√ 1996 B US8C

Cotton Research and Development Corporation Final Report

Project Grant US8C

VERTICILLIUM WILT OF COTTON: GENETIC FINGERPRINTING OF FUNGAL PATHOGENS & WILT- TOLERANT COTTON CULTIVARS

July 1992 - June 1996

1. PROJECT OVERVIEW

1.1. Introduction

Over the last five years the incidence of Verticillium wilt disease in cotton has increased and has cost the industry millions of dollars in lost yield. Information about the pathogenic races of *Verticillium dahliae* prevalent in the cotton growing areas of Australia, their mode of spread and their extent of field persistence has been lacking. Furthermore, although a few cotton cultivars developed by the CSIRO have shown some degree of tolerance to Verticillium wilt in the field, little is known about the number and kinds of defence genes that they possess.

With the advent of new molecular genetic techniques, it is now possible to identify the particular pathogen strain in an outbreak of plant disease, to characterise it, and potentially, to isolate genes associated with pathogenicity. Similarly, such techniques can also be employed to differentiate between plant cultivars and progeny in plant breeding programs and in the identification of genes implicated in improved plant resistance to diseases. In the present investigation, we have applied molecular genetic techniques to strains of *V. dahliae* isolated from cotton plants, and to cotton cultivars tolerant to Verticillium wilt, with the aim of achieving a better understanding of this pathogenic disease in cotton.

1.2. Aims

This project had two major aims consistent with the overall goal of controlling Verticillium wilt in cultivated cotton (*Gossypium hirsutum*). The first aim was to employ molecular genetic techniques to differentiate between isolates of the fungal pathogen *Verticillium dahliae* in order to develop a better understanding of the epidemiology of Verticillium wilt disease in cotton. The second aim was to produce molecular genetic fingerprints of the wilt-tolerant cotton cultivars currently being developed by the CSIRO to assist in the identification and tracking of potential genes for disease resistance in the plant breeding program.

1.3. Stated objectives & achievement summaries

Objective 1: Develop DNA fingerprinting methods to differentiate between isolates of *V. dahliae* and use to study epidemiology of Verticillium wilt in Australian cotton production regions.

Achievements: RAPD-PCR technique chosen for strain differentiation and 99 *V. dahliae* isolates from NSW and Queensland were analysed. Isolates show significant genetic variation but can be classified into 15 different groups. No link is evident between *V. dahliae* genetic group, pathogenicity, region where isolated or host cultivar. Individual cotton fields exhibit broad diversity of isolates which is apparently not influenced by host cultivar.

Objective 2: Clone *V. dahliae* strain-specifc genes and use to detect isolates in plant, water or soil samples. Identify and clone gene markers associated with *V. dahliae* strain pathogenicity.

Achievements: ITS-PCR developed as a potential diagnostic test for the presence of *V. dahliae* in infected plant tissues and soil. RAPD-PCR used to identify strain-specific markers, but these were not developed further as the high level of pathogen diversity necessitates a large number of markers. Fungal genes highly expressed in infected cotton plant roots have been cloned, but as yet no link with pathogenicity has been determined.

Objective 3: Develop DNA fingerprinting methods to differentiate between Australian cotton cultivars and use to determine cultivar relatedness.

Achievements: RAPD-PCR technique chosen for cotton cultivar differentiation and 13 current Australian cultivars analysed. Resulting molecular analysis of genetic relatedness corresponds with known pedigree information, and reveals limited genetic variability between many current cotton cultivars. Cultivar-specific RAPD-PCR products identified for possible plant variety right (PVR) protection.

Objective 4: Develop and use range of marker genes to track potential resistance genes in wilt-tolerant cotton cultivars.

Achievements: Development of pathogenicity assay for determining relative disease tolerance of cotton cultivars and segregating plant progeny. RAPD-PCR used to determine polymorphic markers between two different crosses of Verticillium wilt -tolerant and -susceptible cultivars. Analysis of tolerant and susceptible pools of segregating F2 progeny of the Sicala V-1 and Siokra 1-4 cross identified two potential molecular markers linked with disease tolerance.

1.4. Summary of significant research findings

1. Genetic diversity of *V. dahliae* populations in Australian cotton production regions is high and does not appear to be dependent on geographical location or host cultivar. RAPD-PCR cannot distinguish between isolates with high or low pathogenicity, suggesting that only a few fungal genes are responsible for this character.

2. Pathogenicity tests reveal that a number of field isolates of *V. dahliae* are capable of causing severe disease even on current 'wilt-tolerant' cultivars. This suggests that recent productivity gains due to the introduction of these cultivars may be shortlived unless cultivars incorporating new sources of disease resistance are developed.

3. Molecular genetic techniques reveal that the level of genetic diversity within a number of elite Australian cotton cultivars is low. This suggests that further productivity gains might be made through the incorporation of additional new germplasm into the cotton plant breeding program.

4. Molecular genetic techniques such as RAPD-PCR are capable of detecting numerous polymorphisms between closely related cotton cultivars. Such methods can therefore be employed in strategies designed to identify molecular genetic markers linked with desirable plant traits.

1.5. Summary of other benefits of the research program

1. Full integration with allied molecular plant breeding projects supported by the CRC for Sustainable Cotton Production.

2. Development and utilisation of advanced molecular technologies for the genetic characterisation and analysis of cotton plants and pathogenic fungi.

3. Establishment of scientific and technical capacity which can take advantage of new technologies and developments in molecular plant pathology and molecular plant breeding.

4. Education and training of students and staff in the fields of molecular genetics, molecular plant pathology and molecular plant breeding as they pertain to enhanced and sustainable cotton production.

5. Research maintenance support for five Honours students, of whom three continued with postgraduate study, two in the field of cotton molecular genetics.

6. Research maintenance support for three postgraduate students working on this or associated projects.

7. Research maintenance support for two visiting scientists from China who contributed their knowledge and expertise to the research program.

8. Two publications in international scientific journals (plus three in preparation), three poster presentations at international scientific conferences, numerous proceedings papers and research posters at the biennial cotton research conferences.

2. GENETIC FINGERPRINTING OF FUNGAL WILT PATHOGENS

2.1. Introduction

In a cool, wet season, the incidence of Verticillium wilt disease in cotton can cost the Australian industry millions of dollars in lost yield. Until recently, little was known about the isolates of *Verticillium dahliae* prevalent in the cotton production regions of Australia. As a consequence, it has been virtually impossible to study the population diversity, mode of spread and degree of persistence of these pathogens in the field. However, with the advent of molecular biological techniques such as genetic fingerprinting, it is now possible to identify and characterise the strain of pathogen in an outbreak of plant disease.

The sensitivity of genetic fingerprinting rests in the ability of the procedure to detect the rare or subtle differences that exist between the genes of one individual and another. In a typical experiment, discrete subsets of the genetic material (DNA) of two or more organisms are analysed for genetic similarities and differences, and calculations are made as to the likely relatedness of the organisms based on the observed genetic similarity. In the present investigation, we have applied conventional PCR[1] and RAPD-PCR[2] techniques to strains of *V. dahliae* isolated from cotton plants from a range of production regions in Australia (Table 1). By using genetic fingerprinting to identify different strains of *V. dahliae*, we hoped to achieve a better understanding of the epidemiology of Verticillium wilt disease in cotton.

2.2. An epidemiological survey of *V. dahliae* in Australian cotton

With the assistance of cotton pathologists in NSW and Queensland, isolates of *V. dahliae* were collected from the Bourke area [4 isolates], the Darling Downs (Brookstead and Dalby)[3], the Gwydir Valley (Collarenebri and Croppa Creek)[6], the Macintyre Valley (Boggabilla and Boomi)[3], the Macquarie Valley (Trangie and Warren)[3], the Namoi Valley (Baan Baa, Boggabri, Breeza, Burren Junction, Cryon, Maules Creek and Merah North)[8], the St George area [6], and around Theodore [2]. Two fields of cotton near Narrabri [34 isolates] and two

---

[1] The PCR (Polymerase Chain Reaction) is an *in vitro* procedure for the enzymatic synthesis of DNA, which uses two small DNA pieces known as primers that specifically anneal with opposite strands of a DNA molecule flanking a region of interest. A repetitive series of cycles involving DNA strand separation, primer annealing, and the synthesis of copy DNA results in the production of numerous identical DNA fragments. The amplification is dramatic because the amplified products of one cycle serve as templates for the following reactions and thus after 20 cycles of the PCR, a 10^6-fold amplification (2^{20}) is achieved. For technical details of the procedures employed in this research, see Ramsay *et al.*, 1996.

[2] 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.

TABLE 1. List of *Verticillium dahliae* isolates characterised in this survey[3]

Isolate	Host	Strain	Collection			
No.	Variety	Colour	Farm/Grower	Area	State	Date
1001	Sicala 3-2	White	Northcote	Boomi	NSW	03.90
1002	Deltapine 90	White	Benwerrin	Croppa Creek	NSW	03.90
1003	Siokra S324	White	Drayton	Breeza	NSW	03.90
1004	Deltapine 90	White	Latoka	Bourke	NSW	03.90
1006	Deltapine 61	White	Benwerrin	Croppa Creek	NSW	02.84
1007	Deltapine 61	Black	20 Stone	NW Warren	NSW	02.84
1009	-	W/B	-	Bourke	NSW	02.92
1010	Sicala 33	Black	Iffley	Collarenebri	NSW	03.91
1014	Deltapine 61	White	Benwerrin	Croppa Creek	NSW	02.84
1015	Deltapine 61	White	20 Stone	NW Warren	NSW	02.84
1016	CS 189	White	Kerribee	Merah North	NSW	03.91
1018	Sicala 33	W/B	Iffley	Collarenebri	NSW	03.91
1019	Deltapine 90	White	Burratipi	Trangie	NSW	03.92
1021	Siokra L22	White	Korolea	Boggabilla	NSW	03.92
1029	Sicala V-1	W/B	T. Porter	Brookstead	Qld	02.93
1030	CS 189	W/B	L. Brazzel	Brookstead	Qld	02.93
1031	Siokra L22	W/B	Brownlie	Theodore	Qld	04.93
1032	Siokra 1-4	W/B	Brownlie	Theodore	Qld	04.93
1034	Siokra L22	Black	Cooneah	Dalby	Qld	04.93
1036-1056	Sicala V-2	Black	Auscott 7	Narrabri	NSW	03.94
1057-1069	Sicala V-2	Black	Auscott 8	Narrabri	NSW	03.94
1070-1093	CS 50	Black	Milawa	Warren	NSW	03.94
1094-1099	CS 189+	Black	Milawa	Warren	NSW	03.94
1100	cv 84009-47	Black	Drayton	Breeza	NSW	03.94
1101	Sicala V-1	Black	Undoolya	Boggabri	NSW	03.94
1102	Deltapine 6100	Black	Dundee	Cryon	NSW	03.94
1103	-	Black	Ferguson	North Bourke	NSW	03.94
1104	CS 189+	Black	Hillview	Croppa Creek	NSW	03.94
1105	Sicala 34	Black	Latoka	Bourke	NSW	03.94
1106	CS 50	Black	Retreat	Baan Baa	NSW	03.94
1107	Siokra L22	Black	Northcote	Boomi	NSW	03.94
1108	CS 189+	Black	Waverly	Burren Junction	NSW	03.94
1109	Siokra L23	Black	Warilea	Maules Creek	NSW	03.94
1110	CS 189+	Black	Leopard	St George	Qld.	03.94
1111	Siokra L23	Black	Leopard	St George	Qld.	03.94
1112	Sicala V-1	Black	Plantation	St George	Qld.	03.94
1113	CS 50	Black	Plantation	St George	Qld.	03.94
1114	CS 50	Black	Bilorey	St George	Qld.	03.94
1115	CS 50	Black	Snow Farm	St George	Qld.	03.94

---

[3] Verticillium fungus was isolated from infected cotton plant stems, and cultures were identified using standard morphological and molecular genetic procedures (Ramsay *et al.*, 1996). Variation in colour (white, black or white/black) and growth pattern (fast, slow) was observed among the isolates. This indicated the existence of a complex population structure of *V. dahliae* in these cotton production areas.

near Warren [30] were also targeted for intensive collection of Verticillium wilt infected plants to examine population diversity on a local scale. The host cultivars and collection details of the 99 *V. dahliae* isolates are presented in Table 1.

The genetic fingerprints of all 99 *V. dahliae* isolates were obtained by RAPD-PCR in order to compare their genetic relatedness. This analysis revealed that the isolates could be classified into 15 different RAPD-PCR groups (RGs), with each member of a group showing greater than 80% genetic similarity (greater than 80% of their RAPD-PCR fragments in common) with the other strains in that group (Figure 1). The 15 RGs could be subsequently grouped into 4 major clusters (A, B, C and D) based on a level of 70% genetic similarity.

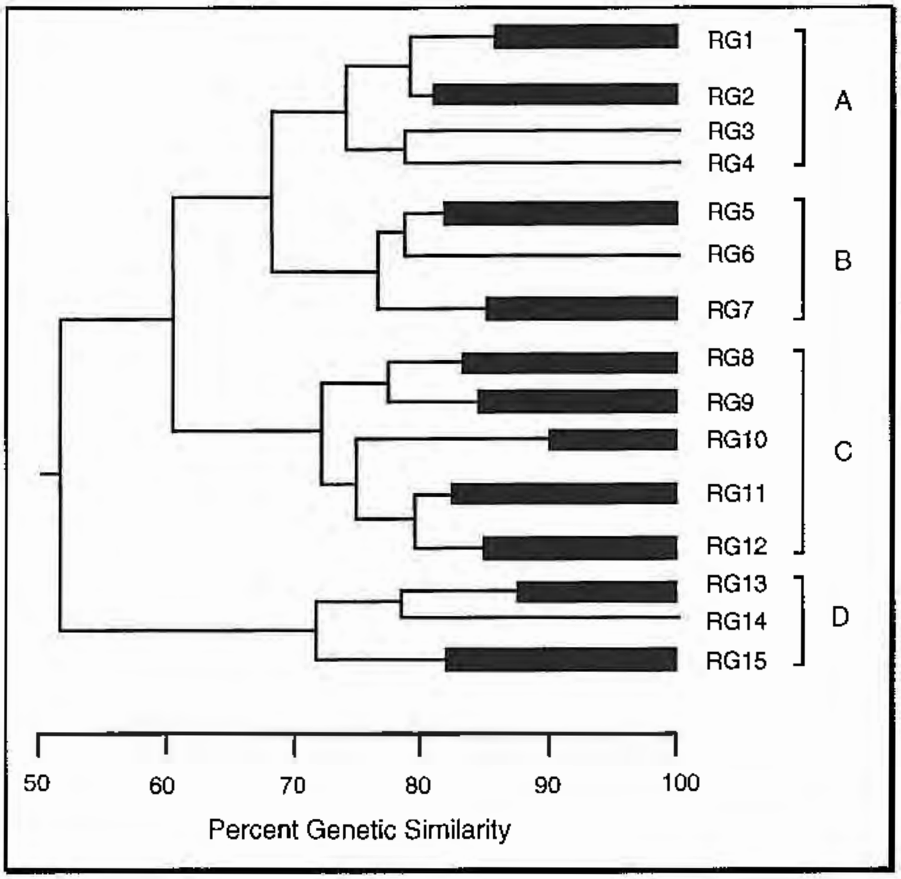

FIGURE 1. Tree diagram showing the percentage genetic relatedness of the *V. dahliae* isolates and their classification into 15 RAPD-PCR groups (RGs). The thick black bars signify that two or more closely-related *V. dahliae* isolates belong to the corresponding RG. Four major clusters (A, B, C and D) are also shown.

2.3. Isolates of *V. dahliae* from different cotton production regions

The largest of the RAPD-PCR groups (RG11), comprised isolates from all eight cotton production regions indicating the widespread nature of this particular genetic type (Figure 2). Representatives of each of the other 14 RGs appeared to be more localised, as they were found in, at most, three different regions. Indeed, members of RGs 2, 3, 4, 6, 7 and 14 were only isolated from a single production region, and so may represent rare genetic types.

Members of RGs 1, 2, 3, 4, 5, 6, 7, 13 and 14, representing almost all of the isolates in the major clusters A, B and D, were only isolated from the intensively-sampled fields in the Macquarie and Namoi production regions. Furthermore, isolates from these surveys were also represented in RGs 8, 9, 10, 11 and 15. These findings imply that *V. dahliae* populations are often extremely diverse and that more intensive sampling of fields in the other production regions may also reveal significant strain diversity.

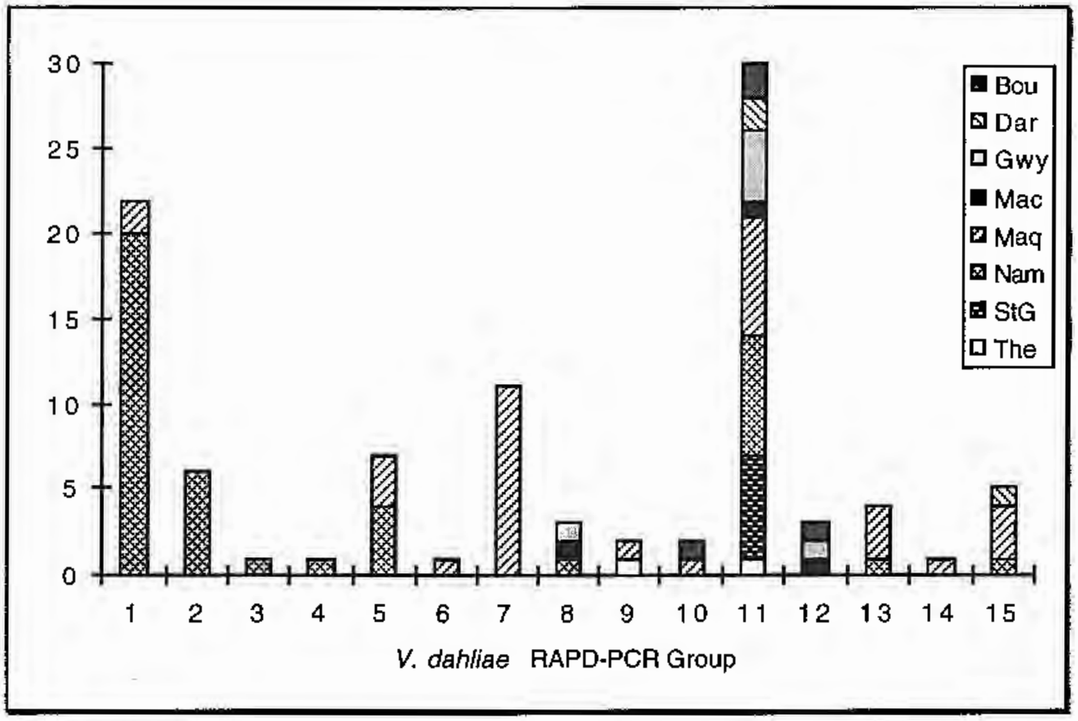

FIGURE 2. Chart comparing the numbers of *V. dahliae* isolates from each RAPD-PCR group found in the eight cotton production regions (Bou = Bourke, Dar = Darling Downs, Gwy = Gwydir Valley, Mac = Macintyre Valley, Maq = Macquarie Valley, Nam = Namoi Valley, StG = St George, The = Theodore).

2.4. Isolates of *V. dahliae* from different cotton cultivar hosts

Strains of *V. dahliae* included in this study were isolated from Verticillium wilt-sensitive cotton cultivars such as CS 50, Sicala 33, Siokra S324, Siokra L22 and Deltapine, as well as from the more disease-tolerant cultivars such as CS 189+, Sicala V-1 and Sicala V-2. Relationships between the host cultivars and RAPD-PCR groups of the *V. dahliae* isolates are presented in Figure 3. Both sensitive and tolerant cultivars were found to be hosts to members of RGs 1, 5, 8, 9, 11, 13 and 15. Moreover, members of the most regionally diverse RAPD-PCR group (RG 11) showed an extremely broad preference for host cultivar. These findings imply that there is little apparent relationship between the genetic type of a *V. dahliae* isolate and its potential to cause disease in a particular host.

2.5. Amplification of variable DNA regions from *V. dahliae*.

We have employed conventional PCR to amplify a variable DNA region, known as the Internal Transcribed Spacer (ITS), from *V. dahliae* strains isolated from cotton plants grown in N.S.W. and Queensland, using conceусus primers. As expected, we found that all of the strains exhibit an amplification product of characteristic size which has been shown by researchers elsewhere to indicate the presence of members of the Genus *Verticillium*. The ITS region of an Australian isolate of *V. dahliae* was cloned and sequenced and was found to be identical to that found in isolates from elsewhere in the world.

Using this DNA sequence, specific primers have been designed which enable the amplification of *V. dahliae* DNA from a variety of infected plant tissues. This result forms the basis for a molecular diagnostic test for the presence of the *Verticillium* pathogen in plant material. Preliminary trials have shown that this test can give an indication as to the level of infection

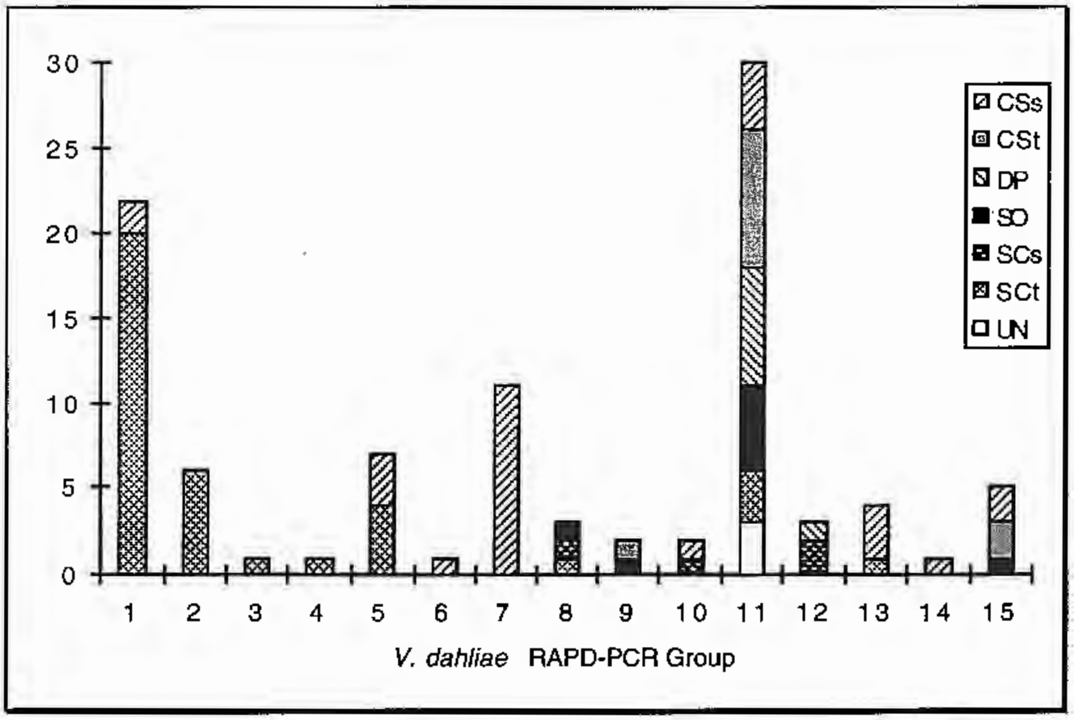

FIGURE 3. Chart comparing the numbers of *V. dahliae* isolates from each RAPD-PCR group isolated from different cotton cultivar types (CSs = CS sensitive, CSt = CS tolerant, DP = Deltapine, SO = Siokra, SCs = Sicala sensitive, SCt = Sicala tolerant, UN = unknown).

present in a plant, and may therefore provide a valuable measure of the relative tolerance of different plant individuals or varieties to infection with different strains of *V. dahliae*. This assay is being further examined for use in the pathogenicity testing of parental and progeny plants in the molecular genetic marker project which succeeded this research (US33C). The test also has the potential to be employed in the detection (and possible quantification) of pathogenic organisms in the soil. However such an assay will only be species-specific and is not capable of differentiating individual strains of the pathogen.

2.6. Identification of strain-specific markers

We have used RAPD-PCR to differentiate between individual isolates of *V. dahliae* with diverse morphologies and/or origins. Ten different RAPD-PCR primers are sufficient to produce over 100 different DNA markers, some of which are common to all strains and others which are unique to particular individuals or groups of isolates. Ideally it would be possible to identify individual strains with important characteristics such as high pathogenicity or field persistence by conducting an assay for a linked strain-specific molecular marker. We have attempted to correlate genetic fingerprints as determined by RAPD-PCR with the pathogenicity characteristics of these isolates, however we have yet to observe an obvious trend. The results reveal that some highly pathogenic strains are apparently closely related to some less pathogenic isolates, while the highly pathogenic strains appear to be genetically unrelated even though they may exhibit the same host specificity.

2.7. Cloning of *V. dahliae* pathogenicity genes

We have turned to the characterisation of potential pathogenicity genes as a means of more directly identifying genetic markers associated with *V. dahliae* pathogenicity. This approach has involved the cloning of fungal genes which are highly expressed during infection of cotton plants. This research utilised the cDNA library prepared by Ms. Melissa Hill as part of the CRC project SU251 and was initiated by Ms. Catherine Keniry as an Honours project in 1995. The work successfully identified 30 genes which have been partially sequenced. These genes do not appear to correspond to any previously characterised pathogenicity genes from other fungal species but they may nonetheless represent unique genes associated with *V. dahliae* pathogenicity. This research continues in 1996/97 as CRDC project US32C.

2.8. Conclusion

Genetic fingerprinting has proved to be an extremely useful technique for the identification of strains of *V. dahliae* isolated from cotton production regions in NSW and Queensland. This survey highlights the significant genetic diversity evident within populations of *V. dahliae* which infect cotton plants, and clearly shows that a variety of organisms are capable of causing disease even in relatively tolerant cotton cultivars. Unfortunately, no obvious link could be established between isolate identity and pathogenicity on a particular host cultivar. Nonetheless, it is believed that the detailed epidemiological and pathological data obtained in this research will be extremely valuable in the development and testing of new cotton cultivars with enhanced tolerance to Verticillium wilt.

3. GENETIC FINGERPRINTING OF COTTON CULTIVARS

3.1. 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.

3.2. 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. 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 4). 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.

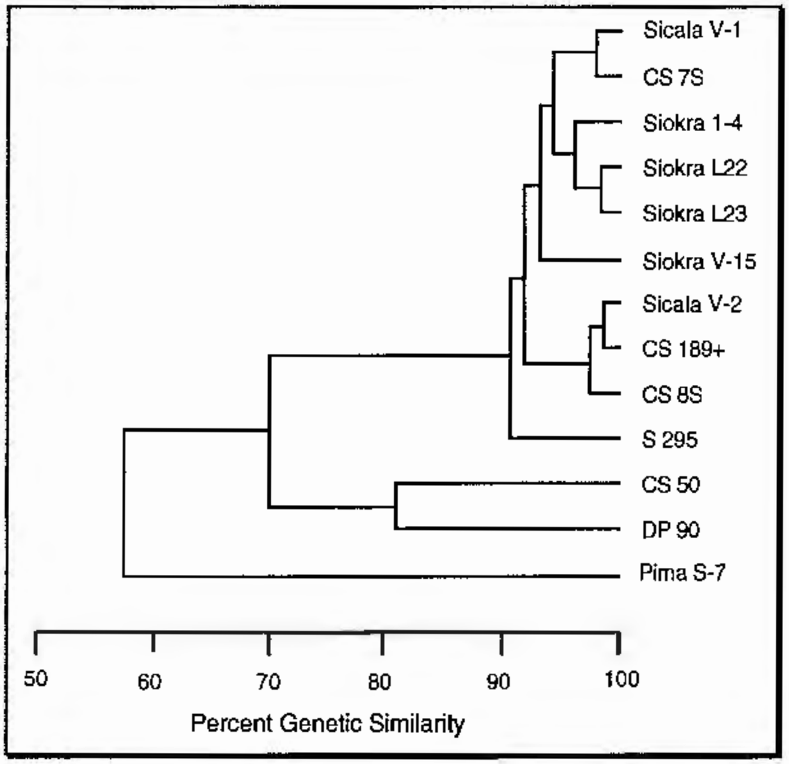

FIGURE 4. Tree diagram showing the percentage genetic relatedness of a number of cotton cultivars subjected to genetic fingerprint comparison.

3.3. Cultivar-specific markers for plant variety rights (PVR) protection

Our results have also confirmed the potential of using RAPD-PCR markers to establish genetic fingerprints which could be used to identify cotton cutivars developed by the CSIRO cotton breeding program. Closely related cultivars such as Sicala V-1 and Sicala V-2, Siokra L22 and Siokra L23, and CS 7S and CS 8S, are readily discriminated, and nine *G. hirsutum* varieties presently under cultivation in Australia can be characterised individually based upon cultivar-specific RAPD-PCR markers. Such markers could be developed as unique 'identifiers' for the particular cultivars as a means to verify plant variety rights (PVR) registration.

3.4. Artificial infection of cotton with *V. dahliae*.

Most of the studies conducted on Verticillium wilt of cotton in Australia have been surveys of the extent of damage to the cotton crop. Although we have successfully differentiated isolates of *V. dahliae* using RAPD-PCR, pathogenicity tests remain the only means of determining the pathological classification (races) of fungal strains present in diseased cotton plants. We therefore aim to determine the reaction of individual fungal strains on a set of cotton cultivars. We have standardised an artificial technique of induction of Verticillium wilt, and to date, have tested 30 independent *V. dahliae* isolates on the five cotton cultivars Siokra 1-4, Deltapine 90, Sicala V-1, Acala Royale and Pima S7. As expected, all but one of these isolates were able to cause significant disease on Siokra 1-4, but more alarmingly, over half of the isolates (17) were capable of causing significant disease on the wilt-tolerant variety Sicala V-1, and three were able to cause significant disease on the more tolerant Acala Royale variety. In addition to establishing a system for the classification of pathological races of *V. dahliae* prevalent in Australian cotton production regions, this work may assist in the identification of plant genes which impart enhanced tolerance to particular strains of the fungus.

3.5. Resistance of selected cotton cultivars to Verticillium wilt

To breed cotton cultivars with enhanced resistance to Verticillium wilt, we must first identify parental plants which display improved disease resistance. Pathogenicity assays currently remain the only means of determining the degree of resistance of different cotton cultivars towards Verticillium wilt disease. We have employed the artificial technique of induction of Verticillium wilt to determine the reaction of selected cotton cultivars to infection with individual fungal strains. The results of inoculating the cotton cultivars Siokra 1-4, DP 90, Sicala V-1, Acala Royale and Pima S-7 with a random collection of 20 previously-identified strains of *Verticillium dahliae* are presented in Figure 5. As was shown by our earlier work, strains of *V. dahliae* in Australian cotton production regions are extremely diverse, both genetically and pathogenically, and a number of these strains are capable of causing significant disease even on the more tolerant cultivars.

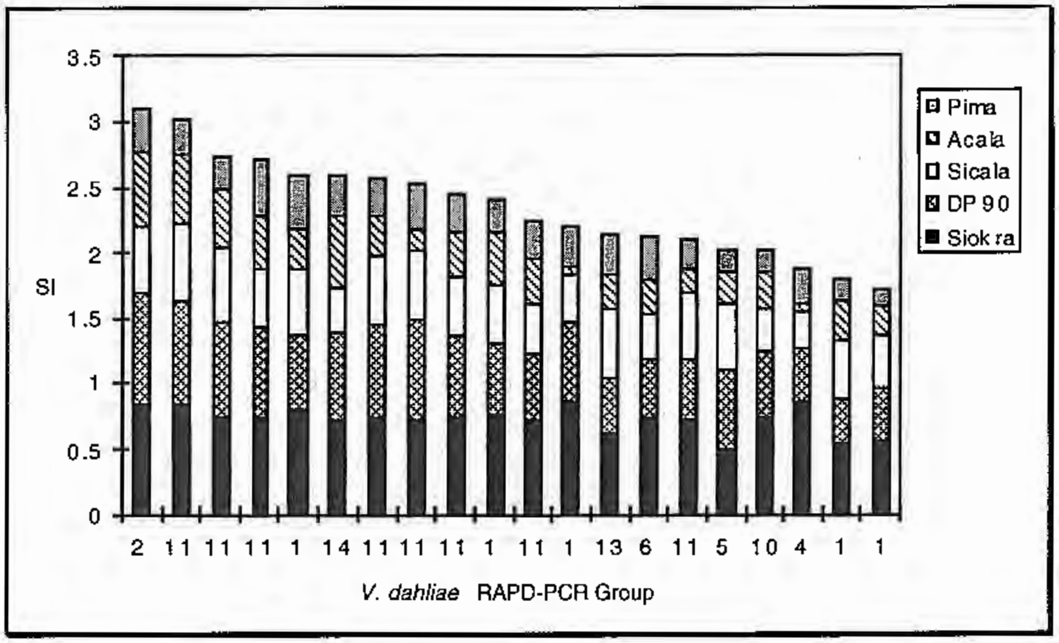

FIGURE 5. Chart showing the severity of disease (SI = severity index) caused by 20 randomly-selected isolates of *V. dahliae* (number = RAPD-PCR group) on the cotton cultivars Pima S-7, Acala Royale, Sicala V-1, DP 90 and Siokra 1-4. The height of each bar segment is proportional to the severity of disease on that cultivar.

3.6. Genetic markers for enhanced resistance to Verticillium wilt

As 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 characters. One useful strategy for such work is segregant fingerprint analysis (Figure 6).

We have employed segregant fingerprint analysis to compare pools of F2 progeny from the cross between the wilt-tolerant cultivar Sicala V-1 and the wilt-susceptible cultivar Siokra 1-4. From more than 2000 RAPD markers, we have found 86 which are polymorphic between the two parental types. This confirms earlier findings that these parents are about 95% isogenic. RAPD primers which produce these polymorphic markers have been screened against the F2 resistant and susceptible pools. One primer (OPG-17) identified a marker in the Sicala V-1 parent and F2 resistant pool which was not present in the Siokra 1-4 parent nor the F2 susceptible pool. Another primer identified a second marker which also seems to follow this pattern, while a third primer identified a marker in the Siokra 1-4 parent and F2 susceptible pool which was not present in the Sicala V-1 parent nor the F2 resistant pool.

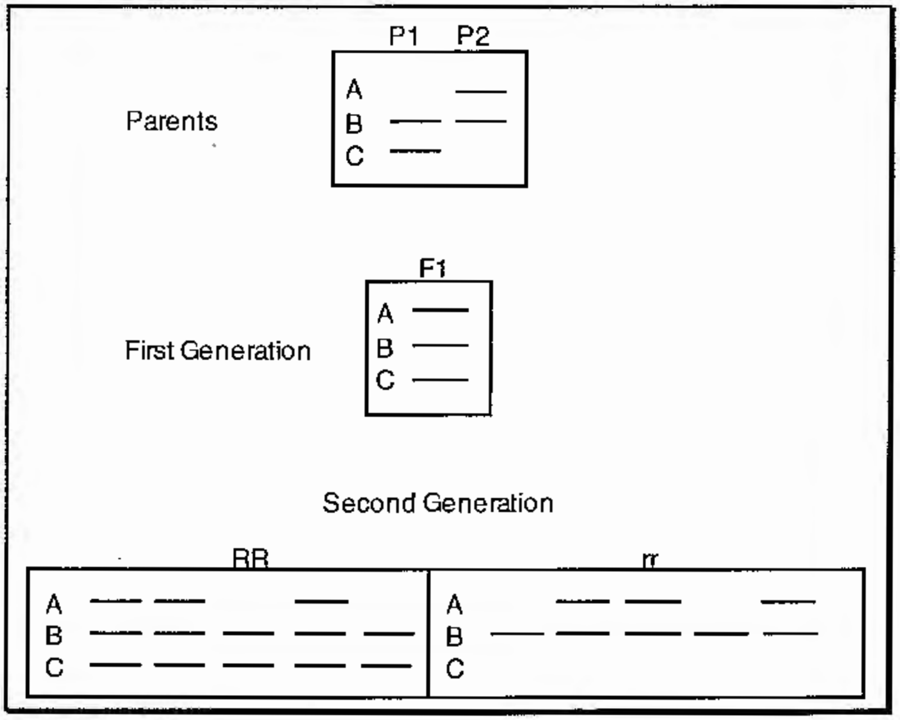

FIGURE 6. Diagram illustrating the principle of segregant fingerprint analysis. Parental plants (P1 and P2) possess different genetic fingerprint patterns. All first generation progeny (F1) possess the combined parental pattern (fragments A, B and C), but second generation progeny (F2) segregate with different patterns. Patterns for F2 progeny that are either disease resistant (RR) or disease sensitive (rr) are compared. If disease resistant F2 progeny always appear to possess patterns containing fragment C, this fragment may be linked to a gene for disease resistance.

We have checked a number of individual F2 plants for the presence of the OPG-17 marker, however, although there is a general correlation between the presence of the marker and disease resistance, the marker was also found to be present in a small proportion of plants that were rated as disease susceptible. Hence, rather than being exclusively associated with disease resistance, the current state of our research suggests that the OPG-17 marker can only be said to be more frequently associated with plants exhibiting enhanced resistance to Verticillium wilt. This finding may result from the inadvertent inclusion of heterozygotes within the F2 pools, or from the possibility that this marker is associated with a quantitative trait locus which is only one of several required for the fully resistant phenotype. Verification of existing markers and a search for additional molecular markers for Verticillium wilt tolerance from this parental cross is continuing in 1996-97 as part of CRDC project US33C.

Analysis of a second cross between the wilt-tolerant cultivar Acala Royale and the wilt-susceptible cultivar CS 50 was commenced in August 1995. A total of 280 random primers have now been tested on the two parents, generating approximately 2000 RAPD markers of which 136 are polymorphic between the two parental types. This suggests that the parents are about 93% isogenic. Pools of F2 progeny are being formulated and assayed in 1996-97 as part of CRDC project US33C.

3.7. Conclusion

Our results have confirmed the practicality and applicability of using genetic markers to establish genetic fingerprints of cotton cutivars developed by the CSIRO cotton breeding program. Closely related cultivars such as Sicala V-1 and Sicala V-2, Siokra L22 and Siokra

L23, and CS 7S and CS 8S, are readily discriminated, and nine *G. hirsutum* varieties presently under cultivation in Australia can be characterised individually based upon cultivar-specific RAPD markers. The future use of the RAPD-PCR technique will aim to establish linkages between molecular genetic markers and Verticillium wilt-tolerant genes in selected cotton cultivars, and this will be augmented by the isolation of genes from cotton plants that are expressed in response to *V. dahliae* infection. Genetic markers identified in this work will be employed to assist in the breeding of cotton cultivars with enhanced levels of tolerance to Verticillium wilt.

4. RESEARCH PUBLICATIONS & PRESENTATIONS

4.1. Scientific Papers

1. Multani DS, and BR Lyon (1995). Genetic fingerprinting of Australian cotton cultivars with RAPD markers. Genome 38: 1005-1008.
2. Ramsay JR, DS Multani, and BR Lyon (1996). RAPD-PCR identification of *Verticillium dahliae* isolates with differential pathogenicity on cotton. Aust. J. Agric. Res. 47: 681-693.
3. Zhu Y, DS Multani, and BR Lyon. DNA polymorphism, geographical origin and host cultivar relationships of Australian isolates of *Verticillium dahliae* from cotton plants. (In preparation).
4. Zhu Y, DS Multani, and BR Lyon. DNA polymorphism and pathogenicity relationships of *Verticillium dahliae* isolates from cotton plants. (In preparation).
5. Kota R, and BR Lyon. Comparative study of DNA marker technologies for genetic fingerprinting and molecular breeding of cotton. (In preparation).

4.2. Published Conference Proceedings

1. Kearney A, OL Chan, JL Bayliss, and BR Lyon (1992). Molecular techniques for the genetic identification of cotton plants and associated soil microorganisms. pp. 381-384, Proceedings of the 6th Australian Cotton Conference, Broadbeach, Australia.
2. Multani DS, MK Hill, JR Ramsay, and BR Lyon (1994). Verticillium wilt of cotton: Application of molecular genetic techniques in fingerprinting and gene cloning. pp. 257-264, Proceedings of the 7th Australian Cotton Conference, Broadbeach, Australia.
3. Lyon BR, Y Zhu, Y-Y Wang, and DS Multani (1996). Verticillium wilt of cotton: Epidemiology of Verticillium dahliae. pp. 657-662, Proceedings of the 8th Australian Cotton Conference, Broadbeach, Australia.
4. Lyon BR, M Akbari, MK Hill, R Kota, AM Mackie, DS Multani, and Y Zhu (1996). Verticillium wilt of cotton: Genetic markers for disease resistance. pp. 663-668, Proceedings of the 8th Australian Cotton Conference, Broadbeach, Australia.

4.3. Conference Presentations

1. Lyon BR, JL Morrow, JR Ramsay, KP Ridgway, and MK Hill (1994). Molecular genetic identification of fungal pathogens and symbionts of cotton. Poster session, World Cotton Research Conference - 1, Brisbane, Australia.
2. Multani DS, MK Hill, JR Ramsay, and BR Lyon (1994). Verticillium wilt of cotton: Application of molecular genetic techniques in fingerprinting and gene cloning. Poster session, 7th Australian Cotton Conference, Broadbeach, Australia.
3. Multani DS and BR Lyon (1995). Random amplified polymorphic DNA (RAPD) markers for genetic fingerprinting of Australian cotton cultivars. Poster session, Plant Genome III, San Diego, USA .
4. Lyon BR (1995). Molecular markers for the genetic analysis of Australian cotton cultivars. Cotton Workshop, Plant Genome III, San Diego, USA .
5. Hill MK, CA Keniry, and BR Lyon (1995). Identification of cDNA clones involved in the interaction between cotton and the Verticillium wilt pathogen *Verticillium dahliae*. Poster

session, 35th Annual General Meeting of the Australian Society of Plant Physiologists, Sydney, Australia.

6. Hill MK, CA Keniry, KP Ridgway, and BR Lyon (1996). Identification of genes involved in the interaction between the cotton plant and the fungal wilt pathogen *Verticillium dahliae*. Poster session, 8th International Congress on Molecular Plant-Microbe Interactions, Knoxville, Tennessee, USA.
7. Keniry CA, MK Hill, KP Ridgway, and BR Lyon (1996). Molecular genetic analysis of the cotton-verticillium interaction. Poster session, 8th Australian Cotton Conference, Broadbeach, Australia.
8. Akbari M, R Kota, and BR Lyon (1996). Identification of molecular markers linked to verticillium resistance . Poster session, 8th Australian Cotton Conference, Broadbeach, Australia.
9. Becerra Lopez Lavalle LA, Y Zhu, and BR Lyon (1996). Verticillium and Fusarium wilt in cotton: Rapid assays for the identification of disease resistant plants. Poster session, 8th Australian Cotton Conference, Broadbeach, Australia.