1689 Breeding for resistance to a new strain of Fusarium wilt in Australia

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A new virulent strain of Fusarium wilt appeared in Australia in the early 1990s and new cultivars were required to allow viable production in the locations where this disease first appeared and where it had spread. This paper describes the screening of germplasm from a wide range of sources and the development of breeding populations and new cultivars. All screening was done in field nurseries and resistance assessment was by measuring survival of plants and absence of symptoms in adult plants. All results of introductions or breeding lines were by comparison with a standard cultivar, Sicot 189. Good sources of resistance were found in Indian and Chinese parents as well as from within existing Australian cultivars. A new cultivar, Sicot F-1 was released in 2004 with double the resistance of Sicot 189. It was concluded good progress was being made but better resistance was required for situations with very high incidence of Fusarium wilt. Although there may be more resistance to be found in *Gossypium hirsutum*, other *Gossypium* species and/or biotechnology may potentially add resistance.

Keywords: Fusarium wilt; field nurseries; resistance breeding.

Introduction

A virulent strain of Fusarium wilt of cotton (*Fusarium oxysporum* f.sp. *vasinfectum*) was first identified in the early 1990s in Australia (Kochman 1995). It has subsequently been shown the strain is unique and substantially different to Fusarium wilt strains in other countries (Kim *et al* 2005). This local Australian strain is common in alkaline clay soils and not associated with nematodes (Colyer 2003).

Although Fusarium wilt does not (yet) cover a significant area in Australia, the extreme virulence and ready transport in fields and across farms in soil, water or in trash raised fear of spread. As local cultivars were highly susceptible, a program was initiated to identify sources of host plant resistance and to develop new cultivars - since an Integrated Disease Management program would rely heavily on cultivar resistance.

This paper aims to outline the activities and progress with developing new Australian cotton cultivars resistant to this strain of Fusarium wilt.

Field methods

Two nurseries have been used for field screening and evaluation of breeding material for resistance to Fusarium wilt. These nurseries are located at ‘Norwin’ and ‘Pampas’ near Brookstead (27°40’S; 151°22’E) in Queensland, Australia. Each field was identified as having a high incidence of Fusarium wilt in the early to mid 1990’s and they have now been used by pathologists and plant breeders for a number of years. The ‘Pampas’ site involves two fields with cotton experiments and commercial grain sorghum rotated in alternate summers. This rotation is aimed at providing uniformity of Fusarium wilt incidence in the cotton experiments. Field plot assays for measuring resistance to Fusarium wilt were done in experiments with two to four replications in row and column designs for later generation experiments; for early generation material, unreplicated nearest neighbor designs were used. Plots were a single 1m row, 10m length sown to approx 120 seeds with a cone seeder. Assessments included: emergence, seedling survival, adult survival and adult severity incidence with stem cut rating on a 1 to 5 scale. Seed cotton yield was measured by harvesting each plot with a plot harvester. Sicot 189 (Reid 1996) was used as the control cultivar in all experiments.

Results and discussion

Evaluation of Australian cultivars

It was quickly established there was cultivar variation for resistance: a few cultivars at the time such as Sicot 189, CS 8S and Delta Emerald had typical field survival of less than 50% under heavy incidence of the disease. Most other cultivars were worse, with survival close to zero in some cases (Figure 1).

Evaluation of introduced *Gossypium* germplasm

An initial screen from the CSIRO germplasm collection in 1994 of over 30 genotypes from the US, South America, Africa, Asia and Europe, showed a few had field survival up to 50% better than the best Australian cultivars, although none had immunity. With promising lines repeated in 1995, a few introductions showed consistently higher survival (Figure 1) and were included in a large crossing and selection program. It was clear that resistance to the US Fusarium race/nematode complex did not confer resistance to the Australian race – survival of Auburn cultivars for example was less than 10%.

Of immediate interest was MCU-5 from India and KC311 from the USA. MCU-5 has been a good parent and has led directly to the development of Sicot F-1 released in 2004 (Stiller and Reid 2004) with double the level of field resistance to Fusarium wilt compared with Sicot 189. Table 1 shows that increased resistance and increased plant survival gave a 32% yield increase in the presence of Fusarium wilt but at the expense of an 8% decrease in the absence of Fusarium wilt. New cultivars due for release in 2007 have overcome the yield penalty. Crosses with KC311 did not produce any progeny with improved resistance and it is assumed that cultivar carried similar resistance mechanisms to those in existing Australian cultivars.

New introductions have been evaluated every year in an effort to identify greater resistance and field survival. To date there have been 134 introductions evaluated in Fusarium wilt nurseries. Some *G hirsutum* race cottons, many *G barbadense* and some diploid *G arboreum*, *G herbaceum* and *G sturtianum* genotypes have been screened in the past 10 years for Fusarium resistance (Table 2). Despite entering targeted *G hirsutum* genotypes, only a very small number of genotypes exhibited resistance. Of interest was the high frequency of resistance found in diploid species. Breeding is being attempted with some of these in crosses with Australian *G hirsutum* genotypes. That research effort is long term as there are difficulties with some crosses and considerable yield drag in all of them.

It is of interest to note MCU-5 has Sea Island *Gossypium barbadense* in its pedigree (Kenniyan and Marappan 1969) and the occasional resistance discovered in our *G barbadense* screening may be the same mechanism(s) as in MCU-5, although no Sea Island accessions have shown good resistance to Fusarium wilt in our screening.

Lessons in evaluation

As the Australian race of Fusarium wilt is so far only present in limited locations, distance between the breeding station and key Fusarium nurseries (450 km) is a considerable constraint. CSIRO currently have about 9,000 plots in Fusarium nurseries and the logistics for sowing, disease assessment and harvest are substantial in time and cost.

Measures in field nurseries have evolved through time. Mortality occurs at any time from the seedling stage to adult plants. The current protocol of assessing survival is the percentage of established plants that have no symptoms of Fusarium wilt in stem cross sections at harvest.

Field Fusarium wilt incidence is strongly affected by climatic conditions such as rain and minimum temperature as well as agronomic management (sowing date, inter-row cultivation), so Fusarium wilt incidence in field nurseries will vary. Low incidence of Fusarium wilt does not allow distinction between medium and high resistance genotypes; very high incidence may kill more than 95% of control genotypes such as Sicot 189, preventing effective screening. However there are significant correlations in the field between seedling survival and adult freedom of symptoms (Figure 2), so seedling survival can be used as a resistance screen in situations of very high incidence. Yield ('yield resistance') in Fusarium wilt nurseries can be used as a selection measure as well.

Bioassays have been developed for screening in the glasshouse/laboratory (Becerra pers comm.). These bioassays have been reliable for identifying very susceptible genotypes, but cannot reliably distinguish between medium and high levels of resistance.

Variability within Fusarium nurseries

Since Fusarium wilt in a field is mostly spread by water moving down rows and along drains (Grinstein et al 1983), the disease incidence varies widely across and down a field. This variability obviously reduces accuracy, so replication in time and space is required to produce reliable measures of Fusarium wilt resistance in breeding material.

Inheritance of Fusarium wilt resistance

Resistance to Fusarium wilt in segregating populations has not been simply inherited and is assumed to be multigenic. Only small proportions of each population have been measured to display high resistance to Fusarium wilt (Figure 3), even when both parents were relatively resistant.

Figure 3 illustrates the uncertainty with segregation where unexpected susceptibility occurs. The populations in Figures 3a and 3b were high x medium resistance parents and produced many medium and some high resistance progeny as expected. Figure 3c was a population with low x medium resistance parents and produced many medium and low resistance progeny as expected but more high resistance progeny than expected. Figure 3d was a population with high x medium resistance parents and produced many medium, some high, but unexpectedly many low resistance progeny.

Because of this uncertainty, we have used large population sizes to ensure the low frequency of resistant lines can be identified. Families of 50 to 80 sister lines from each cross are evaluated at F3 or F4 in replicated experiments at two sites for two generations.

Selection of elite lines is based on adult plant survival and yield. Parallel experiments at Fusarium-free sites are also used for yield and fiber quality selection. The concerted initiative to develop Fusarium wilt cultivars has coincided with introduction and development of Bollgard®II and Roundup Ready® cultivars. Despite three backcrosses in the development of transgenic material, a significant proportion of the transgenic population has been much more susceptible than the recurrent parent (Stiller et al 2006) with frequency distributions similar to Figure 3d. We conclude that having the correct recombination of multiple genes is required for Fusarium resistance.

One line from China (LuMein) was evaluated in 1997 as it had reputed Fusarium wilt resistance in that country and crosses were made at the same time as entry into quarantine prior to evaluation. Field bioassays showed it only had similar survival to local standards (Sicot 189), but on evaluation of crosses involving LuMein, it did appear to have additional resistance in the progeny (Figure 4). This raises the possibility that many cultivars may have additional genes for resistance but cannot be identified without developing and evaluating progeny from many crosses.

The LuMein x Sicot 189 family did not produce a commercial cultivar because of inadequate fiber strength, but excellent parents for combination of high yield and increased Fusarium resistance have been developed from LuMein crosses.

Crossing amongst Australian cultivars has also been able to find improved resistance. For example Sicala 45 (Reid 2003) has 48% better survival than Sicot 189 despite all four grandparents having less or equal survival than Sicot 189, indicating at lest one apparently susceptible grandparent was carrying resistance gene(s).

Verticillium wilt

Verticillium wilt (*Verticillium dahliae*) is widespread in Australia and is a major disease affecting cotton yield (Allen 2006). Good cultivar resistance has been developed in Sicala V-2 (Reid 1994) and other cultivars to reduce yield impact and even reduce the field incidence of Verticillium wilt (Reid et al 1999). There is some correlation across cultivars for Verticillium wilt and Fusarium wilt resistance possibly indicating some common resistance mechanisms (Figure 5). Terpenoid phytoallexins and condensed tannins have been shown to be important in Verticillium wilt defense reactions by resistant cotton cultivars (Bell 1994) and at least some of these chemicals may also protect against Fusarium wilt (Kaufman et al 1981) although not all or identical (Shi et al 1992).

Marker assisted breeding

Difficulties with assays in field nurseries make molecular markers attractive but as the resistance is most likely multigenic with some minor genes, identification of markers for all genes will be difficult and unlikely. However, discovery of major genes would help. Becerra (pers comm.) using a population from a cross between a susceptible *G hirsutum* and a resistant *G barbadense* has identified at least eight Quantitative Trait Loci (QTL) associated with Fusarium wilt resistance, confirming more than one gene is involved in resistance.

Conclusions

We are making good progress in breeding resistant cultivars to a new virulent strain of Fusarium wilt. Sources of resistance have been identified and used and field screening procedures have been improved through experience. The suite of cultivars in Australia has totally changed in response to Fusarium wilt: susceptible cultivars have been removed from the market and new cultivars have an increasing trend for resistance. However in fields with high Fusarium incidence, even the most resistant cultivars may only have 20% survival, so the search for increased resistance levels is necessary. Although molecular markers would help breeding efficiency using existing sources of resistance, they will not discover new genes. We expect wild relatives and/or biotechnology to contribute to further improvement in the long term.

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Table 1. Relative yield and survival of Sicot F-1 compared with Sicot 189. Yield in absence of Fusarium from 19 irrigated experiments in three seasons. Relative yield and plant survival from five field Fusarium nurseries in the same three seasons.

Cultivar	Relative yield in absence of Fusarium	Relative yield in presence of Fusarium	Relative plant survival in presence of Fusarium
Sicot 189	100	100	100
Sicot F-1	92	132	220

Table 2. Numbers of introduced *Gossypium* spp. lines tested for resistance to Fusarium Wilt and nominal frequency of at least moderate resistance observed in field screening.

Gossypium species	Number of lines tested	Percentage of lines showing at least moderate resistance to Fusarium Wilt
G hirsutum	134	4
G hirsutum race	24	21
G barbadense	52	15
G tomentosum, G darwinii, synthetic tetraploids	5	0
G arboreum, G herbaceum, *G sturtianum*	22	91

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Figure 1. Field survival of introduced cotton cultivars under heavy incidence of Fusarium wilt compared with Australian cultivars. Mean data from two seasons: 1994/95 and 1995/96. Grey bars denote Australian origin; open bars US origin; and black bars African or Asian origin

Figure 2. Correlation between seedling survival and adult survival in Fusarium wilt nursery. Data for 76 genotypes in 2005/06.

Figure 3. Frequency distributions of Fusarium wilt survival in Fusarium wilt nursery for four segregating populations. Arrows denote parent values. The average population size was 53 F_3 or F_4 sister lines.

Figure 4. Relative survival in Fusarium wilt nursery for Sicot 189, LuMein and CSX102, a line selected from a cross between Sicot 189 and LuMein.

Figure 5. Association between Fusarium wilt resistance and Verticillium wilt resistance across 40 Australian cotton cultivars. Data from **www.csd.net.au**.

Figure 1

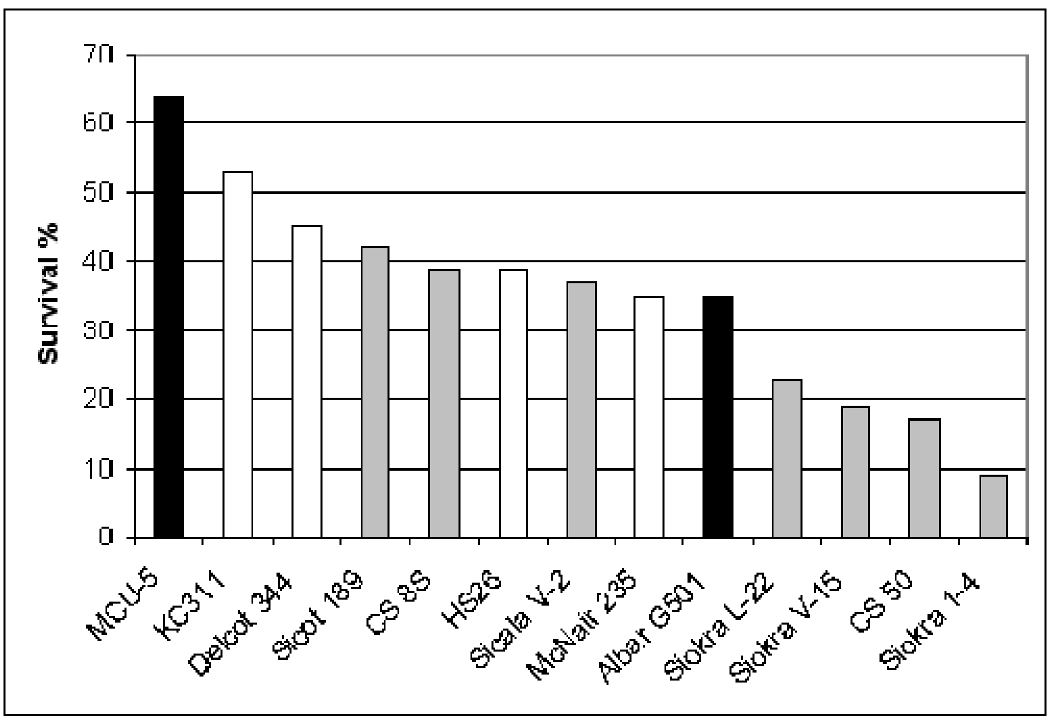

Figure 2

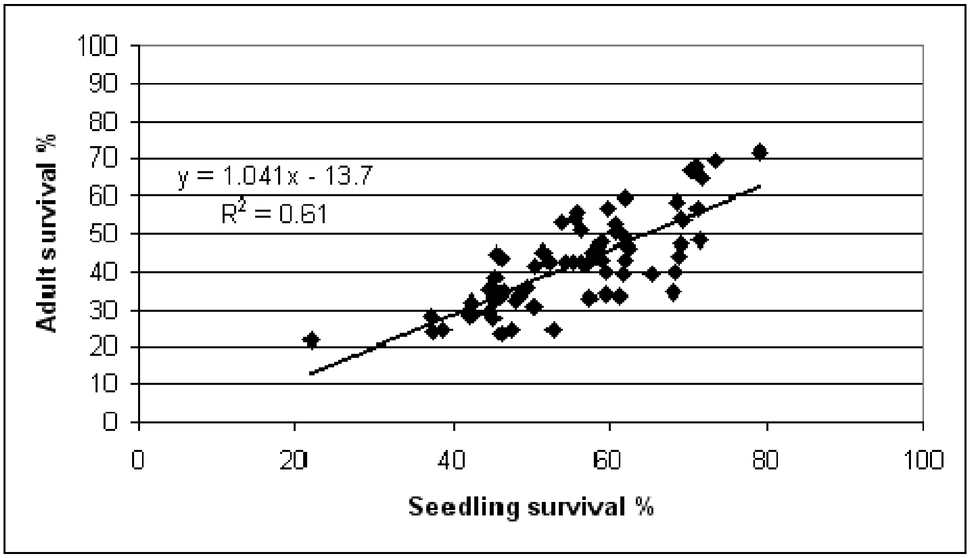

Figure 3

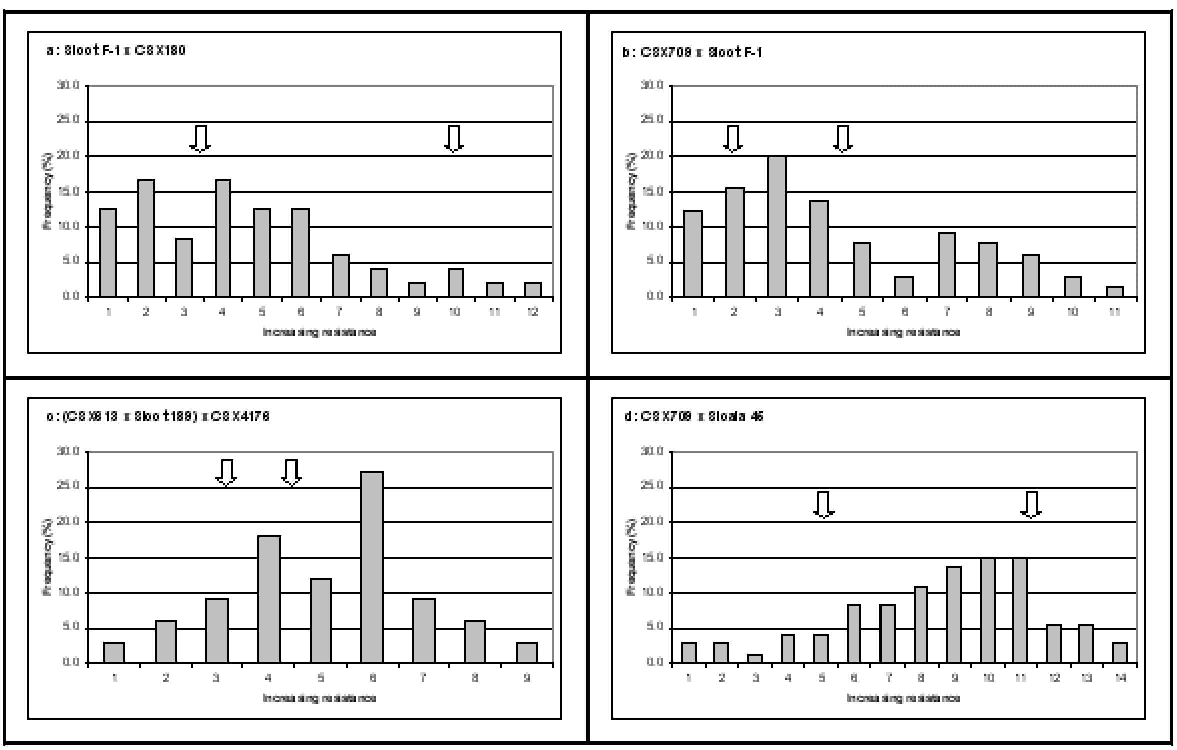

Figure 4

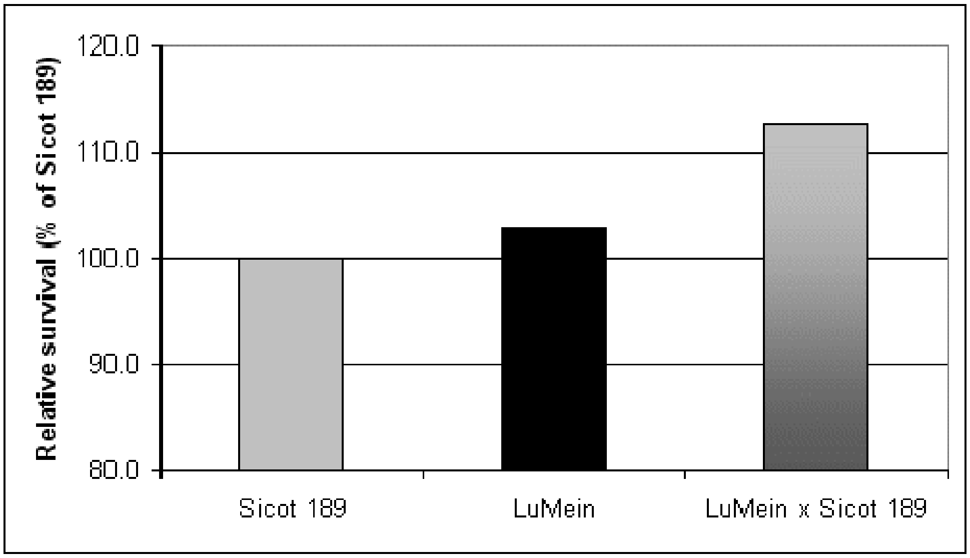

Figure 5