Genotypic differences for traits associated with water-use efficiency and yield in groundnut (*Arachis hypogaea* L.) under rainfed conditions

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This study was undertaken to ascertain the genetic differences in 32 genotypes for pod yield and its component traits, and morpho-physiological characters, namely carbon isotope discrimination (Δ C^{13}), enrichment of oxygen isotopes (Δ O^{18}), specific leaf area (SLA) and SPAD chlorophyll content (SCMR) that are known to influence water-use efficiency (WUE) in groundnut. Highly significant genotypic differences (P<0.01) were observed for Δ C^{13}, Δ O^{18}, SLA, SCMR, harvest index, shelling percent, plant height, and pod, kernel and haulm yields and total dry matter production. A strong inverse relationship of SCMR with SLA (r = –0.626, P<0.01) and Δ^{13}C (r = –0.552, P<0.01) was observed. Very high broad sense heritability estimates (81%) were obtained from SCMR. Δ C^{13} was positively associated with harvest index (r = 0.535, P<0.01) and its association with haulm yield (r = –0.617, P<0.01) and total dry matter (r = –0.522, P<0.01) were negative. The cluster analysis revealed that genotypes ICR 11 and JUG 28 were the most closely related, and GG 20 and JUG 13 were the least related genotypes. This has significance for future breeding due to the potential to produce trangressive segregrants through hybridization of these distant genotypes. The genotypes that involved ICGS 76 as one of the parents in their pedigree scored very high SCMR values.

Keywords: WUE; Δ^{13}C; Δ^{18}O; SLA; SCMR; Groundnut, Cluster Analysis, Genotype differences, Rainfed conditions, Yield.

Reduction in crop yield due to water stress is currently estimated at 65% globally and over 80% in many developing countries. In India, groundnut is the most important oilseed crop, and is grown on about 8.0 million ha. Mostly (80%) groundnut is grown under rainfed situations during *kharif* (June to October) season in the semi-arid regions, and thus the crop is vulnerable to the vagaries of the monsoon. Irrigated groundnut acreage in India comprises about 19% of the total groundnut cultivated area (FAO, 2004) and further increase in the irrigated area under groundnut cultivation appears unattainable due to depleting water resources and increasing domestic water demand with increasing population size. However, irrigation can increase groundnut production by up to 19% over dry land production (Lamb *et al*. 1997). Hence, it is desirable to optimise the water-use by the crop both through agronomic interventions and genetic manipulations.

Water-use efficiency (WUE), defined as the ratio of photosynthesis to transpiration, is an important factor deciding the productivity under water-limited situations (Nageswara Rao and Wright, 1994). At the same time, increased WUE (when transpiration loss is minimum) at the cost of yield has no value in agricultural systems. However, groundnut has the potential to have very high photosynthetic capacity accompanied by low stomatal conductance levels, which transforms into high WUE without sacrificing carbon assimilation and possibly yield (Wright *et al*. 1993). This attribute would allow the successful breeding of highly water-use efficient genotypes while maintaining optimum or even high levels of yield.

Farquhar et *al*. (1982) proposed that the transpiration efficiency of a genotype could be estimated by measuring the carbon isotope discrimination (Δ) in leaves. Transpiration efficiency in groundnut has been reported to be negatively correlated with Δ (Hubick *et al*. 1986, 1988; Wright *et al*. 1988, 1994). Measurement of Δ is rapid but is expensive and may not be applicable in large segregating breeding populations. On the other hand, specific leaf area (SLA, leaf area per unit leaf dry weight) is highly correlated with Δ in groundnut (Nageswara Rao and Wright 1994). Also, SLA (cm^2g^{-1}) is reported to be closely and negatively correlated with WUE in groundnut (Wright *et al*. 1988, 1994). This suggests that SLA can serve as a surrogate while selecting for WUE in groundnut breeding programmes. Nageswara Rao *et al*. (2001) evaluated the use of a hand-held portable SPAD chlorophyll meter for rapid assessment of drought tolerance in groundnut. They observed a significant positive correlation between the chlorophyll meter reading (SCMR) and SLA, and suggested that SCMR could be used as a rapid and reliable measure to identify genotypes with low SLA, and hence high transpiration efficiency in groundnut.

Oxygen isotope enrichment ($\Delta^{18}O$) that occurs during transpiration has emerged as a potential tool for time averaged measurement of stomatal conductance and transpiration rate (Sheshshayee *et al*. 2003). It is inadequate if genotypes possess only high WUE. Since the crop growth rate depends on total water-use, identifying genotypes with high transpiration rate per unit leaf area is important for crop improvement. One of the approaches to identify relatively high water-use coupled with high WUE is by identifying genotypes with low $\Delta^{13}C$ and high $\Delta^{18}O$ (Sheshshayee *et al.* 2003)

The present study was conducted with the objective to detect genetic variation for the morpho-physiological characters, namely $\Delta^{13}C$, $\Delta^{18}O$, SLA and SCMR that are known to influence WUE, as well as variability for pod yield and its component traits in groundnut.

Materials and Methods

Twenty advance breeding lines of groundnut along with eight parental lines involved in their pedigree and four commercial varieties as yield checks formed the experimental material for the study (Table 1). These 20 advance breeding lines were developed at four Indian locations (Junagadh, Jalgaon, Tirupati and Patancheru) for enhanced WUE. A replicated breeding trial with these 32 genotypes was established under rainfed situations at Junagadh (21.31 ºN latitude, 70.36 ºE longitude and at an altitude of 61 m from mean sea level) during 2003. The sowing was done during the second fortnight of June. All recommended agronomic practices were followed (except irrigation) for raising the crop.

After 45 days of sowing, the second fully opened leaf from the apex of five plants selected at random in each replication was used to measure chlorophyll content using a Minolta SPAD chlorophyll meter (Minolta Corp., Ramsey, N.J.) in the morning (0800-0900) hours. Leaf area of these leaves was measured with a LI3100 leaf area meter (LI-COR Inc., Lincoln, NE). Leaves were then oven-dried at 60 ºC for 72 hours and weighed. Specific leaf area was calculated as the ratio of leaf area to leaf dry weight. Leaves were then finely powdered, put through a sieve of 80 mesh and analysed for carbon isotope discrimination ($\Delta^{13}C$) and enrichment of oxygen isotopes ($\Delta^{18}O$) at the National Facility for Stable Isotopes, University of Agricultural Sciences, Bangalore (India).

At the time of harvest, five plants were randomly selected in each replication to record plant height, haulm yield and total dry matter production per plant and harvest index based on pod yield. Plot yield was recorded at harvest and expressed in kg ha^{-1}. Shelling percent obtained based on the five plants was used to derive kernel yield per hectare (kg ha^{-1}).

The replicated data were subjected to the analysis of variance (ANOVA) and descriptive statistics were derived to elucidate the amount of genetic variation available for the traits and in the material studied. Pearson correlations and cluster analysis using Euclidean distances based on mean values for 11 traits (Table 2) were performed using the statistical software SYSTAT 10 to establish genetic divergence among the 32 genotypes studied. Heritability (broad sense) was estimated for six traits, *viz*., pod yield, HI, SCMR, SLA, Δ C^{13} and Δ O^{18} as the percentage of genotypic variance to phenotypic variance.

Results and Discussion

Analysis of variance performed on the mean values collected from the random samples from each replication indicated highly significant

(P<0.01) variances due to genotypes for Δ C^{13}, Δ O^{18}, SLA, SCMR, harvest index, shelling percent, plant height, pod, kernel and haulm yields and total dry matter production. This revealed the existence of considerable genetic variation for leaf morpho-physiological characters (Δ C^{13}, Δ O^{18}, SLA and SCMR) that influence WUE in groundnut, partitioning efficiency (HI) and the yielding ability (pod and kernel yield, shelling outturn, plant height, total dry matter and haulm yield).

Highly significant genotypic differences were also observed for Δ C^{13}; the genotypic mean values ranged from 21.06 ± 0.2‰ to 22.32 ± 0.2‰ with a coefficient of variation (CV) of 1.31%. Least discrimination for the stable isotope of carbon (Δ C) ^{13} was observed in the commercial variety, Somnath, followed by the genotype JAL 03. Significant genotypic variation observed for $\Delta^{18}O$ ranged from 18.55 ± 0.55‰ (ICGS 76) to 21.62 ± 0.55‰ (TIR 16) with a CV of 3.84%. The range indicates that considerable variation does exist in the material for genetic exploitation. The SCMR values ranged from 31.3 ± 1.14 to 41.6 ± 1.14 and SLA ranged from 206 ± 14.48 to 98 ± 14.48 cm^2g^{-1} with CV values of 4.4% and 8.2%, respectively. The lowest SLA was recorded in TIR 16 (206 cm^2g^{-1}) followed by ICGV 86031 (211 cm^2g^{-1}).

Table 1 Details of material used

S.No	Genotype	Pedigree	Originating Centre
Advanced breeding lines			
1	JUG 28	ICGS 76 x CSMG 84-1	Junagadh
2	ICR 27	ICGS 76 x CSMG 84-1	Patancheru
3	JUG 15	ICGS 76 x CSMG 84-1	Junagadh
4	JAL 03	ICGS 76 x CSMG 84-1	Jalgaon
5	ICR 24	ICGS 44 x ICGS 76	Patancheru
6	ICR 10	ICGS 44 x ICGS 76	Patancheru
7	JUG 27	ICGS 76 x CSMG 84-1	Junagadh
8	ICR 11	ICGS 44 x ICGS 76	Patancheru
9	ICR 43	ICGS 44 x ICGS 76	Patancheru
10	ICR 12	ICGS 44 x ICGS 76	Patancheru
11	JAL 17	ICGS 44 x CSMG 84-1	Jalgaon
12	ICR 40	TAG 24 x ICGV 86031	Patancheru
13	JUG 13	ICGS 76 x CSMG 84-1	Junagadh
14	ICR 20	TAG 24 x ICGV 86031	Patancheru
15	TIR 47	K 134 x TAG 24	Tirupati
16	JUG 33	ICGS 44 x CSMG 84-1	Junagadh
17	JAL 05	ICGS 44 x CSMG 84-1	Jalgaon
18	JAL 36	ICGS 44 x CSMG 84-1	Jalgaon
19	TIR 42	ICGV 86031 x TAG 24	Tirupati
20	TIR 16	ICGV 86031 x TAG 24	Tirupati
Parents			
21	ICGS 76	TMV 10 x Chico	Patancheru
22	GG 20	GAUG 10 x R 33-1	Junagadh
23	ICGV 86031	F334A-B-14 x NCAc 2214	Patancheru
24	CSMG 84-1	Selection from MA 10	Mainpuri
25	ICGS 44	Selection from Robut 33-1	Patancheru
26	TAG 24	TGS 2 (TG 18 x M 13) x TGE 1	Trombay
27	GG 2	J 11 x EC 16659	Junagadh
28	K 134	Kadiri 3 x JL 24	Dharwad
Checks			
29	Somnath	Induced mutant of (TG 18A x M 13)	Junagadh
30	JL 24	Selection from ‘EC 94943’	Jalgaon
31	JL 220	JL 80 x VG 77	Jalgaon
32	SB XI	Ah 4218 x Ah 4354	Junagadh

Table 2. Eleven quantitative traits used in the study for cluster analysis

Sl. No.	Trait	Unit of measurement
1	Pod Yield	kg ha^{-1}

2	Kernel yield	kg ha^{-1}
3	Haulm yield	g $plant^{-1}$
4	Total dry matter	g $plant^{-1}$
5	Shelling	%
6	Plant height	cm
7	HI	%
8	SCMR	-
9	SLA	cm^2 g^{-1}
10	$\Delta^{13}C$	‰
11	$\Delta\ ^{18}O$	‰

Table 3 Performance of top ten genotypes for pod yield, harvest index and leaf morpho-physiological characters contributing to WUE in groundnut

Character	Variability Parameters				Potential Genotypes	
	CV %	**Range**	**Population Mean**	**Heritability (%)**	**Top 10 genotypes**	**Range**
Pod Yield (kg ha^{-1})	18	1331- 3407	2459	60	GG 20, CSMG 84-1, JL 220, JL 24, ICR 10, ICGS 76, ICR 24, JUG 27, JAL 17 and JUG 28	2812 - 3407
HI (%)	14	26 - 54	39.19	67	JAL 36, ICR 20, JAL 17, TAG 24, JL 220, ICR 40, JL 24, ICR 24, TIR 42 and TIR 47	43 - 54
SCMR	4.4	31.3 – 41.6	36.39	81	JUG 28, ICGS 76, ICR 27, JUG 15, JAL 03, ICR 24, ICR 10, GG 20, JUG 27 and ICR 11	38.4 - 41.6
SLA (cm^2 g^{-1})	8.2	206 - 298	248.91	69	TIR 16, ICGV 86031, JUG 28, ICR 40, ICR 27, ICR 10, ICR 11, CSMG 84-1, JUG 27 and ICR 20	206.1-234.1
$\Delta^{13}C$ (‰)	1.31	21.06 - 22.32	21.63	71	Somnath, JAL 03, CSMG 84-1, GG 20, ICGS 44, JUG 27, JUG 28, JL 24, ICR 11 and JUG 15	21.06 - 21.41
$\Delta\ ^{18}O$ (‰)	3.84	18.55 - 21.62	20.24	35	TIR 16, ICR 40, JUG 28, ICR 10, ICR 11, ICR 20, JUG 27, JL 220, ICR 24 and TIR 42	20.58 - 21.62

Table 4 Euclidean distance (rounded to second decimal place) between pairs of cultivars based on eleven quantitative traits

Sr. No.	Cluster containing genotypes A and B	Euclidean distance between A and B	No. of genotypes in

Genotype A	Genotype B	genotypes	new cluster
1. ICR 11	JUG 28	7.09	2
2. JAL 03	JUG 15	10.47	2
3. ICR 11	ICR 43	11.72	3
4. JL 220	JL 24	12.43	2
5. TIR 16	JUG 33	13.76	2
6. ICR 10	ICR 24	13.89	2
7. ICR 10	ICGS 76	13.93	3
8. JAL 03	JAL 05	18.90	3
9. ICR 10	JAL 17	19.34	4
10. JAL 03	JAL 36	21.30	4
11. ICR 11	JUG 27	22.76	4
12. GG 2	SB XI	25.70	2
13. JAL 03	ICR 20	26.24	5
14. TIR 47	ICGS 44	26.99	2
15. TIR 47	ICR 40	27.65	3
16. GG 2	TAG 24	30.50	3
17. ICR 11	ICR 10	31.17	8
18. TIR 47	ICR 12	31.33	4
19. Somnath	ICR 27	32.90	2
20. JAL 03	TIR 47	35.71	9
21. JAL 03	Somnath	38.75	11
22. GG 2	TIR 42	40.70	4
23. JAL 03	TIR 16	49.24	13
24. K 134	ICGV 86031	55.39	2
25. K 134	GG 2	55.82	6
26. ICR 11	JAL 03	57.37	21
27. CSMG 84-1	ICR 11	69.93	22
28. CSMG 84-1	JL 220	72.00	24
29. K 134	CSMG 84-1	75.60	30
30. JUG 13	K 134	116.29	31
31. GG 20	JUG 13	121.23	32

Among the genotypes studied, pod yield ranged from 1331 ± 313 to 3407 ± 313 kg ha^{-1}. The variety GG 20 was found to be a high yielder in terms of pod (3407 kg) and kernel (2520 kg) yields per hectare. Harvest index, a measure of partitioning efficiency, ranged from 26 ± 11.24 to 54 ± 11.24%. A sufficient range of variability was also observed for other traits, namely shelling percent, plant height, kernel and haulm yields, and total dry matter production. TIR 47 and K 134 recorded the highest shelling outturn (77%) and ICR 20 was the shortest (27 cm) among the genotypes studied. The top ten genotypes identified with desirable values for pod yield, harvest index and four leaf morpho-physiological traits known to influence WUE in groundnut are given in Table 3.

JAL 17

Apart from assessing genetic variability, the character associations between surrogate measures of WUE were also worked out. The study confirmed the strong inverse relationship of SCMR with SLA (r = –0.626, P<0.01) reported earlier by Nageswara Rao *et al*. (2001), and SCMR with Δ C^{13} (r = –0.552, P<0.01). The SCMR was also found to be positively associated with pod (r= 0.505, P<0.01), kernel (r = 0.384, P<0.05) and haulm (r = 0.401, P<0.05) yields, and total dry matter (r = 0.375, P<0.05). These strong inter-relationships suggest that SCMR can not only potentially be used as an indirect measure of SLA, but also for total dry matter production. The association of Δ C^{13} with haulm yield (r = –0.617, P<0.01) and total dry matter (r = –0.522, P<0.01) were negative, suggesting that selection for low Δ C^{13} might result in production of more dry matter. But the positive association between Δ C^{13} with harvest index (r = 0.535, P<0.01) is an indication to the breeders that selection for higher total dry matter through lower values of Δ C^{13} might not result in enhanced economic yield. Hence, a judicious application of selection strategy by optimising the $\Delta\ ^{13}C$ and harvest index is essential to achieve high yields with improved WUE.

The SCMR that essentially estimates the chlorophyll content in the leaves is a simple and rapid measure, and can very well be incorporated into the selection strategy. The top five genotypes for SCMR values were JUG 28, ICGS 76, ICR 27, JUG 15 and JAL 03 in decreasing order. Of these genotypes, four (JUG 28, ICR 27, JUG 15 and JAL 03), though originating from different Indian locations (Table 1), were derived from a common cross, ICGS 76 x CSMG 84-1. Two genotypes, ICR 24 and ICR 10, ranking sixth and seventh for SCMR values were, derived from a cross ICGS 44 x ICGS 76. Thus, the study suggests that ICGS 76 and its derivatives are important donors of this trait. ICGS 76 has also registered the highest haulm and total dry matter yields.

Estimation of genetic relatedness among the available experimental material after quantification of variability for individual traits would assist the breeders in selecting parents for future hybridisation programmes. Instead of looking at individual traits, a comprehensive grouping of materials based on variability for a number of traits is essential. Accordingly, mean values of 11 traits were used to estimate the relatedness among the 32 genotypes studied. The cluster analysis resulted in many groups (Figure 1) indicating that the genotypes studied were genetically diverse. The Euclidean distances between pairs of genotypes have been given in Table 4. The genotypes, ICR 11 and JUG 28, were the most closely related while GG 20 and JUG 13 were the least related genotypes. Meticulous examination of Table 4 revealed that 11 pairs of genotypes were joined at Euclidean distance of less than 25. Interestingly, all the genotypes of these pairs had at least one parent common in their pedigree while in some cases both the parents were common. For example, the genotype JAL 03 and JUG 15 (ICGS 76 x CSMG 84-1), ICR 11 and ICR 43 (ICGS 44 x ICGS 76), and ICR 10 and ICR 24 (ICGS 44 x ICGS 76) had either ICGS 76, ICGS 44, CSMG 84-1 or combination of any of these parents in their pedigree. As the divergence between the member-genotypes of a pair increased, the genotypes involved in the parentage were different, an exception being ICR 11 and ICR 10, which were joined at 31.17 distance but had the common parentage (ICGS 44 x ICGS 76).

Conclusion

Considerable genotypic variation was observed among the 32 genotypes for leaf morpho-physiological traits allied to water-use efficiency apart from the yield and related parameters. Easily measurable characters such as SLA and SCMR can be used to indirectly select for low carbon isotope discrimination ($\Delta^{13}C$), strong associations of which have already been established in groundnut with WUE. Furthermore, very high broad sense heritability estimates (81%) were observed for this character (SCMR). The genotypes (20 advance breeding lines) owing to selection at diverse locations do exhibit variability; though, common parents were involved in some cases. High values for SCMR, observed in the majority of the genotypes derived from the crosses involving ICGS 76 as one of the parents, offer enough scope for improving SCMR and thereby WUE by using ICGS 76 as a donor for SCMR. This genotype is also endowed with high haulm yield and total dry matter production and offers immense scope for tailoring genotypes with high WUE and enhanced pod yield if genes from partitioning efficient genotypes like TAG 24 are recombined. The grouping of the genotypes based on Euclidean distance will help in future breeding programmes to select parents based on their genetic distance on the dendrogram. A cross between two genotypes that figure in the extreme ends such as JUG 13 and GG 20 would throw out more transgressive segregants which would be further selected for improved WUE through its surrogate measures, high yield and its related attributes.

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