

**COTTON RESEARCH AND DEVELOPMENT CORPORATION****FINAL REPORT**

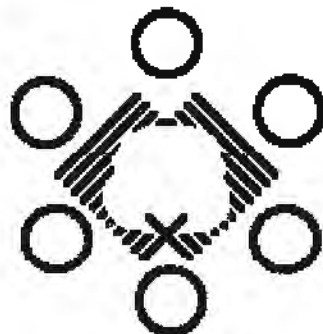
**PROJECT TITLE:** Improving water use efficiency of cotton.

**PROJECT CODE:** CSP55C (CRC Project CS 3.1.2)

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## **Plain English summary**

The increasing area of dryland and partially irrigated cotton prompted research into identifying characteristics of varieties that made them better suited to water stressed conditions.

Water use efficiency (WUE) was measured in a number of ways with a view to identifying a simple method for screening breeding lines. Agronomic WUE was determined using neutron probe measurements. Physiological WUE was determined using gas exchange and isotope discrimination techniques.

The okra leaf trait combined with high adaptation and yield potential conferred good performance under raingrown conditions. This result is consistent with physiological measurements. If morphological characters such as okra leaf type provide improved performance under raingrown conditions, there may be potential for placing selection pressure on other physiological characters such as photosynthesis, transpiration efficiency and carbon isotope discrimination.

Full season types were superior for raingrown yield; earliness was generally associated with lower yield under conditions of water stress. These experiments were for normal sowing dates, late sowings may require early maturing cultivars.

Small scale experiments were able to identify better raingrown genotypes so long as disease incidence was known at each site. CSIRO are evaluating the possibility of increasing the number of dryland sites for evaluation of breeding lines.

Overall, the project has demonstrated the following highlights:

- The okra leaf trait was associated with superior field yield under raingrown conditions. That result is consistent with some leaf physiological water use efficiency measurements.
- Full season types were superior for raingrown yield. Rooting depth was one factor associated with that result.
- Some physiological water use efficiency traits have significant genetic differences and may be of value for use in choosing parents and/or screening segregating populations.

**Background:**

Total area cropped to cotton in Australia over the last three years has exceeded 300,000 ha, compared with 170,000 ha ten years ago. Irrigation water availability, better management techniques and locally adapted varieties have all contributed to the growth of the industry. An important component of this area is raingrown production, increasing from minor areas ten years ago, to over 80,000 ha in northern NSW and Southern QLD. One reason for this increasing interest in growing cotton as a dryland crop has been financial. To compete against raingrown cotton, the price of grain sorghum has to be greater than \$140/tonne, and wheat greater than \$200/tonne.

The vast majority of cotton in Australia is still grown under irrigation. However, unreliable rainfall, liberal allocation of irrigation licences and debate over environmental river flows, have led to the situation where major production regions such as the Macquarie, Namoi, Gwydir and MacIntyre valleys are often restricted in irrigation supply. As a result, some irrigated crops become water stressed. Dryland cotton plantings are more variable, depending largely on stored soil moisture. Without further irrigation schemes being developed, any potential expansion in Australian cotton production will involve expansion and increased yield from raingrown areas.

In 1972, the CSIRO Cotton Research Unit commenced a cotton breeding program at Narrabri which, for more than a decade has produced high yielding, locally adapted varieties. To now, this program has concentrated mostly on producing varieties suited to irrigated conditions, with less emphasis on raingrown or water stressed performance. Given the increased frequency of irrigation restrictions and the expansion of raingrown cotton production, the potential exists therefore, for selection of varieties under raingrown conditions or the selection of characters that would enhance the performance of raingrown cotton.

## Objectives and achievements

- Aims:**
1. Determine differences in physiological and agronomic WUE of Australian and overseas varieties and breeding lines.
  2. Measure heritability of those differences and include the material and information in the CSIRO breeding program.

### **Achievements:**

*Variety and breeding line evaluation.* Variety trials were done, and consistent differences in WUE were found both within and between Australian and Texan varieties. The magnitude of these differences were found to vary between years and sites. This indicates that at least some of the mechanisms controlling WUE are environmentally influenced.

*Heritability measurements.* All heritability experiments on data from the F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub>'s have been analysed. The characters that we are most interested in are; photosynthesis, stomatal conductance to water vapour, intercellular CO<sub>2</sub> concentration, transpiration efficiency (mol CO<sub>2</sub> fixed / mmol H<sub>2</sub>O transpired and delta (ratio of C<sup>12</sup> to C<sup>13</sup> in the plant tissue). Significant genetic variation was detected for these characters. Heritability estimates of between 0.3-0.5 were obtained for WUE characters, indicating progress could be made within a breeding program. The sampling time of peak flowering (January) consistently produced the highest heritabilities, indicating that would be the time producing the greatest response to selection.

### **Methodology**

All of the experiments were located on ACRI except for one experiment at Pecos TX, and another at Dalby Qld.

#### *Treatments*

The genotypes were arranged in randomised blocks with three replicates, except the Texas experiment which had four replicates. In the 1995/96 and 1996/97 seasons cultivar experiments were grown under both raingrown and irrigated conditions. This was achieved by growing experiments side by side with eight metres of planted buffer between them. Plot sizes on the Australian Cotton Research Institute (ACRI) were three rows × 15 metres, at Dalby four rows × 15 metres, and in Texas two rows × 10 metres.

Crosses were also made between Texan and CSIRO genotypes, creating diverse F<sub>2</sub> populations. The individuals in this population were measured for a range of physiological characters. Heritability was estimated for these characters on a single plant basis using a number of methods. When calculated on the raw values, heritability was very low, ranging from 0 to 0.2. However, these heritabilities can be increased to between 0.3 to 0.4 when the data is corrected for day and time of day effects using 'best linear unbiased prediction' (BLUP), a statistical analysis procedure. Plants were then selected that were either high or low for the physiological characters measured. These plants were then taken through to the two subsequent generations as F<sub>3</sub> and F<sub>4</sub> rows and measured for yield in addition to the physiological characters.

### *Gas exchange*

Gas exchange measurements were made using a Li-cor Li-6400 (Li-cor Lincoln, NE) open system portable photosynthesis unit with a 6 cm<sup>2</sup> chamber. Measurements were made on a portion of the youngest, disease free, fully expanded, fully sunlit leaf (usually four nodes from the terminal) where possible. Measurements were taken within the period of three hours either side of solar noon, with leaves held perpendicular to the sun.

### *Carbon isotope discrimination (Delta)*

Leaves that had been measured for gas exchange, or leaves that fitted the same criteria were harvested. Each leaf blade was removed from the petiole, put into a paper packet and placed immediately on ice to stop respiration. As soon as possible they were transferred to a fan forced dehydrator at 80°C for a minimum of 24 hours. These samples were shipped to the Australian National University, Research School of Biological Sciences for analysis.

### *Yield*

The centre row of the three row plots, the centre two rows of the four row plots and both rows of the two row plots were machine harvested with a spindle picker and the seed cotton weighed. A sub sample of approximately 400 g of seed cotton was taken from each plot. These samples were saw ginned in a 20 saw gin to determine lint percentage, and lint yield was calculated as seed cotton multiplied by lint percentage.

### *Quality*

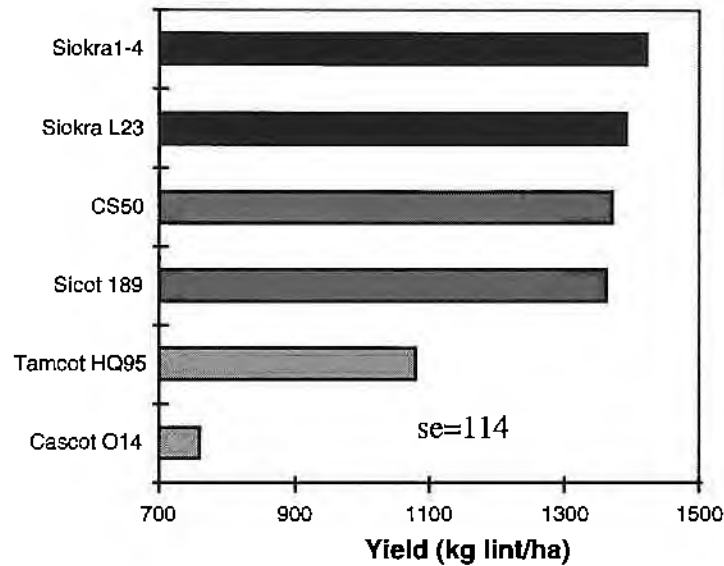
A lint sample from each plot was evaluated for quality using a Spinlab High Volume Instrument (HVI) model 900. Fibre characters measured were upper half mean length (inches), uniformity index (ratio of mean length to upper half mean length), strength (g/tex), extension (%) and micronaire value. On some occasions samples from the Australian experiments were also evaluated for micronaire, fineness (millitex) and fibre maturity (%) using a Shirley Fineness Maturity Tester (FMT3).

## **Results**

Detailed results are attached in the form of three papers prepared for the Australian Agronomy Conference, the Australian Cotton Conference, and the World Cotton Research Conference.

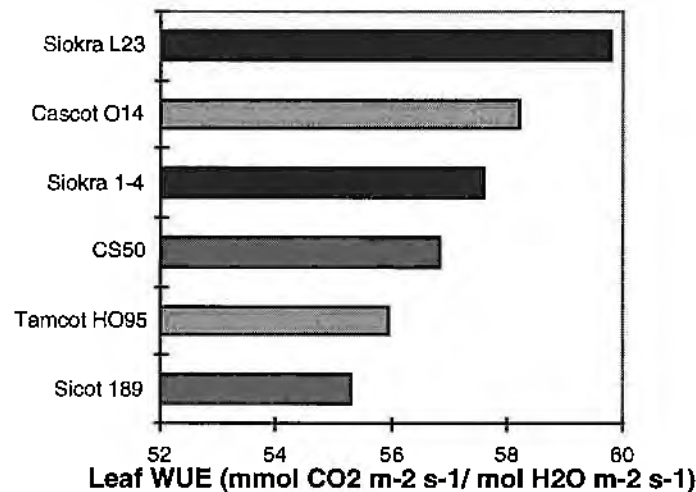
## **Discussion**

This project addresses the issues of selecting genotypes from a breeding population that have improved water use efficiency over commercial cultivars. This is being done by evaluating a range of physiological measurements (photosynthesis, stomatal conductance, intercellular CO<sub>2</sub> concentration, transpiration) backed up with agronomic and morphological measurements. By doing this we hope to take advantage of the larger numbers of individuals in the early generation populations, with a greater chance of selecting better performing cultivars. That approach is in contrast with the present strategy where good breeding lines from irrigated trials are evaluated under dryland conditions. Such an approach may have discarded good raingrown performers in the early stages.



**Figure 1: Three year mean yield of six commercial cultivars grown under raingrown conditions.**

Each season that the project has been running, cultivar trials have been undertaken in a number of locations to determine if genetic variability exists for water use efficiency among commercial cultivars. In each trial a number of cultivars consistently yielded well, while other cultivars were either erratic or poor. Figure 1 shows the mean of three years experiments. While this figure shows that Siokra 1-4 and Siokra L23 averaged the highest yields, it does not show that these two cultivars were consistently the best performers.



**Figure 2: Three year mean leaf water use efficiency (transpiration efficiency) of six cultivars grown under raingrown conditions**

The three year mean leaf water use efficiency of the same six cultivars are shown in Figure 2. This indicates that the cultivars must have good agronomic characters and adaptation, before the water use efficiency advantages are realised in the field. This is best demonstrated by Cascot O14, which although it had a high leaf water use efficiency, it had a low yield.

Carbon isotope discrimination or 'delta' was one of the physiological measurements that was being assessed. In theory, delta gives an estimate of leaf water use efficiency integrated over time. It was hoped that this would be a simple measure for screening plants for water use efficiency. In practice however, this was not the case. Delta was only CSP 55C final report

weakly associated with water use efficiency, and either not associated with yield at all or negatively associated. Indications are that delta may not be better than other physiological water use efficiency measurements for screening.

The other conclusions to be drawn from this series of experiments is that the okra leaf trait was an advantage for raingrown performance. This was expressed through high rates of assimilation and high leaf water use efficiency, together with yield. Early maturity was also examined as a strategy for raingrown performance, but in all experiments lint yield was positively associated with maturity, *ie* later maturity types yielded better.

The second component of the project involved making crosses between Texan and CSIRO genotypes, and creating diverse F2 populations. The individuals in this population were measured for a range of physiological characters. Heritability was estimated for these characters on a single plant basis using a number of methods. When calculated on the raw values, heritability was very low, ranging from 0 to 0.2. However, these heritabilities can be increased to between 0.3 to 0.4 when the data is corrected for day and time of day effects using 'best linear unbiased prediction' (BLUP), a statistical analysis procedure. Plants were then selected that were either high or low for the physiological characters measured. These plants were then taken through to the next generation as F3 rows and measured for yield in addition to the physiological characters. One of the characters, photosynthesis, is shown in Figure 3. These data show a poor association between photosynthesis of F2 and F3 populations, indicating low heritability.

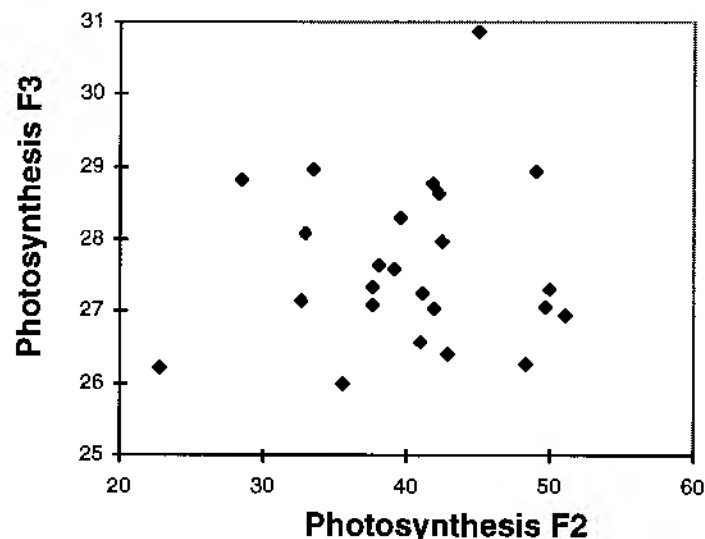


Figure 3: F2 photosynthesis vs F3 photosynthesis

### Recommendations

We propose that the knowledge regarding choice of parents and screening procedures for identifying high performing dryland genotypes be incorporated in the existing CSIRO breeding program. An example of this is to use both gas exchange techniques together with delta measurements to gain a better understanding of the water relations and stress tolerances of potential parents. Another possibility is to use gas exchange techniques to screen for water use efficiency in early generation segregating populations. This however is very labour and time consuming and requires in depth statistical knowledge to make the results useful.

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## Varietal characteristics for adaptation of cotton (*Gossypium hirsutum* L.) to raingrown conditions

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### Abstract

Three year means of large scale seed company trials indicated that okra leaf was a desirable character associated with yield under raingrown conditions. Early maturity was not a desirable character. Small scale experiments indicated a similar ranking. Deviations were associated with disease susceptible cultivars grown in small scale trials at sites with a greater than normal incidence of verticillium wilt. It was concluded that there is scope for greater selection pressure for cotton yield under raingrown conditions.

### Keywords

raingrown cotton, leaf shape, crop maturity, plot size.

### Introduction

The Australian raingrown cotton industry has increased from minor areas ten years ago, to over 80,000 ha in northern NSW and southern Qld. This increased interest has been economically motivated, as the gross margin of cotton is greater than wheat or sorghum.

In 1972, the CSIRO Cotton Research Unit commenced a cotton breeding program at Narrabri NSW which has produced high yielding, locally adapted varieties, with particular emphasis on producing varieties suited to irrigated conditions. Given the increased frequency of irrigation restrictions and the expansion of raingrown cotton production, there is now greater impetus for selection of varieties more suitable for raingrown conditions.

Morphological characters have been utilised in many crops to improve water stress tolerance. Recently, physiological measures such as carbon isotope discrimination (1) have been used to select for greater water use efficiency. Earliness of maturity is often utilised as a trait for increasing yield or yield stability under raingrown conditions. In Texas (USA) there are over 2 million hectares of raingrown or partially irrigated cotton, which generally adopt an early maturity strategy to avoid late season 'terminal' drought (8). When compared with Australian conditions, the Texas High Plains has a shorter growing season when measured in heat units (5).

This paper reports on three seasons of seed company cultivar trials, that compare cultivar performance under raingrown conditions. Because a breeding program needs to develop trial programs which identify genotypes for commercial practice, comparison of relative varietal performance is made with small scale raingrown experiments from the CSIRO breeding program. The importance of earliness of maturity and okra leaf type under raingrown conditions are examined.

### Methods

Each season, Cotton Seed Distributors (CSD) conduct large scale raingrown cultivar trials in all the major cotton growing areas of Australia. Entries in these trials vary between regions. They include the most popular CSD cultivar in the region, a standard CSD cultivar, a Deltapine nominated cultivar as well as one or two lines from CSIRO nearing commercial release. These trials were set out in a 'nearest neighbour' design, with a control cultivar planted between each treatment. Each cultivar had a planted area of about 4.5 ha. CSIRO also conducts small scale cultivar experiments each season in most of the major growing regions, usually with 48 entries. The experiments were laid out in a latinized alpha-

design with five replicates of each cultivar. Each plot had an area of 45 m<sup>2</sup>. All crops were managed with standard commercial practices. The characteristics of the cultivars discussed in this paper are presented in Table 1. The small and large scale sites are not always in close proximity to one another, which may confound interpretation. The small scale site on the Darling Downs was at Dalby and has been compared with the large scale sites at Warra (45 km north-west) and Bongeem (48 km south-east).

Table 1: Cultivars tested in all experiments.

| Cultivar     | Leaf type | Maturity | <i>Verticillium</i> (% plants infested) |
|--------------|-----------|----------|-----------------------------------------|
| Siokra L23   | O         | 8        | 25.6                                    |
| Siokra V-15  | O         | 7        | 10.3                                    |
| Siokra 1-4   | O         | 6        | 36.0                                    |
| Siokra S-324 | O         | 5        | 38.0                                    |
| Sicala V-2   | N         | 8        | 8.2                                     |
| Sicot 189    | N         | 9        | 10.7                                    |
| CS 85        | N         | 6        | 11.1                                    |
| DP 90        | N         | 8        | 31.4                                    |

Leaf type: O=okra leaf, N=normal leaf; Maturity: (relative scale, 1=very early, 10=very late) calculated from date of 60% boll opening when grown at Narrabri (P. Reid, *pers comm.*); and percentage of plants infested with *Verticillium* when grown at Narrabri (S. Allen, *pers comm.*).

Table 2: Mean monthly rainfall (mm) for the three trial years compared with 100 year average (italics) for the three regions.

|       | Narrabri          | Dalby             | Biloela           |
|-------|-------------------|-------------------|-------------------|
| Oct   | 45<br><i>53</i>   | 64<br><i>58</i>   | 57<br><i>107</i>  |
| Nov   | 59<br><i>58</i>   | 49<br><i>73</i>   | 38<br><i>99</i>   |
| Dec   | 93<br><i>66</i>   | 115<br><i>93</i>  | 79<br><i>67</i>   |
| Jan   | 136<br><i>81</i>  | 61<br><i>84</i>   | 79<br><i>51</i>   |
| Feb   | 55<br><i>63</i>   | 106<br><i>78</i>  | 76<br><i>73</i>   |
| Mar   | 39<br><i>58</i>   | 40<br><i>65</i>   | 91<br><i>99</i>   |
| Total | 427<br><i>379</i> | 434<br><i>451</i> | 420<br><i>496</i> |

## Results and discussion

Figure 1 presents the means of varieties that were entered in the CSD large scale trials for the three seasons 1994/95 to 1996/97. The general yield level under raingrown conditions of 1000 kg lint/ha at Narrabri and Darling Downs is about 60% of irrigated yields, and 600 kg lint/ha at Biloela is about 36% of irrigated yields. The lower relative yield at Biloela reflects the higher evaporative demand and lower summer rain (Table 2). Averaged over the four sites, okra leaf cultivars yielded 5.2% more than normal leaf types. At each location the two highest average yielding cultivars were okra leaf types (Fig. 1). The lowest yielding cultivars at Biloela and Bongeem were also okra leaf types, indicating that the okra leaf character has to be combined with adaptation for a site and season together with high yield potential to be an effective performer. Okra leaf is an old and common mutant present in cotton, characterised by deeply cleft and narrowly lobed leaves. Interest in the character has come from reduction in boll rot in humid environments (4) and in host plant resistance to some insect pests (3, 9, G. Fitt, *pers. comm.*). Despite these advantages, the only place where any significant area of okra leaf cottons are grown commercially is in Australia, with up to 50% of total plantings (9). Considerable research has focused on examining the physiological differences between the okra and normal leaf types. Peng and Krieg (6) reported that okra leaf plants had consistently greater canopy photosynthesis per unit leaf area compared with normal leaf plants. Lower stomatal conductances were also reported for okra compared to its normal leaf isolate (7, 10), this, coupled with greater photosynthetic rates gave the okra leaf types 40% greater leaf WUE (CO<sub>2</sub>/H<sub>2</sub>O) over the normal leaf isolines (7). This increase in leaf productivity and efficiency is likely to be an important contributor to the yield advantages observed in these large scale trials.

There was no consistent association between cultivar maturity and yield. At Biloela, there was a trend toward later season cultivars having the best yield. However, the association within a site and especially across sites between yield and maturity was not significant in the large scale trial data set. More detailed studies have actually shown a significant positive relationship between yield and maturity, with later types being superior (W. Stiller, unpub.).

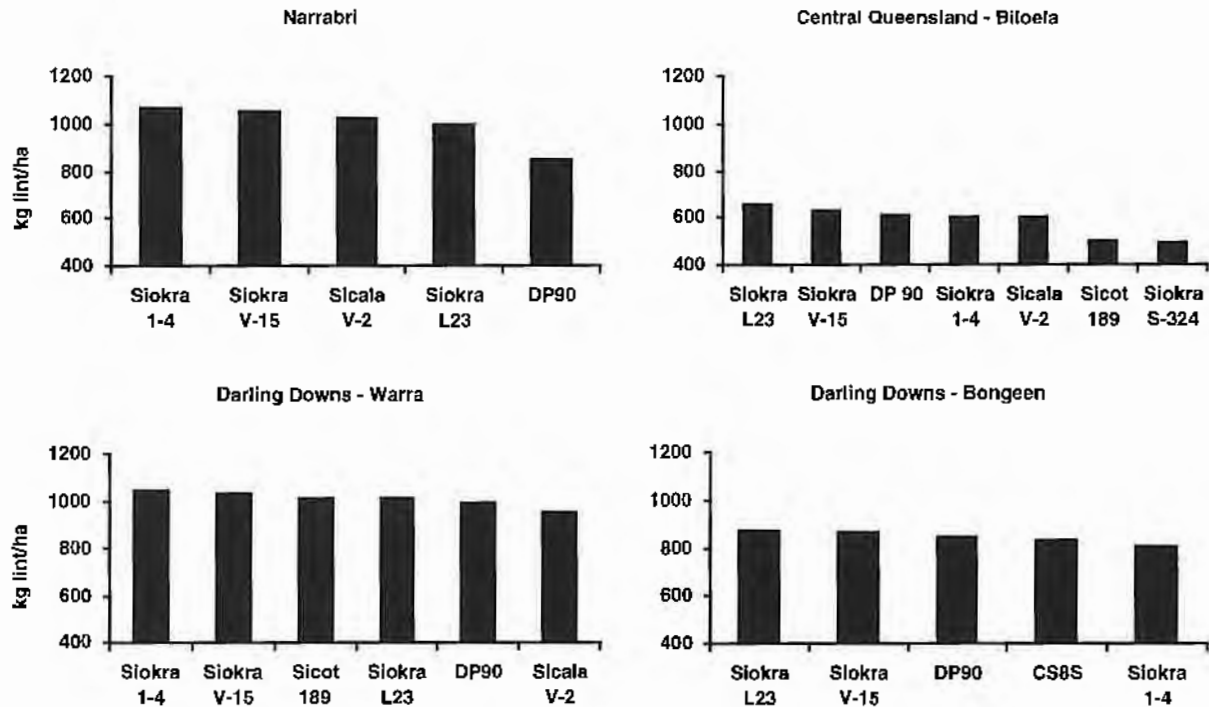


Figure 1: Three year mean lint yields for Narrabri, Central Queensland (Biloela) and two Darling Downs sites (Warra and Bongeen).

There was good general consistency in cultivar rank between small and large scale trials (Fig. 2). At Narrabri, Siokra 1-4 (labelled in the graphs) had poor performance in small scale trials due to high infestation with verticillium wilt. This cultivar has very low tolerance to that disease (Table 1). There was good agreement at Biloela between the two scales of trial size. The Bongeen site agreed closely, however the Warra site was less consistent with the small scale experiment at Dalby. Incidence of verticillium possibly resulted in the poor performance of Siokra 1-4 at Warra. Irrigation is known to encourage the build-up of verticillium wilt in the soil (2). This factor along with rotation crops of non-host species, reduces the severity of disease under raingrown cotton cropping compared with irrigated cropping.

## Conclusions

1. The okra leaf trait combined with high adaptation and yield potential conferred good performance under raingrown conditions. This result is consistent with physiological measurements. If morphological characters such as okra leaf type provide improved performance under raingrown conditions, there may be potential for placing selection pressure on other physiological characters such as photosynthesis, transpiration efficiency and carbon isotope discrimination.
2. Earliness of maturity was not associated with raingrown performance. These experiments were for normal sowing dates, late sowings may require early maturing cultivars.
3. Small scale experiments were able to identify better raingrown genotypes so long as disease incidence was known at each site. It is possible that more experimentation to investigate genotype × environment interactions for raingrown cotton are necessary to more effectively identify selection environments.

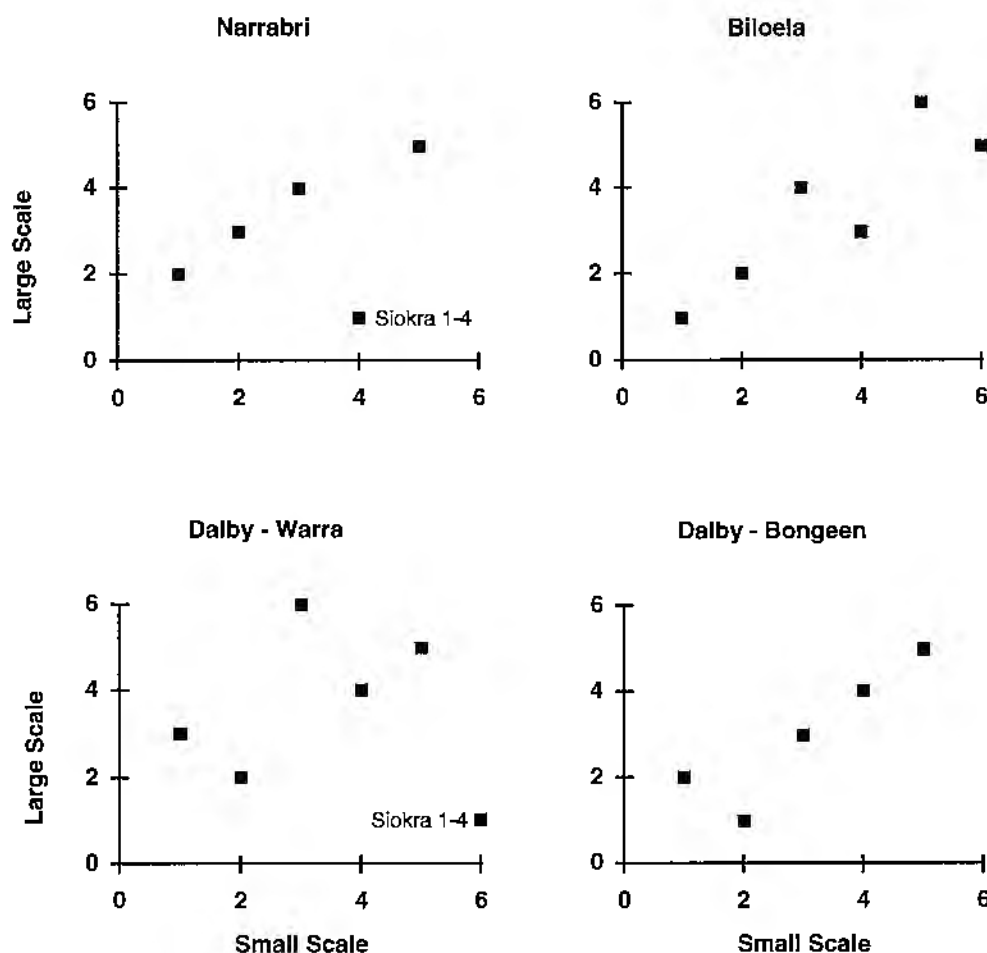


Figure 2: Comparison between small and large scale experiments of varietal ranking for yield.

### Acknowledgments

Partial funding for the small scale experiments through the Cotton Research and Development Corporation is gratefully acknowledged. We also acknowledge the work put into the large scale trials by Bill McDonnell, Louise Mills and David Schulze, and the small scale experiments by Lindsay Heal (Narrabri) and Gavin Mann (DPIQ, Biloela).

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## Variety development for dryland conditions

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### Introduction

Even though the Australian cotton industry is based on a high input irrigated system, there is an increasingly significant area of dryland cotton. Over the last few years there has been increased interest, by researchers and growers alike, in trying to gain the maximum amount of yield from the limited water that is available to them. This limited water occurs both in dryland systems, and in irrigated systems where water allocation from storages is reduced due to low dam levels.

Morphological characters have been utilised in many crops to improve water stress tolerance. Recently, physiological measures such as carbon isotope discrimination have been used to select for greater plant water use efficiency. Earliness of maturity is often utilised as a trait for increasing yield or yield stability under raingrown conditions. In Texas (USA) there are over two million hectares of raingrown or partially irrigated cotton, which generally adopt an early maturity strategy to avoid late season 'terminal' drought. When compared with Australian conditions, the Texas High Plains has a shorter growing season.

With this project we have two major objectives in mind:

1. To determine the range of genetic differences for WUE among commercial Australian and Texan cultivars, and decide on reliable methods for identifying differences in the field.
2. To measure the heritability of traits important for WUE of field-grown cotton, and include the information and genetic material into the existing CSIRO breeding program.

This paper reports on three seasons of trials which compare cultivar performance under dryland conditions. Because a breeding program needs to develop trial programs which identify genotypes for commercial practice, comparison of relative varietal performance is made with small scale raingrown experiments from the CSIRO breeding program. Some yield results are also reported from the breeding strategy carried out in this project. The importance of earliness of maturity and okra leaf type under dryland conditions are examined.

### Methods

Each season, Cotton Seed Distributors (CSD) conduct large scale raingrown cultivar trials in all the major cotton growing areas of Australia. Entries in these trials vary between

regions. They include the most popular CSD cultivar in the region, a standard CSD cultivar, a Deltapine nominated cultivar as well as one or two lines from CSIRO nearing commercial release. These trials were set out in a 'nearest neighbour' design, with a control cultivar planted between each treatment. Each cultivar had a planted area of about 4.5 ha.

CSIRO also conducts small scale cultivar experiments each season in most of the major growing regions (ACCT), usually with 48 entries with five replicates of each cultivar. Each plot had an area of 45 m<sup>2</sup>. All crops were managed with standard commercial practices. The characteristics of the cultivars discussed in this paper are presented in Table 1. The small and large scale sites are not always in close proximity to one another, which may confound interpretation. The small scale site on the Darling Downs was at Dalby and has been compared with the large scale sites at Warra (45 km north-west) and Bongeeng (48 km south-east).

**Table 1: Cultivars tested in all experiments.**

| Cultivar     | Leaf type | Maturity | <i>Verticillium</i><br>(% plants infested) |
|--------------|-----------|----------|--------------------------------------------|
| Siokra L23   | O         | 8        | 26                                         |
| Siokra V-15  | O         | 7        | 10                                         |
| Siokra I-4   | O         | 6        | 36                                         |
| Siokra S-324 | O         | 5        | 38                                         |
| Sicala V-2   | N         | 8        | 8                                          |
| Sicot 189    | N         | 9        | 11                                         |
| CS 8S        | N         | 6        | 11                                         |
| DP 90        | N         | 8        | 31                                         |

Leaf type: O=okra leaf, N=normal leaf; Maturity: (relative scale, 1=very early, 10=very late) calculated from date of 60% boll opening when grown at Narrabri (P. Reid, *pers comm.*); and percentage of plants infested with *Verticillium* when grown at Narrabri (S. Allen, *pers comm.*).

leaf types. At each location the two highest average yielding cultivars were okra leaf types (Figure 1). The lowest yielding cultivars at Biloela and Bongeeng were also okra leaf types, indicating that the okra leaf character has to be combined with adaptation for a site and season together with high yield potential to be an effective performer.

Considerable research has focused on examining the physiological differences between the okra and normal leaf types. Our research has confirmed results from the USA where okra leaf plants had consistently greater canopy photosynthesis per unit leaf area compared with normal leaf plants. Lower stomatal conductances were also found for okra compared to its normal leaf isolate, this, coupled with greater photosynthetic rates gave the okra leaf types 40% greater leaf WUE (CO<sub>2</sub>/H<sub>2</sub>O) over the normal leaf isolines. This increase in leaf

## Results and discussion

Figure 1 presents the means of varieties that were entered in the CSD large scale trials for the three seasons 1994/95 to 1996/97. The general yield level under raingrown conditions of 1000 kg lint/ha at Narrabri and Darling Downs is about 60% of irrigated yields, and 600 kg lint/ha at Biloela is about 36% of irrigated yields. The lower relative yield at Biloela reflects the higher evaporative demand and lower summer rain. Averaged over the four sites, okra leaf cultivars yielded 5.2% more than normal

productivity and efficiency is likely to be an important contributor to the yield advantages observed in these large scale trials.

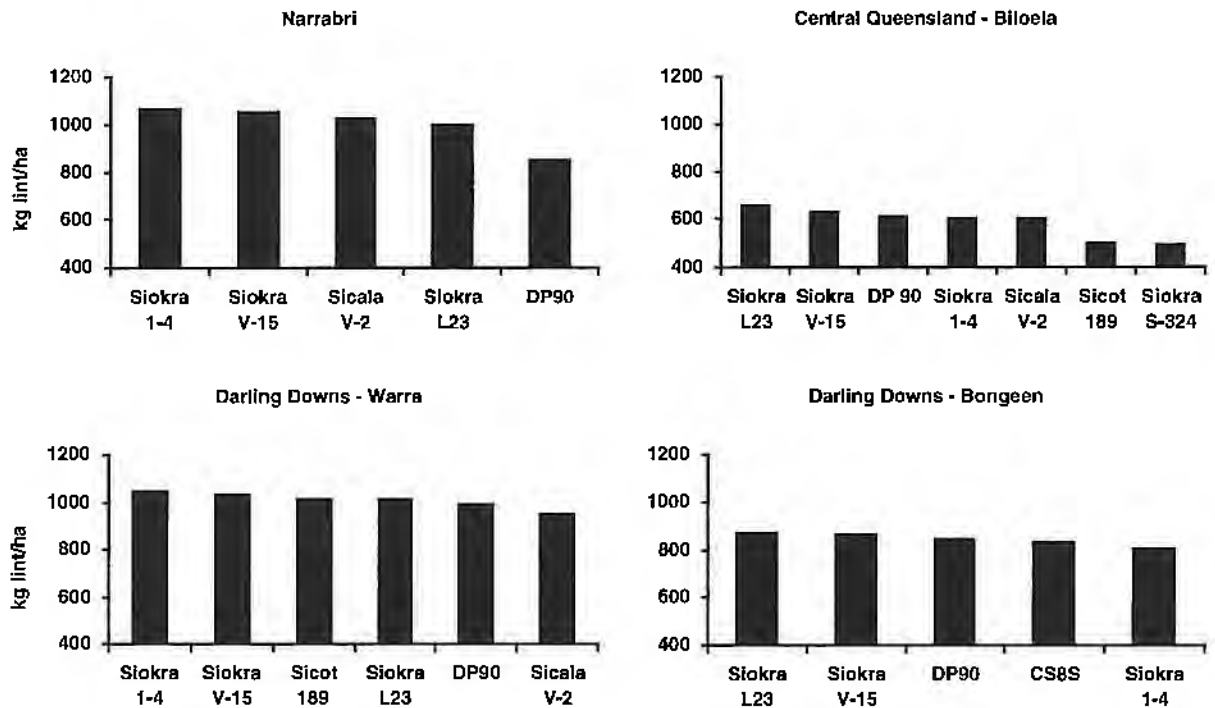


Figure 1: Three year mean lint yields for Narrabri, Central Queensland (Biloela) and two Darling Downs sites (Warra and Bongeem).

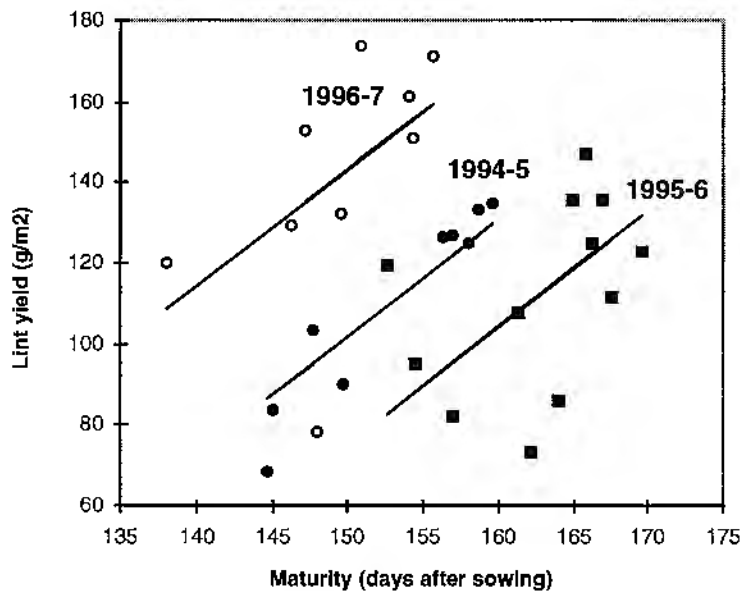
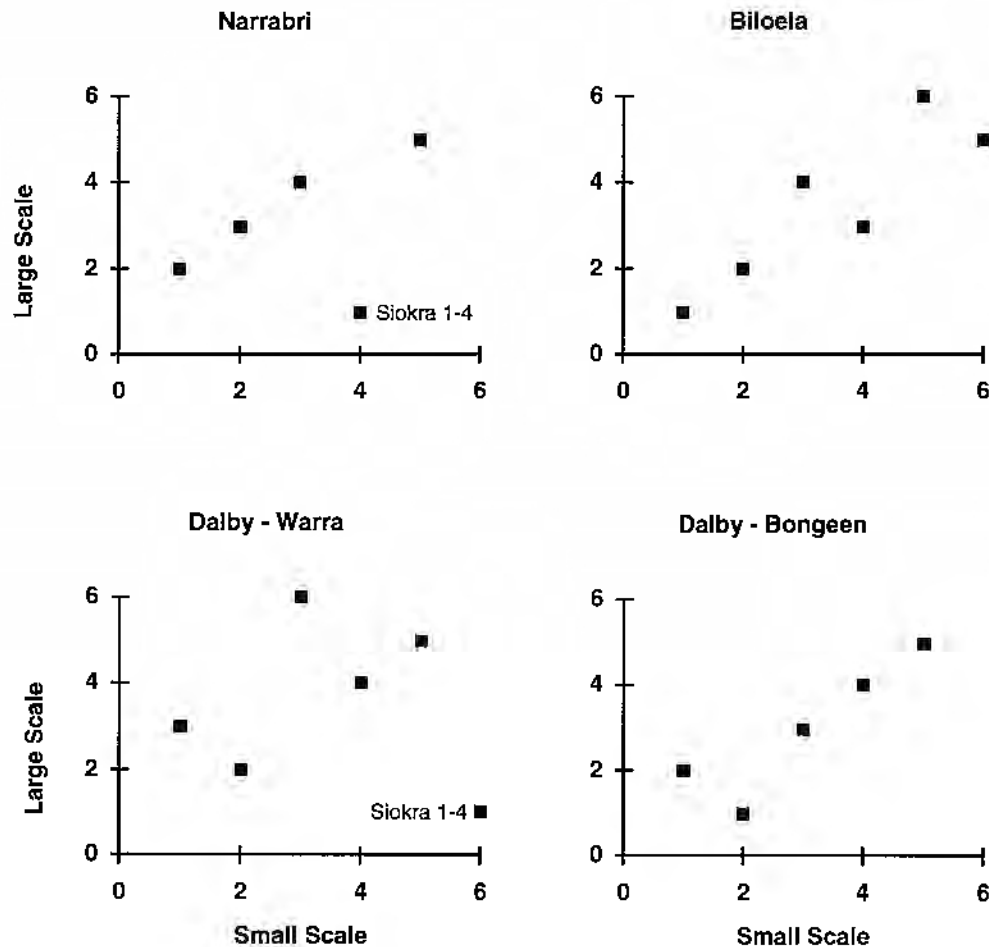


Figure 2: Lint yield vs maturity of the cultivars grown at ACRI over three seasons.

Figure 2 describes the relationship between cultivar maturity and lint yield when the cultivars were grown at ACRI. In all seasons the later maturing cultivars performed best. The regression indicated that the lines have the same slope each season but shift to the left or right predominantly due to the amount of rain heat units received during the growing season. The fitted regression lines indicate that for every one day increase in

cultivar maturity there is a 29 kg/ha increase in lint yield. Obviously this yield increase would only continue while the cultivar maturity is less than the available season length. There was good general consistency in cultivar rank between small and large scale trials (Figure 3). At Narrabri and Bongeem, Siokra 1-4 (labelled in the graphs) had poor performance in small scale trials due to high infestation with verticillium wilt. This cultivar has very low tolerance to that disease (Table 1). This factor along with rotation crops of non-host species, reduces the severity of disease under raingrown cotton cropping compared with irrigated cropping. There was good agreement at Biloela between the two scales of trial size.



**Figure 3: Comparison between small and large scale experiments of varietal ranking for yield.**

As part of this effort to produce varieties better adapted to dryland conditions, progeny testing was carried out using a number of lines, both from this WUE project and the general breeding program. Table 3 shows the results of a dryland trial conducted in the 1997/8 season indicating the improved performance of some of these lines over two controls, Siokra L-23 and Stoneville 474.

**Table 3: Cultivar yields from genotypes selected under dryland conditions**

| Genotype       | Lint yield | Rank in trial |
|----------------|------------|---------------|
| 92238-255      | 562        | 1             |
| 92238-318      | 562        | 2             |
| 92238-395      | 536        | 3             |
| 88201-343-939  | 535        | 4             |
| 92238-556      | 532        | 5             |
| 92238-601      | 529        | 6             |
| 94228-65       | 518        | 7             |
| Siokra L-23    | 492        | 14            |
| Stoneville 474 | 352        | 40            |

## Conclusions

1. The okra leaf trait combined with high adaptation and yield potential conferred good performance under raingrown conditions. This result is consistent with physiological measurements. If morphological characters such as okra leaf type provide improved performance under raingrown conditions, there may be potential for placing selection pressure on other physiological characters such as photosynthesis, transpiration efficiency and carbon isotope discrimination.
2. Earliness of maturity was not associated with raingrown performance. These experiments were for normal sowing dates, late sowings may require early maturing cultivars.
3. Small scale experiments were able to identify better raingrown genotypes so long as disease incidence was known at each site. It is possible that more experimentation to investigate genotype  $\times$  environment interactions for raingrown cotton are necessary to more effectively identify selection environments.

## Acknowledgments

Partial funding for the small scale experiments through the Cotton Research and Development Corporation is gratefully acknowledged. We also acknowledge the work put into the large scale trials by Bill McDonnell, Louise Mills and David Schulze, and the small scale experiments by Lindsay Heal (Narrabri) and Gavin Mann (DPIQ, Biloela).

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Identification of characters for selecting increased water use efficiency in cotton.

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#### Abstract

Water supply is a major determinant of cotton yield. In Australia, unreliable rainfall means that irrigation supplies can not be guaranteed. In addition, 20 % of the industry is raingrown and depends on the same unreliable rainfall.

We aimed to determine if it is possible to identify and select characters that enhance the leaf and crop water use efficiency (WUE) of cotton. We identified that differences in WUE do occur between genotypes. Crosses were made between relatively diverse germplasm and  $F_2$ 's produced. 160 single  $F_2$  plants were measured for a range of gas exchange characters including photosynthesis, stomatal conductance, intercellular  $CO_2$  concentration and transpiration. Physiological WUE was calculated from these measurements. Each  $F_2$  plant was grown in an  $F_3$  row the following year. For the purpose of more intensive measurements, selections were based on the  $F_2$  data, and 24  $F_4$ 's were measured for the same characters as the  $F_2$ 's. Estimates of narrow sense heritability ( $h^2$ ) were also calculated. Relatively low estimates of  $h^2$  were obtained (0-0.5) for gas exchange characters compared with the high  $h^2$  for characters such as lint percentage (0.9). However, some  $h^2$ 's were significant, indicating that it will be possible to select lines with improved physiological WUE. These lines can then be screened for other agronomic characters. We believe this procedure will produce improved raingrown types than has been possible with our previous procedure in testing successful irrigated types for dryland adaptation.

#### Introduction

In 1972, the CSIRO Cotton Research Unit commenced a cotton breeding program at Narrabri NSW Australia, which has produced high yielding, locally adapted varieties, with particular emphasis on producing varieties suited to irrigated conditions. Given the increased frequency of irrigation restrictions and the expansion of raingrown cotton production (now 80,000 ha), there is now greater need for varieties adapted to raingrown conditions.

Breeding for increased water stress tolerance in cotton, as well as many other crops, has occupied a large number of research projects. Ray *et al.* (1974) reviewed research in water use efficiency (WUE) in cotton and concluded that variability existed for numerous traits that enhance WUE. This gives rise to the potential gains to be made through breeding. Roark and Quisenberry (1977) proposed that plant growth habit effects yield and WUE in rain grown cotton crops. Relatively indeterminate varieties tended to have higher yields and higher water use efficiency under conditions of water stress than more determinant varieties. They also suggested that the traits associated with this water use efficiency were heritable and could be accumulated in drought resistant strains. Recently, physiological measures such as carbon isotope discrimination (Condon *et al.*, 1987; Craufurd *et al.*, 1991; Donovan and Ehleringer, 1994; Hall *et al.*, 1994) have been used to select for greater water use efficiency in a range of crop species. The idea of selecting for WUE on physiological characters has

also be put forward by Johnson and Tieszen (1994) in alfalfa, Johnson *et al.* (1995) in lentil, and Singh (1995) in common bean.

This paper reports on an experiment using  $F_4$  progeny rows to examine if selection pressure could be placed on physiological WUE characters in the early stages of a breeding program, with the aim of producing cultivars with improved water stress tolerance.

#### Materials and methods

The experiment was done in the field at the Australian Cotton Research Institute (149°47' E, 30°13' S) in the 1997-8 cotton season. The 24  $F_4$  genotypes previously chosen from a population of 160  $F_2$  plants on the basis of gas exchange measurements were arranged in randomised blocks with three replicates. Plot size was one row  $\times$  15 metres.

Nitrogen fertiliser was applied as anhydrous ammonia, at the rate of 120 kg ha<sup>-1</sup> N. Trifluralin herbicide was incorporated in September before sowing in October. The crop was sown on the 14<sup>th</sup> October into a full soil moisture profile with a cone seeder on rows one metre apart to achieve a plant population of 80 000 ha<sup>-1</sup>. Fluorometuron herbicide was applied after sowing before crop emergence. Hand hoeing and inter-row cultivation were used for all subsequent weed control. The crop was sprayed by aircraft to control insect pests as required. The crop was grown utilising only rainfall. Effective rainfall for the growing season was 335mm compared with an average of 384mm. Rainfall during the month of peak flowering was only 35mm (cf average of 87mm) with an average maximum temperature of 34.6°C (cf long term average of 32°C).

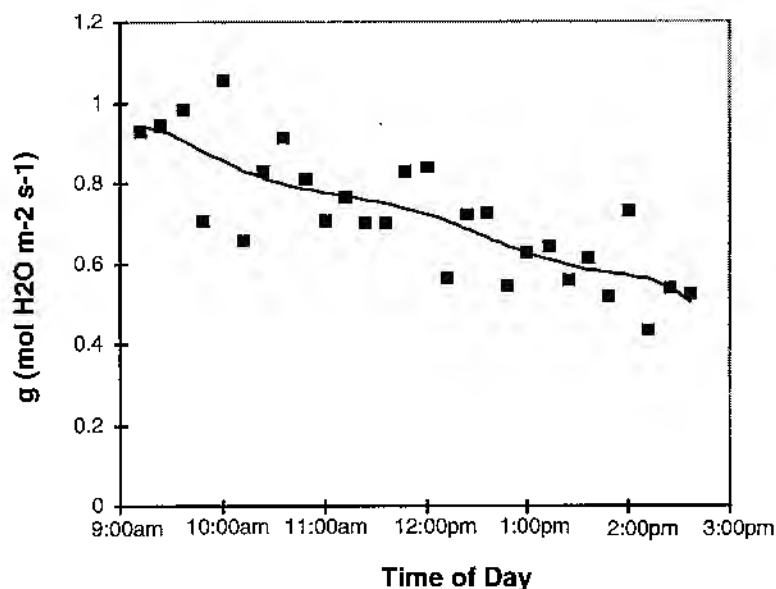
Gas exchange measurements were made using a Li-cor Li-6400 (Li-cor Lincoln, NE) open system portable photosynthesis unit with a 6 cm<sup>2</sup> chamber. Measurements were made on a portion of the youngest, disease free, fully expanded, fully sunlit leaf (usually four nodes from the terminal) where possible. Measurements were taken within the period of three hours either side of solar noon, with leaves held perpendicular to the sun. A control plot was measured every 30 minutes to determine the time of day influences on the measurements.

The yield results were analysed using analysis of variance techniques with the Genstat 5 package (Payne *et al.*, 1987). Differences between means were identified using Fishers protected LSD (Snedecor and Cochran, 1980). Residual Maximum Likelihood (REML) was also used to reduce row and column variation within the trials. Substantial spatial variation for time of day and day of gas exchange measurement existed. This variation was removed using the procedures outlined by Gilmour *et al.* (1997). Estimates of heritability ( $h^2$ ) were also calculated in these analyses by equating the average prediction error variance of a best linear unbiased predictor (BLUP) of a line effect with  $\sigma_g^2 (1-h^2)$  where  $\sigma_g^2$  is the REML estimate of the genetic variance (Cowling *et al.*, 1997). These analyses were conducted using the ASREML program (Gilmour *et al.*, 1996).

## Results and discussion

There was considerable variation during the day for gas exchange data. Figure 1 shows an example of one of the characters (conductance) for one day. The statistical procedure with Asreml was able to make some adjustments to these data for minimising error from time of measurement. These statistical techniques would be required when measuring gas exchange on early generations of segregating material when there are no or few replicates.

**Figure 1: Diurnal variation in stomatal conductance measured on the 24 F<sub>4</sub> genotypes, December 17, 1997.**



Asreml analyses showed significant genetic variance and heritability for net photosynthesis while other gas exchange parameters were not significant (Table 1). The literature contains little information about heritability of photosynthetic or gas exchange traits. Abdullaev (1990) reported narrow sense heritabilities for net photosynthesis of between 0.59 to 0.76 in F<sub>2</sub> populations of cotton (*G. hirsutum*), using coefficients of heritability methods (Mahmud and Kramer, 1951; Warner, 1952). Similar values of between 0.58 and 0.80 were found in inbred lines of maize (Crosbie *et al.*, 1977). However, Asay (1974) reported lower values of between 0.22 and 0.44 in diverse clonal lines of tall fescue. There is little or no data available on the heritability of other gas exchange traits. The heritability value of 0.38 for photosynthesis obtained in our study of F<sub>4</sub> lines is in agreement with the available literature. Table 1 also shows the parent-progeny correlation coefficients. This relationship provides another indication of the heritability of these traits. This approach produces similar heritability values for most traits, except for stomatal conductance where a large improvement was observed. Figure 2 presents the association between the F<sub>4</sub> and F<sub>3</sub> generations for stomatal conductance.

Our data, as with the above mentioned authors, indicates that significant genetic variance does exist, and that progress should be realised from selection for high net photosynthesis. Our data from this experiment also suggests that selection for the other traits listed, may not result in progress. However, other experiments in this project indicate that peak flowering may be a more reliable growth stage to evaluate gas exchange traits.

**Table 1: Range, narrow sense heritability and correlation with F<sub>3</sub> generation associated with five gas exchange traits of F<sub>4</sub> plots measured during early flowering, December 1997. \* and \*\* indicate significance at the 0.05 and 0.01 levels respectively.**

| Trait                                                                               | Units                                                | Range       | h <sup>2</sup> | F <sub>3</sub> :F <sub>4</sub><br>(r) |
|-------------------------------------------------------------------------------------|------------------------------------------------------|-------------|----------------|---------------------------------------|
| Net Photosynthesis (A)                                                              | μmol CO <sub>2</sub> m <sup>-2</sup> s <sup>-1</sup> | 27.1-28.6   | 0.38*          | 0.160*                                |
| Stomatal Conductance (g)                                                            | mol H <sub>2</sub> O m <sup>-2</sup> s <sup>-1</sup> | 0.677-0.735 | 0.13           | 0.483**                               |
| Leaf WUE (A/g)                                                                      | μmol mol <sup>-1</sup>                               | 40.3-42.5   | 0.18           | 0.117                                 |
| Ratio of intercellular CO <sub>2</sub> to ambient (C <sub>i</sub> /C <sub>a</sub> ) |                                                      | 0.724-0.736 | 0.19           | 0.113                                 |
| Transpiration efficiency (TE)                                                       | μmol mmol <sup>-1</sup>                              | -           | 0.0            | -                                     |

**Figure 2: Parent-progeny regression for stomatal conductance measured at early flowering for the 24 F<sub>4</sub> genotypes grown in 1997/8.**

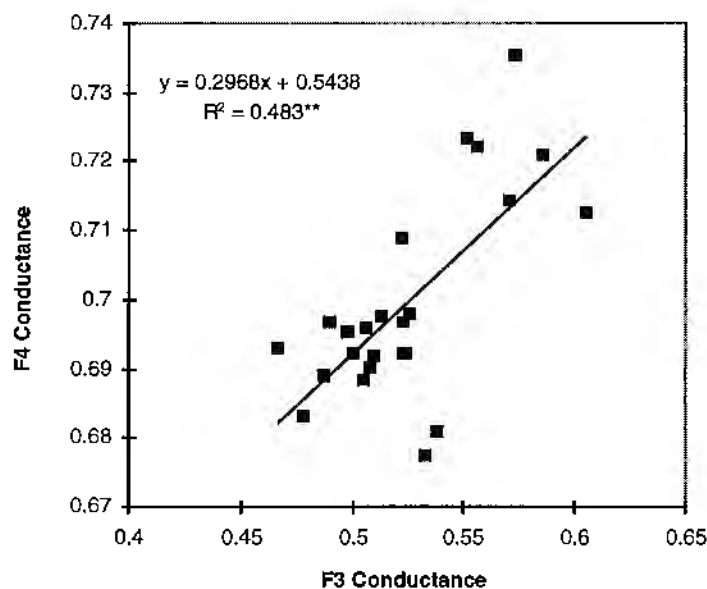
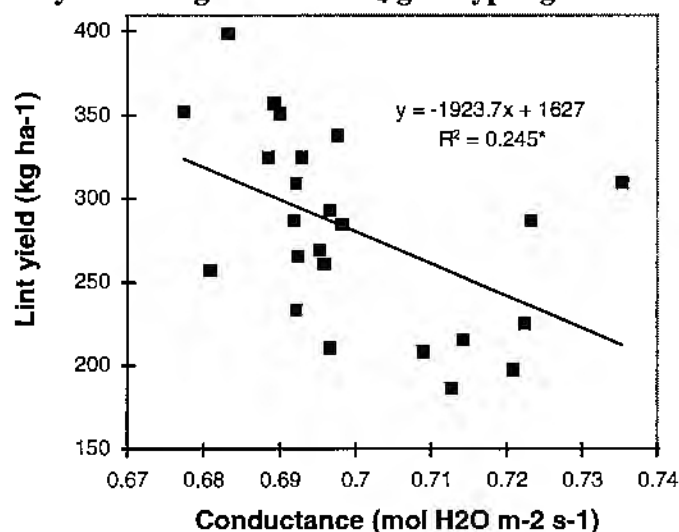


Figure 3 presents the significant negative relationship between stomatal conductance and lint yield. In this particular season, where water was limited during flowering and there was a high evaporative demand, high conductance was an impediment to yield, presumably because the crop used all its available water before the time of yield determination (Singh *et al.*, 1992). However, it is not expected that this would occur every season. In a favourable season, when rains are timely, the crop behaves like an irrigated crop, and genotypes with high rates of conductance and net photosynthesis can take advantage of the unlimited water. This variation between seasons is a problem when deciding on a selection strategy for raingrown genotypes. Genotypes suited to highly stressed conditions may not have the yield potential to perform in a more favourable season. It may be possible however, to select those genotypes that have high conductance and fall above the line shown in Figure 3, and hence select for types that should perform well in a favourable season.

**Figure 3: Relationship between yield and stomatal conductance measured at early flowering for the 24 F<sub>4</sub> genotypes grown in 1997/8.**



#### Conclusions

Because variation within a day makes it difficult to obtain reliable measures of gas exchange traits, robust statistical procedures are required to adjust the data.

Heritability of several gas exchange traits indicate that significant genetic progress should be realised from selection. Care must also be taken not to adopt a selection strategy using gas exchange traits based on conserving water, and thus reducing yield potential.

#### Acknowledgments

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