Carbon Isotope Discrimination, Water-Use Efficiency, Specific Leaf Area Relationships in Groundnut

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THE previous papers have demonstrated that extensive variation in WUE exists among groundnut cultivars. The difficulty in accurately measuring WUE in glasshouse and field situations, however, means it is virtually impossible to include such a trait into large-scale breeding programs. The pioneering work by Professor G. Farquhar and his colleagues (Farquhar et al. 1982) which showed that WUE and isotopic discrimination against ^{13}C during photosynthesis (Δ) were correlated, raised the possibility of using Δ as a rapid and non-destructive surrogate measure for selection of high WUE in large-scale breeding programs.

Subsequent research has further assessed the possibility of using Δ in groundnut breeding programs, by investigating the relationships between WUE and Δ under both glasshouse and field conditions. This paper reports the nature of these relationships. It is imperative that breeders and physiologists be confident that Δ is a reliable predictor of WUE before it can be recommended as a selection trait.

Theory for Associations between Carbon Isotope Discrimination and Water-use Efficiency

Atmospheric CO_2 contains two stable isotopes, ^{13}C and ^{12}C in a ratio of approximately 1:89. During photosynthesis, C_3 plants discriminate against $^{13}CO_2$ and take up less of it compared with $^{12}CO_2$ in relation to the proportions of these stable isotopes in the atmosphere. This discrimination has both physical and biochemical bases: slower diffusion of ^{13}C through the stomates, and lower affinity of the carboxylating enzymes for ^{13}C than ^{12}C (Farquhar et al. 1989). The degree of discrimination, Δ, is defined as:

$$\Delta = a + (b - a)p_i/p_a - d$$

where p_a and p_i are the intercellular and atmospheric vapour pressures for CO_2, and a, b, and d are parameters for isotope effects on discrimination, carboxylation, respiration and other processes [see Hubick et al. (1986) for details].

WUE is defined as the ratio of total dry matter (TDM) to transpiration (T) which can be modelled at many levels:

$$\text{WUE} = \frac{\text{TDM}}{\text{T}} = \frac{\text{A}}{\text{E}} = \frac{p_a(1 - p_i/p_a)}{1.6(e_i - e_a)} \quad \ldots 2$$

Consequently, we can see from equations 1 and 2 that Δ and WUE are both a function of p_i/p_a. They will exhibit a negative, linear association providing p_a is relatively constant and $e_i - e_a$ does not vary (i.e. little variation in external humidity and leaf temperature). When Δ is measured on a carbon sample from a leaf, it provides a time and spatially integrated estimate of WUE.

Relationships in Groundnut

Groundnut cultivar variation in transpiration efficiency and correlation with Δ at the whole plant level

Using medium-sized pots (13 kg capacity) in a glasshouse study, Hubick et al. (1986) showed there was significant variation in WUE among seven *Arachis hypogaea* cultivars and two wild *Arachis* species, ranging from 1.41 to 2.29 g/kg. A close negative correlation ($r^2 = 0.66$) between WUE and Δ was also observed, as expected on the basis of theory and data presented by Farquhar and Richards (1984) (Fig. 1). Differences in photosyn-

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thetic capacity were largely responsible for WUE variation, as dry matter production was negatively correlated with Δ, while water use showed no such relationship with Δ. The lack of a relationship between water use and Δ may be associated with the use of small pots in this study, where plants were forced to use most of the available water, and therefore ended up having the same total water use. Differing responses may occur in the field where access to soil water can be relatively unrestricted.

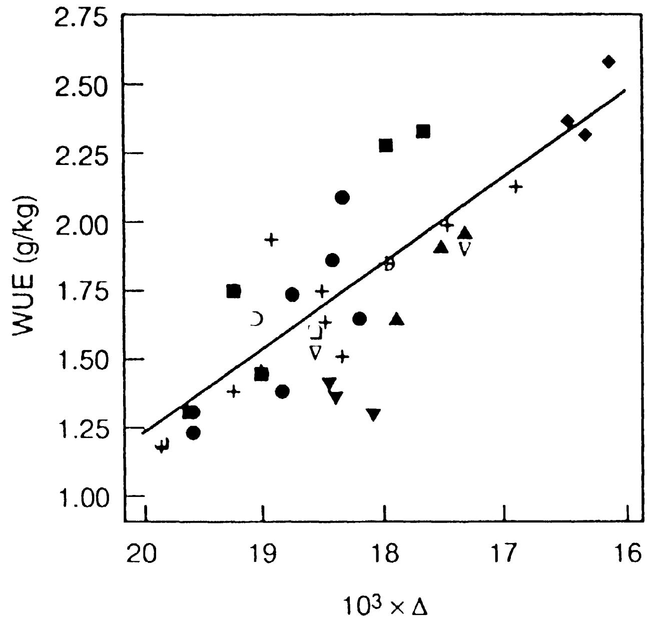

Figure 1. Transpiration efficiency versus carbon isotope discrimination (Δ) in a range of groundnut cultivars

The experimental confirmation that variation in WUE exists among groundnut cultivars, and that a strong relationship between WUE and Δ often exists under glasshouse conditions, suggest that Δ could be used as a criterion to exploit variation in WUE in breeding programs. There are, however, a number of potential sources of discrepancy between results from glasshouse plants in pots and plants grown under field conditions, including the following.

- There are difficulties in correctly apportioning water use into that lost by transpiration and that lost by evaporation. In field studies there are problems in estimating soil evaporation, in contrast to pots where it can be minimised (Turner 1986). Complications can also arise from differences between cultivars in the extent and timing of soil evaporation (Condon et al. 1991).
- There is generally a lack of data on root dry matter in field studies and WUE usually is based on above-ground dry matter. Differences among cultivars in apportioning of dry matter to roots and shoots may lead to erroneous comparisons of WUE defined on this basis. This error may be particularly large in severe drought conditions where total dry matter accumulation may be dominated by roots.
- The aerial environment of field canopies is characterised by complex interactions involving transfer of heat and water vapour, and the interactions are different from those around isolated potted plants. Reduced WUE of isolated plants that occurs because of reduced stomatal conductance may not necessarily be reflected at the canopy level, if the crop boundary layer conductance is relatively small (Cowan 1971, 1977, 1988; Jarvis and McNaughton 1985; Farquhar et al. 1989).

Definitive experiments aimed at assessing variation in WUE among groundnut cultivars, and the correlation between WUE and Δ therefore need to be conducted in canopies under field conditions. This information is essential in order to confirm that WUE variation exists under field conditions, and that Δ can be confidently used as a selection criterion for WUE. Also, this assessment needs to be conducted under both well-watered and water-limited conditions, as it has been shown that the correlation between WUE and Δ may break down under severe plant water deficits (Wright et al. 1992).

Groundnut cultivar variation in transpiration efficiency, and correlation with Δ in field canopies

Two large field experiments using a minilysimeter facility (Wright et al. 1988) were conducted to determine whether cultivar differences in WUE were occurring in small field canopies. One experiment was conducted under full irrigation (Wright et al. 1988), while the other imposed two levels of soil-water deficit (Wright et al. 1994). In both experiments WUE was measured only during the period between full canopy development (ca. 45 days after planting, DAP) and early podfilling (ca. 90 DAP). This was done to minimise the effects of soil evaporation, and avoid any confounding effects arising from maturity differences among cultivars.

The results from experiments clearly indicated that significant differences in WUE existed among groundnut cultivars in the field, under both water non-limiting, and limiting conditions (Table 1). In general, variation in WUE among cultivars was associated with differences in dry matter accumulation rather than differences in transpiration. This result indicates that photosynthetic capacity, rather than leaf/canopy stomatal conductance, was dominating the WUE differences among groundnut cultivars.

Highly significant negative correlations were observed between Δ and WUE under both well-watered (r^2 = 0.67) and water-limited conditions (r^2 = 0.92) (Figs 2a and b). These relationships for field-grown groundnuts support the suitability of Δ as a selection criterion for screening for high WUE.

Changes in p_i/p_a, the ratio of internal CO_2 concentration in the leaf to ambient CO_2 concentration, and Δ can arise from changes in the balance between leaf stomatal conductance and photosynthetic capacity. Where p_i/p_a changes are due to stomatal movements, the relationship between WUE and Δ observed for well-ventilated, isolated leaves may break down in plants grown in canopies in the field because of significant canopy boundary layer resistances to fluxes of water vapour and heat (Cowan 1977, 1988; Farquhar et al. 1989). Where p_i/p_a changes in response to variation in photosynthetic capacity, the problem associated with weak coupling between the crop canopy and atmosphere is not as important, as increased p_i/p_a and Δ arise because of decreased assimilation rate, which causes a relatively small change in the CO_2 concentration in the air above the canopy and no effect on heat and vapour transfer through the boundary layer. The observation that total dry matter production (TDM) was negatively correlated with Δ for the groundnut cultivars examined in the field studies of Wright et al. (1988) and Wright et al. (1994) (Fig. 3) suggests that variation in photosynthetic capacity was the predominant source of variation in p_i/p_a (and therefore Δ).

Genotype × environment interaction and heritability for WUE and Δ

Genotype × environment interaction for WUE appears to be small in groundnut. Wright et al. (1988) found that although there were large differences in WUE and Δ in 'above-ground' as compared with 'in-ground' mini-lysimeters, cultivar ranking in these parameters was largely maintained across the two contrasting environments. Correlation coefficients (r) for WUE and Δ in 'in-ground' versus 'above-ground' mini-lysimeters were 0.91 and 0.83, respectively. Hubick et al. (1986, 1988) also reported that the ranking of WUE and Δ was consistent in a range of cultivars

Table 1. Dry matter (including roots), transpiration, WUE and Δ in groundnut cultivars grown in mini-lysimeters in field canopies under well-watered conditions (Wright et al. 1988) and two levels of water-limited conditions (Wright et al. 1994)

Study	Cultivar	Biomass (kg)	Water use (kg)	WUE (g/kg)	Δ (× 10^3)
Well-watered	Tifton-8	63.1	17.0	3.71	19.7
	VB-81	46.9	16.2	2.90	20.1
	Robut 33-1	55.3	19.0	2.91	20.8
	Shulamit	51.6	16.8	3.07	20.8
	McCubbin	48.6	16.9	2.88	20.8
	Cianjur	43.4	16.3	2.66	20.9
	Rangkasbitung	41.6	16.9	2.46	20.9
	Pidie	47.3	16.6	2.85	20.6
lsd P=0.05		7.0	1.5	0.3	0.55
Water limited (intermittent stress)	Tifton-8	37.5	12.2	3.07	19.4
	Shulamit	35.7	12.8	2.79	19.9
	McCubbin	36.3	13.4	2.71	20.7
	Chico	20.5	11.4	1.80	21.1
(terminal stress)	Tifton-8	31.3	10.0	3.13	18.6
	Shulamit	29.0	9.9	2.93	18.8
	McCubbin	26.8	10.0	2.68	19.4
	Chico	17.8	8.8	2.20	20.9
lsd P=0.05		5.61	1.90	0.38	0.93

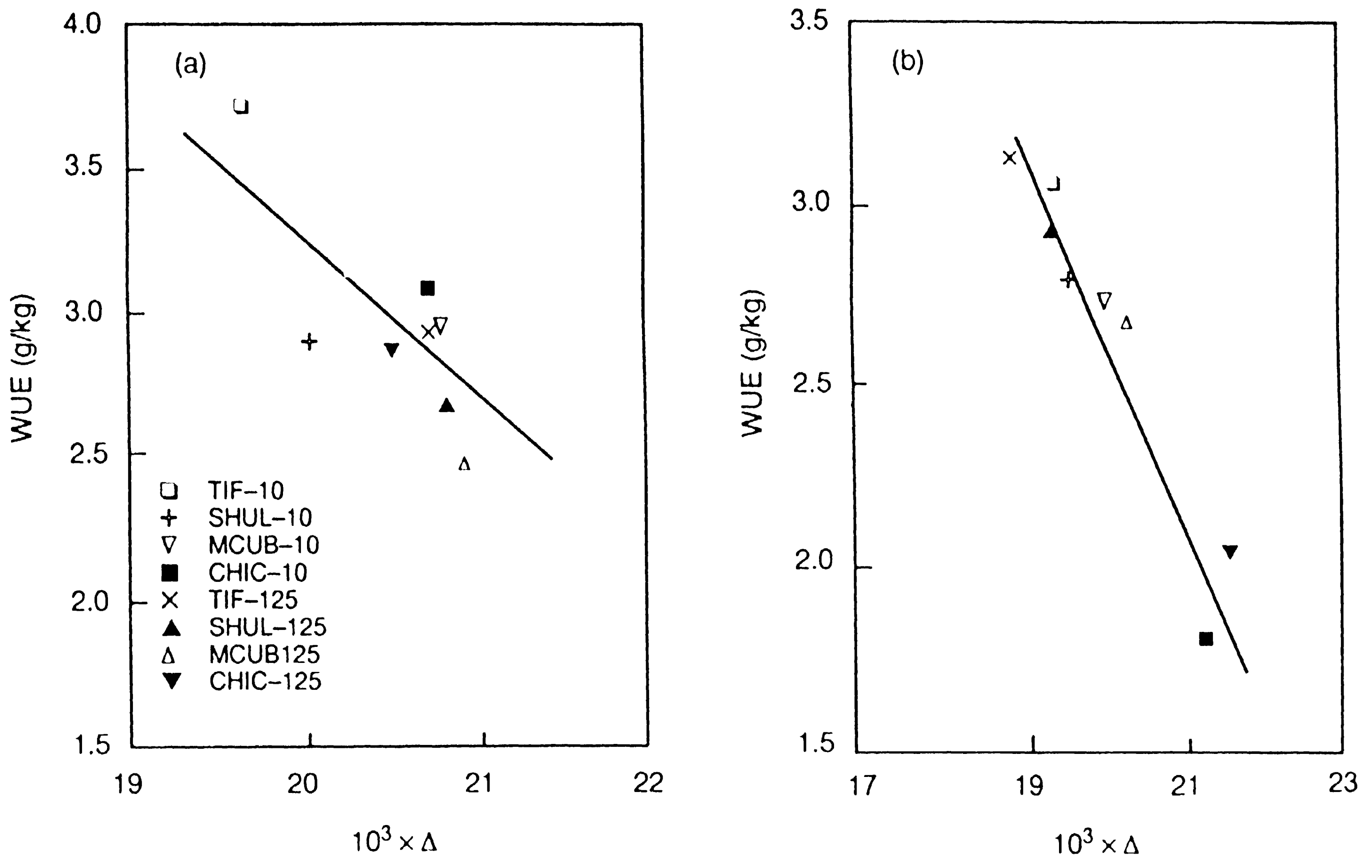

Figure 2. Relationship between water-use efficiency (WUE) and carbon isotope discrimination (Δ) under (a) well-watered and (b) droughted conditions for peanut cultivars grown in the field

under two contrasting water regimes in glasshouse studies. Hubick (1990) showed that although WUE and Δ varied significantly in response to watering treatment and source of nitrogen (mineral N versus nodule N), the ranking of WUE and Δ was similar under each treatment, again indicating there is low genotype × environment interaction for these parameters.

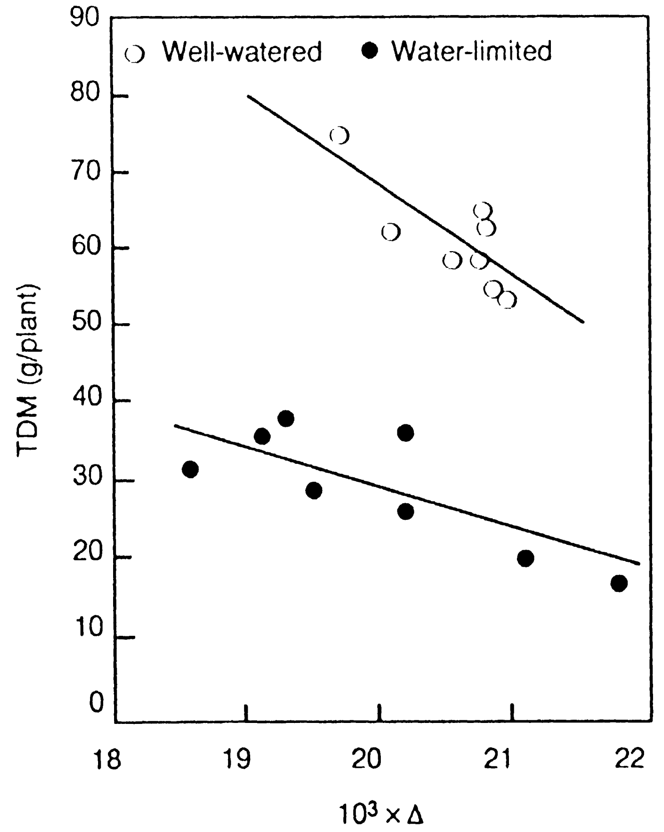

Figure 3. Total dry matter (TDM — roots and shoots) versus carbon isotope discrimination (Δ) in leaves of well-watered and water-limited groundnut cultivars growing in the field

In 16 groundnut cultivars grown at 10 sites with widely different rainfall patterns in sub-tropical and tropical areas of Queensland, Australia, there was significant genotypic variation in Δ, with no significant interaction between genotype and environment (Hubick et al. 1988). The broad sense heritability (ratio of genotypic variance to the total, or phenotypic variance) or repeatability of Δ in this experiment was 81%.

Inheritance of Δ was studied in plants grown in pots using crosses of cultivars with contrasting Δ and WUE (Hubick et al. 1988). The F_1 progeny had Δ values similar to those of the low Δ cultivar, Tifton-8, and considerably smaller than those of Chico, the high Δ cultivar. This response suggests a degree of dominance for small Δ or large WUE in these genotypes. In the F_2 generation, the distribution of Δ exceeded the range between Tifton-8 and Chico, with two F_2 plants having smaller Δ values than those of the low Δ parent, Tifton-8 (Fig. 4). The F_2 distribution for Δ strongly suggested quantitative rather than qualitative inheritance for this trait.

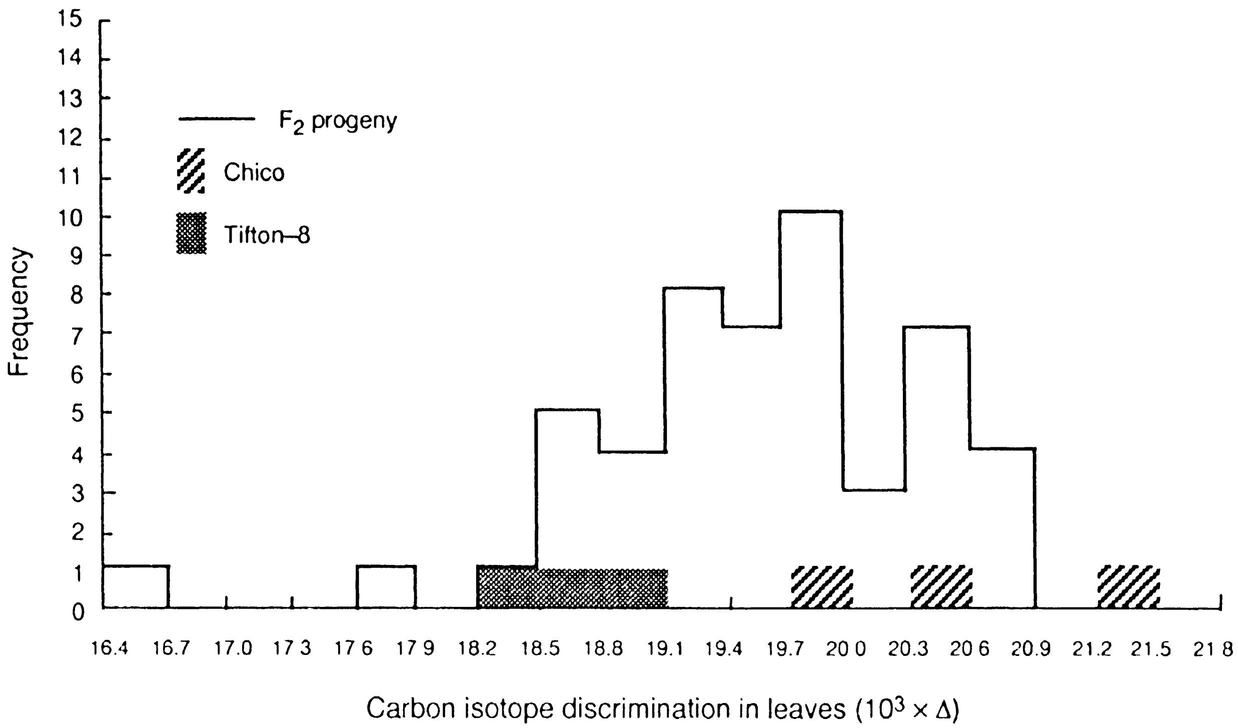

Figure 4. Frequency distribution of carbon dioxide discrimination (Δ) in leaves of well-watered plants of groundnut cultivars Tifton–8 and Chico, and their F_2 progeny, grown together in the same glasshouse environment

The results from the study of Hubick et al. (1988), in combination with the evidence we present here indicating that WUE and Δ have low genotype × environment interaction, suggest that effective selection for Δ, and hence W, could be conducted in a restricted number of environments. Indeed, the results indicate selection could possibly take place in a single environment, be it well-watered or water-limited, and in a glasshouse or field situation.

Relationships between specific leaf area, WUE and Δ

It has been observed over many experiments that specific leaf area (SLA, cm^2/g, which is negatively related to leaf thickness) is closely and negatively correlated with WUE, and also that SLA and Δ are positively correlated. Examples of the relationships between SLA and WUE, and SLA and Δ, measured in the mini-lysimeter study by Wright et al. (1994) are illustrated in Figures 5a and 5b. These observations are consistent with our earlier hypothesis that cultivars with high WUE have higher photosynthetic capacity. If it is assumed that the N:C ratio does not vary among cultivars then it is possible that those cultivars with thicker leaves had more photosynthetic machinery and the potential for greater assimilation per unit of leaf area. Indeed, Nageswara Rao and Wright (1994) have shown that specific leaf nitrogen (g N/m^2) is linearly related with SLA, such that thicker leaves had higher nitrogen contents (data not shown). Similar relationships between WUE and SLA, and Δ and SLA, have been reported elsewhere (Wright et al. 1988). A highly significant relationship between Δ and SLA was also observed for some 300 F_3 plants derived from a single cross of high and low Δ Indonesian cultivars grown in the field (Wright et al. 1992). Thus, there is considerable evidence to support the hypothesis that a very strong association between Δ and SLA exists. This finding has significant implications for breeding programs, where selection for WUE may be practised, as SLA is simple and inexpensive to measure, compared to the more expensive Δ measurement, which requires an isotope ratio mass spectrometer.

An experiment has recently been conducted to determine the generality of the SLA relationship with Δ by growing four cultivars with contrasting Δ in two contrasting temperature environments, under irrigated and rainfed conditions (Nageswara Rao and Wright 1994). The two sites, Kingaroy and Bundaberg, Australia, were similar except for their minimum night temperatures. Mean minimum temperatures during the season were 16°C at Kingaroy compared with 20°C at Bundaberg. Table 2 shows how environment, cultivar and watering regime all influenced the magnitude of SLA and Δ. For instance, SLA and Δ for each cultivar were significantly higher in the warmer

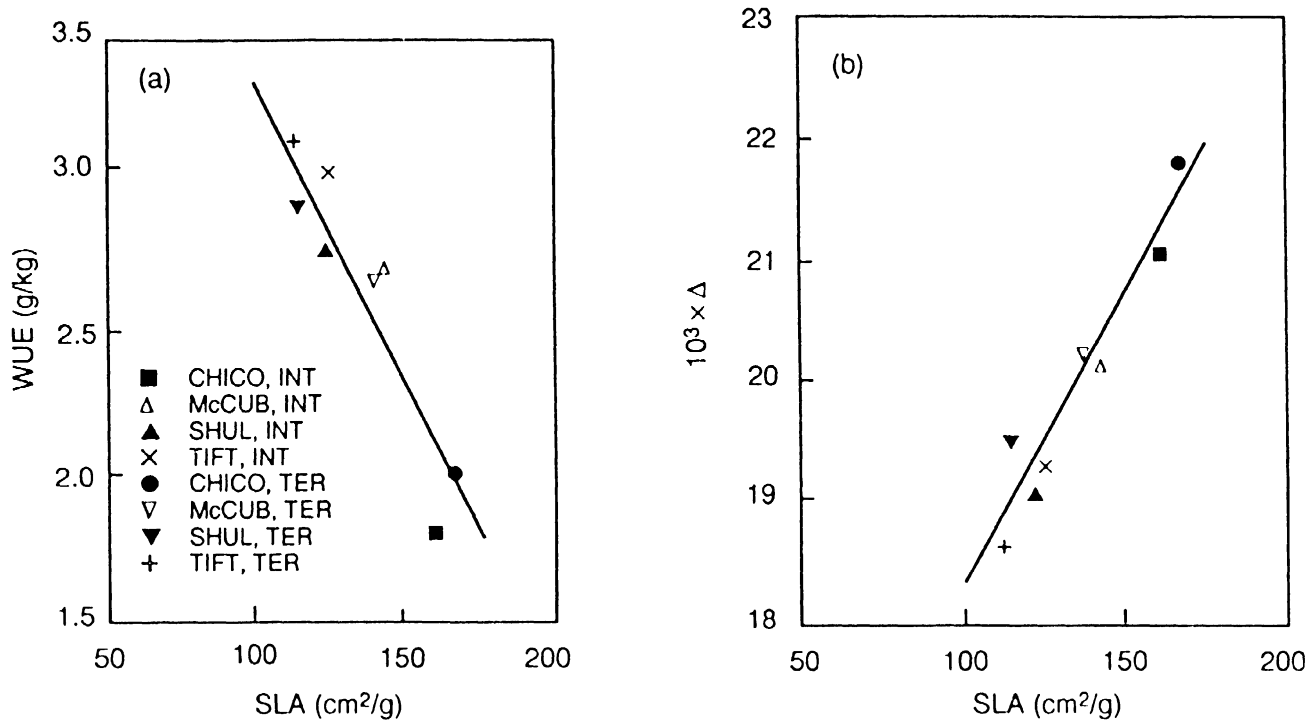

Figure 5. (a) Water-use efficiency (WUE) versus specific leaf area (SLA) and (b) carbon isotope discrimination (Δ) versus SLA for four groundnut cultivars grown under two levels of drought

Table 2. Specific leaf area (cm^2/g) and Δ (‰) measured at maturity for four groundnut cultivars grown at two sites (Bundaberg and Kingaroy) under two watering regimes (irrigated and rainfed).

Site	Treatment	Chico		McCubbin		Shulamit		Tifton	
		SLA	Δ	SLA	Δ	SLA	Δ	SLA	Δ
Kingaroy	Irrigated	155.2	22.44	145.9	21.17	124.0	21.24	117.7	20.40
	Rainfed	138.9	22.15	164.8	21.45	124.9	21.35	132.7	20.77
Bundaberg	Irrigated	184.9	23.21	174.2	22.68	166.8	22.97	148.3	21.50
	Rainfed	186.3	22.29	166.2	21.50	128.5	21.39	136.8	20.84

Bundaberg environment, while water deficits associated with the rainfed treatment tended to reduce SLA and Δ for each cultivar but not in Kingaroy. This effect was particularly apparent at Bundaberg where lower rainfall resulted in greater crop water deficits. The data clearly show that leaves of all cultivars became 'thicker' in response to low temperature and water deficits, possibly due to effects on leaf expansion and translocation of assimilate from the leaf (Bagnall et al. 1988). Of more interest, however, was the observation that cultivar ranking for SLA and Δ remained the same in each environment and watering regime. Indeed analysis of variance showed the main effects of location, irrigation treatments and cultivar were highly significant ($P < 0.05$) for SLA and Δ, while the genotype × environment interactions were non-significant. These results are consistent with the low genotype × environment interactions for WUE and Δ reported earlier.

The strong correlation between Δ and SLA reported previously (Fig. 5b) was again apparent for this data set (Fig. 6) even given the interactions noted above. Interestingly, the data from the contrasting temperature and water stress environment form a universal relationship. Even the data presented in Figure 5b, and other data we have measured elsewhere (e.g. Wright et al. 1992), fit well onto this relationship. The physiological mechanisms involved are unknown, and need further investigation. Nevertheless, the significant application of the relationship is obvious, in that breeders could use the inexpensively measured SLA in lieu of Δ to screen for high WUE among groundnut germplasm within particular environments.

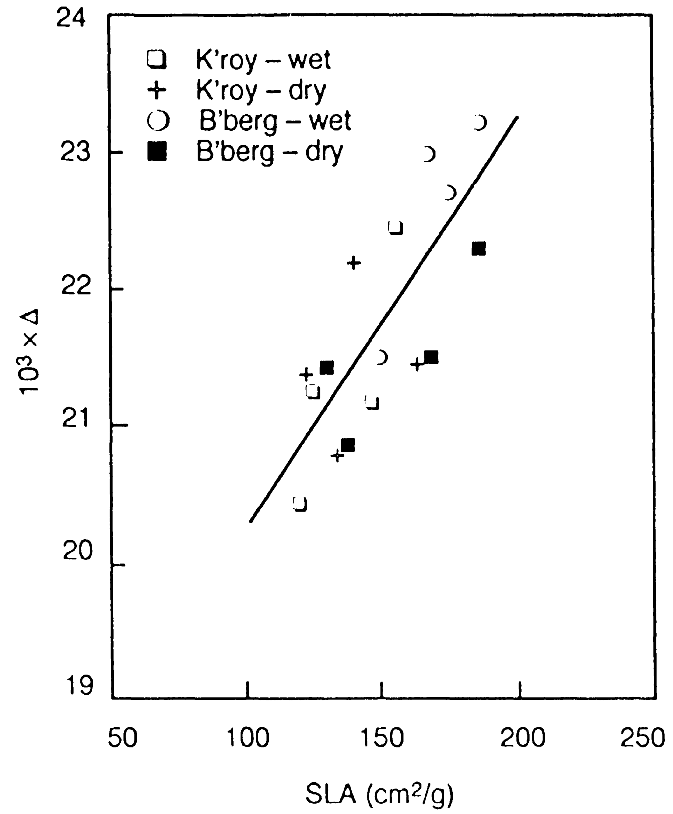

Figure 6. Carbon isotope discrimination (Δ) in leaves versus leaf area for four peanut cultivars grown in the field at Bundaberg and Kingaroy, Queensland, Australia

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