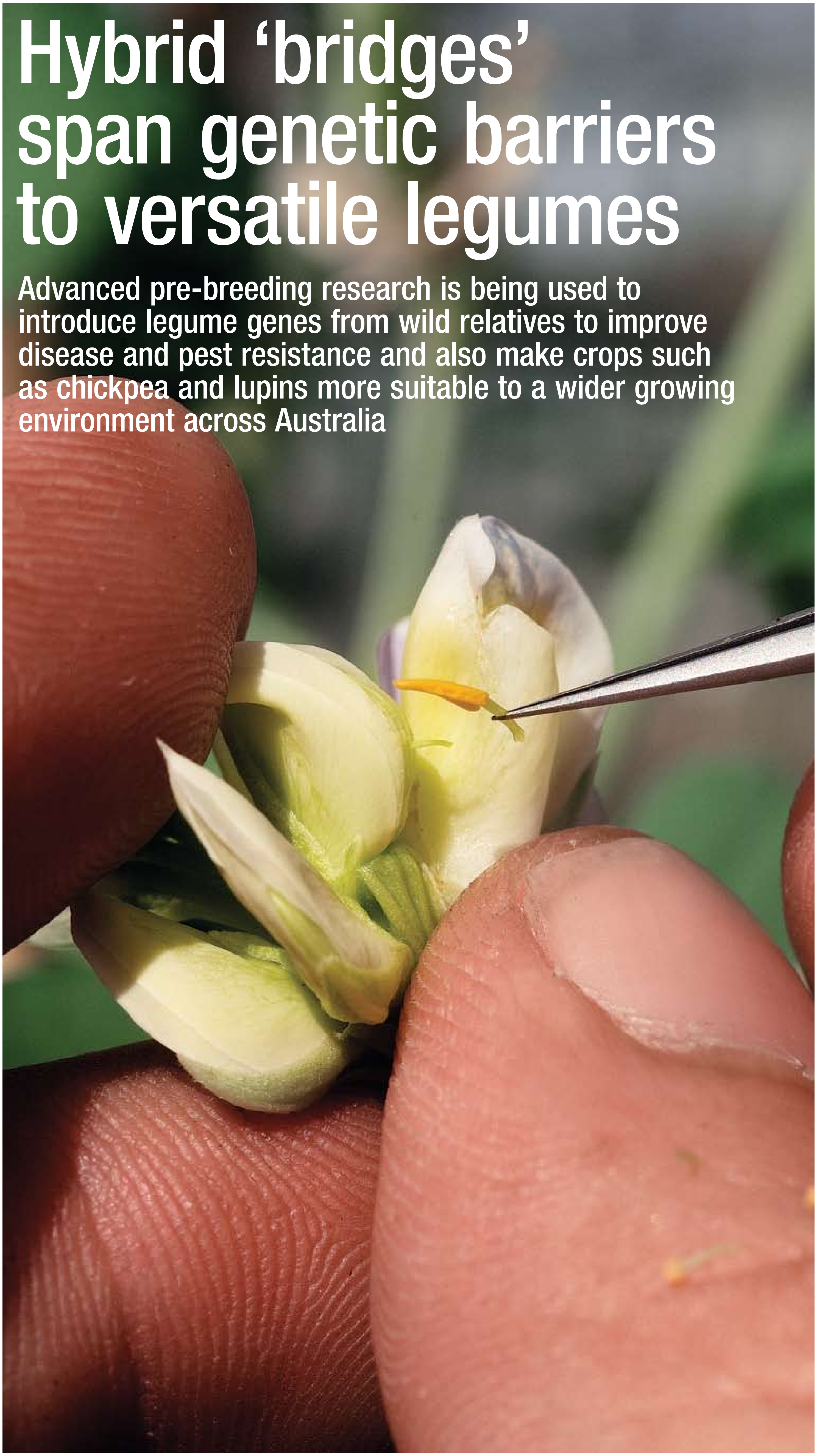
Hybrid 'bridges' span genetic barriers to versatile legumes

Advanced pre-breeding research is being used to introduce legume genes from wild relatives to improve disease and pest resistance and also make crops such as chickpea and lupins more suitable to a wider growing environment across Australia



BY DR GIO BRAIDOTTI

The demand for chickpea cultivars for use as nitrogen-fixing legumes in crop rotations across diverse grain-growing environments is increasing, pushing breeders to deliver more widely adapted, and more disease-resistant, varieties.

However, scientists first need to develop new breeding techniques to overcome genetic constraints hindering the introduction into legume cultivars of valuable traits from wild relatives.

The first constraint is chickpea's narrow gene pool. The genetic heritage needed to combat fungal diseases, frost, salinity and pests has proved to be persistently elusive in domesticated varieties. By contrast, the gene pool of wild relatives in the broader *Cicer* genus includes many of the desired and commercially valuable traits.

The problem is that cultivated chickpeas belong to the species *Cicer arietinum*, for which there is no known wild population from which to source novel genes. This means the genes cannot be introduced using conventional cross pollination. Attempts to do so result in progeny – technically called 'interspecific hybrids' – that are unviable or sterile, bringing further breeding to a halt.

The many worldwide attempts to overcome this barrier have met with only limited success. To date, only two annual *Cicer* species are easily hybridised with cultivated chickpeas: *C. reticulatum* and *C. echinospermum*. Many other annual and perennial species remain inaccessible to breeding programs.

With breeders worldwide unwilling to accept such limited biodiversity, an international collaboration was established with GRDC support to improve gene flow between *Cicer* species. It links Australia's Centre for Legumes in Mediterranean Agriculture (CLIMA) at the University of Western Australia (UWA) to the Crop Development Centre at the University of Saskatchewan in Canada, and the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) in India.

The Australian partner, CLIMA, is home to an R&D program specifically dealing with nitrogen-fixing legumes for both pasture and grain, including native species never before considered for cultivation. CLIMA's new director Professor Willie Erskine says he intends to continue a strong push in pre-breeding research to support national pulse and legume pasture-breeding programs: "Broadening the genetic base of these pulse crops through interspecific crosses is essential for making substantive progress in breeding," he says.

The team working on the chickpea hybrids includes director of the UWA Institute of Agriculture Professor Kadambot Siddique, Dr Heather Clarke and Sabrina Tschirren. The project is an example of the type of research that could deliver advances across legume breeding, since other crops, such as lupins, are genetically restricted in much the same way as chickpea. Ultimately, success in this research would potentially deliver flow-on benefits to the pulse, wheat, forage, processed protein and livestock feed industries.

Dr Clarke says that since 2002, a total of 218 wild *Cicer* accessions from various world gene banks have been brought together in WA. DNA analysis confirmed that genetic diversity among these species far exceeds that within cultivated chickpeas. These wild plants – belonging to eight annual and one perennial species – serve as the source

John Quealy, from the Centre for Legumes in Mediterranean Agriculture, emasculates pearl lupin flowers prior to cross pollination. PHOTO: EVAN COLLIS of novel genetic traits for the project's daring new hybridisation technique.

To kick off the process, CLIMA's team has to firstly stop chickpea plants from self-fertilising. That means removing the flower's tiny pollen-producing anthers with forceps and then fertilising by hand, using pollen harvested from the wild relative. The result is the creation of a hybrid embryo containing a set of chromosomes from both the domesticated and wild parent. Unfortunately for the pre-breeders, the mother plant usually rejects and aborts the carefully cultivated pods.

"We needed a technique to rescue these embryos before they are aborted," Dr Clarke says. "So we ended up removing the hybrid embryo from the mother plant and transferring it to our tissue-culture facilities, where we are learning how to assist the embryos mature and germinate under artificial growth conditions."

This 'embryo-rescue technique' has the biologists hand-rearing the barely viable embryos as they experiment with the use of artificial medium, growth regulators and regeneration regimes. "We also applied plant growth regulators to the flower buds at pollination to promote pod set and nurturing by the mother plant for as long as possible before rescue," she says.

Without the combined interventions, hybrids abort as early as seven days after pollination (in the case of hybrids with wild *C. pinnatifidum*), 14 days (*C. judaicum*) and 21 days (*C. bijugum*).

First developed at ICRISAT, the technique has enabled the Australian team to successfully rescue 14 to 21-day-old hybrids between several Australian cultivars and *C. pinnatifidum*. There has also been some success with the even more immature hybrids with *C. bijugum* and *C. judaicum*.

As the hybrid plantlets increasingly survive to progressively more advanced stages of maturity, the CLIMA team faces the next obstacle towards establishing viable breeding lines.

"Albinism and recalcitrance to rooting are the major factors limiting the transfer of hybrid plantlets to pots in the glasshouse," Dr Clarke says.

"We are currently examining the causes. Once we have them growing in pots, we can quantify the hybrid's pollen viability and fertility, as well as investigate the hybrid's chromosome arrangements."

If fertile and genetically stable hybrids are identified, then early generation breeding lines can be established that make new traits available to breeders.

CLIMA's breeding goals are to improve tolerance to chilling during flowering, and to salinity and to fungal diseases, especially ascochyta blight.

"Since chickpeas make efficient use of water, there has been no screening for additional water-use efficiency," Dr Clarke says.

"On the contrary, since chickpeas can remain green and filling pods after other crops are finished by terminal drought, we might learn from chickpeas how to improve water-use efficiency in other crops."

Overall, the pre-breeder is optimistic that the 'marriage' of plant species (such as chickpea and its wild relatives) can succeed in the long term, but there are obstacles to overcome before traits are likely to start moving into commercial cultivars.

When it happens, one thing is certain. With 313,000 tonnes of chickpeas worth over $100 million cultivated in 2007, mainly in blight-unaffected northern regions, there is a solid bottom line basis – both agronomic and economic – for using such genetic innovations to drive the industry forward. □

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SELF-IMPROVEMENT FOR LUPINS AND PEAS

With parents belonging to different chickpea species, these hybrid embryos would normally be aborted but CLIMA scientists are assisting them to germinate under under artificial growth conditions.

Chickpeas are not alone among legumes in frustrating breeders with a too-limited biodiversity. Since lupins and peas also stand to benefit from an influx of wild genes, CLIMA has extended its hybridisation technology across its legume-breeding programs with GRDC support.

One early success towards this goal is the transfer of traits for pea weevil resistance from a wild pea species into field peas. The resistance stands to save growers up to $16 million in insecticide costs and alleviate the storage threat to the nation's $100 million crop.

This leap in progress was made by CLIMA's Dr Oonagh Byrne and Dr Darryl Hardie from the Department of Agriculture and Food, Western Australia. They have since sent early generation germplasm to Pulse Breeding Australia for use in their program.

"We used a wide cross between different species but the parents were not so distantly related that the embryos needed rescuing," Dr Byrne says. "Given viable progeny, we are in a position to focus on understanding the mechanisms in the resistant peas that stop the weevil burrowing into the pod and seed as a platform for further improved breeding."

Lupins, in contrast, are proving more difficult. They are WA's major grain legume, with production averaging 800,000 tonnes a year, but their value is constrained by seed-quality factors that relegate lupins to the low-value end of the feed market.

Keen to see gains in the rate of lupin improvement, CLIMA's Dr Jon Clements initiated a project to hybridise different lupin species with the involvement of Dr Julia Wilson, John Quealy and Dr Larisa Pirilyuk.

"Unlike chickpeas, there was no need to assemble a collection of wild relatives for lupins," Dr Wilson says. "We already have four species of cultivated lupins, each with distinctive sets of traits. So the hybridisation technique was really about providing a way to combine the desirable traits into new hybrid germplasm."

The four species in the program are:

- narrow-leafed lupin (*Lupinus angustifolius*) that offers wide adaptation, water-use efficiency, tolerance to anthracnose and aphids but lower relative seed quality;
- yellow lupin (*L. luteus*) with good seed quality and tolerance to waterlogging, brown spot, pleiochaeta root rot and acid soils;
- pearl lupin (*L. mutabilis*), whose seed has high protein and oil content, plus a thin seed coat; and
- albus lupin (*L. albus*), also with good seed quality.

"We looked for possible barriers to hybrid formation using a range of microscopy techniques," Dr Wilson says. "This is helping us postulate the best combination of species to cross and the best stage to rescue the embryos."

Attention is beginning to focus on the narrow-leafed and yellow lupin cross. Three weeks after crossing, about 50 per cent of the pods aborted. Of the remainder, five per cent of pods contain viable seed but DNA analysis is required to determine whether these are true hybrids. The remaining 45 per cent of pods die between three and six weeks after crossing and the CLIMA team is setting up an attempt to rescue these embryos.

"Making these interspecific hybrids is a long-term commitment involving a lot of twists and surprises," Dr Wilson says. "However, we think it's worth attempting to improve grain quality, which could help open new lupin markets, including human foods."

Tests at UWA by Dr Jonathan Hodgson, of the School of Medicine and Pharmacology, have found that lupin seeds can be ground into flour with high protein and fibre content, but negligible amounts of sugar or starch. When incorporated into bread, it was found to increase satiety in humans, reduce energy intake and prevent weight gain.

"That profile suggests a role in fighting obesity," Dr Wilson says. "So that's the kind of payoff possible from improving grain quality."

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Dr Julia Wilson, who is developing an embryo-rescue method to allow genes to be brought in from wild relatives.

PHOTO: EVAN COLLIS

THE BLIGHT OVER CHICKPEA PRODUCTION

Globally, ascochyta blight is the most damaging chickpea disease. Caused by *Ascochyta rabiei*, the fungus wiped out the industry around the Wimmera in Victoria in 1996. In WA, it has reduced the area under cultivation since it was first detected in 1999 in the state's northern agricultural area.

Rejuvenating the blight-affected chickpea industry is seen to depend on the development of new varieties with high levels of genetic resistance. Towards that goal, Dr Nader Aryamanesh travelled from Iran to WA to undertake postgraduate studies comparing the nature of resistance in cultivars and wild species (*C. reticulatum* and *C. echinospermum*).

Dr Aryamanesh found that resistance is under the control of recessive genes that must be inherited from both parents (not just one) in order to overcome the dominance of blight susceptibility. Genes with both major and minor effects on resistance were detected. The additive effect of minor genes was found more effective for inheriting the resistance trait.

Importantly for the chickpea industry, the wild *Cicer* species were found to rely on some gene variants not yet found in cultivated varieties. For Australia, that is good news. It paves the way to introduce new sources of resistance into commercial varieties and to pyramid genes together to achieve a level of resistance that could eventually prove decisive in the battle against blight.

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Chickpea shoots derived from crosses between wild and cultivated species are multiplied under artificial tissue-culture conditions. These plants are a crucial stepping stone towards getting novel genes flowing from wild relatives into cultivated chickpeas varieties.