

# Through the glasshouse: Hexima's stylish resistance

Agbiotech often sails under the radar, but Melbourne company Hexima has been making waves with its fungal and insect-resistance technology, and with its deal with Pioneer Hi-Bred International, the agricultural business unit of multinational DuPont. Kate McDonald reports.

**IT WAS SERENDIPITOUS** to say the least that just a month after he took up the role as CEO of listed agbiotech Hexima in July 2008, Josh Hofheimer was able to announce publicly a terrific deal with agriculture giant DuPont. That deal involves the multinational transferring exclusive control of its transgenic anti-fungal protein disease research to a small Melbourne company that started out researching self-incompatibility in plants.

Under the deal, with DuPont's Pioneer Hi-Bred International business, DuPont took a five per cent stake in Hexima, which in turn received exclusive commercialisation rights to Pioneer's library of anti-fungal proteins.

The companies have combined their respective libraries and Hexima is now responsible for identifying and developing anti-fungal resistance traits, while DuPont will take on later-product development and marketing of those traits in corn and soy.

It's not the first time Hexima has worked with a multinational – it is coming to the end of a contract with Dow AgroSciences to perform cotton transformation experiments – but it is a stamp of approval for both the company and its technologies. And in Hofheimer's words, Hexima is one of the few small-cap biotechs in the world that is taking its research "through the glasshouse and into the field" in commercial crops.

"A multi-billion company is going to take its technology, its lengthy investment in genetic anti-fungal protein disease resistance and combine that and give exclusive control over that technology to a \$30 million Australian company," he says. "That DuPont has entered such partnership with Hexima attests to the stature of our scientific team and our technology."

That scientific team includes Professor Adrienne Clarke, one of Australia's best-known scientists and former chair of the CSIRO, who is Hexima's chief scientific advisor and deputy chair; Professor Marilyn Anderson, who discovered the company's insecticidal and anti-fungal molecules and now leads

the company's scientific team and heads the company's research and discovery based at La Trobe University; and Dr Robyn Heath, who established the company's cotton transformation capability and field trials, and is now head of Hexima's product development team based at the University of Melbourne.

These scientists, along with Dr Angela Atkinson, published a series of groundbreaking papers in the 1980s and 1990s that form the basis of the technologies now being commercialised by Hexima.

The IP involves three technologies: the fungal-resistance technology based on defensin proteins; insect-resistance technology based on proteinase inhibitors (PIs); and a multi-gene expression vehicle (MGEV) that does what the name suggests – allows scientists to deliver multiple proteins to a particular cellular site from the same transcript.

## Women's business

Hexima was founded in 1998 but existed as a virtual company until it was spun-out by the University of Melbourne in 2001 and the current group of major shareholders invested. The company listed in August 2007, raising a tidy \$40 million and achieving an initial market capitalisation of \$100 million.

It is not travelling so high now – few biotechs are – but it has excellent cash reserves of almost \$32 million and a spend of only \$7-8 million per annum, which should see it through another four years of R&D. The company even generates revenue through its contract with Dow.

It is also seeking to commercialise its MGEV and PI technologies, and has also begun construction of a new glasshouse, complete with tissue culture facilities and robotics, at La Trobe University.

This is all a far cry from the 1980s when Adrienne Clarke headed the Plant Cell Biology Research Centre at the University of Melbourne, where her team set about cloning the gene that controls self-incompatibility in plants.



Joshua Hofheimer

This is a question that has fascinated plant biologists since the days of Darwin, Clarke says. "This is a very important gene in plants, of course, because the ability of plants to recognise and reject their own pollen was a critical event in the evolution of plants.

"If they couldn't reject their own pollen then they'd have incestuous mating and the problems of inbreeding depression. Then of course they wouldn't flourish and we wouldn't be here today."

Clarke's group received a large grant from the Commonwealth and another from the US company Agrigenetics in the 1980s to fund the cloning work. "With the beginning of the revolution in molecular biology, we thought the time was ripe for a molecular understanding of the basis of self-incompatibility," she says.

Fellow carbohydrate chemist and enzymologist Marilyn Anderson, a graduate of the University of Melbourne and La Trobe University, had by then returned from Cold Spring Harbor Laboratory and brought her skills in molecular biology to the project of cloning the self-incompatibility gene from *Nicotiana glauca*.

They managed to clone the gene – known as the S-gene and one of the most widespread of plant self-incompatibility mechanisms – by looking at the proteins associated with

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specific alleles. The gene turned out to code for a series of ribonucleases expressed in the plant's style, which arrest the growth of the pollen tube.

This work was published in a series of papers in *Nature* in the 80s and 90s, and along the way the team began cloning some of the other genes associated with female sexual tissues. One of these was a gene in *Nicotiana glauca* that was highly expressed in the female stigma and coded for inhibitors of the digestive enzymes trypsin and chymotrypsin.

"But the protein in the stigma didn't precisely match the gene," Clarke says. Robyn Heath was then a PhD student with Anderson, and she and another student, Angela Atkinson, endeavoured to find out what this gene, later called NaPI, really coded for. It turns out that it codes for a 42 kD protein which is then processed to give six separate proteinase inhibitors: two chymotrypsin inhibitors and four trypsin inhibitors.

"Marilyn's team then discovered that the protein circularises, so the ends are folded around like a clasp," Clarke says. "We describe it as having the proteinase inhibitors being linked like beads on a string, like a bracelet, and the clasp holds the bracelet together with three disulphide bonds. The plant then processes this and clips back the linker sequences to release six proteinase inhibitor units."

(This later gave rise to Hexima's name, she says. The team was in a rush to register the company but every name they thought of had already been bagged. "It was about seven o'clock at night and our lawyer said we had to come up with a name that night. So I mentioned hexamer, as in monomer, dimer, trimer etc. Being a lawyer, he just wrote it phonetically, so we became Hexima.")

This work forms two of Hexima's technologies: the proteinase inhibitor technology for insect control and the MGEV. Anderson collaborated with Professor David Craik from the University of Queensland, a world expert in NMR spectroscopy and protein structure, to examine the proteinase inhibitor 'beads on a string'.

"Marilyn's team learned that this particular structure was very flexible around the linker sequence," Clarke says. "That then led to the idea that we could, within this gene, replace units, so you take out one or more proteinase inhibitor genes encoding different molecules. This would enable delivery of multiple proteins to a particular site from the same transcript. That became what we call our multi-gene expression vehicle."



Adrienne Clark

### Resistance with style

*Nicotiana glauca*, the common ornamental tobacco plant, has proved an excellent model to work with for the Hexima scientists. It has very well-defined self-incompatibility, and the female stigma and style are easy to collect.

Female sexual tissues are at the heart of Hexima's science. When the team was in the early stages of research into finding out what the PI gene coded for, they pondered why the plant would put so much effort into producing such large amounts of protein in the female stigma, Clarke says.

"Why was it so? We thought it could be to protect the female tissues from insect attack. On the one hand, the stigma surface is covered with a sticky secretion to enable efficient capture of pollen. On the other hand, this secretion could be a good food source for insects or a good substrate for fungi or bacteria.

"Yet in the field, the stigma of flowers rarely suffers from insect attack or infection. Our question then was, are the proteinase inhibitors involved in protecting the female tissues from insect attack?"

The team discovered that when the proteinase inhibitors were fed to certain insects in an artificial diet, the growth and development of the larvae are severely affected. "Our strategy was then to transfer the genes encoding certain of these inhibitors to crops vulnerable to insect attack, such as cotton, and look for protection against insect attack," Clarke says.

If the plant was expending so much energy protecting its eggs from insects, it would probably have similar weapons against fungal or

bacterial attack. And of course it does – the plant version of the defensin protein family.

One easy experiment was to inoculate the surface of the stigma with fungi or bacteria. If the innoculum seeps on to the petals or leaves, they are quickly infected and the tissues are destroyed, but the stigma and style remain intact and undamaged.

Defensins are part of a very large family of molecules, but Hexima has managed to identify several that are very highly expressed and very potent against fungal disease. The scientists engineered these into cotton, which at that time was the only GM crop allowed to be grown in Australia, with good early results.

The team set up a cotton transformation system and then moved into field trials. This is what first attracted the attention of DuPont, which was interested in the technology's potential in corn. Hexima is now setting up a corn transformation system, with the assistance of DuPont scientists.

The company has conducted two years worth of field trials of the defensin technology in cotton against Fusarium wilt and one year against Verticillium wilt, and a third year of cotton disease trials are underway. Under the deal, DuPont has exclusive rights to the technology in corn and soy, while Hexima retains rights to the other crops.

Early trials with the PIs have been promising, but not quite effective as a commercial alternative to Bt. That's not to say the next generation of PIs won't be more effective, but for the time being the company is looking at whether PIs can be combined with Bt to make an overall more effective product.

"We have identified additional proteinase inhibitors and using the MGEV as an expression tool, we plan to combine multiple PIs and create a more robust trait, which is important for resistance management and for refuge management, and potentially for improving the spectrum of the trait," Hofheimer says.

"We think there is the potential to be a stand-alone product. However, when you combine the PIs with Bt you may have an improved product than from Bt alone. The advantage is that you get multiple modes of action and potentially expand the spectrum of pests that you can target."

"We are exploring partnership opportunities to develop our insect resistance technology, and we think the MGEV has value as a protein delivery vehicle that can help you get traits and multiple proteins in one location."

"With the push towards stacking, these big companies are looking for tools that can help them do that stacking in a controlled way." [ALS](#)