

Designer plants: Manipulating cotton defence chemicals

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One of the challenges facing the Australian cotton industry is maintaining crop yield and real value in the face of shifting world market prices and international agricultural policy. While the fibre is certainly the most valuable component of cotton production, by-products such as cottonseed and the downstream oil and meal products can add extra value to the crop.

Cottonseed prices are currently at high levels, fetching upwards of AUD\$250 per tonne bulk in parts of the country. The majority of Australian produced cottonseed is exported as whole fuzzy seed for ruminant feedlots, with the remaining small proportion being processed locally.

Gossypol: a natural insecticide

Research is underway at CSIRO Plant Industry in Canberra to evaluate the potential for expanding the market for cottonseed products or increasing the base value of cottonseed by attracting a premium for high quality gossypol-free cottonseed.

Gossypol is a terpenoid compound naturally produced by the cotton plant and similar in chemical structure to some plant hormones and pigments. Gossypol is a major component of the purple/brown coloured glands that are visible on the plant.

Gossypol and related chemicals are produced by the plant to provide a degree of natural protection against pests and diseases. Unfortunately the beneficial aspects of gossypol are counteracted by disadvantages for downstream applications of cottonseed products.

Gossypol is toxic to humans and other non-ruminant animals, can result in male sterility, discolours oil and lowers the nutritional value of cottonseed meal. Because of these problems, gossypol must be chemically removed from cottonseed oil before it can be used for human consumption.



Belinda Townsend with Sturt desert rose plants (*Gossypium sturtianum*) in the glasshouse, a potential source of the glandless seed trait. (Photo: D. Llewellyn)

The ideal cotton plant would have significant levels of gossypols in the roots and aerial plant parts to protect the plant from pests and diseases and therefore maintain fibre yield, but would have negligible levels of gossypols in the seed for improved cottonseed applications and reduced processing costs.

Glanded plants with glandless seeds

The Australian native cotton species *Gossypium sturtianum* (Sturt desert rose) has a naturally occurring glanded plant and glandless seed phenotype, but is commercially useless for fibre production. There has been considerable effort over the past 50 or more years by breeders to transfer the glandless seed characteristic into cultivated cotton, but without a great deal of progress.

Australian researchers Curt Brubaker and Tony Brown of CSIRO Plant Industry in Canberra have played an important role in the international efforts to exploit the diversity of Australian native cottons for important traits — such as the glandless seed trait and more recently for *Fusarium* wilt tolerance — but it continues to be extremely difficult because of the large genetic differences between the cultivated and wild species.

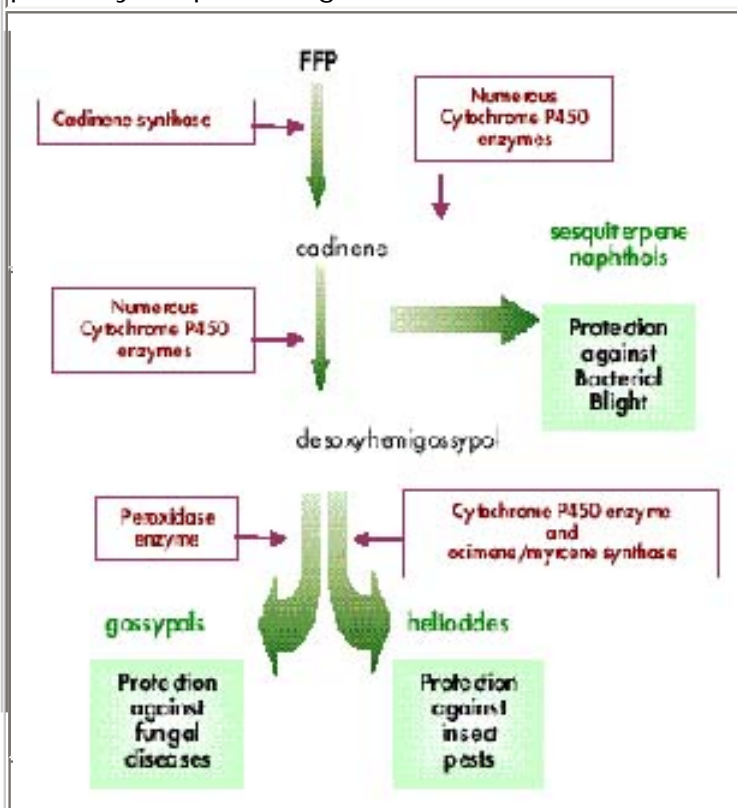
Another source of gossypol-free seed originates from naturally occurring glandless mutants of cultivated cotton. These mutants are similar to standard cultivated cotton except that they have no visible gossypol glands in any plant part including the leaves and bolls — they have no gossypol at all.

Totally glandless plants, while ideal for food and feed applications of cottonseed, are more susceptible than conventional cotton to a wider range of insects and diseases, and therefore have had a limited potential for commercial production. Glandless cotton is currently only grown with significant profit in those parts of Egypt and the US where pest pressures are low.

A transgenic approach

At CSIRO Plant Industry in Canberra, we are undertaking basic research into the biosynthetic pathway that produces gossypols in the cotton plant. This research is part of the CSIRO Biotechnology program and is funded by the Cotton Research and Development Corporation.

FIGURE 1: A schematic view of the biochemical pathway for producing cotton defence chemicals



Seeds from glanded (left) *Gossypium hirsutum* and the glandless mutant (right). There are no purple/brown coloured gossypol glands visible to the naked eye in seeds and bolls of the glandless mutant. (Photo: C. Brubaker and D. Llewellyn)

Using molecular biology and gene technology methods, we are investigating the roles that some of the genes involved in this pathway play in determining the amount and types of gossypols produced by cotton. Four genes are being investigated as candidates for manipulation by genetic engineering.

The steps involved include isolating the genes of interest (usually by comparisons with similar genes and pathways from other plant species) and characterising when and where those genes are turned on and off in different parts of the plant in response to a variety of biological stimuli. Those genes are then used to generate transgenic cotton plants with altered levels of expression of the genes and hopefully with altered spectrums of defence chemicals.

The gossypol biosynthetic pathway is complex and highly branched. In addition to gossypols it is also responsible for producing other important defensive chemicals such as the heliocides essential for protection against insect pests, or the sesquiterpene naphthols, which are critical for protection against diseases like bacterial blight.

As many of the key chemical intermediates and enzymes are shared between the different branches of the pathway, there is a lot of cross-talk both within and between different chemical defence pathways, making targeted alterations in one component more difficult.

One key gene, called cadinene synthase, is involved in the first committed step in the biosynthesis of gossypol and is a strong candidate for altering overall gossypol levels in cotton plants. There are numerous slightly different copies of this gene encoded by the genetic material of cotton.

Gene silencing

Using a technique called 'gene silencing' with one particular cadinene synthase gene we were able to demonstrate that this version of the gene is important in the early defence response against infection with bacterial blight (*Xanthomonas campestris* pv. *malvacearum*). But this silenced copy of the gene did not alter gossypol production in the seed or the rest of the plant and we think that a different copy or copies of the gene are responsible for gossypol production in these tissues.

We have now isolated several different versions of the cotton cadinene synthase and more detailed studies into the expression patterns of these different gene copies are in progress to find the important copy that controls seed gossypol levels. We hope that the next generation of transgenic cotton with silenced seed-specific cadinene synthase genes will generate the required pattern of gossypol-free seed.

A second gene being investigated encodes a peroxidase enzyme, a member of a diverse class of enzymes often involved in plant defence responses. We have isolated one particular sort of peroxidase gene from cotton, but there are still no clues to its exact function except that it is expressed highly in seeds and roots (tissues high in gossypol), and appears to be turned on strongly in response to stem infection with the *Verticillium* wilt fungus.

A peroxidase is known to be involved in the final step in gossypol biosynthesis and our results strongly suggest a possible role for this particular gene — at least in disease induced gossypol biosynthesis. A further analysis of this gene will be undertaken in the future by transgenic methods.

Research into the third gene, a cytochrome P450 enzyme, is at a similar stage of analysis. This gene, as with cadinene synthase, is induced in response to infection with bacterial blight and *Verticillium* wilt, and it too could be a candidate for manipulating gossypol levels in cotton seeds.

Plants contain many cytochrome P450 enzymes with varied roles in both primary and secondary metabolism, so we still need to confirm the exact function of this disease-induced gene. But biochemical studies predict that there may be a number of P450 type enzymes involved in the synthesis of many of the intermediates in the gossypol pathway.

The last gene being studied is some new work aimed at increasing the levels of natural pest repellants produced by cotton and is not involved in manipulating seed gossypol. Ocimene and myrcene are volatile compounds that are structurally related to gossypol.

They are produced at high levels in the older leaves of the plant for protection against herbivores. They are either toxic or released as attractants to predators of the primary insect

pest. The major gene responsible for the production of these compounds, ocimene/myrcene synthase, has only recently been isolated from the model plant *Arabidopsis thaliana*.

The DNA sequence of the *Arabidopsis* gene has been an important tool in allowing us to isolate the equivalent gene from cotton. Once we have the cotton gene involved in generating ocimene and myrcene we aim to increase the level of expression of this gene in cotton using genetic engineering approaches.

Our ultimate aim is to further increase the levels of ocimene and myrcene in the green plant parts that are usually attacked by herbivores and therefore discourage feeding.

Towards the field

These relatively crude beginnings in manipulating biochemical pathways in plants may not always produce the predicted results, but they are bringing us closer to being able to generate desirable characteristics such as gossypol-free cottonseed and cotton plants with improved natural methods of pest and disease control.

Already we have seen that manipulating individual defence chemicals within a plant is difficult as many of the enzymes involved have multiple forms, each with a different role and perhaps different location within the plant. Changing one enzyme may impact on the flow of intermediates into a completely different pathway and compensating responses.

Designer plants are not yet with us, but as our knowledge of the genes and enzymatic networks in plant defence metabolism grows, the trial and error methods that we have to use today will eventually be replaced by more precise and predictable changes.

To be of any real value to the industry these novel metabolic engineering traits will need to be made available in conjunction with other transgenic traits such as Ingard, Bollgard II and/or Roundup Ready. The close links between CSIRO's biotechnology R&D program and its breeding program will ensure that any new developments in biotechnology are quickly made available to Australian growers.