

# Designing rice to improve water productivity for temperate production

Jaquie Mitchell<sup>1</sup>, Christopher Proud<sup>1</sup>, Ricky Vinarao<sup>1</sup>, Wenliu Gong<sup>1</sup>, Brian Dunn<sup>2</sup>, Peter Snell<sup>2</sup> and Shu Fukai<sup>1</sup>

<sup>1</sup>The University of Queensland, School of Agriculture and Food Sciences, St Lucia, QLD, 4072, Email: [jaquie.mitchell@uq.edu.au](mailto:jaquie.mitchell@uq.edu.au).

<sup>2</sup>Department of Primary Industries, Yanco Agricultural Institute, Yanco, 2703, NSW, Australia.

## Abstract

Low water availability and low air-temperature are major constraints to Australian flooded rice (*Oryza sativa* L.) production in the Riverina. With water limitations and increasing irrigation costs, drill-sown aerobic rice production is proposed as a solution to deliver transformational change. Varieties have not been developed for aerobic conditions and as such potential donor varieties, traits of importance and quantitative trait loci (QTL) which contribute to cold and aerobic adaptation need to be identified. Our pre-breeding activities have led to the development of phenotyping systems to enable characterisation of rice genotypes for both cold and aerobic conditions. Putative QTL have been identified for both cold tolerance and root cone angle (RCA, deep rooting), with marker development underway. Genotypes with greater low temperature tolerance and narrower RCA than commercial varieties have been identified and subsequently incorporated into the rice breeding program to maximize productivity of rice adapted to a reduced water input production system.

## Keywords

genotypic variation, water use efficiency, water deficit, cold tolerance

## Introduction

Under permanent water (PW) rice growers in the southern Australian temperate environment of the Riverina region have achieved the highest yielding rice crops in the world, with a five year average (2013-18) of 10.3 t/ha (ABARES 2020). However, due to water limitations and associated high cost, the sustainability of this system is under question. Drill-sown aerobic rice production is proposed as a solution to deliver a step-change in water productivity. Aerobic production, non-flooded, well-watered conditions however, often comes with a yield decline in comparison to PW when unadapted (lowland/flooded) varieties are utilised. Suitable varieties need to be developed to maximise water productivity to make it an economically viable option.

Achieving high yields and yield stability under aerobic conditions firstly requires a significant improvement in the level of cold tolerance. Low temperatures (15-19°C) can occur at any time of the growing season in the Riverina, with annual reductions in yield of around 0.68t/ha, however when they coincide with the most sensitive young microspore (YMS) and flowering stages, the reduction to grain yield can be as much as 2 t/ha (Farrell et al 2001). When not grown under PW, the rice crop is more susceptible to low temperature damage (Ha *et al* 2018). Thus, to be able to maintain high yield and yield stability when grown under high yet transient water availability, there is a critical need to improve cold tolerance of genotypes. Furthermore, low repeatability of cold tolerance screens in the field within the breeding program has led to the desire for a molecular strategy.

The largest varietal limitation to the adoption of aerobic rice production, aside from cold tolerance, is rice's relatively shallow root system. It was hypothesised that increasing the fraction of roots at depth (e.g. >20cm) would decrease the impact of transient water deficit in the surface soil between irrigation events by increasing access to plant available water at depth. Under such conditions, stomata would be kept open and gas exchange enabled without transient decline. This paper reports on the progress of identification of traits and QTL contributing to cold and aerobic adaptation for improved water productivity in the Riverina.

## Methods

### *Germplasm*

Two sets of genetic material have been characterised, they are: 1) the Sherpa/IRAT109 (SHIR) population; and 2) the Yanco core japonica diversity set (YCJDS). The SHIR population, consisting of 252 genotypes, is a mapping population developed by the NSW DPI rice breeding program, where Sherpa is an Australian, high-yielding cold tolerant variety and IRAT109 is a deep rooting variety from the Ivory Coast, Africa. While the YCJDS consists of 300 genotypes that are relevant to the Australian breeding program which includes potential donors for cold tolerance and aerobic adaptation. Additional populations derived from cold donors Norin PL8 (Japan) and Lijiangheigu (Yunnan, China) have been developed for the detection of QTL associated with cold tolerance at YMS. The germplasm was genotyped using the Diversity Arrays Technology (DArT) genotyping-by-sequencing platform (DArTSeq) and the methodology for SNP, linkage mapping and QTL identification is described fully by Vinarao et al (2021). Statistics for both genotyping data and all phenotypic data was analysed by a mix of software but largely ASREML and R package based.

### *YMS cold tolerance*

Cold tolerance phenotyping, as measured by the degree of spikelet sterility, was conducted across two rooms of a controlled-temperature glasshouse facility at The University of Queensland, St Lucia campus (27°23'S, 153°06'E), using the two-set screening method described by Mitchell et al. (2016).

### *Yield evaluations under aerobic conditions*

Most yield evaluations under aerobic conditions were conducted at the University of Queensland's Gatton field site where temperatures rarely fall below critical thresholds during YMS. In general, two irrigation frequencies were deployed to evaluate aerobic adaptation: a well-watered (WW) control and an intermittent water-stress (IWS). WW was used to determine maximum genetic potential of a given genotype, and generally was irrigated three times a week to minimize any transient water deficit. IWS irrigations (typically Monday and Friday) were spaced to induce a mild transient stress event (barring rain). General management practices of the experiments utilised herein are described by Vinarao et al. (2021).

### *RCA and deep rooting*

Soil coring and the utilisation of core break counts (CBCs) was used to quantify the distribution of roots in field experiments, and loosely follows the methods of Wasson et al. (2014). Briefly, a 1m soil core was taken 50mm from an internal row. The core was divided into 100mm segments, with each segment broken in half, with the number of roots on each of the broken faces determined and summed to calculate the CBCs. Summary statistics were generated from the cores such as the deepest root and the deep root percentage below 20cm which is the sum of CBCs below 20cm/ total sum of CBCs.

Methods for quantification of RCA were developed for the field and glasshouse. In the field, after 50% anthesis, single plants were extracted (lifted) from the soil and any loose soil was shaken off. Plants were rotated to identify the two nodal roots with the widest internal angle, and the angle measured. In the glasshouse, a clear pot method inspired by Richard et al. (2015) was used where plants were grown against the wall of a clear ANOVA© pot for 35 to 45 days to allow for the development of nodal roots, and the angle between the two widest roots against the wall measured (Vinarao et al 2021).

### *Canopy to-air temperature differential (CTD)*

CTD as an index of stomatal conductance was determined from canopy temperature data collected using Everest 6210L Agri-Therm III, (Everest Interscience Inc, USA) and RS PRO thermohygrometer (Engtech, UK), between 10:00 and 14:00 on sunny days, collected after canopy closure across several experiments.

## Results and discussion

### *Cold tolerance at YMS*

Seven populations have been screened for cold tolerance derived from Lijiangheigu, Norin PL8 and Sherpa and have been phenotyped and marker association analysis conducted and total of 22 QTL have been identified across the populations. Twelve were identified in Norin PL8 derived lines, five of these were background specific and pose little value for marker-assisted selection (MAS). Two, *qYMCT6.1* and *qYMCT9.1*, have demonstrated an effect across multiple backgrounds, while a third (*qYMCT10.1*) has also been identified across two additional populations suggesting these are prime candidates for MAS, with marker development currently underway. Screening indicated a 10-20% decrease in spikelet sterility per QTL when exposed to 21/15°C (day/night) for 14 days at YMS. Additional QTL from the Lijiangheigu and Norin PL8 are in the process of QTL validation.

### *Yield*

Over 30 yield evaluations have been conducted under aerobic conditions at Gatton with additional trials conducted in the Riverina. The performance of the most widely grown variety, Reiziq, was highly variable with high yield achieved under optimal conditions (G20WW), however even with only mild water deficit significant yield reduction resulted (Table 1). This clearly demonstrated the peril of using lowland varieties under aerobic conditions. Conversely, Sherpa and Lemont consistently performed well in all environments although somewhat variable. IRAT109 on the other hand performed moderately well across all environments although it tended to lodge in high-yielding environments which led to yields below the trial mean in G20 WW.

**Table 1: Genetic values (expressed as deviation from trial means) for grain yield (t/ha) of key Australian and aerobic breeding lines across 4 Gatton (G, QLD) aerobic and 1 Yanco (Y, NSW) drilled permanent water trial conducted between 2018 (18) and 2020 (20). WW= well-watered, IWS= intermittent water stress. Derived from a two-stage unbalanced factor-analytic MET analysis consisting of 129 genotypes in 29 environments across Gatton and the Riverina.**

Variety	G18 WW	G18 IWS	G19 IWS	G20 WW	Y19
IRAT109	1.21	1.43	1.79	-1.75	0.61
LANGI	-0.25	1.22	-0.67	0.34	1.48
LEMONT	2.28	3.45	5.57	3.81	2.38
M205	-0.27	-0.93	-0.95	2.07	2.66
REIZIQ	-0.79	-1.47	-1.30	3.10	1.93
SHERPA	1.51	2.49	3.51	3.21	3.20
Trial Mean	8.72	7.15	8.49	9.00	10.94

### *Deep rooting to improve yield*

Four experiments in 2017/18 and 2018/19 conducted under IWS demonstrated a moderate to high genetic correlation (0.44-0.73) between grain yield and deep root (DR) percentage below 20cm. As expected, this was not observed in 2019/20 under WW due to high frequency irrigation. The DR from WW and IWS experiments with twenty diverse genotypes, was strongly correlated with one another, however, heritability under WW (0.69) was considerably higher than IWS (0.32) suggesting better genetic gains with DR can be made with selection under well-watered conditions.

In 2018/19, experimentation with the SHIR population and a second experiment evaluating 48 diverse genotypes, identified that there was a moderate negative correlation between RCA and the DR. Analysis of RCA across four environments in the SHIR has found it to be highly stable ( $r_g=0.44-0.95$ ) which indicated that RCA can be used for the indirect selection of deep roots even if the environment is not conducive to expressing the deep root phenotype. There is also an advantage in that RCA can be screened in the glasshouse in earlier generations whereas CBCs are limited to plots in the field. Linkage mapping of the SHIR population has revealed stable QTL on chromosome 4 (qRCA4.1) and chromosome 1 (qRCA1.1) with the former accounting for 17-24% of the genetic variance (Vinarao *et al* 2021). Multi-background QTL and marker validation is underway to deliver usable molecular selection tools to the breeding program.

## CTD

The relationship between CTD and grain yield is well-established in the literature, although not as thoroughly in rice, nor in aerobic rice systems. In several experiments CTD had moderate to high negative genetic correlations with grain yield (e.g. -0.42 to -0.87), particularly under IWS. The results suggest that CTD can be used in aiding the selection of aerobically adapted genotypes.

## Conclusion

High yielding SHIR lines have been identified which achieved consistently greater than 12.5 t/ha when grown under aerobic conditions at Gatton, which is comparable to yield obtained under PW in the Riverina. The work has established several phenotyping methods for putative traits and QTL for adaptation to aerobic production. Furthermore, highly cold tolerant genotypes have been identified and several high-yielding, cold tolerant, deep-rooting SHIR lines have since entered the crossing block of the NSW DPI rice breeding program. In the YCJDS, several genotypes have been identified as potential donors of high stomatal conductance/low CTD. Importantly, QTL for cold tolerance and RCA have been validated. These are ready for deployment in the breeding program to improve the genetic gain for cold tolerance and the opportunity now exists to improve water productivity for a sustainable rice production system in the Riverina.

## Acknowledgements

The authors wish to thank AgriFutures (PRJ-011067 and PRJ-009790) for financial support, and the NSW Department of Primary Industries for providing the germplasm used in the experiments. We also wish to thank the technical support provided by those in NSW DPI rice breeding and agronomy teams and technical support staff from UQ glasshouses and crops research unit teams.

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