Verticillium Wilt of Cotton: Genetic Markers for Disease Resistance

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Introduction

A number of techniques have recently become available to assist plant breeders in the development of improved plant cultivars. Many of these tools stem from the expanding field of molecular genetics, and herald a revolution in our ability to characterise and manipulate the genetic material (DNA) of plants. We already know that differences in the performance characteristics of plants often correspond to differences in their genes. Methods such as genetic fingerprinting allow us to differentiate individual plants in a breeding program on the basis of which genes they carry, and can help us to link useful agronomic characteristics such as enhanced disease resistance with the possession of certain genetic markers.

Genetic fingerprinting of cotton cultivars

Improved cotton cultivars which have been developed using traditional plant breeding procedures are mostly selections from intervarietal crosses and backcross programs. As these plants inherit much common genetic material, they often cannot be differentiated on the basis of growth and performance characteristics alone. In such cases, genetic fingerprinting can be employed to identify subtle differences between two closely-related plants, and so enable recognition of a unique cultivar. Furthermore, knowledge of the degree of genetic similarity between individuals is helpful in facilitating efficient utilisation of germplasm sources. For example, the breeder can use this information in selecting diverse parents to cross in hybrid combinations to maximise the expression of hybrid vigour.

To examine the genetic similarity of cotton cultivars available in Australia, twelve cultivars of *Gossypium hirsutum*, together with an American Pima cotton (*G. barbadense*) cultivar, were subjected to genetic fingerprint analysis using the technique of RAPD-PCR[1]. The study revealed that it is possible to differentiate each of the CSIRO cultivars, even though the majority of these cultivars possess greater than 90% of their genetic material in common (Figure 1). As would be expected from their wide pedigrees, cultivars such as CS 50 and DP 90, together with the *G. barbadense* cultivar Pima S-7, reveal much less genetic similarity. From these results we can assume that if such an analysis were extended to cultivars with unknown pedigrees, it would be possible to obtain a detailed picture of their genetic relatedness.

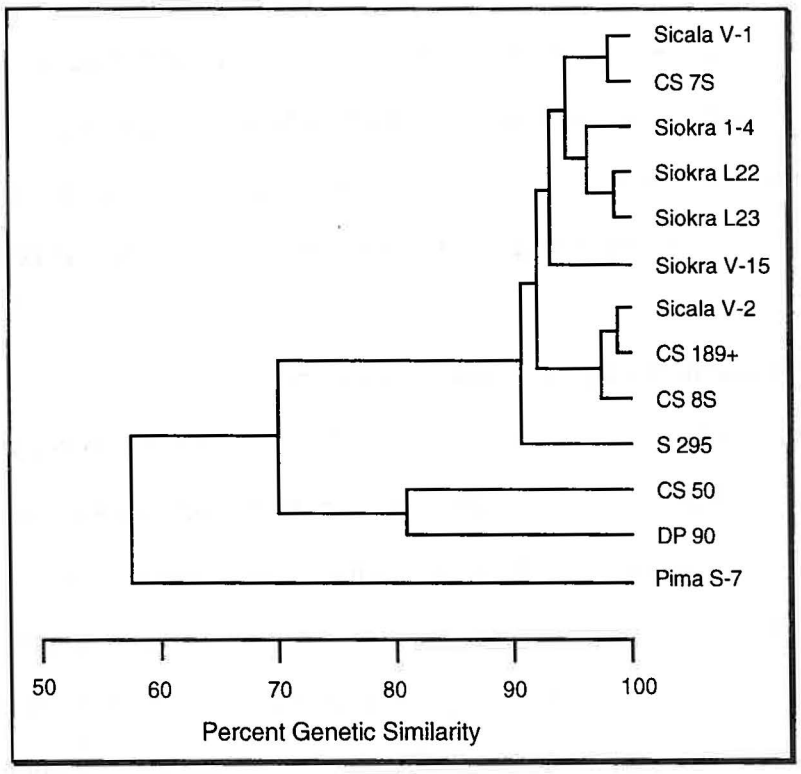

FIGURE 1. Tree diagram showing the percentage genetic relatedness of a number of cotton cultivars subjected to genetic fingerprint comparison.

[1] RAPD-PCR (Random Amplified Polymorphic DNA - Polymerase Chain Reaction) is a rapid molecular genetic technique which samples random regions of an organism's DNA and produces a pattern of DNA pieces or fragments not unlike a supermarket barcode. Alignment of the 'barcodes' of two or more organisms can reveal both similarities and differences between the individuals and can therefore provide a measure of their genetic similarity. For technical details of the RAPD-PCR procedure, see Multani & Lyon, 1995.

Resistance of selected cotton cultivars to Verticillium wilt

In order to breed cotton cultivars with enhanced resistance to Verticillium wilt, parental plants which display improved disease resistance must firstly be identified. Pathogenicity assays currently remain the only means of determining the degree of resistance of different cotton cultivars towards fungal infection. A method for the artificial induction of Verticillium wilt in cotton plants has therefore been employed to determine the reaction of selected cotton cultivars to infection with individual fungal strains[2]. The results of inoculating the cotton cultivars Siokra 1-4, DP 90, Sicala V-1, Acala Royale and Pima S-7 with a broad selection of previously-identified strains of *Verticillium dahliae* are presented in Figure 2.

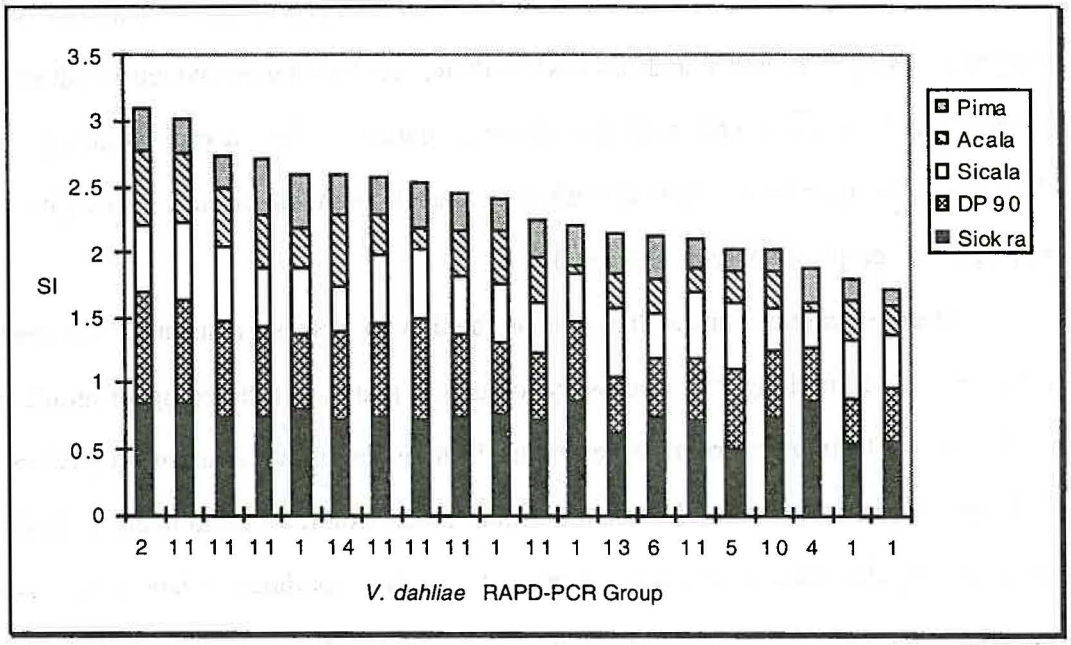

FIGURE 2. Chart showing the severity of disease (SI = severity index) caused by 20 randomly-selected isolates of *V. dahliae* (number = RAPD-PCR group) on the cotton cultivars Pima S-7, Acala Royale, Sicala V-1, DP 90 and Siokra 1-4. The height of each bar segment is proportional to the severity of disease on that cultivar.

As was shown by our earlier research[3], strains of *V. dahliae* from Australian cotton production regions are extremely diverse, both genetically and pathogenically. Furthermore, a number of these strains are capable of causing

[2] For technical details of the pathogenicity assay, see Ramsay, Multani and Lyon, 1996.
[3] See previous paper by Lyon, Zhu, Wang & Multani.

significant disease even on the more tolerant cotton cultivars. Nevertheless, from these tests it can clearly be seen that cotton cultivars such as Sicala V-1, Acala Royale and Pima S-7 are more tolerant to the vast majority of *V. dahliae* isolates than are cultivars such as Siokra 1-4 and DP 90.

Genetic markers for enhanced resistance to Verticillium wilt

As was shown above, pathogenicity assays can be used to determine the level of resistance to Verticillium wilt exhibited by a range of cotton plant cultivars. Such assays can also be applied to the progeny of genetic crosses between disease-resistant parents, in order to determine the pattern of inheritance of the genes for enhanced resistance to Verticillium wilt. Genetic fingerprinting of parents and progeny, using techniques such as RAPD-PCR, can then be employed to identify genetic markers associated with the desired character. One useful strategy for detecting genetic markers linked with a character such as disease resistance is segregant fingerprint analysis (Figure 3).

Once a genetic marker that is associated with disease resistance has been identified, it will no longer be necessary to subject plants in a breeding program to pathogenicity testing in order to determine if they are more tolerant to disease. Instead, a rapid test of the DNA of individual plants could be conducted, with the presence of the genetic marker signifying that the candidate plant possesses enhanced disease resistance. This will be an important time-saving method, as young seedlings can be selected and then propagated in the breeding program without the requirement for their deliberate exposure to infectious organisms.

Our research currently focuses on the identification of genetic markers associated with the enhanced resistance to Verticillium wilt exhibited by the cotton cultivars Sicala V-1, Acala Royale and Pima S-7. We have examined an array of genetic fingerprint patterns for these parental plants, and have begun the process of identifying specific DNA fragments found in progeny plants which have segregated

with disease resistance. The same technique of segregant fingerprint analysis will shortly be applied to the detection of genetic markers associated with improved resistance to Fusarium wilt, and could equally be employed to detect genetic markers for a number of other useful agronomic characters in cotton breeding.

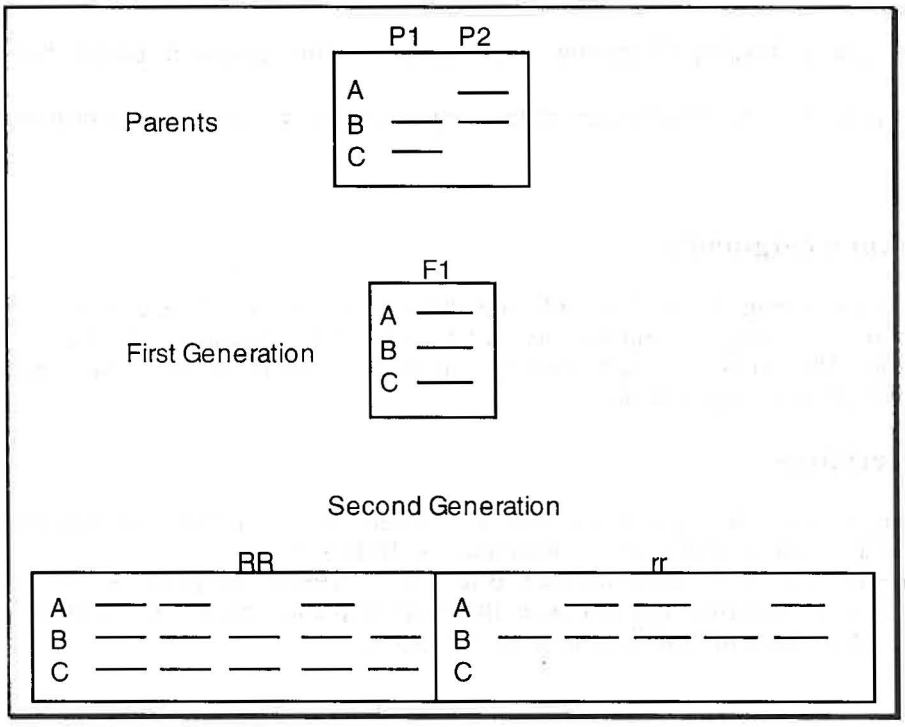

FIGURE 3. Diagram illustrating the principle of segregant fingerprint analysis. Parental plants (P1 and P2) possess different genetic fingerprint patterns. All first generation progeny (F1) possess the combined parental pattern (fragments A, B and C), but second generation progeny (F2) segregate with different patterns. Patterns for F2 progeny that are either disease resistant (RR) or disease susceptible (rr) are compared. If disease-resistant F2 progeny always appear to possess patterns containing fragment C, and disease-susceptible F2 progeny always appear to possess patterns lacking fragment C, then it is reasonable to assume that this fragment is linked to a gene for disease resistance, and could therefore be used as a genetic marker for disease resistance.

Conclusion

Our research has confirmed the practicality of employing genetic fingerprinting in the differentiation of cotton cultivars developed by the CSIRO cotton breeding program, with closely-related cultivars such as Sicala V-1 and Sicala V-2, Siokra L22 and Siokra L23, and CS 7S and CS 8S, being readily discriminated. The technique also shows promise in identifying and determining the

genetic relatedness of plants of uncertain pedigree. Strategies such as segregant fingerprint analysis aim to establish linkages between molecular genetic markers and genes for enhanced resistance to Verticillium in selected cotton cultivars. Genetic markers identified in this work will be used to assist in the breeding of cotton varieties with enhanced levels of resistance to Verticillium wilt. The techniques developed during the course of this research could have broader application in the general agronomic improvement of Australian cotton cultivars.

Acknowledgments

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