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Stability of soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) and their association across varying soil moisture stress conditions in groundnut (*Arachis hypogaea* L.)

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Abstract The complex nature of physiological traits associated with drought tolerance and the difficulties associated with their measurements in segregating populations and large number of genotypes inhibited their use in the past in developing water-use efficient genotypes in breeding programmes. With new knowledge of easily measurable surrogates of transpiration efficiency (TE), a trait associated with drought tolerance—specific leaf area (SLA) and soil plant analytical development (SPAD) chlorophyll meter reading (SCMR), it is now possible to integrate TE through the surrogates in breeding and selection schemes in groundnut (*Arachis hypogaea* L.). As a noninvasive surrogate of TE, SCMR is easy to operate, reliable, fairly stable and low cost. However, in a large-scale breeding program, it is difficult to complete SCMR observations within a specified time. The present study addressed the issue as to what extent the SCMR measurements can be spread over time by evaluating 18 diverse groundnut genotypes for two physiological traits, SCMR and SLA in two postrainy (Nov–Apr) seasons (2002/2003 and 2003/2004) in India. Observations were recorded at different times during and after the release of moisture deficit stress. There was general agreement in genotype and trait performance in both the seasons. Interaction between SCMR and time of observation was significant in only one season (2002/2003) but its variance relative to genotypes and time of observation was very small. ICGV 99029 and ICR 48, which recorded higher SCMR and lower SLA values in both the seasons, will make good parents for water-use efficiency trait in breeding programmes. Other good parents include ICGS 76, TCGS 647 and TCGP 6. SCMR recorded at three different times under differing soil moisture deficit in each season showed highly significant correlation with each other. Similarly, SLA at different times also correlated significantly with each other. SCMR and SLA were significantly negatively correlated with each other and the relationship was insensitive to time of observation. The results of the present study indicated that SCMR/SLA observations can be recorded at any time after 60 days of crop growth, preferably under moisture deficit conditions. This gives groundnut breeders a large flexibility to record these observations in a large number of segregating populations and breeding lines in the field. Thus, making it easy to incorporate these physiological traits associated with drought tolerance in breeding and selection scheme in groundnut.

Keywords Water-use efficiency · Genotypic and phenotypic correlations · Peanut · Repeated measurements · Surrogates traits · Water-deficient conditions

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Introduction

Crop productivity per unit of water has become an important consideration in breeding programmes aimed at developing water-use efficient varieties across the world. Following the simple model proposed by Passioura (1977) in wheat, the yield variation in groundnut under water-limited conditions can be ascribed to three components: T (the amount of water transpired by the crop), transpiration efficiency (TE) (dry matter produced per unit of T) and HI (the ratio of pod weight to total dry matter). Genetic variation for T, TE and HI has been reported in groundnut (Hubick et al. 1986; Wright et al. 1988, 1994; Nageswara Rao et al. 1993; Hebbar et al. 1994; Serraj et al. 2004). From the results of a multi-environment study in India to compare efficiency of physiological trait-based and empirical selection approaches for drought tolerance in groundnut, Nigam et al. (2005) concluded that both approaches were more or less equally effective in developing high-yielding selections. However, the yield advantage in empirical approach based selections came largely from greater T (probably through deeper root system exploiting more water from the soil) and in trait-based approach largely from greater TE (through efficient utilization of the water). Cruickshank et al. (2003), from their study in Australia, also reported trait-based approach as being more efficient in selecting for higher TE. High-yielding selections based on T would not be of advantage in water-deficient environments in rainfed agriculture, where more than 90% of the groundnut crop is grown. These will have to come through either higher TE or HI or both to give high yield under water-limited conditions. However, it is difficult to measure TE, a complex physiological trait, in segregating populations and breeding lines in large-scale breeding programmes. Recent studies indicated that specific leaf area (SLA) and soil plant analytical development (SPAD) chlorophyll meter reading (SCMR), which are easy to measure, are highly correlated with TE. Both traits have considerable genetic variation in groundnut (Serraj et al. 2004; Upadhyaya 2005; Lal et al. 2006; Sheshshayee et al. 2006). SCMR is an indication of the light-transmittance characteristics of the leaf which is dependent on the leaf chlorophyll content (Richardson et al. 2002). The relationship between TE and SLA is negative (Wright et al. 1994; Nageswara Rao et al. 2001; Bindu Madhava et al. 2003) and between TE and SCMR positive (Bindu Madhava et al. 2003; Sheshshayee et al. 2006). SCMR and SLA are negatively correlated (Nageswara Rao et al. 2001; Bindu Madhava et al. 2003; Upadhyaya 2005). Whereas Nageswara Rao and Wright (1994) found these associations relatively stable across environments, Serraj et al. (2004) found them operative only under moisture-deficient conditions. SCMR has provided an easy tool to integrate TE trait in breeding programmes in groundnut (Nageswara Rao et al. 2001; Bindu Madhava et al. 2003; Serraj et al. 2004). As a noninvasive surrogate of TE, SCMR is easy to operate, reliable, fairly stable and low cost (Sheshshayee et al. 2006). SCMR is reported to be more stable than SLA (Upadhyaya 2005). It is also correlated with pod yield in groundnut (Reddy et al. 2004; Upadhyaya 2005). In a large-scale breeding programme, it is difficult to complete SCMR observations within a specified time and crop stage. It would be helpful to breeders if these measurements could be spread over time. In a 2-year experiment, observations on SLA and SCMR (surrogates of TE) were recorded at different times under differing soil moisture conditions on 18 genotypes included in the study. These observations were analyzed to ascertain the period when surrogates could be recorded in a breeding programme engaged in developing groundnut varieties tolerant to water-deficient conditions.

Materials and methods

The experiment was conducted in Alfisols in 2002/2003 and 2003/2004 postrainy seasons at ICRISAT Center, Patancheru, India. It comprised 18 diverse genotypes (Table 2) and was laid out in a randomized complete block design (RCBD) with three replications. The experiment in the 2002/2003 postrainy season was sown on 22 Jan 2003 and in the 2003/2004 on 23 Dec 2003 on flat beds. The plot size was three rows of 4 m length. The inter- and intra-row spacing was 30 cm and 10 cm, respectively. It received 375 kg ha^{-1} of single super phosphate before sowing and was protected from foliar diseases and foliage pests during the cropping period, as and

when necessary, to avoid damage to the leaves. Plots were kept weed free through manual weeding.

The experiment was subjected to mid-season moisture deficit 60 days after sowing (DAS) in the 2002/2003 postrainy season and to end-of-season moisture deficit 80 DAS in the 2003/2004 postrainy season. Before imposition of moisture deficit, it received normal irrigation (5 cm water in each irrigation at 10–12 days interval). In the 2002/2003 postrainy season, the mid-season moisture deficit was released after 44 days (104 DAS) and the regular irrigation was resumed up until the harvest. In the 2003/2004 postrainy season, the end-of-season stress continued up until harvest. Rainout shelters (ROS) were kept in stand by to avoid any interference from rains during the period of stress. In the 2002/2003 postrainy season, the mid-season moisture deficit was partial as water equal to 30% of cumulative pan evaporation was made available to the plots at 10–12 days interval. In the end-of-season moisture deficit, no irrigation was given.

In each season, observations on SCMR and SLA were recorded three times. In the 2002/2003 postrainy season, two observations were recorded during the moisture deficit period- 86 DAS and 96 DAS, and the third was taken after the release of moisture deficit 108 DAS. In the 2003/2004 postrainy season, the first observation was taken at the beginning of the moisture deficit (88 DAS) and the second and third observations were taken during the progressive moisture deficit (98 DAS and 109 DAS). The observations on SCMR and SLA were recorded on the second or third leaf from top on the main stem of randomly selected five competitive plants in each plot following the procedure described by Nageswara Rao et al. (2001). The leaves were plucked from the plants between 8.30 and 9.30 a.m. and brought to the laboratory in zipped polythene bags for recording observations. The SCMR was recorded twice on each leaflet along the mid-rib. After recording SCMR, the same leaves were used to record SLA ($cm^2\ g^{-1}$). After taking the fresh weight, the leaves were submerged in water for 4–6 h to allow them to become fully turgid. Excess water was removed with the help of a blotting paper. The weight and leaf area of turgid leaves were recorded. The leaves were then oven dried at 60°C for 2 days before recording their dry weights. The SLA was calculated using the following formula:

$$\text{SLA} = \text{Leaf area } (cm^2)/\text{Leaf dry wt (g)}$$

Statistical analysis

The mean values of observations in each plot on all the traits taken at different time intervals were subjected to preliminary statistical analysis for each season separately. Due to differing moisture deficit patterns in each season, the combined analysis over seasons could not be done. Simple two- way ANOVA considering genotypes as treatments and replications as the blocking structures was conducted to assess the differences among the genotypes. Interaction between the genotypes and time (the time when observations were taken for each trait) was assessed using the repeated measures analysis of variance of GENSTAT (Genstat, eighth edition) following the procedure of Winer (1962). Genotypic and phenotypic correlations were worked out between the traits.

Results and discussion

Genotype and trait performance

2002/2003 postrainy season

The genotypes differed significantly for SCMR and SLA. The time of observation also showed significant differences for both the traits. But the time of observation × genotype interaction was significant only for SCMR (Table 1). However, its variance was very small (5.155 with $P = 0.032$) in relation to variance for genotype (32 times more with $P < 0.001$) and time of observation (27 times more with $P < 0.001$). During the partial imposed mid-season drought, SCMR showed a significant increase whereas SLA showed a significant decrease between the first and the second observation (Table 2). But 4 days after the release of moisture deficit, SLA showed an increase over the second observation, however, the difference was not significant. The SCMR showed a slight but significant decline.

Among the genotypes, ICGS 76, ICGV 99029, ICR 48, TCGS 647 and CSMG 84-1 recorded the higher values (in descending order of values) for SCMR and CSMG 84-1, ICR 48, ICGS 76, ICGV

Table 1 Analysis of variance for repeated measurements of traits, 2002/03 and 2003/04 postrainy seasons, ICRISAT Center, Patancheru, India

Source of variation	Df	MSS		F value		Probability	
		2002/2003	2003/2004	2002/2003	2003/2004	2002/2003	2003/2004
SCMR							
Genotype	17	165.634	163.966	29.01	31.07	<0.001	<0.001
Residual	34	5.709	5.278				
Time	2	141.588	75.406	46.63	15.99	<0.001	<0.001
Time × Genotype	34	5.155	4.455	1.70	0.94	0.032	0.561
Residual	72	3.036	4.715				
SLA							
Genotype	17	2010.8	969.6	10.46	7.00	<0.001	<0.001
Residual	34	192.2	138.6				
Time	2	484.6	5321.1	4.14	50.74	0.020	<0.001
Time × Genotype	34	110.3	183.1	0.94	1.75	0.565	0.038
Residual	72	117.1	104.9				

SCMR—SPAD Chlorophyll Meter Reading; SLA—Specific Leaf Area

99029 and TCGP 6 lower values (in ascending order of values) for SLA. Genotypes ICGS 76, ICGV 99029, ICR 48 and CSMG 84-1 were common in desirable direction for both the surrogates of TE.

2003/2004 postrainy season

Genotype and time of observation differences for SCMR and SLA were significant (Table 1). Unlike the 2002/2003 postrainy season, the time of observation x genotype interaction was significant only for SLA. However, the variance for interaction was relatively smaller (1.75 with $P = 0.038$) than that of genotype (four times more with $P < 0.001$) and time of observation (29 times more with $P = 0.020$). Wright et al. (1993) also observed very low G × E for SLA along with TE and carbon isotope discrimination and suggested that selection for these traits could be done in single environment, be it sufficiently watered or water limited and in the greenhouse or the field. The SCMR initially increased significantly as the moisture deficit progressed, then, it declined significantly (Table 2). SLA showed a progressive increase as the moisture deficit increased, but the difference between the second and third observations was non-significant. Among the genotypes, ICGV 99029, ICGS 76, ICR 48, TCGS 647 and ICGV 86590 recorded higher SCMR (in descending order of values) and ICGV 99029, CSMG 84-1, ICGV 86031, ICR 48 and TCGP 6 lower SLA values (in ascending order of values). Genotypes ICGV 99029 and ICR 48 were common in desirable direction for both the surrogates of TE.

There was a general agreement in genotype and trait performance between 2002/2003 and 2003/2004 postrainy seasons (Table 1). The pattern of change in SCMR over time of observation was similar irrespective of differing soil moisture deficit between the two seasons (Table 2). In the 2002/2003 postrainy season, the experiment was subjected to partial mid-season drought with subsequent release of moisture deficit up until harvest. Whereas in the 2003/2004 postrainy season, the drought was progressive as the experiment did not receive any irrigation after 80 DAS up until harvest. The pattern of change in SLA was different between the two seasons and it appeared to be more sensitive than SCMR to differing soil moisture deficit. A few differences in genotype and trait performance and differing patterns of change in traits over time between the two seasons could be due to their differing moisture deficit patterns.

In both the seasons, ICGV 99029 and ICR 48 were high in SCMR and low in SLA values. They will make very good parents for water-use-efficiency trait in breeding programmes. Other good parents include ICGS 76, TCGS 647 and TCGP 6.

Table 2 Mean values of SCMR (SPAD Chlorophyll meter reading) and SLA (specific leaf area), 2002/2003 and 2003/2004 postrainy seasons, ICRISAT Center, Patancheru, India

	SCMR 1		SCMR 2		SCMR 3		SLA 1		SLA 2		SLA 3	
Genotype	2002/2003	2003/2004	2002/2003	2003/2004	2002/2003	2003/2004	2002/2003	2003/2004	2002/2003	2003/2004	2002/2003	2003/2004
ICGV 86590	44.10	48.50	48.37	53.43	44.20	49.10	151.67	125.73	147.90	125.50	152.77	129.13
ICGV 89104	39.77	42.70	44.03	46.03	42.93	43.30	159.57	127.17	140.77	138.30	149.37	142.43
ICGV 93280	39.10	44.87	44.57	47.07	43.77	47.20	145.97	104.77	136.73	122.37	133.03	129.57
ICGV 93291	33.07	39.90	35.83	40.83	35.90	35.33	156.27	111.33	157.97	132.83	147.73	136.87
ICGV 95322	39.10	45.40	38.83	45.80	40.93	44.27	133.80	117.37	145.43	125.13	137.53	129.97
ICGV 95492	38.77	42.87	42.17	45.13	39.53	42.10	153.83	118.23	147.77	128.10	142.77	138.30
ICGV 99029	49.43	57.23	50.10	57.20	53.00	53.90	129.50	94.60	122.73	111.53	115.40	112.53
J 11	40.43	43.83	42.10	47.20	40.07	42.57	155.03	115.87	149.03	133.57	144.87	142.50
ICR 48	44.67	50.47	51.67	52.27	48.47	51.70	117.17	91.10	107.10	114.00	106.00	116.93
TCGS 647	44.90	50.93	50.83	50.03	46.00	50.67	142.47	115.23	143.17	120.20	137.97	128.47
ICGV 86031	42.97	49.37	46.37	50.77	44.70	50.60	143.70	104.47	131.60	106.47	134.53	110.17
ICGS 76	50.63	52.00	52.63	53.57	52.17	54.07	121.73	99.83	115.93	152.47	113.27	112.00
ICGS 86158	39.03	43.17	41.07	45.93	37.73	42.20	155.03	124.23	152.17	141.23	149.10	142.53
ICGV 91284	40.60	44.97	42.27	47.17	40.23	43.07	138.37	116.90	151.13	135.73	157.00	137.73
TCGP 6	41.33	48.63	45.20	49.87	42.77	49.33	136.87	108.83	125.57	120.23	141.23	120.37
CSMG 84-1	44.47	50.00	46.93	50.83	47.20	49.10	106.87	97.77	101.50	108.80	117.50	113.60
JL 24	39.10	44.43	43.20	45.30	43.23	43.90	163.60	114.80	145.23	133.50	156.43	156.97
TMV 2	39.47	44.83	42.87	48.33	41.87	44.53	151.50	119.80	137.63	129.27	148.73	144.97
Mean	41.72	46.89	44.95	48.71	43.59	46.50	142.4	111.60	136.6	126.60	138.1	130.30
SEM (±)	1.07	1.10	1.14	1.52	1.15	1.13	6.02	3.82	6.51	8.54	8.07	4.75
CV (%)	4.43	4.08	4.40	5.39	4.58	4.23	7.33	5.93	8.25	11.68	10.13	6.32
LSD (at 5% level of *P*)	3.06	3.17	3.28	4.36	3.31	3.26	17.31	10.97	18.70	24.54	23.21	13.66
F Prob.	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	<0.001	<0.001

SCMR 1, 2 and 3 and SLA 1, 2 and 3 are observations taken at different times in the experiments (details given in Materials and methods)

Correlations

SCMR recorded at three different times under differing soil moisture deficit in each season (2002/2003 and 2003/2004) showed highly significant correlation with each other (genotypic $r = 0.949–1.000$; phenotypic $r = 0.778–0.858$). Similarly, the genetic correlation between SLA recorded at different times was also highly significant (Table 3). The negative association between SCMR and SLA reported earlier (Wright et al. 1994; Nageswara Rao et al. 2001; Bindu Madhava et al. 2003) and also observed in the present study was insensitive to time of observation.

From the above results it is clear that SCMR/SLA observations can be recorded at any time after 60 days of the crop growth. However, as suggested by Serraj et al. (2004), these measurements should be recorded after imposition of moisture deficit and particularly at mid-way through stress. This gives a large flexibility to breeders who have to record observations in a large number of segregating and breeding populations in the field. The significance of time of observation × genotype interaction in one year and non-significance in the other year for both SCMR and SLA, should not limit the application of this conclusion as the variance for significant interaction for both the traits was much smaller than the variance for genotypes and time of observation for both the traits. Further, SCMR showed similar pattern of change over time of observation in both the years inspite of the time of observation × genotype interaction being significant in one year. However, it would be advisable to give attention to both, SCMR and pod yield, in developing high-yielding, water-use efficient genotypes in groundnut. In experiments, where precise estimates are required for characterization or phenotyping for TE, direct measurement of TE remains the best option. As both ICGV 99029 and ICR 48 were high in SCMR and low in SLA values in both the seasons, they will make good parents in a drought resistance breeding programme. ICGV 99029 belongs to the Spanish botanical group and ICR 48 to the Virginia botanical group. In various on-station yield trials at ICRISAT Center during 1999/2000–2003/2004 postrainy seasons, both genotypes ranked among the top 10 with dry pod yield ranging between 4 and 6 t ha^{-1}.

Table 3 Genotypic and phenotypic correlations between specific leaf area (SLA) and SCMR (SPAD Chlorophyll meter reading) in 18 genotypes in 2002/2003 and 2003/2004 postrainy seasons, ICRISAT, Patancheru, India

Trait pair	Genotypic correlation		Phenotypic correlation	
	2002/2003	2003/2004	2002/2003	2003/2004
SCMR 1-SLA1	–0.665**	–0.709**	–0.635**	–0.601**
SCMR 1-SLA2	–0.771**	–0.717**	–0.556*	-0.303^{NS}
SCMR 1-SLA3	–0.833**	–0.845**	–0.525*	–0.645**
SCMR 1-SCMR 2	0.958**	0.998**	0.842**	0.778**
SCMR 1-SCMR 3	0.971**	0.998**	0.858**	0.830**
SCMR 2-SLA1	–0.631**	–0.650**	–0.472*	-0.384^{NS}
SCMR 2-SLA2	–0.769**	–0.607**	–0.598**	-0.236^{NS}
SCMR 2-SLA3	–0.824**	–0.825**	–0.477*	–0.589**
SCMR 2-SCMR 3	0.949**	1.000**	0.819**	0.798**
SCMR 3-SLA1	–0.721**	–0.661**	–0.587*	–0.538*
SCMR 3-SLA2	–0.863**	–0.544*	–0.673**	-0.332^{NS}
SCMR 3-SLA3	–0.932**	–0.836**	–0.606**	–0.669**
SLA1-SLA2	0.994**	0.652**	0.675**	0.404^{NS}
SLA1-SLA3	0.989**	0.845**	0.650**	0.618**
SLA2-SLA3	0.998**	0.781**	0.662**	0.367^{NS}

**Significant at 1% level of *P*; *Significant at 5% level of *P*; ^{NS}Non significant

SCMR 1, 2 and 3 and; SLA 1, 2 and 3 are observations taken at different times in the experiments (details given in Materials and methods)

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