PRELIMINARY EVIDENCE FOR THE CYTOPLASMIC CONTROL OF TRANSPIRATION EFFICIENCY IN SUNFLOWER.

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Summary

We initiated a study to evaluate the cytoplasmic effect on transpiration efficiency (TE-ratio of dry matter production to water transpired) in sunflower. Using carbon isotope discrimination (delta) as a surrogate for TE (high delta = low TE) we identified variation for leaf delta in a set of HA89 alloplasmic lines (same nucleus different cytoplasm) from the USDA program in Fargo USA. One line CMS HA89-MAX1 had significantly higher delta than the others. In a glasshouse experiment the TE of CMS HA89-MAX1 was only 80% that of CMS HA89-PET1 and HA89-ANN1. The lower TE of CMS HA89-MAX1 was associated with thinner leaves and lower photosynthetic capacity. There were no significant differences in plant height, leaf area and stomatal conductance between the lines. Because these lines share the same nuclear genome we hypothesize that TE may be, in part, cytoplasmically controlled in sunflower. Data supporting this hypothesis is presented from delta signatures of hybrids and their parental lines where there were strong significant correlations between the female parent and the hybrid ($r > 0.7$) but not between the male parent and the hybrid ($r = 0.0$) We suggest sunflower breeders may need to identify and incorporate new cytoplasmic variation in their breeding programs to enhance drought tolerance in sunflower.

Introduction

Since the discovery of cytoplasmic male sterility (CMS) in sunflower, breeders around the world have exclusively used the PET1 and ANN1 cytoplasms to develop commercial sunflower hybrids. The vulnerability of using a narrow range of cytoplasms has been recognized and has led to the search for several other CMS systems. To date over 40 CMS systems have been derived and characterized (Jan 1997). We have initiated a study to evaluate the cytoplasmic effect on transpiration efficiency (TE) of sunflower.

Carbon isotope discrimination (delta) and can be used as an indicator of TE in several C3 species including wheat (Farquhar and Richards, 1984), peanut (Hubick *et al.* 1988) and sunflower (Virgona *et al.* 1990, Virgona and Farquhar 1996, Chapman *et al.* 2000). Studies in wheat (Rebetzke *et al.* 1999) have shown that low delta iso-lines (i.e. high TE) are up to 10% higher yielding in rain-fed Australian environments compared to their high delta counterparts. The units of delta are per thousand (per mil), and for wheat a unit difference is associated with a 15% improvement in TE. Two factors are potentially associated with improved TE in crop plants i.e. improved photosynthetic capacity (A) (fixation of CO_2) and/or stomatal conductance (g) (the prevention of water loss through stomates). In sunflower it appears that differences in photosynthetic capacity underlie the variation for TE (Virgona and Farquhar 1996, Chapman *et al.* 2000).

In this study we examined the effect of the cytoplasm on TE by examining the delta signatures of a set of HA89 alloplasmic lines, i.e. lines that share a common nuclear genome but have different cytoplasms. We also examined the delta signatures of hybrids and their parents from an Australian commercial breeding program and from the CSIRO drought tolerance germplasm enhancement program.

Materials and Methods

(i) *Alloplasmic material*
Nine alloplasmic lines with the HA89 nucleus were sampled initially in the USDA breeding nursery of Dr Jerry Miller at Fargo, USA. Subsequently, CSIRO imported seven HA89 alloplasmic genotypes (Table 1) from USDA. As each of these lines is male sterile they were maintained by pollinating them with HA89B, initially in the CSIRO quarantine glasshouse at Samford, Queensland, Australia.

Field evaluation of variation in carbon isotope discrimination
Leaf samples were collected from the following sources: (a) Fargo, USA: survey of lines from unreplicated rows in the USDA breeding nursery; (b) Gatton, Australia: Two field trials, sown in spring and summer 1999, of the seven alloplasmic genotypes introduced to Australia. Each of these field trials used a two replicate alpha-lattice design.

A fully expanded sunlit leaf was sampled from five plants per single-row-plot. Leaf material was dried and ground to a fine powder for analysis. Carbon isotope analyses were performed in the Isotope ratio mass spectrometer at the Research School of Biological Science Australian National University.

Glasshouse evaluation of carbon isotope discrimination and other traits
The experiment was grown in a glasshouse in September/October 1999. Day temperatures averaged 26.5°C (max of 34°) while night temperatures averaged 23°C (min of 18°). Four seeds of each genotype (Table 2) were planted in pots (0.15 m diameter, 0.6 m high) containing 11 kg of air dry soil with a field capacity of 18% and a lower limit (after free drainage) of 11%. Four replicate pots were planted per genotype and the pots were spaced 5 pots wide in the glasshouse in a randomised complete block design that also incorporated an alpha-lattice.

The soil mix included a complete nutrient fertiliser. The pots were watered to 14 kg with water added to keep them at that level until 21 days after planting. At that time, the plants were thinned to one per pot. The pots were weighed and watered to field capacity and the surface covered to 0.02 m depth with plastic polyethylene beads that reduced evaporation from the soil surface to negligible amounts. By monitoring several pots of different plant size, water was added every 2 to 3 days in 100 to 300 mm quantities. The pots were weighed each week and at the end of the experiment. Total water use was computed by the subtracting the final pot weight from the initial pot weight and adding the amount of water that had been put into the pots.

Over several days in the 2 weeks prior to final harvest, a Li-Cor 6400, was used to measure leaf transpiration and assimilation of CO_2. Sunlit leaves were equilibrated for up to 30 s in the leaf chamber before readings were made.

At harvest (5 Nov 1999), the plants were separated into stems and leaves and oven-dried after determination of leaf area. The soil was washed from the pots to recover roots, which were then also dried and weighed. Transpiration efficiency was calculated by dividing total dry matter growth (final – initial weight) by total water use. Specific leaf weight was calculated as leaf weight/leaf area (g/m^2 leaf).

(ii) *Variation in carbon isotope discrimination in hybrids and their parental lines*
Several commercial and pre-commercial hybrids and their parents (Table 3) were surveyed from un-replicated plots in the breeding nursery of the Australian seed company Agseed in spring 1998. In addition, several CSIRO experimental hybrids using the common male PAR-1673-2 and a range of females (Table 4) were evaluated in the field at Gatton, Queensland, Australia in spring 1999. An alpha lattice design with two replicates was used. Leaf sampling of these hybrids and their parents was as described above.

Results and Discussion

(i) Variation in carbon isotope discrimination among alloplasmic lines
The survey of HA89 alloplasmic lines in the USDA nursery identified large variation for leaf delta (19.0-22.3) (Table 1). This result was surprising given that all these lines share a common nuclear genome. These preliminary results suggested that TE may be, in part, cytoplasmically controlled. When we tested a subset of this HA89 alloplasmic series under Australian field conditions, one line with cytoplasm from *H. maximiliani* had significantly higher delta, and hence lower TE, than other members of the alloplasmic set. (Table 1). The magnitude of difference observed is unprecedented in studies of delta in C3 species.

To confirm these observations and to understand the physiological basis of the large delta differences, the lines CMS HA89-MAX1, CMS HA89-PET1 and HA89-ANN1 were grown

in a replicated glasshouse experiment to obtain accurate measures of TE. These data (Table 2) indicated that the TE of CMS HA89-MAX1 was only 80 % that of CMS HA89-PET1 and HA89-ANN1, confirming the previous observed delta signatures. The lower TE of CMS HA89-MAX1 was associated with significantly lower specific leaf weight (SLW) (thinner leaves) and lower photosynthetic capacity (A) (Table 2). However, there were no significant differences in plant height, leaf area (LA) and stomatal conductance (g) among the lines (Table 2). Line CMS HA89-MAX1 was visually indistinguishable from other members of the alloplasmic set when grown in the field and glasshouse under favorable water and nutrient status. Hence we believe the high delta signature of CMS HA89-MAX1 is independent of the reduced vigor effect (Jan 1992) that can occur when cultivated genomes are backcrossed into perennial cytoplasms such as MAX1.

Table 1 Delta signatures (per mil) for a set of HA89 alloplasmic lines grown in several field locations in the USA and Australia.

Genotype	**Nuclear genome**	**Cytoplasm**	**Pollen fertility**			
				Fargo, USA	**Gatton, Australia**	**Gatton, Australia**
				field	**field**	**field**
				1997 summer	**1999 summer**	**1999 spring**
CMS HA89-GIG1	HA89	*H. giganteus*	Sterile	20.6	21.0 ± 0.49	19.7 ± 0.27
CMS HA89-MAX1	HA89	*H. maximiliani*	Sterile	22.3	24.4 ± 0.21	22.7 ± 0.27
CMS HA89 -PEF1	HA89	*H. petiolaris ssp fallax*	Sterile	19.3	21.0 ± 0.07	19.5 ± 0.27
CMS HA89 -PET1	HA89	*H. petiolaris*	Sterile		20.8 ± 0.07	19.7 ± 0.27
HA89-ANN1	HA89	*H. annuus*	Fertile		21.2 ± 0.21	19.7 ± 0.27
CMS HA89-ANN2	HA89	*H. annuus*	Sterile	19.6	20.8 ± 0.14	19.5 ± 0.27
CMS HA89-ANN3	HA89	*H. annuus*	Sterile	19.4	21.2 ± 0.35	19.2 ± 0.27
CMS HA89-ANN7	HA89	*H. annuus*	Sterile	19.4		
CMS HA89-ANN8	HA89	*H. annuus*	Sterile	19.0		
CMS HA89-ANN9	HA89	*H. annuus*	Sterile	19.8		
CMS HA89-ANT1	HA89	*H. annuus texanus*	Sterile	19.6		
No. reps				1	2	2
Range				19.0-22.3	20.8-24.4	19.2-22.6
Lsd (0.05)					0.56	0.75

Table 2 Physiological and morphological data for three alloplasmic lines grown in a replicated glasshouse experiment in Brisbane, Australia 1999. (Delta-carbon isotope discrimination, TE-transpiration efficiency, SLW-specific leaf weight, LA-leaf area, A-photosynthetic capacity, g-stomatal conductance).

Genotype	**Delta (per mil)**	**TE (gDW/kgH_20 transp.)**	**SLW (g/m^2 leaf)**	**Plant Height (cm)**	**LA (cm^2)**	**A (μmol CO^2 $m^{-2}s^{-1}$)**	**g (mol H_20 m^2s^1)**
CMS HA89-MAX1	24.0 ± 0.21 a	4.52 ± 0.22 (100) a	35.8 ± 1.2 a	50.7 ± 2.2	1299 ± 82	23.0	0.58
CMS HA89-PET1	21.9 ± 0.18 b	5.52 ± 0.20 (122) b	38.8 ± 1.1 b	49.4 ± 1.9	1296 ± 71	37.7	0.56
HA89-ANN1	21.8 ± 0.18 b	5.55 ± 0.20 (123) b	40.4 ± 1.1 b	49.0 ± 1.9	1423 ± 71	40.1	0.61
Lsd (0.05)	0.48	0.49	2.4	5.4	206		

Because the alloplasmic lines carry the same nuclear genome we hypothesize that differences in TE amongst these lines are likely to be associated with differences in the cytoplasmic genomes (chloroplastic and/or mitochondrial). While CMS HA89-MAX1 has significantly lower TE than traditional versions of HA89 we see this difference as valuable for understanding the mechanism of TE in sunflower. Consequently, we have commenced DNA analysis of the chloroplastic and mitochondrial genomes of the MAX1 cytoplasm.

(ii) Variation in carbon isotope discrimination among hybrids and their parental lines.

A preliminary indication of the inheritance of TE was obtained by examining the delta signatures of several Agseeds commercial and pre-commercial hybrids and their parents (Table 3) and of CSIRO experimental hybrids and their parents (Table 4). In the survey of Agseed hybrids and parents, although the parental range for any particular hybrid was relatively narrow, the delta value of the hybrid was generally lower than either parent. However delta values for the CSIRO experimental hybrids were generally between the two parents although closer to the female parental value. The latter was particularly so for the hybrid involving the high delta line HA89-MAX1 as the female parent. For both sets of hybrids, delta values were significantly correlated ($P< 0.05$) with those of their female parents but not their male parents (Agseeds $r_{f/h} = 0.71$, $r_{m/h} = 0.0$, CSIRO $r_{f/h} = 0.99$, $r_{m/h} = 0.0$), adding further support to our hypothesis of a maternal effect on delta and hence TE. Based on these results we have made an additional set of hybrids for testing. We crossed the seven alloplasmic HA89 lines introduced to Australia with a panel of males representing high medium and low delta signatures. These hybrids will be assessed under field conditions at several locations to provide better estimates of the maternal effect.

Table 3 Delta signatures of several commercial and pre-commercial hybrids and their component parent lines from Australian seed company Agseeds , grown on the Darling Downs, Australia spring 1998.

P1 (female parent)	P2 (male parent)	P1	P2	P1 X P2 Hybrid
Agseeds female 1	Agseeds male 1	18.4	18.4	17.9
Agseeds female 2	Agseeds male 2	18.6	19.2	18.0
Agseeds female 2	Agseeds male 1	18.6	18.4	18.0
Agseeds female 3	Agseeds male 3	18.4	18.4	18.0
Agseeds female 4	Agseeds male 4	17.7	17.6	18.4
Agseeds female 5	Agseeds male 2	18.8	19.2	18.4
Agseeds female 6	Agseeds male 5	19.6	18.6	18.9
Agseeds female 6	Agseeds male 6	19.6	19.0	19.1

Table 4 Delta signatures of several CSIRO experimental hybrids and their component parent lines grown in a replicated field trial in Gatton, Australia 1999. Delta signature of the common male parent P2 (genotype PAR-1673-2) = 19.1 ± 0.27.

P1 (female parent)	P1	P1 X P2 Hybrid
CMS HA89-GIG1	19.7 ± 0.27	19.6 ± 0.27
CMS HA89-MAX1	22.7 ± 0.27	22.6 ± 0.27
CMS HA89-PEF1	19.5 ± 0.27	19.2 ± 0.27
CMS HA89-PET1	19.7 ± 0.27	19.5 ± 0.27
HA89-ANN1	19.7 ± 0.27	19.7 ± 0.27
CMS HA89-ANN2	19.5 ± 0.27	19.5 ± 0.27
CMS HA89-ANN3	19.2 ± 0.27	19.3 ± 0.27
Lsd (0.05)		

Conclusion

These results provide preliminary evidence that there is a cytoplasmic influence on delta and hence TE in sunflower. If substantiated, sunflower breeders may need to consider selection for cytoplasmic genomes as well as nuclear genomes to improve adaptation in dry environments. In this regard, we consider cytoplasms of species adapted to dry environments eg *H. argophyllus*, *H. anomalus* and *H. deserticola* as worthy candidates for further investigation.

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