# FINAL REPORT

CSP319

# A novel approach to improve water use by grain crops

## PROJECT DETAILS

| | |
|---|---|
| **PROJECT CODE:** | CSP319 |
| **PROJECT TITLE:** | A NOVEL APPROACH TO IMPROVE WATER USE BY GRAIN CROPS |
| **START DATE:** | 01.03.2001 |
| **END DATE:** | 30.09.2004 |
| **SUPERVISOR:** | NEIL TURNER (CHIEF RESEARCH SCIENTIST) |
| **ORGANISATION:** | CSIRO PLANT INDUSTRY |
| **CONTACT NAME:** | NEIL TURNER |

## Summary

Aquaporins or water channels are pores in the membranes of cells that have been shown to affect the rate of water movement through plant membranes. This project aimed to determine whether the aquaporins influenced the movement of water through the roots of wheat and lupins. By using a blocker of water channel activity, it was shown that the channels controlled water movement through wheat roots, but not lupin roots in which water moved around the cells, not through the membranes. Waterlogging appeared to activate the aquaporins in wheat, but had little effect in lupin, particularly in yellow lupin that is waterlogging tolerant.

## Report Disclaimer

This document has been prepared in good faith on the basis of information available at the date of publication without any independent verification. Grains Research & Development Corporation (GRDC) does not guarantee or warrant the accuracy, reliability, completeness or currency of the information in this publication nor its usefulness in achieving any purpose. Readers are responsible for assessing the relevance and accuracy of the content of this publication. GRDC will not be liable for any loss, damage, cost or expense incurred or arising by reason of any person using or relying on information in this publication. Products may be identified by proprietary or trade names to help readers identify particular types of products but this is not, and is not intended to be, an endorsement or recommendation of any product or manufacturer referred to.



Level 4, East Building | 4 National Circuit, Barton ACT 2600 | PO Box 5367, Kingston ACT 2604 | t. +61 6166 4500 | f. +61 2 6166 4599 | grdc@grdc.com.au | grdc.com.au

Other products may perform as well or better than those specifically referred to. Check www.apvma.gov.au and select product registrations listed in PUBCRIS for current information relating to product registration.

## Copyright

Grains Research and Development Corporation. This publication is copyright. Apart from any use as permitted under the Copyright Act 1968, no part may be reproduced in any form without written permission from the GRDC.

## Old or Archival Reports (Projects that concluded in 2007 or earlier)

The information contained in these older reports is now several years old, and may have been wholly or partially superseded or built upon in subsequent work funded by GRDC or others. Readers should be aware that more recent research may be more useful for their needs. Findings related to agricultural chemical use are also potentially out of date and are not to be taken as a recommendation for their use.

## Conclusions

While the roots of wheat, narrow-leafed lupin and yellow lupin clearly have water channels (aquaporins) that are active, water movement in both lupin species and to a lesser degree in wheat is largely in the apoplast. This means regulation of aquaporin activity will have little effect on the water movement, water use and water-use efficiency of these crops. Although there has been considerable research on the molecular basis of aquaporins in plants, it is not appropriate to follow a genetic route for controlling water use by altering water channel activity. Furthermore, the study suggests that while waterlogging tolerance may be related to aquaporin activity, it has other bases and much more needs to be known about the role of aquaporins and the genetic variation in their activity in relation to waterlogging before a genetic solution based on knowledge of specific aquaporins can be contemplated.

## Outcomes

The primary aims of this project were to provide a basic understanding of the role of water channels (aquaporins) that reside in the membranes of plant cells and their influence on water movement through the roots of three crop plants; wheat, narrow-leafed lupin and yellow lupin. These three species were chosen as previous work showed differences between wheat and narrow-leafed lupin in the rate of water movement through the roots of these species. Yellow lupin was included because of its tolerance to waterlogging, an environmental factor for which the role of water channels was unknown. A second aim was to train a PhD student in modern methods of studying water relations of crops of interest to the grains industry.

The expected benefit was therefore primarily one of capacity building. The PhD student training, conducted at both the University of Western Australia (UWA) and Adelaide University, involved use of the pressure chamber, root pressure probe and cell pressure probe, all of which proved difficult in developing protocols for the studies. The student also studied the anatomy of roots and explored several microscopic techniques. The project has not been without its difficulties and the student (Mrs Bramley) has learned that research is not always easy and requires innovation to overcome some of the difficulties. The student has also had to present seminars at the University of WA and Adelaide University and has participated by presenting her research results at the annual meeting of the Australian Society of Plant Scientists. She also participated in the International Conference on Plant Anaerobiosis held in Perth in September 2004. Therefore, Mrs Bramley is well trained to undertake research for the grains industry either in a university or CSIRO.



Level 4, East Building | 4 National Circuit, Barton ACT 2600 | PO Box 5367, Kingston ACT 2604 | t. +61 6166 4500 | f. +61 2 6166 4599 | grdc@grdc.com.au | grdc.com.au

A second outcome of the project is a clear understanding of the role of water channels in water transport of roots. The water channels in wheat are active in water movement, but in both lupin species water movement through the root occurs through the apoplastic without passing through membranes. This suggests there is little to be gained from pursuing a genetic approach to manipulating water channel activity in crops. While species differ in their response to blocking of water channels in the cells and appear to play a role in the response to waterlogging in wheat, the effects are not great. This suggests they do not play a significant enough role in water movement to warrant breeding for their up-regulation. It also suggests that support of further research on water channels is not warranted by the grains industry at this stage.

## Achievements/Benefits

The aims of this project were:

1. To determine the role of aquaporins (water channels) on the hydraulic conductance and water use of roots and leaves of grain crops, with emphasis on wheat and lupin.
2. To determine the effect of environmental factors (drought, waterlogging, salinity, temperature extremes) on the activity of aquaporins and the potential to alter water use and water use efficiency of crops.
3. To train a PhD student in a rapidly-expanding field and introduce new skills to the Western Region.

The project aimed to provide strategic new knowledge that will ultimately enable improved management of the hydrological balance through crop rotations and germplasm to better utilise water to benefit crop yields and reduce deep drainage. The second major aim was to increase the skills base by training a young scientist in an emerging new field of value to the grains industry.

Background

Most agricultural systems in Australia are constrained by limited rainfall. This is particularly true of the grains industry in the Western Region where crops are grown under rainfed conditions and terminal drought frequently limits production. Crops have been carefully selected over the past century with adaptations that fit them to particular environmental and edaphic niches. In particular, plant breeders have selected short-season crops that have shown small, but steady, increases in yields throughout most of the Western Region. Recent studies also demonstrated that significant yield increases can be achieved by improving the water use and/or water use efficiency of cereals and pulses. These characteristics are now being selected in traditional breeding programs. Despite terminal drought being a major limitation to yield, waterlogging can also limit yields, in particular cereals, when waterlogging during the tillering window can reduce the number of fertile tillers at maturity. Considerable understanding has also been gained in recent years on the factors that control water use by plants, so that rotations and management practices can be developed that enable better use of rainfall. For example, earlier planting to match the pattern of water use to rainfall input has led to improved crop yields and to greater use of the rainfall resulting in smaller losses through soil evaporation and drainage. Similarly, two or three years of lucerne production can dry the profile sufficient to reduce waterlogging during the tillering window.

A discovery in the 1990s provided an opportunity to explore new ways to potentially control the water use and water use efficiency of crops. Water channels were discovered in the membranes of all plant and animal cells. These channels comprise proteins called aquaporins that span the membrane and regulate movement of water across cell membranes. This potentially provides an exciting new opportunity to genetically manipulate the hydraulic conductance of crops and achieve significant advances in improving water use and water use efficiency. Prior to the commencement of the project, research emphasis had been on the molecular biology and activity of aquaporins in cell membranes. Work had not been conducted on the influence of aquaporins on the hydraulic conductance of whole transpiring plants, particularly roots. Since there are pathways of water movement in the roots and leaves of plants other than through cell membranes, and as transport in the xylem is largely a process controlled by differences in water potential, any change in aquaporin activity at the level of the cell may not affect the transpiration of whole plants.

Major activities

The project was initiated to study the role of aquaporins on the hydraulic conductance and waterlogging tolerance of roots of three important species of crops; wheat, narrow-leafed lupin and yellow lupin. Wheat and narrow-leafed lupin were chosen as previous work in Dr Neil Turner's laboratory showed that the hydraulic conductance of wheat and lupin differed markedly, even though both species grow in similar environments and use similar amounts of water from the soil. Yellow lupin was included as previous work in co-supervisor Dr David Turner's laboratory had shown that it was much more waterlogging tolerant than narrow-leafed lupin. The basis of this tolerance was unknown and may have been related to aquaporin activity.

A PhD student was recruited from Flinders University at the end of 2000 and commenced studies on the project in March 2001. After an initial literature review and development of a research proposal (a requirement for all PhD students at the University of Western Australia), Mrs Helen Bramley commenced research in CSIRO Plant Industry in Perth in October 2001. Initial experiments confirmed that narrow-leafed lupin roots had twice the hydraulic conductance of wheat roots and showed that the hydraulic conductance of yellow lupin was similar to narrow-leafed lupin. When mercuric chloride[#], a chemical that is known to block the water channels in plants, was applied to the soil around the roots, the hydraulic conductance of wheat roots was reduced by 20-50%, whereas narrow-leafed and yellow lupin roots were unaffected. This suggests that the water channels were active in controlling the flow of water in wheat roots, but not in lupin roots. Before concluding that this implied that the water in lupin roots bypassed the cells and moved through the apoplast (free space between the root cells), it was necessary to demonstrate that the mercuric chloride entered the root and was not absorbed to the soil or prevented from entering the root.

Mrs Bramley then moved to Adelaide to work in the laboratory of co-supervisor Professor Stephen Tyerman who has pressure probe facilities for studying the hydraulic conductivity of individual plant cells and a root pressure probe for detailed measurements of the hydraulic conductivity and permeability of detached roots at Adelaide University. Measurement of individual cells within the root showed that the permeability of cortical root cells was very high in all three species. Use of mercuric chloride to inhibit the activity of the water channels had a marked effect on the permeability of the wheat root cells and a small effect on the lupin roots. Cells close to the outside of the root cortex of lupin were affected to a greater extent to those four or five cell layers deep. Therefore, it is concluded that the cortical cells of both lupin species have active aquaporins, but that water movement across the root is largely in the apoplast outside the cell membranes.

Initial studies in CSIRO Perth in 2001 and early 2002 showed that waterlogging of the plants, particularly lupins, in the pressure chamber filled the root apoplastic spaces with water, making it impossible to measure the hydraulic conductance of roots during a waterlogging event. Therefore the root pressure probe was used at the University of Adelaide to investigate the effect of waterlogging on detached whole roots of the three species. Measurements were carried out before and after imposing 30 minutes of mild hypoxia on the root tissue to simulate the effects of waterlogging. Hypoxia did not affect the hydraulic conductivity of wheat roots, but during re-aeration the hydraulic conductivity was faster than pre-treatment rates by 1.8 times. This suggests that aquaporins may have been activated by the waterlogging treatment. The lupins were affected differently reflecting their tolerance to waterlogging. Hypoxia caused a small reduction in hydraulic conductivity of yellow lupin, which is known to be more tolerant to waterlogging than narrow-leafed lupin. This reduction recovered during re-aeration. The hydraulic conductivity of narrow-leafed lupin was also reduced by a small amount, but most roots could not be measured when re-aerated. The small reduction in hydraulic conductivity observed for lupin roots also supports the conclusions of the pressure chamber experiments that water transport across lupin roots may be mostly apoplastic.

Major achievements

A student has been trained in modern methods of studying plant water relations in crops. The studies suggest that water channels are active in wheat and to a lesser extent in both narrow-leafed lupin and yellow lupin. However, this has little effect on water transport through the root of lupins. This indicates that the water bypasses the cells and moves through the apoplast in these species. Waterlogging appears to have little effect on water movement across the root in yellow lupin, kills the roots in narrow-leafed lupin and activates the water channels in wheat.

## Other research

Dr Neil Turner was invited to present a paper on 'Sustainable production of crops and pastures under drought in a Mediterranean environment' at an international conference in Spain organised by the Association of Applied Biologists in March 2003 and a paper on 'Agronomic options for improving rainfall use efficiency of crops in dryland farming systems' at an international conference on Water-Saving Agriculture and Sustainable Use of Water and Land Resources in Yangling, China, in November 2003.

## Intellectual property summary

Students at the University of Western Australia retain control over all intellectual property generated while a student for a

# Refer to 'Report Disclaimer'

period of 12 months after the thesis is lodged in the university library. It is anticipated that all the research results will be published in international journals so that the research results are available to the wider scientific community.

## Additional information

Tyerman, S. D., Niemietz, C. M. and Bramley H. 2002. Plant aquaporins: multifunctional water and solute channels with expanding roles. Plant Cell and Environment, 25, 173-194.

Bramley, H., Turner, D.W., Turner, N.C., and Tyerman, S.D. 2004. The measurement of water flow through roots. Are hydrostatic pressure gradients telling us the full story? Combined Conference Abstracts ComBio2004, Perth, Western Australia, September 2004. SYM-30-04, p. 54.

Bramley H, Turner D., Tyerman S.D. and Turner N.C. 2004. The influence of hypoxia on root hydraulic conductivity of wheat and lupin. Abstracts of the International Conference of Plant Anaerobiosis, Perth, September 2004.



Level 4, East Building | 4 National Circuit, Barton ACT 2600 | PO Box 5367, Kingston ACT 2604 | t. +61 6166 4500 | f. +61 2 6166 4599 | grdc@grdc.com.au | grdc.com.au