RICE CRC

RESEARCH REPORT

Title of Project:	Molecular basis of cold-induced pollen sterility in rice.
Project Reference number:	3201 (incorporating project 3209)
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Final report – project number: 3201

1. Background to the Project.

Cold-induced pollen sterility is the most yield-affecting problem for the Australian rice industry, leading to yield losses of 20-40%, or A$15-29 million, on average every 3-4 years. The molecular basis of the problem remains largely unknown, and breeding efforts have been largely unsuccessful due to the lack of reliable selection techniques in the field/glass house, and the lack of molecular markers for selection of cold-tolerant lines. Identification of the molecular basis of the problem may lead to the identification of markers that will greatly facilitate breeding of cold-tolerant Australian rice varieties. Our starting point was to study the accumulation of sucrose that occurs in anthers after cold treatment. We investigated whether cold affects expression of a gene involved in sucrose metabolism, thereby introducing a metabolic block in sucrose metabolism.

2. Objectives.

- Detailed study of the effect of cold on sucrose metabolism in rice anthers.
- To identify the gene involved in sugar metabolism that is affected by cold in rice anthers.
- The study the effect of cold on the expression of this sugar metabolism gene, in order to identify the signaling mechanism used by cold to affect sucrose metabolism.
- To identify molecular markers that could be used in marker-assisted breeding.
- To study effect of cold on sugar metabolism in cold-tolerant rice varieties.

3. Introductory technical information concerning the problem or research need

- Breeding of cold-tolerant rice varieties is difficult because cold spells cannot be predicted in the field, adequate glasshouse infrastructure for large-scale selection experiments is not available, and glasshouse cold tolerance is not always reproducible in the field.
- The availability of objective molecular markers for cold tolerance would greatly facilitate the work for breeders.
- Cold tolerant rice varieties from Hungary and China have now been identified. These can be used to identify gene loci that could be used as a marker for cold-tolerance in breeding populations.

- Improving our knowledge about how cold affects sucrose metabolism may lead to the identification of molecular markers suitable for breeding

4. The Methodology - including a description and justification.

- Grow rice plants until flowering stage and harvest anther material for experiments.

- Biochemical quantification of components of sucrose metabolism to identify the problem caused by cold treatment of anthers: measurement of sucrose levels, starch content and invertase enzyme activity in cold-stressed and normal anthers.

- Cloning and sequencing of invertase genes in rice and identification of the gene encoding cell wall invertase that is critically affected by cold in anthers.

- Studying invertase gene expression at the RNA level by northern blot hybridization and RT-PCR. This enables us to study the effect of cold and other stimuli (hormones) on invertase gene expression, carry out time course experiments, study tissue location of invertase expression, and ultimately to identify the right gene.

- Make invertase promoter-reporter gene constructs for transformation to rice.

- Produce transgenic rice plants containing promoter-reporter gene constructs. This allows us to study precisely in what cells the gene of interest is expressed and what the effect of cold on this expression is.

5. Detailed results - including the statistical analysis of results.

- We found that cold treatment at the young microspore stage results in a dramatic depletion of starch accumulation in the pollen grains at anthesis. Starch is essential in pollen grains, as storage carbohydrate for energy supply during pollen germination and fertilization.

- Measurements of sucrose content showed that sucrose levels increased in anthers that were cold-treated at the young microspore stage.

- Consistent with these results, we found that the enzyme activity levels of cell wall invertase, but not soluble invertase decrease upon cold treatment at the young microspore stage only.

- These results suggest that cold induces a reduction of cell wall invertase activity, which then leads to a blockage of sucrose supply to the developing pollen grains. This blockage is explains the absence of starch synthesis in the pollen grains at maturity.

- We have cloned and sequenced four rice invertase genes (OSINV1-4). While all of these genes are expressed in the anthers, only the OSINV4 gene was

specific for anthers. The expression of OSINV4 and OSINV2 are repressed by cold.

- More detailed expression analysis showed that OSINV4 is expressed for a short period at the young microspore stage, where it is down-regulated by cold. This is the critical stage for induction of pollen sterility. It is also present at high levels immediately after the first mitotic division until late in development.

- Preliminary results suggest that the expression of OSINV4 messenger RNA is higher in some tolerant lines than in Doongara, but in one of two tolerant lines investigated so far, there was no effect of cold on the expression of the gene. Both the higher expression levels and the fact that cold does not repress expression of OSINV4 in one of the lines may contribute to cold-tolerance.

6. Discussion of results including an analysis of research outcomes compared with the objectives.

- The finding that sucrose metabolism, and more specifically the expression of the OSINV4 gene is affected by cold is an important step forward in the identification of the molecular basis of cold-induced pollen sterility in rice.

- OSINV4 is expressed for a very short time at the critical stage of pollen development, the young microspore stage, which makes this gene a critical target for cold treatment.

- Cell wall invertase is essential for providing the developing pollen grain with sucrose. Cold-induced reduction in invertase activity at that stage may affect energy supply to the young microspore and a disruption of normal pollen development.

- OSINV4 expression at the young microspore stage may be essential for providing the pollen grains with sucrose to complete the first stages of pollen development. Failure to do so will result in premature abortion of pollen development.

- The effect of cold may not be as drastic after the first mitotic division stage, when OSINV4 expression is higher and occurs for a longer period. At this stage, the enzyme may be required for starch biosynthesis, which occurs at the later stages of pollen development.

- The fact that OSINV4 expression is higher or, in one of the varieties tested, not affected by cold, provides good prospects to use the gene as a marker in breeding experiments.

7. Implications and recommendations - where possible, a statement of relevant costs and benefits to the Australian industry should be provided.

- The finding that OSINV4 is an anther-specific cell wall invertase gene that is a target of cold stress is important and will enable us to look for molecular markers for breeding.

- To achieve this, we need to acquire more detailed expression data of the OSINV4 gene, more specifically about where (exact cell types) the gene is expressed and how the gene is influenced by stimuli such as hormones (abscisic acid, gibberellic acid) and sucrose.

- The availability of cold-tolerant lines will also allow us to study what is different about the expression and regulation of this gene in those lines.

- OSINV4 is located in chromosome 4 of rice. Cold-tolerance loci have previously been mapped on this chromosome. It would therefore be useful to identify the cold-tolerance loci in the cold-tolerant varieties. Dr. X. Zhao, Univ. Sydney, is currently making double haploid lines from a cross of Doongara and two tolerant varieties. Using microsatellite mapping we will be able to identify these loci in the future and see whether the OSINV4 gene on chromosome 4 is involved.

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

- Identification of molecular markers based on our OSINV4 research will greatly facilitate breeding of cold-tolerant rice varieties. Molecular markers could be made available to breeders as easy to use PCR markers or diagnostic kits.

- It may also enable us to design strategies for genetically engineering cold-tolerance in existing rice varieties, which could save a lot of time in producing tolerant varieties without changing other important traits, eg. grain quality.

- This may enable us to apply for IP protection in the future and commercial applications on the long term.

9. Recommendations on the activities or other steps that may be taken to further develop, disseminate, or to commercially exploit the results of the project.

10. Relevant References.

- "Chilling-induced pollen sterility in rice is associated with sucrose accumulation and repression of apoplastic invertase expression." – SN Oliver, JT Van Dongen, P Geigenberger, HS Saini, SF Fernandes, ES Dennis and R Dolferus. Plant Physiology. Submitted to CSIRO Plant Industry editorial panel.

- "Cold-induced sterility in rice: a sweet affair." - SN Oliver, ES Dennis, R Dolferus. Rice Farmers Newsletter (submitted).

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