Progress continues for tolerant cereal

LEFT: *Collaborators from University of Tokyo with UWA and CRC staff inspecting* Hordeum marinum *in the glasshouse. Tim Colmer (UWA), Hiro Takahashi (U Tokyo), Mikio Nakazono (U Tokyo), Lukasz Kotula (U Tokyo) and Mike Ewing (FFI CRC).*
(Photo: Greg Lawrence, Future Farm Industries CRC)

By Lucy Kealey
Kondinin Group

A salt- and waterlogging-tolerant cereal moves a step closer to reality with the inclusion of two more breeding lines in field trials this year as part of a Grains Research and Development Corporation (GRDC) Future Farm Industries CRC project.

Plant physiologist Professor Tim Colmer, University of Western Australia (UWA), sees significant market potential for a productive cereal with these key attributes.

"The most recent crosses have used long-season grazing wheats because the greatest potential market for a salt- and waterlogging-tolerant cereal is in alley-farming system with perennials, such as saltbush, to produce fodder on saline land," Prof Colmer said.

"The new cereal will provide some bulk and lower salt-content feed."

Researchers are using the same principles and techniques by which triticale was developed — crossing two species (wheat and rye). Here the cross is between sea barleygrass (*Hordeum marinum*) and wheat.

key points

- Two salt- and waterlogging-tolerant hybrids of wheat and sea barleygrass will be tested in a field trial near Darkan, WA as part of a 'proof of concept' trial for developing crop options for saline, waterlogged land
- A fully-fertile hybrid is anticipated to be produced by the end of 2010, as a result of backcrossing work
- Collaborative work between Future Farm Industries CRC and Tokyo University will assist the project with the identification of the genes regulating waterlogging tolerance in sea barleygrass, and the new hybrids.

Like triticale, the new plant will contain the full chromosome sets of both parents, thus making it an amphiploid hybrid.

Ground-truthing the new hybrid

Dr Rafiq Islam, a cytogeneticist at the University of Adelaide, has successfully crossed different sea barleygrasses with several wheat cultivars. The resulting amphiploid hybrids have been screened in the glasshouse, but field testing is required to determine their ability to grow and yield in a commercial production environment.

One of the hybrids produced by a cross between sea barleygrass sown from an international germplasm collection and Westonia wheat has been tested in the field for two years at Lake Grace, WA.

"In the first year of field work, the new hybrid performed really well at germination — it was equivalent to barley, which is quite tolerant to salinity at germination," Prof Colmer said.

"There was quite a lot of late rain that year and it looked pretty showy. This particular amphiploid hybrid has low fertility, so the objective of the trial was just to assess biomass production."

"In the second year, barley looked a lot better than our material because its shorter growing season meant it avoided some of the stresses at the end of the year, linked to increasing salt levels during grain fill."

Field testing the amphiploids

During 2010, two amphiploids — one from a long-season grazing wheat that produces 'better fertility' hybrids and one from Westonia — will be going into the field.

While salt tolerance has been promising in the glasshouse, the hybrids have not been put to the test for waterlogging under field conditions because of recent dry seasons.

In search of a wetter site, field testing of the next two hybrids will be undertaken in the higher-rainfall zone near Darkan, in the south of WA.

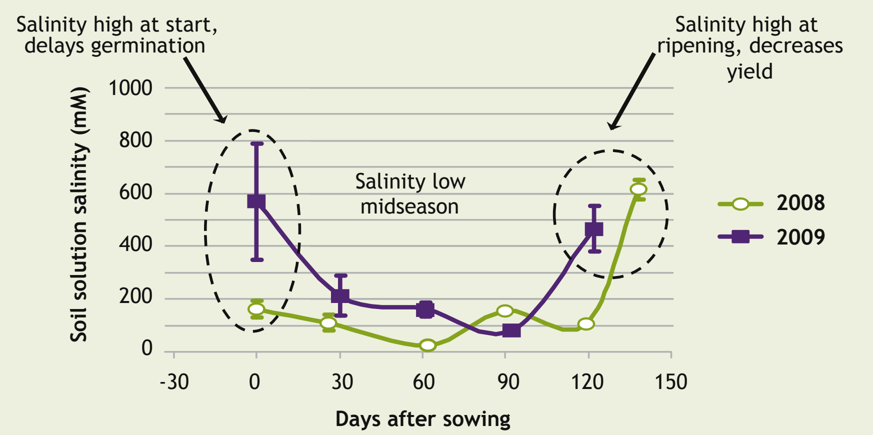
Dr Ed Barrett-Lennard (DAFWA) has characterised how salinity level changes with moisture content through the growing season and highlighting the importance of developing the right phenology for new plants on saline land (see Figure 1). Plants that flower early and fill grain early will likely have an advantage in these drier, saline areas (for more information see *Focus on Perennials* Issue 10).

He is also confident this moderately saline site will experience some waterlogging.

In the meantime, all 16 of the wheat-sea barleygrass hybrids continue to be screened under controlled conditions at UWA and at CSIRO in Canberra, with the cooperation of Dr Rana Munns.

Under controlled conditions of moderate salinity and waterlogging, the group has seen the expression of major useful traits in the hybrids. One trait is the potassium to sodium ratio (K:Na) in the shoots, which is higher

Figure 1 Soil salinity changes on the saline field plot near Lake Grace during the 2008-09 growing season



Source: Barrett-Lennard & Altman (unpub)

RIGHT: Small field plots at Lake Grace, where the amphiploid hybrid was tested during 2008 and 2009. (Photo: Left to right: Imran Malik (UWA), Katsuhiro Shiono (U Tokyo), Prof Tim Colmer (UWA), Michael Lloyd (Grower), Dr Ed Barrett-Lennard (DAFWA), Mikio Nakazono (U Tokyo), Hiro Takahashi (U Tokyo)).

in the hybrids than in the wheat parents — indicating better ability to withstand high salinity (see Figure 2).

Backcrossing to restore fertility

Fertility of the resulting hybrid progeny is a constant challenge for researchers, impacting significantly on potential grain yield. The first generation hybrids have low fertility — particularly so in the Westonia hybrids, although much better in the hybrids with long-season wheats.

"Restoring fertility is a crucial step if we are going to end up with a commercial feed cereal," Prof Colmer said.

"A key issue is that the cross has to be done using wheat as the male parent because sea barleygrass has tiny anthers and insufficient pollen yield.

"The hybrids contain sea barleygrass cytoplasm and this leads to some cytoplasmic-induced male sterility.

"Dr Islam is currently backcrossing the new hybrid plants to revert to wheat cytoplasm — this means checking the genetic material of every backcross made and selecting the plants with all the required chromosome complement. It is a painstaking process but it is really important to improve fertility.

"What Dr Islam is doing is a similar sort of strategy used to develop triticale and some of the challenges have been similar, including this low fertility in the first generation of hybrids."

The issue of low fertility slows down progress in field trials. Firstly, enough seed has to be produced in the greenhouse to enable field trials. Secondly, field measurements have had to be taken as biomass production and not grain yield.

The 'transfer back to wheat work' has been ongoing throughout the project and it is anticipated there will be a hybrid with restored fertility by the end of 2010.

While the process of backcrossing has been technically complex and time consuming, there have already been some benefits with the isolation of useful chromosome addition lines. These lines contain specific chromosome pairs from sea barleygrass in a wheat cytoplasm, so they more closely resemble wheat. Some chromosomes contain genes for traits associated with salt tolerance, and so could become useful pre-breeding materials for bread wheat, although these are less tolerant than the full hybrids.

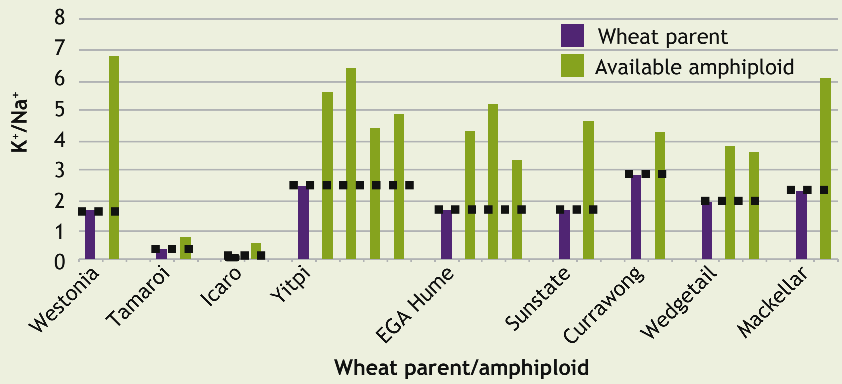
International interest

The genetic material produced during the project has attracted international attention and collaborative work with Japanese scientists interested to characterise the genes involved in waterlogging tolerance has started.

This gene discovery work will improve understanding of regulation of key traits of interest, and might aid additional breeding work if the key genes can be identified.

Such knowledge would further enhance the possibility of a productive crop option for saline agricultural land.

Figure 2 Glasshouse screening for salt tolerance in wheat versus amphiploids



Glasshouse screenings conducted under 200 mM NaCl (approx 20 dS/m), show all amphiploids (green bars) have improved salt tolerance (K/Na selectivity) compared with their wheat parents (purple bars). Data from experiments conducted at CSIRO, Canberra.

Source: Munns *et al*. (unpub)

More information

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Collaboration helps hybrid development

At the outset of the work to develop a salt- and waterlogging-tolerant cereal, Australian researchers looked to rice as a model for waterlogging tolerance.

Two key features of the rice plant impart waterlogging tolerance. The rice has a 'snorkel-like' feature, called aerenchyma — gas channels that enable oxygen to move from the shoots and down into the roots.

Rice plants also develop a physical barrier (suberin deposits) on the outside of their roots to stop oxygen leaking out. These traits have been well described in scientific literature for rice and many wild wetland species, but researchers found the same traits for waterlogging tolerance in sea barleygrass when screening wild relatives of wheat (tribe *Triticeae*).

This work caught the attention of a group of scientists from Tokyo University in Japan with particular expertise in looking at gene expression in specific tissues and cells. The Japanese team uses laser micro-dissection techniques to isolate a specific tissue or cells from plants and then looks at profiles of gene expression.

The group at Tokyo University will evaluate roots of the wheat-sea barleygrass hybrids for gene expression of the traits associated with waterlogging tolerance. They will be able to find out whether or not the same genes are operating in sea barleygrass, as those in rice. Levels of gene expression in roots of the hybrids will then be compared.

The collaborative work helps the Tokyo University group in its work of gene discovery and helps the Future Farm Industries CRC project team understand what regulates traits for waterlogging tolerance.

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