Leaf Carbon Isotope Discrimination in *Gossypium hirsutum* under Drought Conditions

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ABSTRACT

The breeding programme at CIFA Las Torres-Tomejil (Andalucia, Spain) has aimed at developing cotton cultivars with economic yield for conditions of restricted water supply since 1989. Increasing water-use efficiency may be one way for obtaining greater crop yield with limited water. The determination of carbon isotope discrimination (Δ) in plants provides time-integrated measures of physiological activity and plant interaction with the environment. The relationship found between Δ and water-use efficiency (WUE) supports the possibility of using Δ as a criterion to select for WUE in breeding programmes. In this study, we investigated the effects of the environment on Δ and gas exchange parameters in different cultivars of G. hirsutum. Cotton genotypes were cultivated under irrigation in Las Torresand dryland in Tomejil. Gas exchange measurements and samplings for Δ determination were carried out under different levels of water availability. The relationship between gas exchange parameters and Δ changed across the environments. No relationship was found between Δ and estimates of WUE after witholding irrigation but a positive association was found between Δ and photosynthesis/transpiration ratio (A/E) in dryland. Several factors affect gas exchange measurements that make them unreliable for estimating WUE, but variability of Δ across environments requires further studies for its use as a surrogate for WUE in breeding programmes.

Introduction

Water-use efficiency (WUE) is considered an important component of drought tolerance. Increasing crop water-use efficiency may contribute to improved crop yield in water-limited environments (Condon and Richards, 1993; Gerik *et al.*, 1994). Carbon isotope discrimination (Δ) has been proposed as an estimate for WUE (Richards and Condon, 1993). This approach is based on discrimination against ^{13}C by leaves during photosynthesis (Farquhar *et al.*, 1982; Farquhar and Richards, 1984). Analysis of Δ has been considered as a valuable selection trait for higher yield (Condon *et al.*, 1987; Richards and Condon, 1993) and has been studied in several crops (Morgan *et al.*, 1993ab; Wright *et al.*, 1993; Menendez and Hall, 1995). In cotton, a linear correlation between WUE, based on seed cotton yield and total water applied and Δ was found (Yakir *et al.*, 1990). Some researchers detected differences in Δ of progenies from interspecific crosses (*G. hirsutum* x *G. barbadense*) (McDaniel and Dobrenz, 1994). A positive association between Δ and seed cotton yield over a range of environments and years was found by Gerik *et al.* (1996). In Pima cotton, Lu *et al.* (1996) found genetic variability for Δ and a positive correlation with stomatal conductance and yield that could be used for improved cotton yield in environments of high temperature and irradiance and no water limitation.

This report presents the results of field experiments with cotton genotypes grown in conditions of different water availability to study the genotypic variability in Δ and its relation with gas exchange parameters.

Material and Methods

Experiment 1995. Cotton cultivars (*Gossypium hirsutum* L.) were planted at Las Torres (province of Seville, SW Spain) on a sandy loam soil (Typic Xerofluvent). Soil water contents, in % weight on fraction, at 30, 60 and 90 cm depths may range from 22.4, 22.7 and 20.4 % at -0.33 MPa to 9.1, 9.1 and 7.5 % at -1.5 MPa respectively. The soil was brought to field capacity by irrigating before planting. An additional irrigation of 120 mm was provided at flowering because of the low soil water retention capacity and the extreme air vapour pressure deficit. Leaf gas exchange determination and leaf samplings were performed 15 days after the second irrigation and after 72 days. Analysis of Δ for different genotypes was performed on bulked leaves collected from different replications at both sampling dates. Analysis of variance could not be made for estimating genotypic variance.

Experiment 1997. A dryland trial was carried out at Tomejil in 1997 on a clay soil (Typic Chromoxerert). Soil water contents at 30, 60 and 90 cm depths may range from 30.5, 29.5 and 27.4 % at -0.33 MPa to 21.6, 19.9 and 18.6 % at -1.5 MPa respectively. Leaf gas exchange determination and leaf samplings were performed at 77 and 98 days after sowing. Analysis of Δ for different genotypes was performed on leaves collected separately from 3 replications at 98 days after sowing.

Gas exchange parameters. Measurements of single-leaf photosynthesis (*A*), transpiration (*E*) and stomatal conductance (g_s) were made with an open system using a portable infrared gas analyser at saturating photosynthetic photon flux densities (*ca*. 2000 µmoles m^{-2} s^{-1}) on the youngest fully-expanded leaves. The instantaneous WUE was calculated as *A/E*. A related term (A/g_s) (or intrinsic WUE) was calculated for reducing the effects of ambient vapour pressure between measurements on transpiration (Morgan and LeCain, 1991).

C isotope analysis. Dried leaves were ground to fine powder and C isotope composition was analysed by Mass Spectrometry in the laboratory of Prof. G. Farquhar (Environmental Biology Group, RSBS, Australian National University).

Experimental design and statistics. The design was a completely randomised block design with six replications. The plots were 15 m long, with rows spaced 0.75 m (Tomejil) and 0.95 m (Las Torres). After emergence, seedlings were thinned to 6 plants m^{-1} of row. All parameters were statistically analysed. Statistical differences among genotypes for traits were tested with Fischer's least significant difference test ($P<0.05$). Relationships between traits were determined using Pearson's simple correlation test.

Results and Discussion

In the irrigated trial (Las Torres 1995), the increase in water deficit experienced by plants from 15 to 72 days after the last irrigation led to a reduction in Δ (Figure 1) except in cultivars Victo and CNPA3H. The change in Δ between sampling dates associated with drought intensity was differently expressed by the genotypes. Cv. Za407 showed higher reduction in Δ at 77 days while cvs Ac77 and MMar showed little reduction in Δ. The reduction in water available to the crop determined an increase in A/g_s but a reduction in *A*/*E* (Figure 2). No significant relation were found between Δ and the estimates of instantaneous WUE (A/g_s and *A*/*E*). Similar results were obtained when considering more genotypes (Leidi *et al.*, 1998).

Genotypic variation for Δ was observed in dryland conditions (Tomejil 1997) in leaf Δ determinations 98 days after sowing (Figure 3). A group of four genotypes (MMar, Victo, Py792, Tashk9) showed higher Δ values than the others (Za407, Prec1, CNPA3, Ac77). No significant relation of genotypic ranking for Δ in 1995 or 1997 was observed, suggesting an important environmental effect on Δ. In the dryland trial, the increase in drought intensity from the first to the second sampling date (from 77 to 98 days after sowing) led to an increase in A/g_s and *A*/*E* (Figure 4). A positive association was found between Δ and *A*/*E* (Figure 4).

These results are in contrast with previous reports in other species (Wright *et al.*, 1993; Virgona and Farquhar, 1996). This can be explained by the use of different methodology for the determination of WUE. Lu *et al.* (1996) found a positive relationship in Pima cotton between Δ and stomatal conductance in hot, irrigated environments. In the dryland conditions at Tomejil, genotypes with high *A*/*E* presented high Δ. However, the same genotypes did not show statistically significant relations with WUE or gas exchange parameters in a different water-stress treatment in Las Torres in 1995. These results reflect the complexity in the relationship between Δ and WUE pointed out by Hubick and Gibson (1993) and Gerik *et al.* (1994). Several factors may be affecting the relationship between WUE and Δ (Masle and Farquhar, 1988; Hubick and Gibson, 1993; Gerik *et al.*, 1994, 1996). As some authors have indicated (Hubick and Gibson, 1993; White, 1993), differences in root growth may be one the factors changing the relationship between WUE and Δ. Previously research found that differential root growth between genotypes was an important trait related to maintaining photosynthesis, transpiration and higher yield under dryland conditions (López *et al.*, 1995). The genotypes included in this study have differences in rooting pattern (Leidi E.O., unpublished) that might partially explain the variable effect of the environment on Δ and gas exchange. Probably the most variable parameter are gas exchange measurements affected by the environment and growth stage (López *et al.*, 1995; Leidi *et al.*, 1998).

A positive correlation between Δ and cotton yield has been observed in different environments (Gerik *et al.*, 1996; Leidi *et al.*, 1998), making Δ an interesting trait for use in cotton breeding but more studies are required for understanding and overcoming the factors involved in the genotypic variation of Δ across diverse environments.

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Figure 1. Carbon isotope discrimination (Δ) by cotton leaf samples at different dates and different levels of soil water availability (Las Torres 1995).

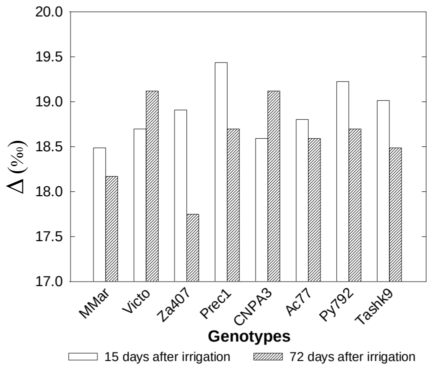

Figure 2. Variation in *A*/*g*s and *A*/*E* in cotton leaves in determinations performed at 15 days and at 72 days after the last irrigation (Las Torres 1995).

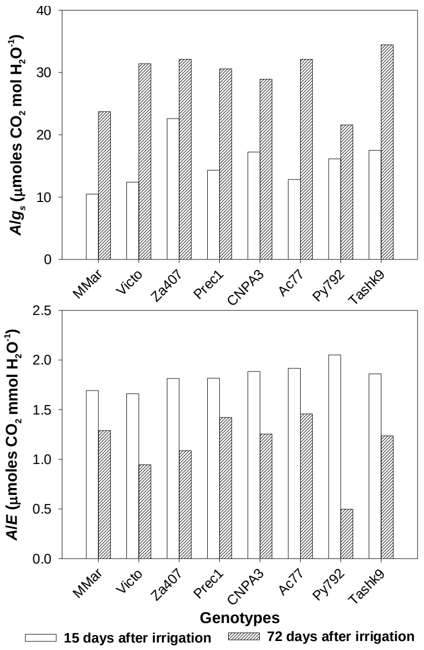

Figure 3. Carbon isotope discrimination (Δ) in leaves of cotton genotypes at 98 days after sowing. Vertical bars indicate standard error of the mean (Tomejil 1997).

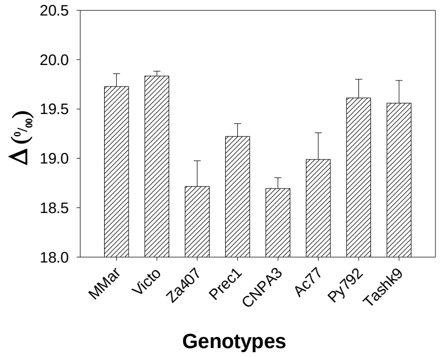

Figure 4. Variation in A/gs and A/E in cotton leaves in determinations performed at 77 days and at 98 days after sowing (Tomejil 1997).

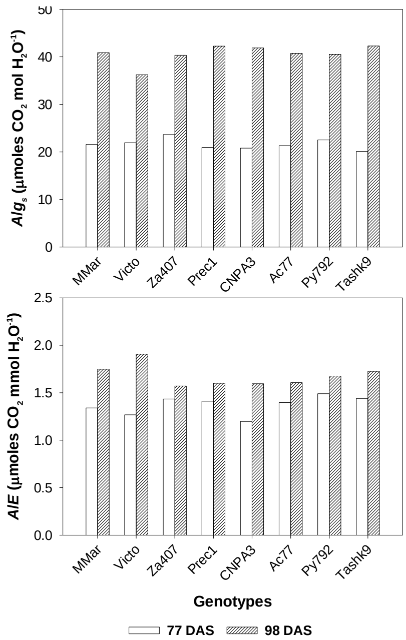

Figure 5. Relationship between instant water-use efficiency (*A*/*E*) and carbon isotope discrimination (Δ) in leaves of dryland cotton at 98 days after sowing (Tomejil 1997).