CARBON ISOTOPE DISCRIMINATION IN SUNFLOWER CORRELATES WITH TRANSPIRATION EFFICIENCY AND IMPROVED PERFORMANCE IN DRY ENVIRONMENTS

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Abstract

Plants incorporate isotopes of carbon into their tissue at different rates because of discrimination against 13C relative to 12C. This difference in discrimination has been negatively correlated with transpiration efficiency (TE) in many C3 species and so carbon isotope (13C/12C) discrimination (Δ) of leaf tissues has been proposed as a potential tool for selecting genotypes with improved performance under water-limited conditions. While the relationship between Δ and TE in sunflower has been described this relationship has not been investigated with material selected from a segregating population. In this study, forty hybrid combinations made by crossing lines from a population segregating for Δ to two tester lines were evaluated in a rainout shelter experiment. For this hybrid set a genetic correlation between grain yield and Δ of -0.9 was observed. Grain yield evaluations under field conditions of hybrids contrasting for Δ showed that low Δ hybrids had a yield advantage between 22-35% under dry environments (yield < 2t/ha). While this yield advantage is not expected from commercial breeding programs, computer simulations suggest that 10-15% yield improvements may be possible.

Introduction

In Australia, the majority of sunflower (*Helianthus annuus* L.) is grown in the summer in dry subtropical environments (<500 mm rainfall) on soils that vary in plant available water holding capacity from 90 to 250 mm. These production environments are also characterised by unreliable rainfall across years, large evaporative demands and maximum temperatures frequently exceeding 35C. Water availability is the major factor (abiotic and biotic) limiting the growth and yield of sunflower. Average yields in Australia are about 990 kg/ha (1993-2002; FAOSTAT, 2003).

Plants incorporate isotopes of carbon into their tissue at different rates because of discrimination against 13C relative to 12C. This difference in discrimination has been negatively correlated with transpiration efficiency (TE) in many C3 species and so carbon isotope (13C/12C) discrimination (Δ) of leaf tissues has been proposed as a potential tool for selecting genotypes with improved performance under water limited conditions (Farquhar and

Richards, 1984; Hubick et al., 1988; Condon and Richards, 1992; Condon et al., 1993; Rebetzke et al., 2002). Rebetzke et al. (2002) demonstrated that early generation divergent selection for Δ affected grain yield among high and low Δ, BC2F4:6 progeny of wheat (*Triticum aestivum* L.). The low Δ lines had a yield advantage compared to the high Δ lines of up to 11% in low rainfall Australian environments.

Generally, a negative correlation exists between leaf Δ and TE (see review by Hall et al., 1995). Virgona and Farquhar (1996) observed a negative correlation ($r = -0.98$, $P<0.001$) between leaf Δ and TE for a range of sunflower genotypes grown in a glasshouse. Lambrides et al. (2004) surveyed 161 sunflower genotypes of diverse origin and revealed a large and unprecedented range of genetic variation for Δ (19.5-23.8‰). Fourteen inbreds that spanned the observed range and five commercial hybrids were grown in a glasshouse where a strong negative genetic correlation (*rg*) between TE and Δ ($rg = -0.86$, $P<0.001$) was observed. While these studies showed a strong relationship between TE and Δ they need to be verified using segregating populations. A study of the relationship between TE and Δ in segregating populations will provide valuable information on heritability and the manipulation of Δ in a breeding program. Lambrides et al. (2004) identified several sunflower inbred lines that differed significantly for TE. Two of these, HAR4 and SA52 have been crossed to develop a set of recombinant inbred lines (RILs). A pot experiment grown under a rainout shelter was designed to study the relationship between TE and Δ and their inheritance in a set of HAR4 x SA52 RILs and a set of hybrids made by crossing these RILs with two female nuclear male sterile tester lines. In the present study we will investigate the relationship between TE and Δ, and yield data for the hybrid set.

Materials and Methods

Rainout Shelter Experiment. A set of 20/243 HAR4 x SA52 RILs that spanned the range of Δ based on field-grown evaluations were selected and crossed to two genetic male sterile females (Pac-tester1 and Pac-tester 2) supplied by Pacific Seeds Australia. This set of 40 hybrids was evaluated for transpiration efficiency under a rainout shelter.

This experiment was conducted during March and April 2002. Several seeds of each genotype were planted (March 20-21, 2002) in 25 L easi-lift black, polypropylene planter bags that had been covered in aluminium foil to reduce the heat load. The bags were filled with 26 kg of a sandy alluvial loam obtained from the Lockyer valley, Queensland and placed in a large plastic bag to prevent drainage. Four replicate bags were planted per genotype, and bags were placed in rows one meter apart with bags 20 cm apart within rows. The planting arrangement was a randomized complete block design that also incorporated an alpha lattice.

The soil surface was covered with 800 grams (about 2 cm deep) of white alcathene (polyethylene) beads that minimized evaporation from the soil surface. The bags were watered to about nine tenths field capacity by adding 4 litres of water. Nineteen days after emergence each bag was thinned to two plants per pot and weighed. By monitoring several bags with different plant size, water was added every two to three days in 594 ml or 1020 ml quantities using bottles with a narrow spout. Total water use (WUSE) was estimated by subtracting final bag weight (less above ground plant) from initial bag weight (less the weight of two seedlings removed at the thinning stage) and adding the amount of water that had been applied to the pots.

Plants were harvested 33-34 days after sowing when plants were at the 20 to 30 leaf stage. On the final day, plant height (HGT) was measured from the top of the apical meristem to the base of the stem. Plants were cut at ground level, separated into leaves and stems and dried to estimate leaf dry weight (LDW), stem dry weight (SDW) and above ground dry weight (AGDW= LDW + SDW). Leaf area (LA) was determined on fresh leaves prior to drying. TE was calculated by dividing AGDW (minus initial plant weight at 19 days after sowing) by WUSE. Specific leaf weight (SLW) was calculated as LDW /LA.

After dry weights were recorded leaves were ground through a 0.5 mm screen to a fine powder for carbon isotope analysis. All analyses were done by mass spectrometry at the Research School of Biological Sciences, Australian National University, Canberra, Australia. Delta (Δ) is a measure of the 13C/12C ratio in plant material relative to the value of the same ratio in ambient air and was calculated according to Farquhar and Richards, 1984. The units of Δ are per mil. (%).

Chlorophyll content of each genotype was estimated with a SPAD meter (Minolta, Osaka, Japan). Measurements were made at the time of sampling for Δ on all replicates.

Yield Evaluations. Hybrid combinations were made by crossing sets of F3, F5 and F7 HAR4 x SA52 segregates, contrasting for delta, to at least one of two genetic male tester lines, Pac tester 1 and Pac-tester 2. The hybrids were yield tested using standard micro-plot techniques using 3-6 m single or two-row plots. Trials with were grown at Gatton, Australia in 2000 and Capella, Australia in 2000, 2001, and 2002. The trial location at Capella in 2002 failed due to a lack of rainfall.

Statistical Analysis. Variance components were estimated with the COVTEST option of PROC MIXED (SAS Institute). Estimates of broad sense repeatability on a genotype mean basis ($h2$) were calculated from variance components as follows:

$$h2 = \sigma_g^2 \Big/ (\sigma_g^2 + \sigma_e^2 / r)$$

where r is the number of replications, $\sigma^2(g)$ is the genetic variance, and $\sigma^2(e)$ is the environmental variance. These estimates may be biased upward because $\sigma^2(ge)$ was confounded with the estimate of $\sigma^2(g)$. Genetic correlations (*rg*) were calculated using variance components. For example:

$$rg = Covg(xy) / \sqrt{\sigma^2 g(x) \sigma^2 g(y)}$$

where $Covg$ is the genetic covariance between traits x and y.

Results and Discussion

A strong negative genetic correlation between TE and Δ was observed, *rg* = -0.90 (Table 1), consistent with a previous study of sunflower using a set of unrelated materials (Lambrides et al, 2004). TE was also correlated to two measures of photosynthetic capacity, i.e., leaf thickness (SLW) and leaf chlorophyll content (SPAD) (Table 1) underlying the importance of photosynthetic capacity as a component of TE in sunflower. SLW and SPAD may be useful, less expensive surrogates of TE that could be used to discard materials in the early stages of a breeding program aimed at developing drought- tolerant material.

Table 1. Genetic correlations among traits measured on forty hybrids grown under a rainout shelter in Gatton, Queensland in 2002. Broad sense repeatability (h2) on a genotype mean basis is also presented for each trait.

Trait † x	Trait y	Genetic correlation *rg* (xy)	se ‡ (*rg*)	h2 x	h2 y
TE	Δ	-0.90***	0.01	0.70	0.28
TE	SLW	0.69**	0.06	0.70	0.58
TE	SPAD	0.55**	0.08	0.70	0.82

** Significant at *P* <0.01, *** Significant at P <0.005

† Δ = 13C/12C discrimination (‰), TE = transpiration efficiency (g DW kg/H20), SLW = specific leaf weight (g /m2), SPAD = a measure of chlorophyll content.

‡ se = standard error.

Yield evaluations of hybrids contrasting for Δ showed that the low Δ pools of hybrids significantly out-yielded the high Δ pools in three dryland locations by 22-35% (Table 2) suggesting that selection for low Δ can significantly improve grain yield in dry environments that are characterised by yield levels of less than about 2 t/ha. While this yield advantage is not expected from commercial breeding programs, our computer simulations (data not shown) suggest that yield improvements of 10-15% may be possible. The ten-year average yield of the commercial sunflower crop in Australia is 1 t/ha, therefore, TE is likely to be a valuable trait in breeding programs designed to develop drought-tolerant hybrids. Wheat varieties selected for low Δ have now been released in Australia and selection for TE looks to be equally promising for the development of drought-tolerant sunflower hybrids. Low Δ material selected from the population HAR4 x SA52 has been distributed to private seed companies for further evaluation.

Table 2. Grain yield (t/ha) and Δ (per mil) for low vs high Δ pools of experimental top-cross hybrids made from (i) F3 segregates of the population HAR4 x SA52 and evaluated in 2000 at Gatton and Capella, Australia and (ii) F5 segregates of the population HAR4 x SA52 evaluated in 2001 at Capella.

	Gatton 2000 Irrigated Yield (t/ha)	Gatton 2000 Irrigated Δ per mil	Gatton 2000 dryland Yield (t/ha)	Gatton 2000 dryland Δ per mil	Capella 2000 dryland Yield (t/ha)	Capella 2001 dryland Yield (t/ha)	Capella 2001 dryland Δ per mil
High Δ pool	0.82 (100) †	20.7	0.48 (100)	22.0	1.01 (100)	1.58 (100)	20.4
Low Δ pool	0.89 (108)	20.3	0.65 (135)	21.6	1.37 (135)	1.93 (122)	20.1
Pr > F	0.52 ns	***	**	***	**	**	**

† Percentages given in parentheses are relative to the high delta group. **P<0.01, *** P< 0.005

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