



## Identification of Characters for Selecting Increased Water Use Efficiency in Cotton

W.N. Stiller and G.A. Constable

CRC for Sustainable Cotton Production, CSIRO Cotton Research Unit,  
Locked Bag 59, Narrabri NSW 2390 Australia

### 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. The aim to determine if it is possible to identify and select characters that enhance the leaf and crop water use efficiency (WUE) of cotton. The occurrence of differences in WUE between genotypes was identified. Crosses were made between relatively diverse germplasm and F<sub>2</sub>'s produced. 160 single F<sub>2</sub> plants were measured for a range of gas exchange characters including photosynthesis, stomatal conductance, intercellular CO<sub>2</sub> concentration and transpiration. Physiological WUE was calculated from these measurements. Each F<sub>2</sub> plant was grown in an F<sub>3</sub> row the following year. For the purpose of more intensive measurements, selections were based on the F<sub>2</sub> data, and 24 F<sub>4</sub>'s were measured for the same characters as the F<sub>2</sub>'s. Estimates of narrow sense heritability (h<sub>2</sub>) were also calculated. Relatively low estimates of h<sub>2</sub> were obtained (0-0.5) for gas exchange characters compared with the high h<sub>2</sub> for characters such as lint percentage (0.9). However, some h<sub>2</sub>'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 determinate 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 been 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<sub>4</sub> 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<sub>4</sub> genotypes previously chosen from a population of 160 F<sub>2</sub> plants on the basis of gas exchange measurements were arranged in randomized blocks with three replicates. Plot size was one row × 15 metres.

Nitrogen fertilizer 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 utilizing 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 analyzed 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 minimizing 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.

Asreml analyses showed significant genetic variance and heritability for net photosynthesis while other gas exchange parameters were not significant (Table 1). The literatures contain 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 realized 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.

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.

## 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 indicates that significant genetic progress should be realized from selection. Care must be taken not to adopt a selection strategy using gas exchange traits based on conserving water, and thus reducing yield potential.

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



