

## DNA Markers for Resistance to Fungal Diseases in Cotton

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### Summary

Limited genetic variation in cotton has presented a significant challenge for the isolation of DNA markers linked with valuable traits such as resistance to *Verticillium* wilt and *Fusarium* wilt. Nevertheless, effective new techniques are now yielding DNA markers that can be used by cotton breeders to select for disease-resistant varieties in the absence of the pathogens.

### Verticillium & Fusarium wilts of cotton

Diseases of cotton such as *Verticillium* wilt and *Fusarium* wilt have the potential to inflict serious losses on cotton crops. Both diseases are caused by fungal organisms that invade the vascular tissues of plants, causing leaf necrosis, plant stunting and death. *Verticillium* wilt was first observed on cotton in the Namoi Valley, NSW in 1959, and is now present in most cotton production regions in Australia. On the other hand, *Fusarium* wilt is a relatively new disease of cotton in this country. The disease was first detected in the Darling Downs district of Queensland in the early 1990's, but is steadily spreading to other regions.

There are a number of strategies that can be employed to reduce the destructiveness of plant diseases. These include improved farming practices to reduce the build-up of pathogens in the soil, and the nurturing or inoculation of selected soil micro-organisms, known as bio-control agents, that compete with or kill plant pathogens. However, one of the best defences against fungal diseases has been to select for disease-resistant plant varieties. For example, the use of varieties that possess one or more genes for tolerance towards *Verticillium* wilt has dramatically reduced the impact of this disease in Australian cotton crops.

### Breeding for disease resistance

DNA markers are being used during the breeding of numerous crop species to identify genes for important characters such as increased yield, improved product quality and disease resistance. By making DNA 'fingerprints' of parental plants and their progeny, DNA markers can be identified that originate from a given parental plant. Some of these markers may indicate those offspring that have inherited useful genes from that parent, and could be used by plant breeders to select offspring with the desired parental character.

The potential value of DNA markers in breeding for resistance to plant disease in cotton would be significant. DNA markers linked with disease resistance would enable the selection of resistant plants without the need to actually infect plants with the pathogen. Using DNA markers, smaller numbers of plants could be selected at an earlier growth stage, thereby reducing the logistical problems associated with large plant breeding programs. DNA markers would also enable selection for multiple genes directed against a single pathogen, and so result in the pyramiding of resistance genes directed against that organism.

## DNA markers for cotton

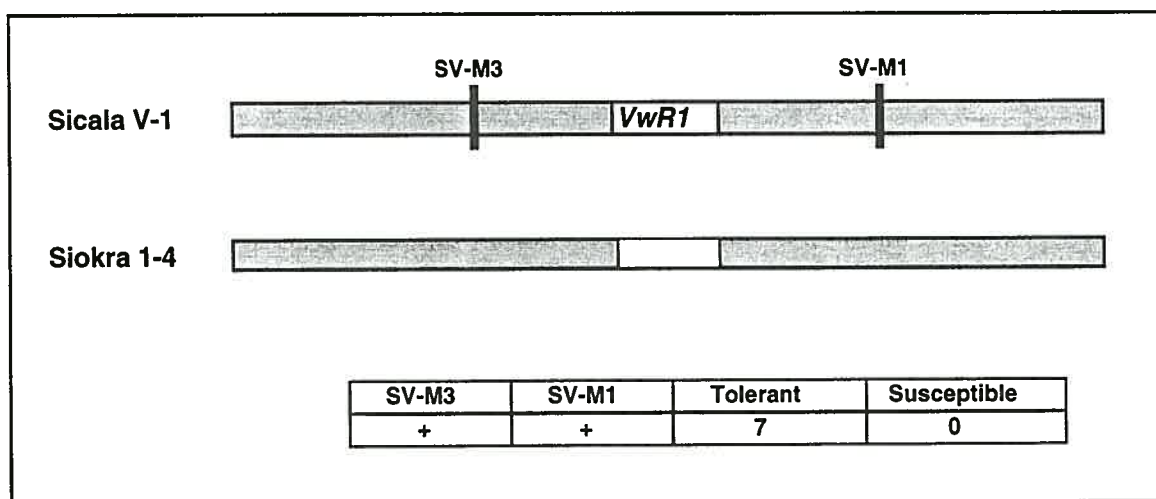
DNA markers have not been widely used in cotton breeding in the past due to the difficulty in identifying adequate genetic variation (DNA polymorphism) in the genes of cotton. Cotton is an inbreeding species, and the past conduct of breeding in this crop has not encouraged outcrossing to obtain greater levels of genetic variation. As a consequence, DNA polymorphism between individual plants is relatively rare, and the likelihood of identifying DNA markers linked with the gene for a particular character is correspondingly low.

However, we have applied a new DNA marker technology, known as amplified fragment length polymorphism (AFLP), which shows greater potential for the detection of DNA polymorphism in cotton. Our research objectives are to employ DNA markers to identify genes for resistance to fungal wilt diseases in cotton. Such markers would be used by plant breeders to accelerate the selection of elite cotton varieties that carry genes for improved resistance to these potentially devastating diseases.

## DNA markers for resistance to *Verticillium* wilt

Disease grading for the classification of disease resistant or susceptible plants was conducted as a prelude to screening cotton plants for DNA markers associated with disease resistance. For *Verticillium* wilt, grading was on parental and segregating ( $F_2$ ) progeny plants from the cross between Sicala V-1, which is resistant to the disease, and Siokra 1-4, which is disease-susceptible. These tests revealed that plant height and vascular discolouration of the stem are the best indicators of plant resistance or susceptibility to infection.

Enhanced resistance to *Verticillium* wilt in Sicala V-1 was found to be due to the action of a single major gene, designated *VWR1*. By comparing progeny plants that exhibit extremes of disease response, we have identified a handful of DNA markers that are linked with *VWR1*. The locations of the two closest DNA markers relative to the position of the putative *Verticillium* wilt-resistance gene are depicted in Figure 1.



**Figure 1.** Diagram of AFLP markers linked with the *Verticillium* wilt-resistance gene *VWR1* in the Australian cotton cultivar Sicala V-1. DNA markers SV-M1 and SV-M3 bracket *VWR1* such that all progeny plants that were shown to possess these two markers are resistant to *Verticillium* wilt.

The identified DNA markers are now ready to be developed for use by plant breeders to ensure that new varieties of cotton carry the *VWRI* gene for resistance to Verticillium wilt.

### **DNA markers for resistance to Fusarium wilt**

For Fusarium wilt, grading was on parental and segregating ( $F_2$ ) progeny plants from the cross between MCU-5, which is resistant to the disease, and Siokra 1-4, which is disease-susceptible. A relationship between plant height and vascular discolouration of the plant stem was also found to be suitable for the classification of cotton plants exhibiting resistance to Fusarium wilt. Disease grading by this method has confirmed the value of the novel Indian cotton variety MCU-5 as a potential source of Fusarium wilt resistance for the breeding of disease-resistant Australian cotton varieties.

Enhanced resistance to Fusarium wilt in MCU-5 was found to be due to the action of a single major gene, designated *FWR1*. AFLP analyses for the identification of DNA polymorphisms that may distinguish disease-resistant and susceptible parental and progeny plants have identified at least six DNA markers that appear to be linked with *FWR1*. One such DNA marker is depicted in Figure 2.

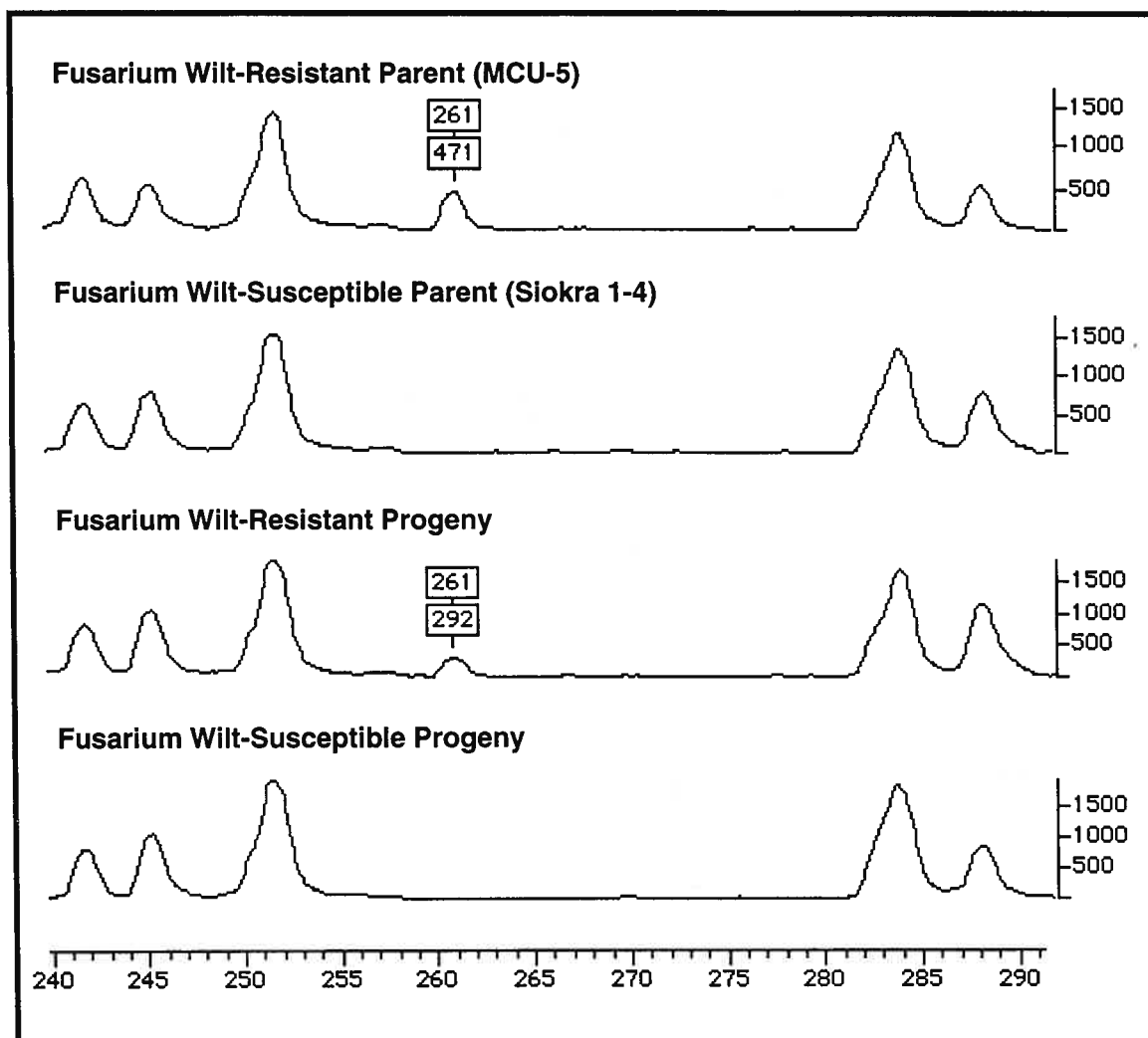
### **Rapid DNA marker assays**

Our research is employing DNA markers to identify linkage with gene(s) for enhanced resistance to fungal wilt diseases in cotton. For efficient utilisation in plant breeding programs, candidate molecular markers need to be converted into stable markers that can be easily assayed. This process, currently under way for the candidate molecular markers linked with resistance to Verticillium wilt, involves the cloning and sequencing of the candidate marker DNA, and the design of PCR primers that allow the allele-specific amplification of that DNA. To further simplify the assay, small samples of tissue would be assayed soon after plant germination without affecting plant viability, and signal molecules such as fluorescent dyes, which can be detected in a fully automated manner, would be used to indicate the presence of the marker.

### **Molecular breeding of cotton**

The utilisation of DNA markers will facilitate the selection of cotton varieties carrying different genes for disease resistance, and could enable plant breeders to pyramid several combinations of such genes in the one plant variety. This would produce varieties with higher disease tolerance to a range of microbial pathogens, and might forestall the appearance of more virulent organisms.

DNA marker technology holds much promise for the accelerated breeding of elite cotton varieties with enhanced resistance to a number of diseases and pests. Furthermore, the technology could help cotton breeders to refine other agronomic characteristics such as more efficient water use, increased yield and improved fibre quality. The door is now open for DNA markers to become integrated in the molecular breeding of cotton to best exploit the superior genes possessed by Australian and exotic cotton varieties.



**Figure 2.** Fluorescent AFLP patterns produced during analysis of DNA from the cotton varieties MCU-5 and Siokra 1-4, and two progeny plants that differ with respect to resistance to Fusarium wilt. Wilt-resistant progeny possess an AFLP marker identical to the wilt-resistant parent MCU-5, which is absent in the wilt-susceptible parent Siokra 1-4 and wilt-susceptible progeny. This DNA marker can be used to identify those plants that inherit a gene for resistance to Fusarium wilt from MCU-5.

### Acknowledgments

This research is being conducted in the Plant Molecular Genetics Laboratory, School of Biological Sciences A12, The University of Sydney. The assistance of Stephen Allen, David Nehl and Joe Kochman, together with the financial support of The University of Sydney, the Australian Cotton Cooperative Research Centre and the Cotton Research and Development Corporation, is gratefully acknowledged.

### Reference

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