



Precision breeding of cold tolerant rice

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in a rice hull

- This project is investigating a number of techniques to better understand the molecular nature of cold tolerance in rice plants, as well as identifying methods and technologies to speed up the breeding of cold tolerant varieties for the Australian rice industry

The ongoing viability of the Australian rice industry relies on maintaining high productivity while improving water use efficiency. Improvement of cold tolerance in rice varieties is therefore very important in terms of providing constant and reliable production, and also reductions in input costs through less reliance on deep water at panicle initiation.

According to previous research, cold tolerance is controlled by many different genes. The mechanism of how those genes control cold tolerance is still not clear. Improvement of cold tolerance by conventional breeding techniques is very difficult and time consuming. It requires expensive facilities for screening and it takes many breeding cycles to bring together all the important agronomic, physiological and quality traits.

In this project, we are trying to develop marker technologies to improve breeding efficiency and reduce the time for the delivery of new cultivars.

New sources of cold tolerance

Several cold tolerant rice varieties have been introduced into Australia from overseas (China, Japan, California and Hungary). The Chinese varieties were sourced from regions where rice is grown traditionally at high altitudes, where average day temperatures in the growing season range from 15°C to 18°C, and the average minimum temperatures range from 8°C to 15°C. These varieties have demonstrated good cold tolerance in Australia under various field and glasshouse testing conditions.

Integration of cold tolerance from the introduced varieties into Australian cultivars will help sustain Australian rice varieties under cold temperatures with reduced water use. We have made seven crosses between Australian cultivars and the cold tolerant varieties for genetic research and breeding.

New technologies

A technology to produce true-breeding lines in one generation by microspore culture using young rice pollen is under development. This technology has great potential in rice

breeding and will significantly cut the breeding time required for advancing generations. Many true-breeding lines have been produced from several crosses. We have also optimised rice growing conditions in the glasshouse to produce two to three generations per year for the development of recombinant populations. Two such populations have been developed from crosses between the Australian variety Doongara and two Chinese cold tolerant landraces, and are now ready for evaluation of cold tolerance as well as genetic analysis and selection within breeding populations.

The two Chinese landraces have very strong cold tolerance (Figure 1). These two populations will be treated at 13°C for four days at the pollen microspore development stage and the cold tolerance of each individual plant will be scored. This cold tolerance information will be used for genetic research, molecular marker linkage analysis and breeding. The cold tolerance testing will also be carried out under different temperature regimes at different locations. Other crosses between another Australian variety Reiziq and cold tolerant varieties will be treated under the same low temperature conditions for cold tolerance testing as soon as true-breeding populations are available. The cold tolerance information of these lines will be very useful for breeders, both for selection and for making further crosses in order to combine the complex characters.

The requirement for expensive temperature controlled rice growing conditions to provide reliability for cold tolerance screening within large breeding populations has been an impediment to the breeding program for a long time. The solution would be marker-assisted breeding, but first a better understanding of the molecular basis of cold tolerance is required.

Sugar delivery underlies fertile pollen

To further the molecular understanding of cold tolerance, some Australian and international rice varieties have been used to understand the mechanism of cold tolerance and to develop molecular markers.

Comparison of the cold sensitive variety Doongara with the cold tolerant Chinese variety Lijianheigu indicated that



sugar metabolism was disturbed by cold in the sensitive Doongara but not in the cold tolerant Chinese variety. Doongara plants accumulated large amounts of sugar in the anthers pollen grains; the pollen grains were sterile and hence after pollination no seed was produced.

We found that low temperature turns off the genes responsible for sugar transport into the pollen grains and therefore starch cannot be produced in the Doongara pollen in cold conditions. Cold did not cause repression of sugar delivery in cold tolerant Chinese rice and fertile pollen was still produced following cold treatment. The sugar metabolism genes also continued to function normally during cold treatment of cold tolerant rice.

Gene expression profiling using DNA chips indicated that large numbers of genes are differentially expressed under cold stress. We identified 242 unique genes that are expressed differentially between cold sensitive and cold tolerant rice. These genes are involved in processes such as senescence, cell death, male sterility and plant hormone response.

We found that the plant hormone *abscisic acid* plays an important role in the induction of pollen sterility, accumulating in cold sensitive anthers and repressing the sugar transport genes. In cold tolerant rice abscisic acid levels are much lower and no accumulation of the hormone occurs in response to cold treatment.

We are currently investigating how the production of abscisic acid and the sensitivity of the plant to it, are controlled in cold tolerant rice, and what genes are involved in this mechanism. Also, we are investigating whether the difference in abscisic acid sensitivity at the seedling stage between cold sensitive

and tolerant varieties can be exploited as a selection method that can replace cold treatment. This could greatly facilitate and speed-up selection efforts in breeding.

A molecular approach called Diversity Arrays Technology (DART) was used to locate molecular markers controlling cold tolerance in the rice genome. Four markers have been identified that are strongly linked to the cold tolerance loci of the Chinese cold tolerant landrace Lijiangheigu. We are currently mapping the cold tolerance genes that are linked to these markers.

Better understanding of cold tolerance

Our work has laid the foundation for a better molecular and genetic understanding of the cold tolerance mechanism in rice and this improved knowledge will pave the way for the development of molecular markers and improved methodologies for cold tolerance screening.

The technologies developed by this project will significantly improve breeding efficiency, resulting in rapid and accurate incorporation of cold tolerance genes into Australian rice varieties. Rice farmers and the rice industry in general will greatly benefit from the outcomes of this project. Cold tolerant rice will provide stable yield potential under adverse weather conditions, as well as improved water-use efficiency and overall improved sustainability. 🌱

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Figure 1: Low panicle fertility of the cold sensitive Australian variety Doongara, compared with the cold tolerant Chinese varieties Lijiangheigu and Banjiemang. The three varieties were cold treated at 13°C for four days at the critical pollen microspore stage of flowering.