described in Chapter 4 of the thesis. An analysis was also performed to compare line means within each population. Genetic coefficients of variation and genetic correlations were also calculated to compare the genetic variation between traits.

4.3 *Phenological response to temperature and daylight*

Five field experiments were conducted in consecutive seasons from 1998/99 to 2002/03 at YAI to evaluate the phenological response of cultivars to temperature and day length and to determine the effect of low temperature during reproductive development on engorged pollen number per anther, spikelet sterility and yield among cultivars in the field.

Sets of 30, 28, 7, 50 and 50 cultivars were included in field experiments from year 1 to 5 respectively. Seven cultivars of diverse origin with different cold tolerance levels were common to all five field experiments (Table 5-1 of the thesis).

In year 1, the experiment was arranged in a modified split-split-plot design with 2 replicates. Sowing date was the main plot (8 sowing dates), while sub plot was water depth (shallow water or deep water) and sub-sub-plot was cultivars (30 cultivars). In year 2, the experiment design was the same but with 6 sowing dates and 28 cultivars. In year 3, the experimental design was also a modified split-split-plot design with 2 replicates, with sowing date (7 sowing dates) as the main plot, pre-flood nitrogen treatments (0, 150, 300kgN ha^{-1}) as the sub-plot and cultivar (7 cultivars) as the sub-sub-plot. The experiment in year 4 was a split-plot design with 2 replicates, with sowing date (5 sowing dates) as the main plot and cultivar (50 cultivars) as the sub-plot. However, the final sowing date had only one replicate with 50 cultivars. The year 5 experiment was very similar to year 4 except that there were 4 sowing dates that were replicated twice.

In year 1 and 2, the seeds were drill sown at a rate of 140kg ha^{-1} whilst in year 3, 4 and 5 seeds were sown with a single row hand planter at a rate of 190kg ha^{-1}. The dimension of each plot in year 1 and 2 was 1.4m x 3m with 7 rows, 0.2m apart. The plots in the year 3, 4 and 5 experiments comprised of two 4m rows, 0.2m apart. The soil type was the same as that described in section 4.1 of this report. Urea at a rate of 150kgN ha^{-1} was applied to the year 1 and 2 experiments before flooding. In the year 3 experiment, N application before flooding was according to the treatment design, whilst 200kgN ha^{-1} as urea was applied to the year 4 and 5 experiments as pre-flood nitrogen (PFN). In addition, 30kgP ha^{-1} and 15kgZn ha^{-1} was applied before flooding in the year 4 experiment. All general cultural operations such as flushing with water, weed control and blood worm control were completed. A detailed description is given in Chapter 5 of the thesis.

Air, soil, water and panicle temperatures were monitored and recorded throughout the trial duration. Air temperature was taken 1m above the soil surface, whilst soil temperature was taken 7cm below the surface. Water temperature was measured 2cm above the soil surface. Panicle temperature was measured by placing a thermocouple close to the developing panicle.

Flowering date in all plots was recorded when 50% of the panicles had 50% of spikelets with extruded anthers. Date of panicle initiation and the young microspore stage were estimated by observing panicles under a microscope. The development rate (DVR) was calculated for the period from sowing to flowering in relation to temperature and day length as described in Chapter 5 of the thesis. In order to determine the period to be used to estimate the effect of day length, a number of day length windows were tested and correlation coefficients (multiple-linear regression) were calculated between temperature and 13 different day length periods.

Plant development was recorded in selected plots by randomly sampling approximately 20 tillers twice every week from PI to head emergence. Plant

height, culm length, panicle length, auricle distance and the stage of panicle development were measured and recorded for each tiller.

At flowering, anther length, width and area, stigma length and area, number of engorged pollen grains per anther and germinated pollen were measured as described in Chapter 6 of the thesis. At physiological maturity, harvest index and spikelet sterility were determined.

Statistical models were developed for each year separately as layouts and treatments varied from year to year and are described in Chapter 6 of the thesis.

4.4 Screening for cold tolerance

Cold water experiments were established in 2000/01 (year 3), 2001/02 (year 4), 2002/03 (year 5) and 2003/04 (year 6) to examine the mechanisms of cold tolerance relating to male and female reproductive organs and to evaluate the reliability of screening for cold tolerance with cold water in comparison with cold air and field screening.

Approximately 200 cultivars from different origins and with different levels of cold tolerance were screened using the cool water facility at YAI, across all years. In year 3 six replications of each cultivar was exposed to the cold water treatment with 2 control replications. In year 4 there were 2 replications with two pots per replicate for cold water treatments as well as for the control. In year 3 control pots were kept in the polyhouse for the duration of the experiment. However, in year 4, the control pots were moved to a control tub adjacent to the cold water tubs. A square plastic tub (1.2m wide and 0.8m high) was used for the cold treatment in year 3 and 4. Rice plants were established in plastic pots (13cm diameter and 14cm height) containing topsoil of a transitional red/brown earth (Birganbigil clay loam) (Van Dijk 1961) from the rice growing area at YAI. Fifteen seeds were planted in each pot at a depth of 2cm. Planting was staggered according to growth duration (late, mid and early). For example, late maturing cultivars were planted first and vice versa. Additional pots were included to monitor the growth stages and to detect panicle initiation. Pots were kept well watered until the three leaf stage and thinned to four uniform plants per pot. Every two weeks pots were rotated to minimise the effects of temperature and light gradients within the polyhouse.

In year 5 the capacity of the cool water system was increased with the installation of three large circular tubs (6.84m^2) that held 20.5m^3 of water. This enabled more cultivars to be tested for cold tolerance. Cultural management remained the same as for year 3 and 4 experiments. However, the cultivars were exposed to 42cm of water and N fertiliser as urea was added at a rate equivalent to 200kgN ha^{-1}. Only treatment plants were included in the year 5 experiment.

Due to excessive amounts of cold damage in Year 5, the plants were exposed to a water depth of 30cm compared to 42cm in Year 6. Controls and treatments were included, with controls remaining in the polyhouse for the duration of the experiment and treatments being exposed to cold water during the reproductive phase. The year 6 experiment was managed in the same way as the year 5 experiment.

In year 3 and 4, N fertiliser (150kgN $ha^{-}1$) as urea was added at the three to four leaf stage. Permanent water to a depth of 2 to 3cm was established by placing the pots in tubs. Following panicle initiation (panicle length 10 to 20mm), pots were moved to the cool water system and placed under cold water ($19^{o}C$) at a depth of 30 cm above the shoot base. At head emergence plants were returned to the original tubs in the polyhouse. A complete randomised block design with 3 replicates was used.

Measurements such as number of engorged pollen grains per anther, anther length, width and area and stigma size, were taken at flowering. The plants were harvested at physiological maturity and plant height, harvest index and percent sterility were determined. Spikelet sterility and harvest index were used to assess the level of cold tolerance for the cultivars. Moreover, the relationship between flowering characteristics and the level of cold tolerance was investigated.

5. Detailed results

5.1 Genotypic variation in response to low temperature during reproductive development.

Low temperature treatment slowed reproductive development in all cultivars. Low temperature treatments delayed heading by an average of 14 days relative to high temperature treatments. However, there were significant differences in duration of delay among cultivars due to low temperature treatment. Some cultivars such as Langi and Yunlen 16 doubled their duration of reproductive development under cold treatment whereas cultivars such as HSC55 did not show a significant delay in reproductive development. Effect of N on delay of duration of reproductive development due to low temperature treatments was negligible.

Low temperature treatment reduced the number of engorged pollen grains per anther by 35%, anther length by 9% and anther width by 3% (Table 3-7 of the thesis) in experiment 1. In experiment 2, a 24% reduction in the number of engorged pollen grains and a 5% decrease in anther length in response to low temperature treatments were observed. There were significant differences among cultivars in number of engorged pollen grains per anther and anther width and length. For example HSC55 had the most number of pollen grains per anther (1721). HSC55 (2.4mm) and YRL39 (2.5mm) had longer anthers than all other cultivars. In contrast, Nipponbare and Sasanishiki had less

engorged pollen grains per anther (415 and 452 respectively) and a shorter anther length (1.4mm and 1.6mm respectively) than all other cultivars. Anther length was significantly reduced from the high to low temperature in cultivars Plovdiv 22, Hungarian 1 and Amaroo, whilst anther length remained relatively unchanged for cultivars such as HSC236, Akihikari and HR 4856-1-1-1-1-2. The pattern for anther width was generally stable across temperature treatments, although China 1039 showed a small reduction from high to low temperature.

HSC 55, Plovdiv 22 and Millin produced over 1200 engorged pollen grains per anther on average across the three temperature treatments in experiment 2. The cultivar Sasanishiki had the lowest number of engorged pollen grains per anther and the shortest anther length (Table 3-8 of the thesis). There was a significant reduction in the number of engorged pollen grains per anther due to low temperature treatments. Akihikari, Hitomebore and Langi had approximately half the number of engorged pollen grains in low temperature compared to high temperature. Whilst Sasanishiki and Doongara had a similar number of engorged pollen grains across all temperature treatments. Significant reductions in engorged pollen number also occurred for cultivars such as Plovdiv 22, Akihikari and Langi from medium to low temperature treatments, whereas the engorged pollen number for Haenukai, M103 and Amaroo remained unchanged.

Very similar trends occurred for anther length when cultivars were subjected to low temperature treatments. The anther length for Calrose and Namaga remained relatively constant across all temperature treatments, whereas the anther length was reduced in some cultivars such as HSC55 and Langi from the high to low temperature treatment. Anther length was significantly reduced for HSC55, Plovdiv 22 and Akihikari from the medium to low temperature treatment. Whilst the anther length for M103, Amaroo and Namaga were unchanged.

There was no significant effect of N or interaction between N and cultivar on number of engorged pollen grains per anther and anther length.

There was a significant and positive linear relationship between the total number of engorged pollen grains per anther and the temperature (r^2=0.46**) and between anther length and the temperature (r^2 = 0.72**). There was also a highly significant relationship (r^2 = 0.68**) between anther length and number of engorged pollen grains per anther in all temperature treatments (Figure 3-4c of the thesis). However, anther length is less affected by low temperature than the number of engorged pollen grains.

Low temperature treatments significantly increased the spikelet sterility of all the cultivars. Spikelet sterility at low, medium and high temperature treatments were 35%, 19-21% and 13–16% respectively in experiment 1 and

2. The relationship between visually estimated and measured spikelet sterility was highly significant ($r^2 = 0.85^{**}$). There was a significant variation (ranging from 13-79%) in spikelet sterility among cultivars in low temperature treatments. Among Australian cultivars, Langi and Doongara gave the highest spikelet sterility at low temperature treatments. The cultivar Amaroo was more tolerant to low temperatures than Doongara, with approximately half the level of spikelet sterility at low temperature (39%). A low range of spikelet sterility in HSC55 and M103 across all temperature treatments (6-18%) suggests that both cultivars are cold tolerant.

Harvest index was also significantly reduced due to low temperature treatment. However, cold tolerant cultivars such as HSC55 and H473 maintained relatively constant HI at all temperature treatments. Temperature effect on spikelet sterility and harvest index is shown in Table 3-9 and 3-10 of the thesis. There was a highly significant negative relationship ($r = -0.68^{**}$) between harvest index and spikelet sterility at low temperature. Spikelet sterility also had significant negative relationships with number of engorged pollen grains per anther and anther length. Spikelet sterility was significantly higher in Australian and Californian cultivars than other cultivars, although there was no significant difference between them for number of engorged pollen grains per anther. However, Australian and Californian cultivars did have a significantly longer anther than that of other cultivars from different origins. There was a strong relationship between spikelet sterility and harvest index between experiments (Figure 3-5 of the thesis) indicating high levels of repeatability.

Despite a significant interaction between temperature and nitrogen for spikelet sterility, the effect of N on spikelet sterility was relatively small. Doubling the nitrogen rate from 75kgN ha^{-1} to 150kgN ha^{-1} resulted in an increase in spikelet sterility by 1 to 2% across all temperatures.

5.2 Genetic control of cold tolerance

Duration of cold treatment varied greatly among cultivars due to their differences in panicle growth rate under low temperature. There was a significant relationship ($r^2 = 0.42^{**}$) between the duration of cold treatment and spikelet sterility. Therefore, the data was adjusted using covariate analysis to accommodate this influence of duration of low temperature treatment.

Differences between populations containing reciprocal crosses (between HSC55 and Millin and HSC55 and Illabong) were not significant for spikelet sterility, harvest index or duration of treatment. This suggests that maternal genetic effects were unimportant on expression of these traits in these crosses.

Four parents showed a significant variation in spikelet sterility and harvest index under low temperature treatment. Cultivars HSC55 and Plovdiv22 had a significantly lower level of spikelet sterility and higher harvest index under cold treatment compared to Illabong and Millin. Under the cold treatment, the Millin/Plovdiv22 crosses had higher spikelet sterility and lower harvest index compared to the other combination of crosses (Table 4-8 of the thesis). On average HSC55 crosses had 48% spikelet sterility under cold treatment compared to 61% for Plovdiv22 crosses.

The distribution of spikelet sterility among lines in each of $F_{5:7}$ populations are shown in Figure 4-2 of the thesis. Parental mean for spikelet sterility is also shown. There was significant variation for spikelet sterility among the lines within each population. There was no transgressive segregation identified for spikelet sterility among the four populations since the distribution of lines was within the range of parents. However, populations with HSC55 as a parent were skewed towards low spikelet sterility at low temperature treatment. In comparison, populations where Plovdiv22 was a parent had normally distributed spikelet sterility levels.

The distribution of harvest index among $F_{5:7}$ lines are shown in Figure 4-3 of the thesis. There was significant variation in harvest index distribution among the lines in each population.

The duration from sowing to PI was variable among lines for each population and there was no significant relationship between spikelet sterility under cold treatment and duration to PI. However, some lines from the Millin/Plovdiv22 population with shorter duration to PI showed less spikelet sterility under low temperature. Fourteen lines were identified as cold tolerant as they had low levels of spikelet sterility similar to HSC55 and Plovdiv22. All these lines had HSC55 as their parent but not Plovdiv22.

HSC55 populations had higher narrow-sense heritability of spikelet sterility (0.62 to 0.66) compared to that of Plovdiv22 populations (0.41 to 0.48) indicating the reflection of higher genetic variance relative to the error variance in HSC55 populations as compared to Plovdiv22 populations. The genetic coefficient of variation was also higher in HSC55 populations (18-20%) than in Plovdiv22 populations (11-15%).

Heritability estimates of harvest index for HSC55 populations were similar to those for spikelet sterility (Table 4-11 of the thesis). However, the low heritability of the Illabong/Plovdiv22 populations was associated with a low genetic variance relative to error variance. In contrast, the Millin/Plovdiv22 populations had a similar genetic and error variance but a higher heritability of 0.78. The phenotypic and genetic correlations for all populations except Millin/HSC55 were similar (Table 4-12 of the thesis). Genetic and phenotypic

correlations for the Millin/HSC55 population were low compared to other populations.

5.3 Phenological Response to temperature and daylight

Mean daily temperature from 1st of October to 31st of March in year 1 (1998/99), year 3 (2000/01) and year 5 (2002/2003) was above the long term average. The mean daily temperature during the same period in year 2 (1999/2000) and year 4 (2001/02) was below the long term average (Figure 5-2 of the thesis). Year 4 was the coolest among 5 years of field experimentation. The minimum temperature in year 4 was 2^{o}C below the long term average for the area and 4^{o}C below year 1 and 3. The diurnal variation of soil and water temperature in year 4 follows the diurnal variation of air temperature.

A diurnal variation of 10^{o}C to 15^{o}C occurred from 8th to 12th February in year 4. This is similar to the long term average diurnal variation (15.3^{o}C) for the region. However, due to cloud cover and chilling wind, the maximum air temperature during the 4th to 6th February was reduced from 28^{o}C to 18^{o}C decreasing the water and soil temperature by 10^{o}C and 7^{o}C respectively. Figure 5-3b of the thesis shows the temperature pattern of panicle, water and air for the 20th and 21st January 2000. Air temperature showed the highest diurnal temperature variation whilst water showed the lowest. At night the soil and water temperature was approximately 6^{o}C and 8^{o}C greater than air temperature, respectively. The minimum temperature of the panicle was 6^{o}C lower than that of water. Relationships between mean air and water temperature show that deep water was 2^{o}C higher than shallow water at all temperatures.

On the other hand, the maximum water temperature in deep water was lower than maximum air temperature when maximum air temperature exceeded 26^{o}C. When the maximum air temperature was 37^{o}C, the temperature for deep water was 30^{o}C. Thus deep water could provide potential protection to the panicle from high temperature.

There was no significant effect of water depth on duration from sowing to flowering in year 1 and 2. In year 3, high N rates delayed flowering significantly. The addition of 150 and 300kgN ha^{-1} delayed flowering on average across all cultivars by 3.3 and 4.9 days respectively, compared to 0kgN ha^{-1}.

There was a significant and positive linear relationship between sowing date and flowering date for all cultivars. There was a significant linear relationship between mean air temperature and development rate (DVR) from sowing to flowering among seven cultivars (Figure 5-6 and 5-8 of the thesis). DVR of M103 and HSC55 had the highest correlation coefficient ($r^2 = 0.85^{**}$ and

0.84** respectively) with mean air temperature suggesting temperature is the main factor that determines growth during the period from sowing to flowering. Correlation coefficients between DVR of Amaroo and Millin and mean temperature was low suggesting that they had mild day length response compared to other cultivars. This may be the reason for shorter duration to flowering for Amaroo, as sowing was delayed. At a day length of 15 hours, Amaroo was 15 to 19 days later to flower than Doongara at all mean temperatures. However, when day length was reduced to 13 hours and mean temperature was 18°C, Amaroo was 12 days earlier to flower than Doongara. At mean temperatures of 25°C, the reduced day length (from 15 to 13 hours) almost halved the duration of reproductive development in Amaroo from 35 to 18 days. Similar reductions were observed in Millin (from 42 to 19 days) and to a lesser extent M103 (from 34 to 22 days). Results also suggest that Doongara, Sasanishiki, and Hitomebore are weakly photoperiod-sensitive.

Estimate of base temperature (the minimum temperature at which growth occurs) ranged from 1.4°C for HSC55 to 6°C for Amaroo. October sown crops generally had a lower DVR than December sown crops for a given temperature.

The duration from PI to flowering, from PI to young microspore stage and from young microspore stage to flowering for each cultivar in year 3 and 4 experiments are given in Table 5-5 of the thesis. There was significant genotypic variation for the duration from PI to flowering and PI to microspore stage in year 3, with the cultivar HSC55 having a significantly shorter duration from PI to flowering than all the other cultivars. High temperature conditions in year 3 resulted in shorter duration in each of the three developmental periods compared to year 4. However, significant differences were observed in duration of developmental stages of each cultivar between years. For example, the cultivars Sasanishiki and Hitomebore had similar duration from PI to flowering in year 3 (approximately 25 days), but Sasanishiki (35 days) had a shorter duration from PI to flowering than Hitomebore (42 days) in year 4.

Daily temperature data from 1955 to 2002 was used to analyse the risk of encountering minimum temperature of below 15°C during reproductive development (Figure 5-9 of the thesis). The probability of encountering the minimum temperature is at its lowest (15%) during early February. The probability of exposure to minimum temperatures less than 15°C during reproductive development across five sowing dates was different for all cultivars. 1st October and 1st December sown Amaroo crops had a 33% chance of encountering minimum temperature below 15°C during the critical reproductive period compared to 15th October and 15th November sown crops where the risk was reduced to 18%. The likelihood of two short duration cultivars (HSC55 and M103) encountering low temperature during reproductive development is more than doubled if they are sown on 1st October compared to 1st December.

Results indicate that the probability of Amaroo encountering minimum a temperature less than 15^{o}C during microspore and flowering in an average year was lowest for the 15th October sowing (42%) and increased for 1st October and 1st November sowing (47%) (Figure 5-12a of the thesis). The risk increased rapidly as sowing was delayed until 15th November (63%) and 1st December (87%). However, in a cold year this risk was lowest for 1st and 15th October sowings but increased sharply (94%) for 1st November, 15th November and 1st December sowings (Figure 5-12b of the thesis). In a warm year, the risk of encountering low temperature conditions was lowest for 1st November sowing (Figure 5-12c of the thesis) and the highest was for 1st October sowing.

Deep water treatment increased plant height (835 to 893mm) and culm length (204 to 241mm) compared to shallow water on average across all cultivars at the young microspore stage in year 1. However, in year 2, there is no significant effect of water depth on plant development.

In year 2, sowing date had a significant effect on plant development among seven cultivars. There was an increase in mean plant height (686 to 867mm), culm length (165 to 251mm) and panicle length (78 to 200mm) at the young microspore stage from the first sowing (8th October) to the sixth sowing (31st December). As a consequence, the panicle was completely exposed to air temperature for the final two sowing dates. Whereas, plants sown on the 8th October had their panicle approximately half submerged in deep water. However, there was no significant effect of sowing dates on plant development in year 1. In year 3, Amaroo had a small increase in plant height and a significant increase in culm length with the delay in sowing. However, panicle length at the young microspore stage was relatively unchanged with the delay in sowing.

In year 2, the mean plant height across six deep water treatments at the young microspore stage varied between cultivars with HSC55 the tallest (886mm) and Millin the shortest (697mm) (Table 6-3). Some cultivars had shorter culm lengths but longer panicle lengths.

Application of 150 and 300kgN ha^{-1} in year 3 significantly increased the plant height and culm length in Amaroo (Table 6-4 of the thesis). As a result, all of the panicle and part of the culm were exposed to air temperature due to shallow water in year 3. A shallow water depth of approximately 70mm did not provide any direct protection to any part of the developing panicle even in a semi-dwarf cultivar like Millin.

There was a significant linear relationship for Amaroo (r^2=0.91**) between auricle distance and the stage of development in year 3 (Figure 6-1 of the thesis). However, sowing date had no significant effect on this relationship.

Results indicate that the critical young microspore stage of Amaroo corresponds to an auricle distance of approximately -7 mm.

A PFN application of 300kgN ha^{-1} significantly reduced the number of engorged pollen grains per anther and pollen interception by stigma, anther size (length, width and area) and stigma area (Table 6-6 of the thesis). There was a significant interaction between cultivar and PFN application on anther length and number of intercepted pollen grains per anther. For example, as PFN increased from 0 to 300 kgN ha^{-1}, anther length was significantly decreased in cultivars such as Sasanishiki (1.78mm to 1.53mm) and Doongara (2.06mm to 1.85mm). However, anther length remains relatively stable with an increase in PFN for cultivars such as Amaroo (2.33m to 2.30mm) and HSC55 (2.56mm to 2.47mm). Similarly, the effect on the number of engorged pollen grains per anther was variable across cultivars.

The deep water treatment in year 2 significantly increased anther length and width, number of engorged pollen grains per anther, intercepted pollen grains and germinated pollen grains per anther (Table 6-5 of the thesis). For example, HSC55 had 743 engorged pollen grains per anther in shallow water compared to 1043 engorged pollen grains per anther in deep water. This suggests that plants in shallow water suffered low temperature damage.

On average, anthers were longer and contained more pollen grains in warmer years (year 3) compared to cooler years (year 2 and 4). Of the seven common cultivars tested, Millin had the highest average number of engorged pollen grains per anther (1431) across all years. HSC55, M103 and Amaroo also had over 1250 engorged pollen grains per anther in year 2, 3 and 4. Sasanishki had significantly fewer pollen grains per anther than the other six cultivars in all years except Doongara in year 4. Mean anther length of cold sensitive cultivars, Sasanishki (1.61mm) and Doongara (1.76mm), was approximately 25% smaller than the mean anther length (2.18mm) of the remaining cultivars. Whereas, in all years, the cold tolerant cultivar HSC55 had a significantly greater anther length compared to the other six cultivars, except Millin in year 4 (Table 6-7 of the thesis). HSC55 also produced the largest anther area, stigma length and stigma area among the other common cultivars in year 3 and 4. The only exception was that the stigma area of HSC55 (0.48mm^2) in year 4 was lower than that of Sasanishki (0.51mm^2). Additionally, Doongara had a significantly smaller stigma area in year 3 (0.33mm^2) and year 4 (0.21mm^2) compared to the other six cultivars. The pollen grain interception efficiency was significantly lower in HSC55 and Doongara (0.92 and 1.17% respectively), compared to the other five cultivars in year 3. However, HSC55 has consistently shown low interception efficiency, even in warmer years. Sasanishiki had a high interception and germination efficiency to compensate for a low number of engorged pollen grains per anther. In year 4 when minimum temperatures were generally low there were significant reductions in anther length, width and area, number of engorged pollen grains per anther and stigma area.

Mean spikelet sterility among cultivars in year 4 was 54% which was more than double the spikelet sterility measured in year 1 to 3 (Table 6-11 of the thesis). This is mainly due to the lower average minimum temperature (13.7^{o}C) experienced from the 20th December to 20th March in year 4, compared to average minimum temperatures (16.3^{o}C to 17.2^{o}C) during the same period in year 1, 2 and 3. In year 4, HSC55 and M103 had low spikelet sterility levels (28 and 34% respectively) compared to other cultivars suggesting that they are more tolerant to low temperature than other cultivars. Hayayuki had the lowest spikelet sterility (21%) and Doongara had the highest spikelet sterility (79%) among cultivars when the data was adjusted by covariate analysis using reproductive stage temperature to account for the temperature variability during reproductive development (Table 6-12 of the thesis). Hayayuki, Banat 725, HSC55 and Hungarian 1, had low spikelet sterilities which coincided with a higher number of engorged pollen grains per anther and larger anther and stigma sizes.

Minimum temperatures of 20^{o}C during reproductive development induced less than 19% spikelet sterility in the seven common cultivars and Hayayuki, when spikelet sterility was estimated using the regression between minimum temperature during reproductive development and spikelet sterility for each cultivar (Table 6-13 of the thesis). However, when the minimum temperature fell to 14^{o}C, the spikelet sterility of Doongara and Sasanshiki increased to 67% and 65% respectively. HSC55, M103 and Hayayuki gave the lowest levels of spikelet sterility when the minimum temperature was 14^{o}C during reproductive development.

The correlation between spikelet sterility and flowering characteristics was stronger in year 4 than in other years (Table 6-14 of the thesis). This may be attributed to low temperatures during reproductive development in year 4. In year 4, spikelet sterility had the highest correlation (r= -0.67**) with the number of engorged pollen grains per anther. Moreover, spikelet sterility was significantly correlated to anther length, width and area and stigma area. Anther area and stigma area in a multiple-linear regression explained 68% of the variation in spikelet sterility. The results in year 2 and 4 were similar, but correlation coefficients were generally lower in year 2. The relationship between number of engorged pollen grains per anther and spikelet sterility in year 4 was weaker in Australian and Californian cultivars (r^2=0.32**) than for cultivars from other origins (r^2=0.61**). The 95% confidence interval showed that Californian and Australian cultivars were less efficient in producing filled grains when the number of engorged pollen grains per anther was in the range of 585 to 1250 and when anther area was greater than 0.52mm^2 compared to cultivars from other origins (Figure 6-7 of the thesis).

Reproductive stage temperature was significantly correlated with harvest index, yield (in years 1 and 2) and spikelet sterility in all years at all water depth treatments, with the exception of the year 1 deep water treatment (Table 6-17 of the thesis). In year 2, there was a small but significant effect of

shallow and deep water treatment on harvest index (37% and 41% respectively) and grain yield (8.0 and 8.6t ha^{-1} respectively). In year 4, there was a strong relationship between reproductive stage temperatures and harvest index (r=0.60**). The relationships between spikelet sterility and both harvest index and yield were stronger in shallow water treatments compared to deep water treatments.

There was a highly significant effect of sowing date on grain yield, harvest index and biomass in years 1 and 2. A delay in sowing in years 1 and 2, lead to a significant decrease in biomass accumulation and yield. Amaroo, Millin and M103 showed a plateau in yield up until early March flowering and declined thereafter (Figure 6-8a of the thesis). However, cultivars such as HSC55, Doongara, Sasanishiki and Hitomebore had a steady decline in yield as flowering was delayed.

Due to above average temperatures during the 2002/03 season, very little cold damage was observed in the year 5 experiment. The mean harvest index for the first three sowing dates was 38%, indicating low temperature was not an issue during the critical reproductive period. However, the mid December sowing resulted in low harvest index for some cultivars. This may be due to the inability of some cultivars to finish flowering and hence no fertilisation occurs. Therefore, the number of engorged pollen grains, anther length, anther width, anther area and spikelet sterility were not measured.

5.4 Screening for cold tolerance

Despite the fluctuations in air temperature, water temperature in tubs, remained mostly between 18^{o}C and 21^{o}C (Figure 7-1 of the thesis). Cold treatment delayed flowering in all cultivars by ten days compared to the control. In the control and cold water treatments, Amaroo flowered ten and six days later than the mean of all cultivars. There was a significant interaction between treatment and cultivar for duration of exposure. The duration that cultivars were exposed to cold water ranged from 20 to 42 days in year 3 and 4 and 16 to 52 days in year 5 and 6.

In year 3, cold water exposure significantly reduced the number of engorged pollen grains per anther by 69%, anther length by 23%, anther width by 15% and anther area by 35% compared to the control (Table 7-5 of the thesis). On average across the two treatments (cold and the control), HSC55 had the highest number of engorged pollen grains per anther whilst Nippon Bare had the lowest. There was a significant interaction between treatment and cultivar for number of engorged pollen grains per anther and anther length, width and area. For example, Amaroo, Millin and HSC55 had a similar number of engorged pollen grains (approximately 1900 grains per anther) under control conditions. However, after cold water treatment, HSC55 had 921 engorged pollen grains per anther compared to Amaroo and Millin with 570 and 560 engorged pollen grains per anther, respectively. The number of engorged

pollen grains per anther and anther width, length and area of the 8 cultivars investigated in the year 3 experiment is shown in Table 7-5 of the thesis.

Significant reductions in the number of pollen grains per anther and anther width, length and area were also observed under cold water treatment in year 4 (Table 7-6 of the thesis). The number of engorged pollen grains per anther was reduced from 1615 in the control to 811 across all cultivars following cold water exposure. M103 and Paragon produced over 1650 engorged pollen grains per anther in the control but following cold treatment M103 had 1310 engorged pollen grains per anther whilst Paragon had 176 grains per anther.

Combining the results in both years produced a positive and significant relationship between total number of engorged pollen grains per anther and anther length ($r^2 = 0.76^{**}$), width ($r^2 = 0.48^{**}$) and area ($r^2 = 0.86^{**}$) (Figure 7-3 of the thesis).

In year 4 there was a significant decrease in pollen interception (by 47%), stigma length (by 10%) and stigma area (by 30%) due to cold water treatment compared to the control. The pollen interception by the stigma was significantly reduced by cold water exposure in cultivars such as Calrose, Honenwase, Namaga and Langi. Whereas, M103, Jyoudeki and Akihikari maintained similar pollen interception levels under cold water treatment and the control. The results indicate that the shape of the stigma is different among cultivars (Table 7-7 of the thesis). In the control, HSC55 ($0.46mm^2$) had a significantly larger stigma area than Langi ($0.33mm^2$) even though their stigma lengths were similar, 0.88mm and 0.86mm, respectively.

When cultivars were grouped according to their origin, Australian and Californian cultivars had a larger anther area containing more engorged pollen grains and a smaller stigma area than cultivars from other origins. However, this difference was not significant under cold treatment.

Cold treatment reduced harvest index and increased spikelet sterility significantly in year 3 and 4 (Table 7-9 and 7-11 of the thesis). There was a significant interaction between cultivar and treatment for harvest index and spikelet sterility. M103, Jyoudeki and HSC55 were the 3 most cold tolerant cultivars, with spikelet sterility of less than 35% and harvest index of approximately 40% under the cold treatment. In year 4, spikelet sterility was significantly correlated with the number of engorged pollen grains per anther, anther area and stigma area (Figure 7-4 of the thesis). Moreover, a strong negative correlation existed between harvest index and spikelet sterility ($r = -0.85^{**}$). Reduction from control to the cold water treatment of engorged pollen grains per anther and anther length was also significantly related to spikelet sterility (Figure 7-4 of the thesis). Results also indicate that cultivars with greater reduction in stigma area due to the cold water treatment tend to have a high level of spikelet sterility. The variation in anther area and stigma

area explained 72% of the variation in spikelet sterility in a multiple regression analysis. A small but significant increase in spikelets per panicle in some cultivars due to the cold water treatment compared to the control was observed.

In year 5 there was an excessive amount of cold damage with 136 of the 200 cultivars tested having greater than 90% spikelet sterility. M103 and HSC55, cultivars consistently identified as cold tolerant even had high spikelet sterilities (66% and 82%, respectively). The water depth (42cm), to which plants were exposed may have been a contributing factor for the high spikelet sterilities recorded, compared to previous investigations. Despite this, there was a highly significant relationship between visually estimated spikelet sterility and measured spikelet sterility ($r^2=0.80^{**}$). This suggests that visual estimates of spikelet sterility by trained personnel can separate tolerant, moderate and susceptible cultivars for cold tolerance.

The results obtained in year 6 were similar to those in year 3 and 4. In year 6, the cold water treatment significantly reduced the number of engorged pollen grains per anther (by 39%), anther length (by 11%), anther width (by 5%), anther area (by 14%), stigma length (by 3%) and stigma area (by 18%), compared to the control. Harvest index was also significantly decreased (48%) by the cold water treatment, leading to a 71% increase in spikelet sterility.

When cold tolerant cultivars (Jyoudeki, M103, M104) and cold sensitive cultivars (Sprint, Nippon Bare, Reiziq) are grouped separately, the effect of cold water treatment was more pronounced on sensitive cultivars. The number of engorged pollen grains per anther, anther length, anther width and harvest index were significantly decreased in sensitive cultivars due to the cold water treatment (Table 5-1, 5-2, 5-3 and 5-4). The number of engorged pollen grains per anther was the only character which was significantly reduced in tolerant cultivars by the cold water treatment. However, M104 showed an increase in the number of engorged pollen grains per anther in the cold water treatment compared to the control.

Even though tolerant cultivars and sensitive cultivars produced a similar number of engorged pollen grains per anther in the control treatment, their engorged pollen number per anther differed greatly in the cold water treatment. For example, Jyoudeki, a tolerant cultivar reduced its engorged pollen number per anther from 1266 in the control, to 977 in the cold treatment (11% reduction). Whereas, Reiziq, a sensitive cultivar, reduced its engorged pollen number per anther from 1162 in the control, to 538 in the cold treatment (54% reduction).

Spikelet sterility in year 6 was significantly correlated to the number of engorged pollen grains per anther and anther length, width and area. The relationship with spikelet sterility was strongest for the number of engorged

pollen grains per anther (r^2=0.36**). Visually estimated sterility was again highly correlated to measured spikelet sterility (r^2=0.91**).

The number of engorged pollen grains per anther was significantly correlated to anther length (r^2=0.39**), anther width (r^2=0.23**) and anther area (r^2=0.39**) indicating more engorged pollen grains are produced in larger anthers.

Table 5-1 Number of engorged pollen grains per anther in control and cold water treatment for six cultivars in year 6.

Cultivar (cv)	Cold Tolerance	Control	Cold	% Reduction
Jyoudeki	Tolerant	1266	977	13
M103	Tolerant	1678	1155	32
M104	Tolerant	1275	1416	+11
Sprint	Sensitive	954	551	42
Nippon Bare	Sensitive	1156	378	67
Reiziq	Sensitive	1162	538	54
Tolerant Mean		1406	1183	16
Sensitive Mean		1091	489	66
Treatment x cv lsd (p=0.05)	279			

Table 5.2 Anther length (mm) in control and cold water treatment for six cultivars in year 6.

Cultivar (cv)	Cold Tolerance	Control	Cold	% Reduction
Jyoudeki	Tolerant	1.87	1.78	5
M103	Tolerant	2.26	2.00	12
M104	Tolerant	2.06	2.17	+5
Sprint	Sensitive	1.71	1.33	23
Nippon Bare	Sensitive	1.86	1.28	31
Reiziq	Sensitive	1.99	1.73	13
Tolerant Mean		2.06	1.98	4
Sensitive Mean		1.85	1.45	22
Treatment x cv lsd (p=0.05)	0.24			

Table 5.3 Anther width (mm) in control and cold water treatment for six cultivars in year 6.

Cultivar (cv)	Cold Tolerance	Control	Cold	% Reduction
Jyoudeki	Tolerant	0.62	0.61	2
M103	Tolerant	0.86	0.68	21
M104	Tolerant	0.75	0.84	+12
Sprint	Sensitive	0.56	0.40	29
Nippon Bare	Sensitive	0.62	0.35	44
Reiziq	Sensitive	0.71	0.55	22
Tolerant Mean		0.74	0.71	4
Sensitive Mean		0.63	0.43	32
Treatment x cv lsd (p=0.05)	0.17			

Table 5.4 Harvest index (%) in control and cold water treatment for six cultivars in year 6.

Cultivar (cv)	Cold Tolerance	Control	Cold	% Reduction
Jyoudeki	Tolerant	43	40	7
M103	Tolerant	52	44	15
M104	Tolerant	56	52	7
Sprint	Sensitive	40	7	82
Nippon Bare	Sensitive	33	6	82
Reiziq	Sensitive	54	14	74
Tolerant Mean		51	45	12
Sensitive Mean		42	9	79
Treatment x cv lsd (p=0.05)	9.3			

6. Discussion of results

6.1 Genotypic variation and consistency for cold tolerance

Genotypic variation for cold tolerance was identified for over 50 cultivars and 206 lines in temperature-controlled rooms, cold water facilities and field experiments. Cold tolerant cultivars were identified as those having low levels of spikelet sterility at low temperatures in the temperature-controlled rooms, cold water facilities and in the field (e.g. M103 and HSC55). Conversely, those cultivars with high spikelet sterility were identified as susceptible to low temperature (e.g. Doongara and Langi).

In the temperature-controlled rooms, day/night temperatures of 27°/13°C and 25°/15°C induced less than 20% spikelet sterility in cultivars such as M103 and HSC55 (Table 3-9 of the thesis). By comparison, the same day/night temperature regimes induced more than 50% spikelet sterility in cultivars such as Doongara and Langi. Results also indicate that variability in cold exposure period among cultivars in temperature-controlled rooms was not related to spikelet sterility among cultivars and hence the levels of spikelet sterility were true genotypic variation for cold tolerance. This genotypic variation for spikelet sterility that was identified in temperature-controlled rooms was confirmed in the field experiments. Therefore, genotypic variation for cold tolerance during reproductive development reflects differential responses to the severity and duration of low temperature events.

The incidence of spikelet sterility was highly repeatable among cultivars that were common to the two experiments (year 1 and 2) in the temperature-controlled rooms (r^2=0.75**) and the year 3 and 4 experiments in the cold water facility (r^2=0.73**). During the cold water treatment, the outside air temperature was higher in year 3 (25°C) than year 4 (22°C), which suggests that cold water screening did not appear to be affected by variation in air temperature. There was a highly significant relationship for spikelet sterility among the common cultivars in the temperature-controlled rooms and cold water facility (r^2=0.63**). After adjusting for temperature during the reproductive development stage in the field in year 4 (2001/02) the spikelet

sterility of 50 cultivars was determined and was significantly related to spikelet sterility in temperature-controlled rooms (r^2=0.52**) and the cold water facility (r^2=0.53**). This indicates that the genotypic consistency of cold tolerance is maintained across the three screening environments despite the variability of cold exposure period in temperature-controlled rooms and the cold water facility and variable temperature conditions in the field.

Considering the nature of spikelet sterility, it is unlikely that a perfect relationship would occur for cultivar performance across three screening environments even in the absence of a differential response of cultivars. In the screening experiments the performance of cultivars was relatively consistent across screening environments, although the relationship was less than perfect because of the differential response of some cultivars in some screening environments. For example, in the first experiment in temperature-controlled rooms Amaroo had 34% spikelet sterility, which would be considered a moderate response compared to the mean spikelet sterility of 35% among all cultivars. In the field (year 4) Amaroo had a spikelet sterility of 57%, which was also considered moderate relative to the cultivar mean of 54%. However, in the cold water screen, Amaroo was identified as tolerant having a spikelet sterility of 36% compared to the mean of 55%. The low spikelet sterility of Amaroo after cold water exposure in experiment 1 may have been due to early exposure causing partial escape from low temperature during reproductive development. The cold damage in Amaroo after cold water exposure in experiment 2 was expressed more in harvest index than spikelet sterility. The variable response of some cultivars across the three screening environments may be related to factors such as the effect of screening under constant (e.g. cold water) versus varying temperature (e.g. temperature-controlled rooms and field) and cold damage at flowering. For example, the roots of plants exposed to the cold water facility are cooler compared to the temperature-controlled rooms. Recent results by Gunawardena *et al.* (2003) showed that both root and panicle temperature had an additive effect on spikelet sterility in Amaroo caused by low temperature during reproductive development. The varied responses of some cultivars highlight the need to use the different screening environments in combination to identify cold tolerance in rice.

6.2 Flowering traits associated with genotypic differences in cold tolerance

In the temperature-controlled rooms, the cold water facility and the field, several components of the stamen and pistil organs were measured under various temperature conditions and correlated to spikelet sterility to examine the important mechanisms of cold tolerance. Spikelet sterility following cold treatment was significantly correlated to the number of engorged pollen grains per anther and anther length of cold treated plants. Therefore, these flowering traits appear facultative in nature as the relationship with spikelet sterility was identified only after cold water exposure and did not exist under non-stressed conditions. However, stigma area in the control and cold treatment was related to spikelet sterility after cold water exposure suggesting that stigma area appeared to be a constitutive trait. High levels of spikelet sterility in the

cold water facility may have contributed to a stronger relationship between spikelet sterility and anther length, width and area, stigma area, number of engorged pollen grains per anther and intercepted pollen compared to other screening methods. The importance of these flowering traits were confirmed in the field in year 4 where spikelet sterility was negatively correlated to engorged pollen number per anther, anther length, width and area and stigma area.

The level of spikelet sterility for any given number of engorged pollen grains per anther was higher in Australian and Californian cultivars compared to cultivars from other origins. This pattern was shown in experiments in temperature-controlled rooms and in the field. For example, in the temperature-controlled rooms the production of 600 engorged pollen grains per anther in the low temperature treatment resulted in 54% spikelet sterility in Australian and Californian cultivars compared to 35% spikelet sterility for the same number of engorged pollen grains per anther in cultivars from other origins. These efficiencies are lower than previous findings (Satake 1991), which suggested that more than 600 engorged pollen grains per anther should result in 10% spikelet sterility. The reduced efficiency of producing filled grains with similar quantities of engorged pollen grains per anther by Australian and Californian cultivars indicates an additional factor such as pollen viability is limiting fertilisation in these cultivars.

The measurements of flowering traits in cultivars from experiments in the temperature-controlled room and the cold water facility were combined to further examine their relationship with spikelet sterility in the field in year 4 (this included 8 Australian, 4 Californian and 20 cultivars from other origins). There was a significant negative relationship between spikelet sterility in the field and the number of engorged pollen grains per anther, anther length and anther area in the control and in cold exposed plants of cultivars other than Australian and Californian cultivars. However, the relationships between spikelet sterility and flowering traits in the cold treatment and the control were not significant for Australian and Californian cultivars. Moreover, Australian and Californian cultivars had a significantly smaller stigma area (0.23mm^2 in the cold water exposed and 0.33mm^2 in the control) compared to that of cultivars from other origins (0.32 and 0.44mm^2 respectively). The small stigma area of cultivars from Australia and California might partly contribute to their lower efficiency in filled grain production compared to cultivars from other origins, even though Australian and Californian cultivars have a similar number of engorged pollen grains per anther and the same anther size as other cultivars.

Despite no relationship being identified between pollen interception and stigma area in the cold water exposed plants (year 4) both had a highly significant relationship with spikelet sterility (Table 7-12 of the thesis). Therefore, it is unclear how a large stigma area increases the likelihood of producing filled grains when low temperatures coincide with reproductive

development in plants exposed to cold water. However, the importance of stigma size after exposure to cold has generally been overlooked. More recently, a negative correlation has been found between stigma length and spikelet sterility in both control and cold treated F_2 plants (Suzuki 1985). Nevertheless, the results indicate that it is the stigma area and not stigma length that is related to spikelet sterility.

6.3 Screening for cold tolerance

Cultivars that can tolerate low temperatures during reproductive development have been successfully identified in this project using temperature-controlled rooms, a cold water facility and field experiments. Understanding the strengths and weaknesses of these three screening methods will assist in developing strategies to produce cold tolerant cultivars in future.

6.3.1 Temperature-controlled rooms

Results indicate that cold screening in temperature-controlled rooms at day/night temperatures of 26.7°/13.0°C (experiment 1) and 28°/13.3°C (experiment 2) and cold exposure of 32 days (experiment 1) and 24 days (experiment 2) during reproductive development can successfully screen cultivars for cold tolerance and obtain comparable results too the other two methods tested here. Therefore, screening for rice cold tolerance could be successfully completed in temperature-controlled rooms by exposing whole plants to day/night temperatures of 25°/12°C during reproductive development (PI to 50% heading of the main culm). Cultivars would need to be separated into three phenology groups (early, middle and late) and grown under high nitrogen application (~200kgN ha^{-1}).

However, differences exist between this recommendation and physiological research and screening previously done in Japan. Most physiological investigations on reproductive cold tolerance have imposed cold treatments of 12°C for approximately four to six days using single culm plants (de-tillered plants) during the young microspore stage (Ito 1976; Ito 1978; Nishiyama 1978; Heenan 1984). Day/night temperature treatments of 25°/12°C during reproductive development more closely reflect the low temperature conditions experienced under field conditions in Australia. Although, a good mechanism of temperature control is required as the high outside temperature during the day can modify the day/night temperature regimes in temperature-controlled rooms. It is currently difficult to maintain day/night temperature treatments of 25°/12°C in the temperature-controlled rooms at YAI.

6.3.2 Cold water screening

A cold water facility was developed to expose plants to cold water (19°C) derived from a shallow aquifer. A deep (25cm) cold water

treatment of 19°C for an extended duration was successful in screening cultivars for cold tolerance. In years 3 and 4 the duration to which plants were exposed to the cold water treatment was 25 and 28 days, respectively. The deep cold water treatment induced a mean spikelet sterility of 53% in year 3 and 55% in year 4. Therefore, it could be recommended to expose plants to deep cold water during their reproductive development (PI to 50% heading) to screen for cold tolerance. Again as for screening in the temperature-controlled rooms, it is useful to group the material into three phenology groups (early, middle and late) to ensure a similar treatment period, and to grow the plants with high N levels (e.g. 200kgN ha^{-1}).

The major advantage of this system is that water from shallow aquifers can maintain a constant temperature of 19^{o}C throughout, irrespective of air temperature and it is also less expensive to maintain. In addition, it has the capacity to screen cultivars more tolerant than M103 which had 30% spikelet sterility in year 4. The size limitations of the current cold water system at YAI and cultivar differences in sensitivities to cooling of roots are the major weaknesses of the system. However, the size limitation could be easily overcome by increasing the size of the cold water facility or moving to a permanent field based system similar to Japan (Nagano 1998) and South Korea (Cho *et al.* 1990). Cultivars may require further testing in the field for confirmation of cold tolerance because of the effect of differential root sensitivities to cold water (Gunawardena *et al.* 2003).

6.3.3 Field screening

In order to overcome the seasonal temperature variations, a number of strategies such as sequential sowing times, shallow water and high N were examined. Over the four year period, the mean spikelet sterility varied from 13% in year 3 to 54% in year 4. The highest correlation between spikelet sterility and the average minimum temperature for a nine day period (from one day prior to the young microspore stage to seven days after) was low (r^2=0.31**). Therefore, screening under field conditions is difficult. To accomplish a good outcome, screening for cold tolerance in the field should include sequential sowing dates (e.g. five times from October to December). Moreover, genotypes should be grown at high N (e.g. 200-300kgN ha^{-1}) and in shallow water (~5cm). Flowering date should be recorded in order to estimate the date of the young microspore stage. The date of the young microspore stage is then used to calculate the temperature during the reproductive stage for the nine day period (as a covariate) in the analysis of spikelet sterility to identify cold tolerant cultivars (described in the thesis).

Field screening for cold tolerance is more valid than the other screening methods since the conditions are representative of the target environment (e.g. farmers' fields) and the capacity is unlimited. The

major difficulty of field screening is the uncertainty of encountering low temperature during reproductive development to induce adequate spikelet sterility for discrimination among cultivars since low temperature conditions in Australia occur approximately one year in four. This can be compensated for by using sequential sowing times, but this requires increased resources with often no return. In addition, statistical adjustment of field data can be time consuming and rice plants in the field are more vulnerable to other abiotic (e.g. high temperature) and biotic stresses (e.g. stem borer) than under temperature-controlled environments.

6.3.4 Developing a low temperature screening facility at Yanco

Given the current facilities at YAI, the reliability and cost effectiveness of water at the desired screening temperature (19°C), the cold water facility is recommended over the temperature-controlled rooms. In addition, the cold water facility has increased potential to screen large amounts of genetic material compared to temperature-controlled rooms.

In order to develop a cold water facility that can screen the number of lines required for a breeding program, three large screening tubs (diameter of 3m and depth of 0.8m) were recently developed at YAI (Plate 8-1 of the thesis). This facility has the potential to screen 400 cultivars or lines with three replications. The entire flow rate from one of the smaller spearhead bores is used to maintain deep water (>25 cm) at a constant temperature of 19°C. It is also possible to have two runs of cold water treatment about one month apart to increase the capacity to screen more genetic material. Sowing one pot of each cultivar or line approximately 25 days prior to the sowing of treatment pots could be a useful strategy to guide the beginning of exposure. When the first pot flowers the treatment pots are approximately at PI (panicle length 10 to 20mm) and should then be dissected to identify whether they are at the correct stage for cold exposure.

The flowering date of the pot that remains in the polyhouse should be recorded and may be used later in the analysis as a covariate to account for variation in duration of cold treatment. In order to screen larger numbers it is also important to increase the number of check entries to make comparisons across phenology groups and different runs. The checks should include the short duration cultivars HSC55 (tolerant) and Hayayuki (tolerant); the medium duration cultivars Sasanishiki (susceptible), Jyoudeki (tolerant), Millin (moderately tolerant) and M103 (tolerant); and the longer duration cultivars Doongara (susceptible), Amaroo (moderately tolerant) and YRL39 (susceptible).

The system described above is relatively labour intensive. However, the possibility exists to have a cold water system that is permanently based in the field and similar to those used in Japan and South Korea, which would reduce running costs. The development of such a system would also reduce the manual labour required to shift pots. Although, a permanent system may be limited by the availability of cold water and the increased risk of damage to plants by ducks, mice and other pests. Thus moving from a semi to a permanently based field screen would require further testing, particularly in relation to water depth to investigate whether the water depth used in Korea (5cm) or Japan (20cm) is most suitable. It is more likely that the deep cold water used in Japan is more suited to cold water screening in the field, given the success of deep cold water to induce high levels of spikelet sterility and the potential of high day temperatures in the Riverina to reduce the impact of low temperature. Notwithstanding the good correlation between cold water and the field, there remains a need to combine screening in the cold water facility with field evaluation.

6.4 Selection criteria for screening

Spikelet sterility was found to be the best and the easiest criteria to use for routine selection for cold tolerance. It is more practical for plant breeders to make selections based on characteristics such as spikelet sterility, which is a more direct measure of yield rather than counting the number of engorged pollen grains per anther and measuring anther size at flowering. Spikelet sterility takes approximately 15 minutes to measure. According to the results, less than 50% spikelet sterility under cold water exposure could be recommended as the baseline to accept a cultivar for cold tolerance. Cultivars with less than 50% spikelet sterility should be further evaluated in sequentially-sown field trials. The visual estimation of spikelet sterility by a trained operator was found to be highly correlated with measured spikelet sterility (r^2=0.85 **). It only requires 20 seconds to estimate the spikelet sterility visually. Thus, visual estimates of spikelet sterility may be appropriate to initially separate lines into susceptible, moderate and tolerant cultivars and then measured spikelet sterility may be calculated within the tolerant group to separate them further.

This project has also shown that cultivars with many engorged pollen grains per anther and a long anther with a large area are generally more cold tolerant except in Australian and Californian cultivars. For cultivars from other origins, the relationship between anther area (in the control plants) and the spikelet sterility in the field in year 4 had the most significant relationship (r^2=0.79**). Therefore, it is possible to incorporate these criteria into a routine selection program under field conditions in the absence of a cold event. Image analysis can increase the speed of measuring these flowering characteristics. Measuring these flowering traits in the polyhouse under warm conditions could be used to pre screen cultivars from other origins (apart from Australia and California) for high pollen number and large anthers prior to exposure in the cold water facility. This finding agrees with Suzuki (1982)

who suggests that plants with long anthers and/or long stigmas were cold tolerant. However, the finding refutes Sawada (1978) who implies that identifying cold tolerance is not possible under normal temperature conditions.

6.5 Selection of parents for cold tolerance

Many of the cold tolerant cultivars identified (Table 4-9 of the thesis) from this investigation originated in cooler climates, such as HSC55 (Hungary) and Plovdiv 22 (Bulgaria). Therefore, these cultivars tend to have short growth durations when grown in warmer climates like the Riverina. The $F_{5:7}$ populations that were developed between HSC55 and Plovdiv 22 and two Australian cultivars, Illabong and Millin, showed that cold tolerance is independent of early flowering (Figure 4-4). Therefore, it is recommended that cold tolerant cultivars from international origins be crossed with high yielding Australian cultivars that have good grain quality. The cold tolerant materials that have been identified in this project need to be further tested for cold tolerance under field conditions. The challenge for plant breeders is to capture the cold tolerance in cultivars of appropriate growth duration. This is a reasonable breeding objective given the high heritability and high frequency of progeny having cold tolerance observed in this investigation.

6.6 Optimum sowing date to reduce the cold damage in Australia

An optimum sowing date must consider two important aspects in relation to reducing cold damage. Firstly, an optimum sowing date must minimise the likelihood of encountering low temperature during reproductive development, and secondly grain yield must be maximised in warm years with no cold damage. One of the major factors that determine sowing time is ensuring the reproductive stage coincides with the warmest time of year (NSW Agriculture 2001). Typically, this occurs between late January and early February (Figure 2-1 of the thesis). Therefore, the time of sowing depends on the duration of a cultivar. Longer and shorter duration cultivars are recommended to be sown in early October and early November, respectively.

Crop phenology data from a range of sowing dates across four years (1999-2002) of field experiments provided 24 different conditions of temperature and daylength, enabling the development of phenology models for seven cultivars. Shorter duration cultivars such as HSC55 and M103 had double the risk of encountering minimum temperatures ($<15^{o}C$) during the young microspore stage when sown on October 1st than when sown on December 1st (Figure 5-9 of the thesis). The optimum sowing date for Amaroo was October 15th considering the low temperature sensitivity at the young microspore and flowering stages. However, according to the results, it is possible to sow Amaroo as late as November 1st when the seasonal temperatures are average. Even a good Amaroo crop could be expected, if sown as late as November 15th in a warm season. *Ricecheck* is a holistic management package for growers (NSW Agriculture 2001) and the current recommended sowing window for a combine sown Amaroo crop is 1st to 15th October. Despite this, sowing Amaroo in the early part of the recommended window may result in the crop

developing too quickly and having a high probability of encountering low temperature during the young microspore and flowering stages. Sowing cultivars during mid October reduces cold damage and has the additional benefit of improving water use by increasing the quantity of grain per unit of water used.

A later sowing date causes a delay in flowering and in the absence of low temperature there were two general yield responses among the seven common cultivars that were examined. Doongara, Sasanishiki, Hitomebore and HSC55 showed a tendency for a yield decline as flowering date was delayed. However, Amaroo, Millin and M103 showed a tendency for a yield plateau for flowering till March 5th and then a yield decline. In both cases, the yield decline was associated with a reduction in biomass and a stable harvest index. A combine sown Amaroo crop sown on November 15th would flower on February 28th if seasonal temperatures were average. Therefore, an optimum sowing date of October 15th for Amaroo would minimise low temperature damage whilst still maintaining high yield potential.

6.7 *Photoperiod sensitive cultivars to increase sowing flexibility*

Increasing the photoperiod sensitivity of cultivars is one strategy to increase the flexibility of sowing time for growers in Australia. The advantage of increased photoperiod sensitivity is that flowering occurs faster as daylength shortens, compared to photoperiod insensitive cultivars. When a delay in sowing occurs, growers could reduce the risk of encountering low temperature by either sowing a short season cultivar that is less sensitive to changes in daylength (e.g. HSC55) or a photoperiod sensitive long duration cultivar (e.g. more sensitive than Amaroo). The disadvantage of less photoperiod sensitive cultivars such as HSC55 is that sowing cannot occur early and hence the sowing window is narrow. Therefore, growers must be well informed of the optimal time of sowing for cultivars that are less sensitive to changes in daylength.

Several benefits of increasing the photoperiod sensitivity of Australian cultivars were identified. These benefits include greater sowing flexibility, reduced risk of low temperature damage during the reproductive stage and reduced water use. The photoperiod response of Construct 2 (Section 5.3.2.4 of the thesis) reduced the chance of encountering low temperature for a mean sowing date between October 1st and December 1st, compared to Amaroo. Breeding of mildly photoperiod sensitive cultivars (such as Construct 2) may therefore have the potential to replace the breeding of shorter duration cultivars. Reduced water use associated with shorter growth duration was highlighted by Reinke (1993) as one of the major benefits of a delayed sowing for shorter duration cultivars. Similar benefits of reduced water use are applicable to photoperiod sensitive cultivars. The low standard deviation of the phenological development stages of photoperiod sensitive cultivars such as Amaroo (Table 5-8 of the thesis) enables the risk of encountering low temperature to be minimised. The selection of cultivars that are slightly more

photoperiod sensitive than Amaroo in NSW rice growing conditions is one possible way to reduce the likelihood of late sown cultivars encountering low temperature during the young microspore stage.

7. Implications

Maximising rice production requires continued research in managing abiotic stresses such as low temperature. The present study provides new knowledge and selection strategies for the development of improved cold tolerance in rice. In concluding this project it can be stated that:

1. Consistent genotypic variation in low temperature tolerance was found using three different screening methods in over 50 cultivars from diverse origins. Cultivars were identified that were more cold tolerant than the Australian commercial cultivars in the field and in cold air screening.

2. Three screening methods which included temperature-controlled rooms, a cold water facility and the field were identified. Protocols to screen for cold tolerance using these three methods were developed and recommendations have been made to improve the efficiency of screening for cold tolerance.

3. Cold tolerant lines with optimum growth duration were developed from crosses of introduced cold tolerant cultivars with short duration (HSC55 and Plovdiv 22) and locally adapted Australian cultivars (Millin and Illabong). There was no relationship between growth duration and spikelet sterility among these lines and hence cultivars can be developed to optimise growth duration that would be high yielding. Crossing cold tolerant cultivars from international sources such as Hayayuki and Jyoudeki with cultivars of appropriate duration is one way of improving the cold tolerance of Australia's commercial cultivars.

4. The major advantage of photoperiod sensitive cultivars such as Amaroo, benefits farmers by increasing sowing flexibility. An October 15th sowing date was recommended for Amaroo to reduce the risk of encountering low temperatures during the young microspore and flowering stage. Sowing of Amaroo could be further delayed as late as November 1st with a low risk of cold damage when seasonal temperatures are average. Additional increases in levels of photoperiod sensitivity can further increase the sowing flexibility and reduce water use when sown late.

5. The level of cold tolerance of cultivars can be identified under both control and low temperature conditions by their flowering traits including the number of engorged pollen grains per anther, anther length, width and area and stigma area. Percent sterility and harvest index under cold exposure are the best tools to identify the cold tolerance level of a cultivar.

8. Description of the Project Intellectual Property and of any commercially significant developments arising from the Project.

This project has not as yet provided any commercially significant developments. However, the findings in this project will assist the rice breeding team at Yanco to develop cold tolerant cultivars in future years.

9. Recommendations

9.1 Increasing stigma area for improved cold tolerance

Further investigations are required to determine the effect of stigma area on pollen interception and subsequent fertilisation. If a positive effect of stigma area can be shown then increasing the stigma size of Australian rice cultivars may be a useful breeding objective to improve the likelihood of fertilisation during low temperature conditions.

9.2 Sensing organ in rice plants

Since cultivars showed variable response when different parts of the plant were exposed to low temperature, the method of selection for cold tolerance can be affected. Gunawardena *et al.* (2003) showed that both low root and panicle temperature during reproductive development had an additive effect on spikelet sterility in one cultivar (Amaroo). Therefore, further research is required to find out the most important sensing organ of commercial cultivars. Deep irrigation water would not protect the plant from cold, if the organ that sensed cold temperatures was something other than roots.

9.3 Flowering stage stress

The flowering stage is the second most sensitive stage to low temperature following the young microspore stage (Matsuo *et al.* 1995). The threshold temperature for low temperature stress at the flowering stage is not well understood. Research directed towards improving our understanding of the threshold temperature at flowering will increase the accuracy of determining an optimum sowing date using long term temperature data.

9.4 Yield penalty when flowering is delayed

Further examination of the relationship between the delay in flowering and yield is required to confirm the results identified on a plot level. The *Ricecheck* database could be used for this purpose since it contains large quantities of data across years, including PI dates and yields for many commercial cultivars. The temperature and photoperiod coefficients could be used to predict flowering dates (from PI dates) and then be related to yield in farmers' fields. A good first step would be to limit the database to the cultivar Amaroo, grown with deep water at the young microspore stage. Moreover, incorporating this phenological data in crop simulation models such as the Temperate Rice Yield Model (TRYM), ORYZA and CERES rice would be a powerful tool to improve the understanding of crop responses to the

environment and hence optimise management of crops to maximise yields. These results would test the potential benefits of photoperiod sensitive cultivars to the Australian rice industry. Following examination of the *Ricecheck* data, targeted investigations in farmers' fields across several years can then be used to further examine the yield penalty associated with a delay in flowering.

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