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RESEARCH ARTICLE

Leaf chlorophyll concentration relates to transpiration efficiency in peanut

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SPAD chlorophyll metre reading (SCMR); specific leaf area (SLA); specific leaf nitrogen (SLN); transpiration efficiency (TE).

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Abstract

Two pot experiments were conducted in two different seasons at the University of Agricultural Science, Bangalore, India, to study (a) the relationship between chlorophyll concentration (by measuring the leaf light-transmittance characteristics using a SPAD metre) and transpiration efficiency (TE) and (b) the effect of leaf N on chlorophyll and TE relationship in peanut. In Experiment (Expt) I, six peanut genotypes with wide genetic variation for the specific leaf area (SLA) were used. In Expt II, three non-nodulating isogenic lines were used to study the effect of N levels on leaf chlorophyll concentration–TE relationship without potential confounding effects in biological nitrogen fixation. Leaf N was manipulated by applying N fertiliser in Expt II. Chlorophyll concentration, TE (g dry matter kg^{-1} of H_2O transpired, measured using gravimetric method), specific leaf nitrogen (g N m^{-2}, SLN), SLA (cm^2 g^{-1}), carbon isotope composition ($\Delta^{13}C$) were determined in the leaves sampled during the treatment period (35–55 days after sowing) in the two experiments. Results showed that the leaf chlorophyll concentration expressed as soil plant analytical development (SPAD) chlorophyll metre reading (SCMR) varied significantly among genotypes in Expt I and as a result of N application in Expt II. Changes in leaf N levels were strongly associated with changes in SCMR, TE and $\Delta^{13}C$. In both the experiments, a significant positive relationship between SCMR and TE with similar slopes but differing intercepts was noticed. However, correction of TE for seasonal differences in vapour pressure deficit (VPD) between the two experiments resulted in a single and stronger relationship between SCMR and TE. There was a significant inverse relationship between SCMR and $\Delta^{13}C$, suggesting a close linkage between chlorophyll concentration and $\Delta^{13}C$ in peanut. This study provides the first evidence for a significant positive relationship between TE and leaf chlorophyll concentration in peanut. The study also describes the effect of growing environment on the relationships among SLA, SLN and SCMR.

Introduction

Peanut (*Arachis hypogaea* L.) is an oilseed, food and cash crop grown under both rain-fed and irrigated conditions between 40°N and 40°S latitudes. Two thirds of the global production occurs in rain-fed regions of the Semi-Arid Tropics where unpredictable drought stress is the most important constraint for peanut production under rain-fed conditions (Smartt, 1994). With water becoming an increasingly scarce commodity globally, genetic enhancement to maximise crop production per unit input of water has been a major research thrust of many crop improvement programmes.

Conventional breeding for improved drought adaptation in peanut has been based on selection for pod yield in a given drought environment. While the direct

selection for yield can be effective for specific adaptation, its application in larger breeding programmes catering for a wider range of environments is becoming increasingly limited. This is perhaps the result of the high resource requirement and poor repeatability of the results due to the large genotype × environment (G × E) interaction for yield (Branch & Hildebrand, 1989; Cooper & Hammer, 1996; Jackson *et al.*, 1996; Araus *et al.*, 2002).

Therefore, improving the adaptability to a given environment depends on a comprehensive understanding of the nature and extent of G × E interactions for the physiological traits contributing to yield, especially under water-limited environments (Ludlow & Muchow, 1990; Sheshshayee *et al.*, 2003).

Simple analytical crop models can provide a framework for the understanding of genotypic variation in yield and the effects of environment on the physiological processes contributing to yield. The model proposed by Passioura (1977) describes Yield (Y) as a product of total transpiration (T), transpiration efficiency (TE, defined as the biomass produced per unit of water transpired) and harvest index (HI, the proportion of economic yield in total biomass).

Significant genotypic variation for T, TE and HI, in peanut has been demonstrated in pot (Hubick *et al.*, 1986; Wright *et al.*, 1988) and field studies (Nageswara Rao *et al.*, 1993; Wright *et al.*, 1994). Rapid and accurate assessment of the genetic variability in TE was made possible with the demonstration of the utility of the carbon isotope discrimination ($\Delta^{13}C$) as a surrogate for TE (Farquhar & Richards, 1984; Farquhar *et al.*, 1989). This technique has also been effectively adopted for the assessment of genetic variability in TE among several crop plants, including peanuts (Hubick *et al.*, 1986; Wright *et al.*, 1988, 1994; Roy Stephen, 1995; Udayakumar *et al.*, 1998*a*; Shashidhar *et al.*, 2003; Sheshshayee *et al.*, 2003). However, determination of $\Delta^{13}C$ is cost intensive; hence, there is a need to develop more economic surrogate screening techniques.

Studies by Wright *et al.* (1994) and Nageswara Rao & Wright (1994) demonstrated a positive correlation between specific leaf area (SLA, ratio of leaf area to leaf dry weight) and $\Delta^{13}C$ (r = 0.90–0.93** relationship statistically significant at 1% confidence level.) in peanut and a negative relationship between SLA and TE, suggesting that SLA can be used as a rapid alternate to estimate genetic variability in TE among peanut genotypes.

Although a close correlation between SLA and $\Delta^{13}C$ (and thus with TE) has been established in controlled experiments, the strength of correlation varied with '*r*' ranging from 0.71 to 0.94 over a range of peanut genotypes and environments (Wright *et al.*, 1994). It can therefore be inferred that SLA might be influenced by factors such as time of sampling, leaf age (Wright & Hammer, 1994; Nageswara Rao *et al.*, 1995, 2001) as well as accuracy of the measurement.

Nageswara Rao *et al.* (2001) emphasised the importance of sampling protocols when using SLA as a selection tool in large-scale peanut breeding programmes. This study demonstrated that in peanut, SPAD chlorophyll metre readings (SCMR) were closely correlated with parameters such as SLA and specific leaf nitrogen (SLN, the ratio of leaf nitrogen content to leaf area). The SPAD metre that determines the leaf light-transmittance characteristics has been shown to be a good estimate of leaf chlorophyll concentration (Takebe *et al.*, 1990; Balasubramanian *et al.*, 2000). Further, Dwyer *et al.* (1995) and Chapman & Barreto (1997) demonstrated that SCMR is related to the leaf nitrogen status. Therefore, the close relationships observed among SCMR, SLA and SLN (Nageswara Rao *et al.*, 2001) implied that SCMR could be used as a rapid, low cost and *in situ* technique to screen for TE in large breeding populations.

There have, however, been no specific studies to examine the relationship between SCMR and TE and it's stability across environments. The major aim of the present investigation was therefore to examine the relationship between SCMR and TE in selected peanut genotypes in two contrasting environments.

Materials and methods

Two pot experiments (Expt I and II) were conducted during the 2000 rainy and 2001–02 postrainy seasons at the Crop Physiology Department, University of Agricultural Sciences, Bangalore, India, to assess the relationship between SCMR and TE. In Expt I, six selected genotypes, viz. ICGS 44, ICGV 86031, TAG 24, TMV-2, ICGS 76 and ICG 476 were used. In the second experiment (Expt II), three non-nodulating isogenic lines of peanut (ICGL-2, ICGL-4 and ICGL-5) were used to study the SCMR–TE relationships under varying levels of leaf N. The seed material for both experiments was procured from the International Crop Research Institute for Semi-Arid Tropics (ICRISAT) in India.

Experiment I

The experiment I was conducted between September and November 2000. Seeds of the six genotypes were sown in carbonised rubber containers of dimensions 45 × 15 × 20 cm and filled with 30 kg of red sandy loam soil and farmyard manure in a ratio of 3:1. Seeds treated with Bavistin 2% (against seedling infections) were sown in excess of four seeds per container and later

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thinned to two healthy seedlings per container. Each genotype was grown in 12 containers (replications). These containers were arranged randomly under a mobile rain-out shelter, to prevent interference from rain during the experimental period (Chauhan *et al.*, 1997). The soil surface in the containers was mulched with carbonised rubber pieces to minimise soil evaporation. At 35 days after sowing (DAS), plants from six containers were carefully harvested along with the roots and were separated into leaves, stems and roots. The total plant leaf area was determined using a leaf area metre (ΔT Devices, Burwell, UK) before oven drying the samples at 80°C for 4 days. Dry weight (including the roots) was determined after completely drying the samples.

Transpiration was determined using a gravimetric approach (described below) for the period between 35 and 55 DAS. Plants from the remaining six containers were harvested at 55 DAS, and measurements on leaf area and total plant oven dry weights were determined.

Experiment II

The experiment II was conducted between December 2001 and February 2002, in which three selected non-nodulating isogenic lines, that is, ICGL-2, 4 and 5, were used to study the effect of leaf N levels on SCMR and TE. The isogenic lines facilitated the manipulation of leaf N, without interference from genotypic effects.

The non-nodulating lines were grown in carbonised rubber containers as described in Expt I. The Expt II was laid out as a complete randomized block design (RBD), with the 3 non-nod lines × 3 basal doses of N with eight replications. The three N treatments were applied as basal dose by mixing 0, 1.3 and 2.6 g urea in 30 kg of soil (in each container) to give a N rate of 0, 25 and 50 kg ha^{-1}. After seedling establishment, plants in each container were thinned down to two plants. On the 35th day, plants from three out of eight replications (containers) were harvested to determine the leaf area and the oven dry weight of plants. The remaining five replications were used for the determination of transpiration and growth rates through the next 30 days. The experiment was terminated at 55 DAS after final harvest.

Measurement of transpiration efficiency

Evapo-transpiration (ET) from each container was determined during the treatment periods (35–55 DAS) for both Expt I and Expt II, using gravimetric methods (Udayakumar *et al.*, 1998*b*). Containers were weighed to determine the ET on a daily basis. Soil evaporation (Es) from containers was computed from the successive weight differences of three bare pots containing similar quantities of soil but without plants.

All containers were maintained at field capacity by replacing the amount of water lost through ET on a daily basis. The total water added to each container over the duration of the experiment was summated to compute the cumulative ET, while the transpiration component of ET, referred here as cumulative water transpired (CWT), was computed as CWT = $\sum$(ET − Es).

Transpiration efficiency (TE g kg^{-1}) was computed as $(DM_2 - DM_1)/CWT$, where DM_1 and DM_2 were total plant dry matter recorded on 35 and 55 DAS, respectively.

To enable TE comparison over the two seasons, adjusted TE was calculated using the formula proposed by Turner & Sinclair (1983) as follows.

$$TE = k/(e_i - e_a)$$

where, e_i and e_a are water vapour pressures of leaf and air, respectively, and k is the constant of proportionality. The daily climate data during the experimental periods were obtained from the Agro-meteorological station located at <1 km from the experimental site. Leaf and atmospheric water vapour pressures were computed from the leaf, and ambient temperatures were measured using a portable photosynthesis system (CIRAS-1, PP System, Hitchin, Herts, UK).

Leaf transmittance and chlorophyll content

The leaf transmittance characteristics were measured using a SPAD Chlorophyll metre (SPAD-502, Minolta Corp., Ramsey, NJ, USA). The third fully expanded leaf from the apex was sampled around 09:00 h to record the SCMR, with four readings taken on each of the four leaflets. After recording the SCMR, leaf areas of individual leaflets were measured. The two leaflets (from one side of the rachis) were oven-dried at 80°C for 48 h before determining the leaf dry weight for estimating SLA and were later used for the determination of leaf N and carbon isotope composition. The SLA was calculated as the ratio of leaf area to leaf dry weight. The other two leaflets were sampled to determine the chlorophyll content.

Chlorophyll content was determined for the peanut genotypes of the Expt I only using a solvent extraction procedure (Hiscox & Israelstam, 1979). Chlorophyll was extracted from the leaflet samples by submerging them in tubes containing 15-mL mixture of acetone (80%) and dimethyl sulphoxide (1:1 v/v) and keeping the tubes in the dark for a period of 15 h. After the leaf pieces were completely bleached, the absorbance of the extract was measured at 652 nm using a spectrophotometer (Spectronic Genesys-2, Milton Roy, Ivyland, PA 18974, USA).

Leaf N and carbon isotope discrimination ($\Delta^{13}C$)

In Expt I, leaf N was measured using an elemental analyzer (NA 1110 Carlo Erba Instruments, Thermo Electron S.p.A, Strada Rivoltana 20090, Rodano (Milan), Italy) at the Research School of Biological Sciences, Australian National University, Canberra, Australia.

In Expt II, elemental N content and carbon isotope composition ($\delta^{13}C$) in leaf samples were measured at the National Facility for Stable Isotope studies, Department of Crop Physiology, University of Agricultural Sciences, Bangalore, India.

The leaf N content was analysed using a Flash Elemental analyzer (NA 1112, Carlo Erba Instruments), and the carbon isotope composition ($\delta^{13}C$) was determined using the Stable Isotope Ratio Mass Spectrometer (Delta-Plus, Thermofinnigan, Bremen, Germany) interfaced with the Flash Elemental analyser (NA 1112) via a continuous flow device (Conflo III). The analytical uncertainty while measuring $\delta^{13}C$ was within 0.1‰. Carbon isotope discrimination ($\Delta^{13}C$) was computed assuming the ^{13}C isotopic composition of atmospheric air ($\delta^{13}C_a$) to be −8‰ as follows (Farquhar *et al.*, 1989):

$$\Delta^{13}C = \left[\delta^{13}C_a - \delta^{13}C_p\right] / \left[1 + \delta^{13}C_p/1000\right]$$

where $\delta^{13}C_a$ and $\delta^{13}C_p$ are carbon isotopic composition of atmospheric air and leaf biomass samples, respectively.

Specific leaf nitrogen was computed as the ratio of the N content per unit leaf area and expressed as mg N m^{-2}.

Statistical analysis

The results were analysed for statistical significance using MSTATC software. The analysis was performed for a simple RBD with five replications for Expt I and a two-factor factorial RBD design with five replications for Expt II.

Results

Season

The weather during Expt I (September–December 2000) was characterised by temperatures of 22 ± 2°C, rain episodes with mild vapour pressure deficits (VPDs) ranging from 10 to 12 mbar. During the Expt II (December 2001–February 2002), there was no rain and both air temperature and VPD rose from 11 mbar in December 2001 to >15 mbar in February 2002, as the season progressed (Table 1). The mean VPDs during the Expt I and II were 10.5 and 14 mbar, respectively.

Experiment I

Genotypes tested in the Expt I used similar amounts of water (10–11 kg) during the treatment period (35–55 DAS) except TMV-2, which used 14.2 kg of water (Table 2). However, total biomass produced by the genotypes varied from 29 to 45 g $plant^{-1}$ as a result of a significant genotypic variation in TE (range 2.76–3.58 g kg^{-1}) (Table 2). SLA ranged from 222 (ICGV 86031) to 293 cm^2 g^{-1} (ICG 476) and SLN between 1.47 (ICG 476) and 2.24 g N m^{-2} (ICGV 86031), representing a significant genotypic variation for these parameters. The SCMR recorded at 55 DAS varied significantly ranging from 33 to 42 among the genotypes (Table 2). The TE was positively correlated with SLN and negatively with SLA (Fig. 1). Furthermore, SCMR was positively correlated with chlorophyll content as well as with TE (Fig. 1).

Experiment II

In Expt II, three non-nodulating isogenic lines were grown under three soil N levels so that the relationship between TE and SCMR could be studied at a range of leaf N levels, without the confounding effects of genotype. Increments in leaf N levels resulted in systematic increase in TDM, CWT, TE, SCMR and SLN, with corresponding

Table 1 Summary of weather data during 2000 rainy (Expt I) and postrainy 2001–02 (Expt II) seasons

Season	Month	Maximum T (°C)	Minimum T (°C)	Sun Shine (h)	Es (mm)	VPD (mbar)	Rain (mm)
Expt I							
2000	September	27.7	19.3	6.0	3.6	10	239.8
	October	27.1	18	5.1	3.6	10	168.4
	November	26.9	16.5	6.3	3.7	11	5.8
Expt II							
2001	December	25.3	14.3	5.7	2.7	11	13.8
2002	January	27.3	15	7.3	4.1	13	0
	February	28.7	15.4	7.9	4.9	15	0

Es, soil evaporation; VPD, vapour pressure deficit.

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Table 2 Variability in gravimetric and growth parameters among genotypes grown during the rainy season of 2000 in Expt 1

Genotype	TDM (g plant^{-1})	CWT (L)	TE (g kg^{-1})	SCMR	SLA (cm^2 g^{-1})	SLN (g N m^{-2})
ICGS 44	42	11.5	3.41	38.0	265	1.93
ICGV 86031	36	10.1	3.58	39.0	222	2.24
TAG 24	33	10.2	2.99	37.7	250	1.92
TMV-2	45	14.2	2.95	34.3	271	1.64
ICGS 76	36	10.9	3.18	41.0	244	1.84
ICG 476	29	10.9	2.76	33.0	293	1.47
SEM	1.273	0.161	0.078	0.435	7.935	0.583

TDM, total dry matter accumulated during the experimental period; CWT, cumulative water transpired (total transpiration); TE, transpiration efficiency; Δ^{13}C, carbon isotope discrimination; SCMR, SPAD chlorophyll meter reading; SLA, specific leaf area; SLN, specific leaf nitrogen.

decreases in Δ^{13}C and SLA in all the three non-nodulating genotypes (Table 3).

There was a significant inverse relationship between Δ^{13}C and TE ($R^2 = 0.72$; $P < 0.01$) and a positive relationship between SCMR and TE ($R^2 = 0.72$; $P < 0.01$). The inverse relationship between SCMR and Δ^{13}C (Fig. 2) suggests that SCMR could be a surrogate of Δ^{13}C (and hence TE) in peanut. Basal application of N to non-nodulating isogenic lines resulted in an increase in leaf N, with SLN ranging from 1.5 to 2.5 g N m^{-2}.

There was a significant positive relationship ($R^2 = 0.77$, $P < 0.01$) between SCMR and SLN, over a range of leaf N measured in both experiments, suggesting that SCMR is closely related to leaf N content (Fig. 3). This finding is further supported by the significant positive correlation between SCMR and TE in Expt I and II (Fig. 4a). Although the slopes of TE and SCMR relationships were similar in both experiments (the standard error for the slope was 0.11), the regressions were clustered separately for Expt I and II, with differing intercepts (Fig. 4a).

However, this study provides the first evidence for a significant positive relationship between TE and SCMR in peanut. The TE was generally greater in Expt I compared to Expt II, indicating the significant effect of environment

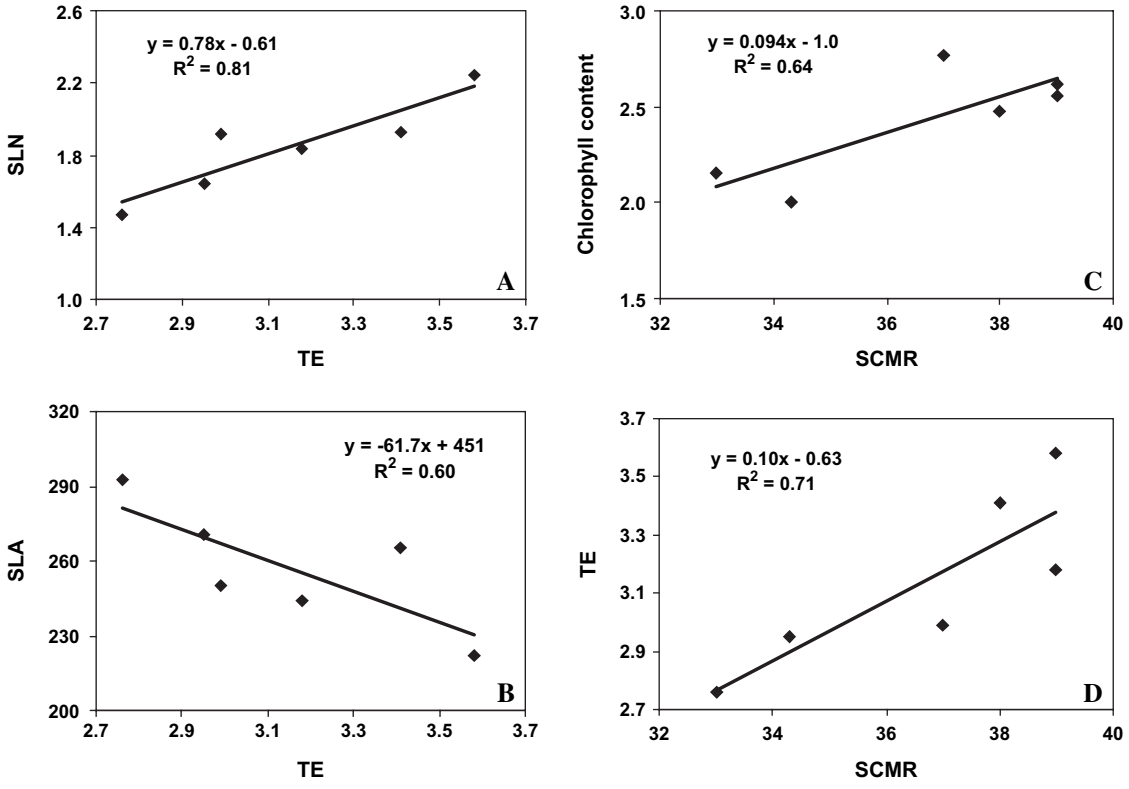

Figure 1 Relationship of TE (g kg^{-1}) with (A) SLA (cm^2 g^{-1}), (B) SLN (g N m^{-2}) and that of SCMR with (C) chlorophyll content and (D) TE among six peanut genotypes.

Table 3 Variability in TE and related physiological parameters among three non-nodulating isogenic lines of peanut grown under three levels of N during the 2001–02 season (Expt II)

Genotype	N level	TDM (g pot^{-1})	CWT (L)	TE (g kg^{-1})	Δ^{13}C (‰)	SCMR	SLA (cm^2 g^{-1})	SLN (g N m^{-2})
ICGL-2	0 N	50	14.4	2.00	19.31	40.3	146	1.79
	1.3 g N	60	11.5	2.60	19.02	45.8	147	2.37
	2.6 g N	73	13.3	2.73	18.95	46.8	139	2.51
ICGL-4	0 N	52	10.7	2.07	19.76	35.3	150	1.47
	1.3 g N	59	11.8	2.02	19.90	36.2	164	1.69
	2.6 g N	62	14.6	2.10	19.40	41.6	157	1.85
ICGL-5	0 N	59	10.4[a]	2.05	19.44	35.4	156	1.52
	1.3 g N	69	15.6	2.63	19.17	41.1	142	1.94
	2.6 g N	82	14.4	2.86	19.29	42.8	141	2.20
SEM		2.393	0.317	0.058	0.075	0.688	3.8	0.64

TDM, total dry matter accumulated during the experimental period; CWT, cumulative water transpired (total transpiration); TE, transpiration efficiency; Δ^{13}C, carbon isotope discrimination; SCMR, SPAD chlorophyll meter reading; SLA, specific leaf area; SLN, specific leaf nitrogen.

on TE but not on SCMR. Adjustment of TE for seasonal variation in VPD (k) could account for most of the seasonal variation in TE (Fig. 4b). Similarly, significant positive relationship between TE and SLN was observed in both the experiments (Fig. 5a), although the regressions clustered separately. However, the relationship between SLN and TE was much stronger once TE was corrected for seasonal differences in VPD (Fig. 5b).

Discussion

Physiological models provide a framework for assessing the traits associated with genotypic performance under water-limited conditions. In the model proposed by Passioura (1977, 1988), TE has been identified as one of the important physiological traits for improving drought adaptation of crops. In peanut, a significant genetic variability for TE has been documented (Hubick *et al.*, 1986; Wright *et al.*, 1988, 1994; Nageswara Rao *et al.*, 1993; Roy Stephen, 1995; Shashidhar *et al.*, 2003; Sheshshayee *et al.*, 2003). Although TE can be rapidly and effectively assessed using carbon isotope discrimination (Δ^{13}C) in leaves (Hubick *et al.*, 1986; Farquhar *et al.*, 1989; Wright *et al.*, 1994), there has been limited application of this tool in peanut breeding programmes because of the high cost and lack of ready accessibility to the isotope ratio mass spectrometers. Earlier studies demonstrated that parameters such as SLA and SLN, which are closely related with photosynthetic capacity, could be used as indirect and cost-effective selection tools for assessing TE in peanut (Nageswara Rao *et al.*, 1993, 1995, 2001). Further, Nageswara Rao *et al.* (2001) demonstrated that SCMR could be used as a rapid nondestructive and indirect measure of SLA and SLN. However, there have been no studies to confirm the direct relationship between SCMR and TE in peanut. It was therefore necessary to assess and explain SCMR–TE relationships in a wide range of peanut genotypes and environments before embarking on using SPAD chlorophyll metres in large-scale breeding programmes.

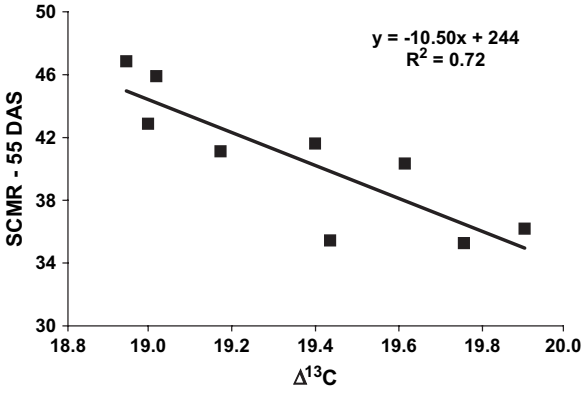

Figure 2 Relationship between SCMR measured at 55 DAS and Δ^{13}C (‰) in three non-nodulating isogenic lines of peanut grown under irrigated conditions.

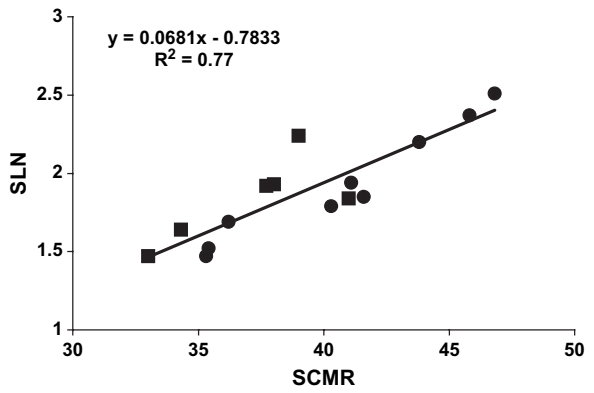

Figure 3 Relationship between SCMR and SLN (g N m^{-2}) in six genotypes (squares) and three non-nodulating isogenic lines (circles) of peanut grown under irrigated conditions.

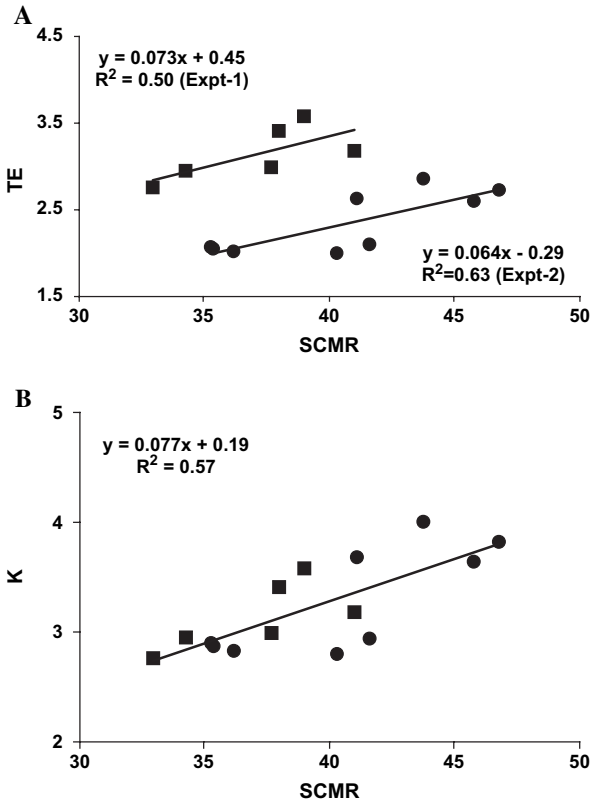

Figure 4 (a) Relationship between SCMR and TE (g kg^{-1}) in six genotypes (squares) and three non-nodulating isogenic lines of peanut grown under irrigated conditions (circles). (b) Relationship between SCMR and *k* (adjustment of TE based on seasonal variations in VPD) in six genotypes (squares) and three non-nodulating isogenic lines (circles) of peanut grown under irrigated conditions.

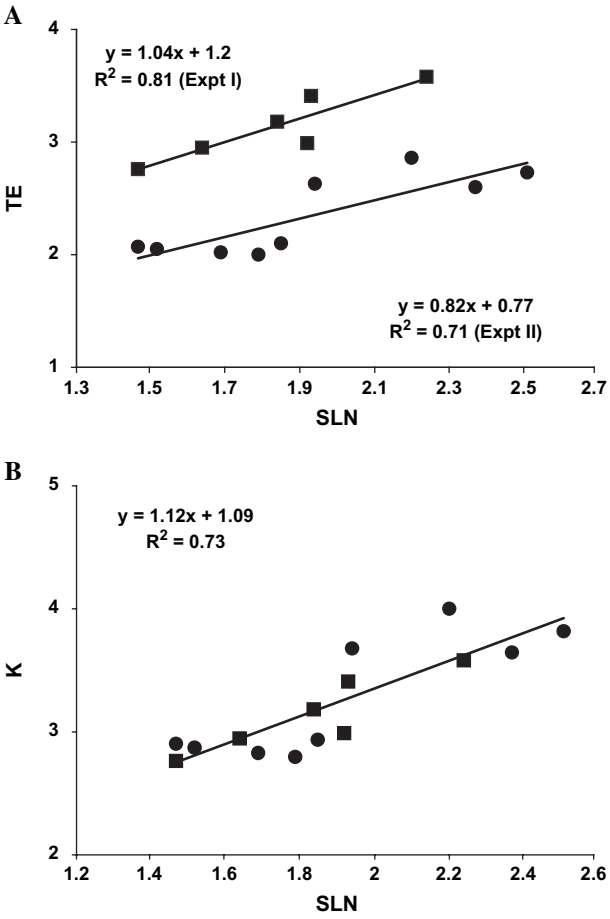

Figure 5 (a) Relationship between SLN (g N m^{-2}) and TE (g kg^{-1}) in six genotypes (squares) and three non-nodulating isogenic lines (circles) grown under irrigated conditions. (b) Relationship between SLN (g N m^{-2}) and *k* (adjustment of TE based on seasonal variations in VPD) in six genotypes (squares) and three non-nodulating isogenic lines (circles) of peanut grown under irrigated conditions.

In the present investigation, a significant genotypic variability was observed for SCMR, TE and other related parameters for a range of genotypes examined in two separate experiments (Tables 2 and 3). The lower TE of ICG 476 (Chico) observed in this study is consistent with the findings of Nageswara Rao *et al.* (1993) and Wright *et al.* (1994), as well as the significant negative relationships observed between TE and SLA (Nageswara Rao & Wright, 1994; Wright *et al.*, 1994; Roy Stephen, 1995).

The SCMR is an indication of the light-transmittance characteristics of the leaf, which is dependant on the leaf chlorophyll content (Richardson *et al.*, 2002). The chlorophyll content in turn is related to the N status of the leaf (Nageswara Rao *et al.*, 1995, 2001). The significant positive correlation between SCMR and chlorophyll content across genotypes in the present study (Fig. 1) clearly demonstrates that SCMR could be a rapid tool to assess genotypic variation for leaf chlorophyll content.

Earlier studies have shown that the major cause of variation in Δ^{13}C (and TE) in peanuts was photosynthetic capacity rather than stomatal conductance (Hubick *et al.*, 1986; Wright *et al.*, 1994; Nageswara Rao *et al.*, 1995; Udayakumar *et al.*, 1998). The observed inverse relationship between TE and Δ^{13}C across N levels was evidently because of variation in chlorophyll content, which would have directly contributed to the variation in photosynthetic capacity. Clearly, leaf N content either coming directly from root uptake or biological N fixing capacity of the plant is an important determinant of TE, within a given environment. It is possible that in Expt I, genotypic differences in biological nitrogen fixation (BNF) could have caused variation in leaf N content (and thus TE and SCMR). However, the use of non-nodulating isogenic lines and the manipulation of leaf N

in Expt II provided an opportunity to assess SCMR–TE relationships, without the potential confounding genotypic effects in BNF variation. The data from Expt II clearly showed that addition of elemental N to non-nodulating isogenic lines resulted in increases in leaf N, TE and SCMR, with a corresponding decrease in $\Delta^{13}C$ (Table 2 and Fig. 2).

A strong positive relationship between SCMR and SLN across the two experiments (seasons), genotypes and N levels (Fig. 3), suggested that SCMR could be used as a rapid selection tool for assessing leaf N in peanut (and thus TE), as suggested by Nageswara Rao *et al.* (2001).

Transpiration efficiency ranged from 2 to 2.75 g kg^{-1} in Expt I and from 2.8 to 3.6 in g kg^{-1} in Expt II. Although there was a strong positive relationship between TE and SCMR in both experiments, the seasonal effect on TE was significant, which resulted in different intercepts for Expt I and II (Fig. 4a). Turner & Sinclair (1983) suggested that TE among crop species and varieties could be compared across variable VPD environments by adjusting TE to the prevailing ambient mean VPD of the test environment. In the present study, VPD varied significantly between Expt I and II, with mean VPD during the experimental period being 10.5 and 14 mbar for Expt I and II, respectively. Use of the VPD-adjusted TE (k) therefore allowed examination of SCMR–TE relationships independent of VPD. This analysis revealed that k values ranged from 2.8 to >4 demonstrating significant genotypic differences. Furthermore, an overall strong positive relationship between SCMR and k when data was pooled across genotypes and seasons (Fig. 4b) clearly indicates the potential of SCMR in assessing the variations in TE among peanut genotypes.

A similar analysis conducted on the SLN–TE relationship highlighted the critical role of VPD on TE (Fig. 5a and Fig. 5b). Thus, while the seasonal VPDs had a significant effect on TE, they did not affect SCMR or SLN suggesting that these measurements are more stable across environments.

The significant relationship between SCMR and TE suggests that SCMR could be a reliable, low cost and non-destructive tool to assess TE in peanut. The results from the present investigation also confirmed the earlier findings of Wright *et al.* (1988) and Nageswara Rao *et al.* (1995, 2001) that leaf nitrogen status (and thus photosynthetic capacity) is the predominant determinant of TE in peanut. The relevance of SCMR as a noninvasive surrogate for TE was examined using container-grown plants of peanut genotypes in this study. The plants often experience restrictions for the root growth in such container experiments and hence might alter the observed relationships when examined under field conditions. While SCMR has been shown to be a stable parameter across seasons (Nageswara Rao *et al.*, 2001), a low G × E interaction is reported for TE (Ashok *et al.*, 1999; Impa *et al.*, 2005). These observations preclude the possible concern on the significance of SCMR as a surrogate for TE under field conditions as well. However, this aspect needs to be examined before employing SPAD chlorophyll metre in large-scale breeding programmes while screening for the variability in TE among peanut genotypes.

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