Molecular Biology of Gossypol Synthesis in Cotton

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Cotton's Defence Chemicals

Although growers spend much of their time and resources in protecting their cotton crop against attack by insect pests and diseases the plant itself is already well armed against these invaders and your task might be hopeless if not for the complex array of natural chemicals produced by the plant. Of particular note are the terpenoid aldehydes, such as gossypol, found in the oil rich gossypol glands all over the plant. These glands are characteristic of cotton and its wild relatives and are full of an oil that is rich in different terpenoid chemicals. When an insect eats some of a leaf these glands burst and release a toxic array of chemicals, some of which have been shown to have a high potency against insects. Besides this constitutive defence against herbivory, the plants also have an inducible defence mechanism that can detect and respond to disease pathogens with the production of more terpenoids and other antifungal chemicals as a first line of defence to limit their invasive capacity.

Such chemical defenses can be effective and in the wild do a reasonably efficient job of protecting isolated plants, but in agricultural systems where we grow plants as monocultures and require high yields and minimal plant damage, these systems of host plant resistance are unable to cope and growers must resort to externally applied chemicals. The importance of these defence chemicals is highlighted by the glandless mutants of cotton that fail to produce any gossypol glands in the above ground parts of the plant and hence contain none of the toxic chemicals. Such glandless plants are completely susceptible to attack by insect pests and although they were developed into cultivars to provide seed meal for animals free of gossypol, they have not found any great commercial use because of their insect susceptibility.

If we could understand more about the chemicals, their roles in plant defence, and how they are made and compartmentalised in the plant we might be able to manipulate these chemicals to improve host plant resistance. We have targeted the pathways of sesquiterpene production in cotton, including the production of gossypol, to isolate specific genes encoding the enzymes of the pathway, to learn more about how the synthesis of the different chemicals is controlled at a molecular level and to investigate how we might use gene technology to change the types and relative levels of specific defence chemicals in cotton.

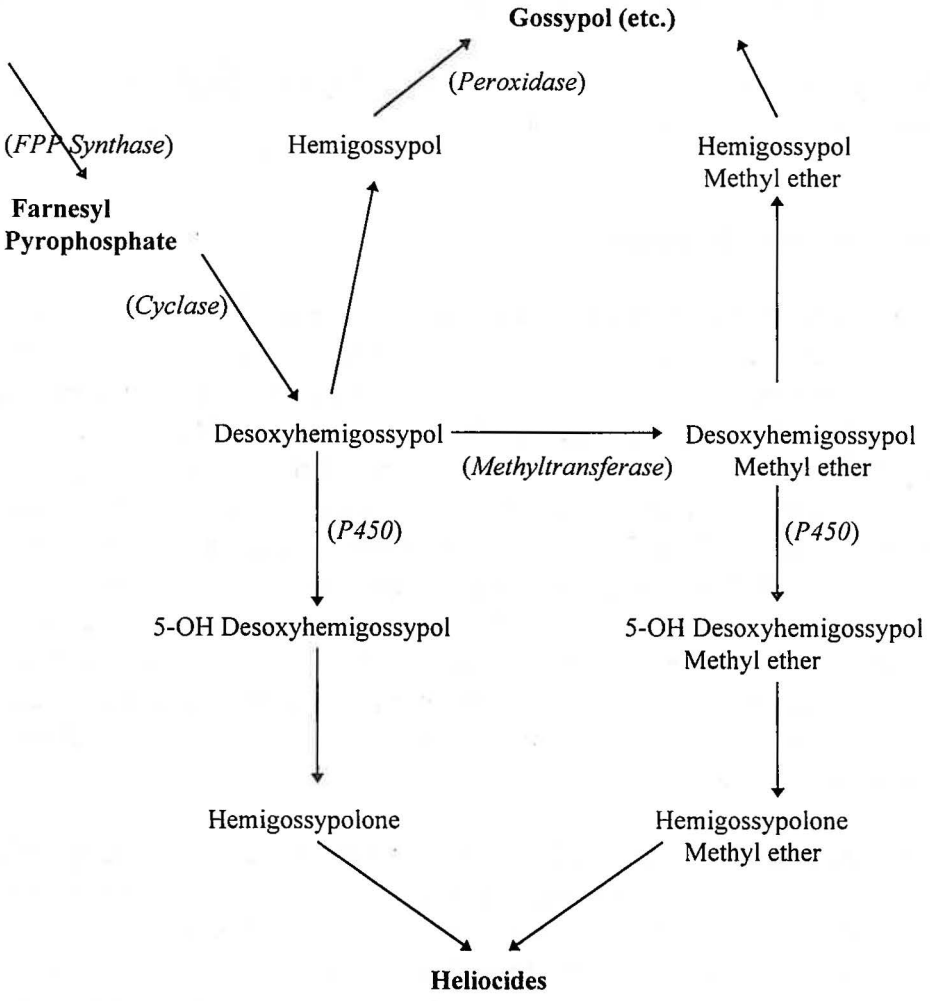

Figure 1: Biosynthetic pathway for gossypol production
(enzymes are indicated in brackets)

Pathways of Sesquiterpene Production in Cotton.

The pathways for the production of the sesquiterpenes in many plants are not completely characterised although many of the main steps are known and the intermediates characterised chemically. Gossypol and its related terpenoid aldehydes are synthesised from the isoprenoid precursor farnesyl pyrophosphate (Figure 1.) that is cyclised by a specific sesquiterpene cyclase enzyme that sets the core structure that characterises the cotton terpenoid aldehydes. Different plants have different cyclases that form different ring structures which are characteristic of different plant genera. The core intermediate desoxyhemigossypol is then acted upon by different hydroxylases,

dehydrogenases and methyltransferase enzymes to form an array of methyl ethers and hydroxy derivates of desoxyhemigossypol. These intermediates can then be dimerised by a peroxidase enzyme to form gossypol, methyl gossypol or dimethyl gossypol. In the green tissues where the glands are exposed to light there are other conversions that result in the formation of quinone derivatives such as hemigossypolone and its methyl ethers. These quinone derivatives can spontaneously condense with the monoterpene oils to form the well known heliocides that are very toxic to *Helicoverpa* species.

All of the interconversions and modifications of the basic core structure should allow us to manipulate this pathway by modifying specific enzymes or their patterns and levels of expression in the plant. Blocking the peroxidase(s) that dimerises desoxyhemigossypol in the seed for example, might allow us to produce transgenic cotton plants similar to some of the Australian natives that have undeveloped glands in the seed that contain no gossypol but upon germination the glands mature and fill with terpenoids to provide protection against insect herbivory.

Cloning Specific Genes for Sesquiterpene Biosynthesis

The target genes we have chosen to clone from cotton are the farnesyl pyrophosphate synthase (the enzyme producing the primary precursor for this and related pathways), the specific sesquiterpene cyclases that characterise the cotton terpenoid aldehydes, the hydroxylases specific for desoxyhemigossypol and related chemicals and the terminal peroxidase(s) that dimerise hemigossypol to gossypol. The project has only just started but we have developed strategies for each of our target genes and have some initial promising results.

The strategies for cloning the different genes are similar and use the technique of polymerase chain reaction (PCR) to amplify specific regions of genes lying between a pair of DNA primers. The primers are designed to correspond to regions of the particular gene that are conserved in the enzymes across different species or for structurally related enzymes. There have been several farnesyl pyrophosphate (FPP) synthase genes cloned from both plants (White lupin, Arabidopsis and rubber tree) and animals (rats, humans and cows), each different but with regions that are similar enough that we can use these to design combinations of primers that might also identify the cotton FPP synthase gene. The hydroxylases that add a hydroxyl group to one of the rings of the desoxyhemigossypol molecule are most likely to be members of a super family of enzymes called the cytochrome P450s from which many hundreds of examples have been characterised. These are often the enzymes that carry out many of the conversions of aromatic compounds in both plants and animals. The P450s have small very highly conserved domains, but are generally very different in the regions surrounding these domains. We have used a nested PCR approach to begin to clone P450 genes that are expressed in glanded cotton and not glandless mutant cotton (and hence ones that should have something to do with the

constitutive synthesis of terpenoids in the gossypol glands) and ones that are expressed in bacterial blight infected seedlings, but not in healthy cotton (and these should have something to do with the induced defence response for terpenoid production). So far, two P450 PCR fragments from cotton have been cloned and are being characterised at the molecular level. The first gene shares similarity with a cytochrome P450 gene from periwinkle, that is probably involved in geraniol hydroxylation, a sesquiterpene induced by infection. The second P450 gene appears most similar to an avocado P450 gene involved in the early stages of fruit ripening. A comparison of these amino acid sequences in Figure 2 displays the level of identitiy and similarity between those from cotton and from periwinkle and avocado. The expression of the genes will be characterised in cotton and we will try to determine their function by expression in a model system, such as bakers yeast, or by putting the genes back into transgenic plants in sense and antisense orientation, a procedure that could either elevate or suppress the activity of the gene in a transgenic plant. Monitoring the changes in terpenoid composition of the plants will tell us what role each gene may have and how we might manipulate the various terpenoid pathways to modify the host plant resistance of cotton.

	Amino Acid Sequence	% Identity	% Similarity
Cotton 1 Periwinkle	ERFAINETKTALSMILQRYTISLSPAYVHA QNFAMLEAKIALSMILQNFSFELSPSYSHA	60	73
Cotton 2 Avocado	DNAVDFKGQHFELLPFGGGRRGCP NNSVDFKGQDFQLIPFGAGRRGCP	75	91

Figure 2: Comparison of the amino acid sequences of the two P450 PCR fragments obtained from cotton (Cotton1 and Cotton 2), and the most similar plant P450 amino acid sequences (Periwinkle and Avocado).