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Testing the significance for difference in mean performance for growth, yield and WUE linked traits using t-test in F_7 and F_8 RILs of groundnut (*Arachis hypogaea* L.)

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Abstract
The RILs of high and low performing class of SCMR and SLA were tested for their performance for all the traits using t-test, non-significance of which for most of the traits suggest that their performances are stable across the generations. However, the trait days to 50 *per cent* flowering significantly differed for two generations, F_7 and F_8 which may be attributed to the seasonal influence on flowering since the earlier is grown in *kharif* 2014 and the latter in *summer* 2015.

Keywords: Groundnut, Recombinant Inbred Lines (RILs), Water use efficiency (WUE), Soil Plant Analysis Development (SPAD) Chlorophyll Meter Reading (SCMR), Specific Leaf Area (SLA), Correlation and Path analysis, paired t-test.

1. Introduction
The possible insufficiency of land and water resources to meet the needs of humanity, particularly those of agriculture, is a pressing issue that is currently affecting roughly a third of the world's population [1-4]. Numerous efforts to mitigate drought through breeding resilient varieties are underway across the world. Progress is, however, hampered because drought tolerance is a complex trait that is controlled by many genes and its full expression is affected by the environment. Since physiological traits associated with drought tolerance is complex new knowledge of easily measurable surrogate traits of water use efficiency i.e. SLA and SCMR can be integrated in breeding programme and selection schemes in groundnut.
Groundnut being one of the rich energy contributors, having 48–50 per cent oil content and protein per cent of 25–28 in its kernels [5] is also under threat since two thirds of the global peanut production occurs in rainfed regions where rainfall is erratic and insufficient [6]. In the present study the F_7 RILs obtained by crossing two genotypes followed by repeated selfing or sibling mating were used for the study and analysis using paired t-test. A paired t-test is used to compare two population means where you have two samples in which observations in one sample can be paired with observations in the other sample.

Material and method
The experiment was carried out in the interim of *kharif* 2014-2015 at GKVK, Bangalore. Experimental material consisted of mapping population of 230 RILs of cross NRCG 12568 × NRCG 12326 segregating for Water Use Efficiency and the checks included TMV-2 and KCG-2 genotypes. The 230 F_7 generation RILs were sown in augmented design with 4 checks including parents during *kharif* 2014 for phenotypic evaluation. Each RIL sown in a single row of 1.5m with a spacing of 30 cm between rows and 10 cm between plants within the row. Recommended fertilization application and agronomic practices as per the package of practices mentioned for this region were followed. Data were recorded on randomly selected five plants from each RIL and average value was used for the statistical analysis for 10 characters viz., days to 50% flowering, plant height (cm), number of primary branches per plant, number of mature pods per plant, pod yield per plant (g), sound mature kernel (SMK) percentage, kernel yield per plant (g), shelling percentage, SPAD chlorophyll meter reading and specific leaf area (cm^2/g).
Observations of above quantitative traits were recorded in F_7 and F_8 generation and were subjected to statistical analysis.

Statistical analysis

t-test: The performance of selected RILs of extreme classes of F_8 and F_7 generations were compared using t-test

$t = (\bar{X}_1 - \bar{X}_2) / [\sqrt{sp^2 (1/n_1 + 1/n_2)}]$ [7]

Where,

$\bar{X}_1$ = mean of sample 1

$\bar{X}_2$ = mean of sample 1

Results and Discussion:

Means for the top performers in each of low and high performing class of SCMR and SLA (fig. 1) were calculated (Table 1, Table 2, Table 3, and Table 4) and these means were compared using t-test.

1. Low performing class of SCMR

Non-significance in the performance of the genotypes of low SCMR performing class was observed between F_7 and F_8 for the traits such as primary branches per plant (0.512), SCMR (0.295), SLA (0.013), pods per plant (0.349), kernel yield per plant (g) (0.080), shelling percentage (0.041), SMK percentage (0.068) and pod yield per plant (0.021). However, significant difference in the performance of the genotypes for days to 50 *per cent* flowering (37.147) was observed between F_7 and F_8 generations (Table 5).

2. High performing class of SCMR

Non-significance in the performance of the genotypes of high SCMR performing class was observed between F_7 and F_8 for the traits such as primary branches per plant (1.574), SCMR (0.034), SLA (0.010), pods per plant (0.136), kernel yield per plant (g) (2.471), shelling percentage (0.228), SMK percentage (0.010) and pod yield per plant (0.719). On the other hand significant difference in the performance of the genotypes for days to 50 *per cent* flowering (28.107) was observed between F_7 and F_8 generations (Table 5).

3. Low performing class of SLA

Non-significance in the performance of the genotypes of low SLA performing class was observed between F_7 and F_8 for the traits such as primary branches per plant (1.212), SCMR (0.149), SLA (0.020), pods per plant (0.102), kernel yield per plant (g) (0.578), shelling percentage (0.031), SMK percentage (0.120) and pod yield per plant (2.004). Howbeit, significant difference in the performance of the genotypes for days to 50 *per cent* flowering (37.990) was observed between F_7 and F_8 generations (Table 5).

4. High performing class of SLA

Non-significance in the performance of the genotypes of high SLA performing class was observed between F_7 and F_8 for the traits such as primary branches per plant (0.269), SCMR (0.037), SLA (0.052), pods per plant (0.168), kernel yield per plant (g) (0.019), shelling percentage (0.015), SMK percentage (0.009) and pod yield per plant (2.004). However, there was significant difference in the performance of the genotypes for days to 50 *per cent* flowering (32.967) between F_7 and F_8 generations (Table 5).

Considering the above results the discussion clubbed for high and low classes of SCMR and SLA describes that the non-significant differences in the mean performance of the genotypes of all the four classes for all the traits except for days to 50 *per cent* flowering reinforces that the genotypes performed stably across seasons. Significant difference in the mean performance of genotypes for days to 50 *per cent* flowering may be attributed to interaction of this trait with changing day length and other environmental parameters in two different seasons in *kharif* 2014 and later in *summer* 2015.

As each RIL is an inbred strain, and so can be propagated eternally, a panel of RILs has a number of advantages for genetic mapping: one need to genotype each strain only once; one can phenotype multiple individuals from each strain to reduce individual, environmental, and measurement variability; multiple invasive phenotypes can be obtained on the same set of genomes; and, as the breakpoints in RILs are more dense than those that occur in any one meiosis, greater mapping resolution can be achieved [8]. So, in this study the non-significance for most of the traits in the paired t- test justifies the characteristic features of RILs and the traits under study have stabilized over generations and thus can be utilised for the mapping programmes.

Table 1: Mean performance of the genotypes of low SCMR class related to growth, yield and surrogate traits related to WUE in F_7 and F_8 RILs of cross NRCG 12568 × NRCG 12326 in groundnut

RILs No.	D to 50 *per cent* flowering		Primary branches per plant		Pod number per plant		Pod yield per plant (g)		Kernel yield per plant (g)		SCMR		SLA (cm^2/g)		Shelling percentage	
	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8
473	31	47.0	2.0	2.0	8.40	16	3.28	13.9	2.18	7.1	32.96	33.7	186.16	190.56	66.46	51.08
123	31	48.0	2.0	4.0	18.00	32	11.64	17.8	5.78	10.5	33.70	34.68	273.21	165.18	49.66	58.99
246	34	48.0	4.0	3.0	7.20	13.2	4.08	8.54	3.28	6.22	35.78	34.08	183.53	181.26	64.57	72.83
248	32	46.0	2.0	2.0	14.00	11.4	29.22	19.02	26.48	15.82	35.80	36.02	57.44	66.58	70.28	86.69
222	32	48.0	4.0	2.0	8.40	17	27.48	15.1	24.30	9.6	35.92	32.98	82.81	92.85	57.49	63.58
249	31	47.0	3.0	3.0	5.00	16	3.54	14.5	3.50	11.1	36.02	35.48	180.39	196.14	77.09	76.55
11	31	47.0	3.0	3.0	6.10	5.4	4.62	3.36	2.66	2.08	36.14	36.88	274.74	283.45	70.39	61.90
84	32	48.0	2.0	4.0	8.10	7	7.36	6.4	6.14	3.5	36.14	37.68	200.70	198.56	59.27	39.06
233	32	46.0	4.0	2.0	10.00	16.6	6.22	9.32	2.82	7.42	36.22	34.7	150.85	177.15	66.82	79.61
42	33	48.0	3.0	2.0	14.00	9.6	12.20	7.22	6.96	4.9	37.82	37.88	127.99	110.68	57.05	67.86
S.Ed	0.14	0.14	0.13	0.12	1.15	1.53	1.81	1.08	1.76	0.83	1.04	2.76	10.68	13.01	2.19	1.87
CD@ 5%	0.28	0.28	0.27	0.25	2.33	3.09	3.65	2.19	3.57	1.68	2.10	5.58	21.62	26.29	4.43	3.79

Table 2: Mean performance of the genotypes of high SCMR class related to growth, yield and surrogate traits related to WUE in F_7 and F_8 RILs of the cross NRCG 12568 × NRCG 12326 in groundnut

RIL No.	D to 50 *per cent* flowering		Primary branches per plant		Pod number per plant		Pod yield per plant (g)		Kernel yield per plant (g)		SCMR		SLA (cm^2/g)		Shelling percentage	
	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8
176	32.00	47.00	4.00	2.00	20.00	5.40	29.04	23.92	27.92	21.92	56.12	48.69	157.67	168.59	65.96	63.20
182	33.00	45.00	4.00	3.00	21.60	18.60	19.58	16.86	15.18	12.71	52.14	51.84	85	91	62.21	64.23
181	33.00	46.00	4.00	4.00	33.20	29.00	30.12	14.10	26.20	14.10	52.88	47.92	198.00	210.00	65.61	65.89
51	32.00	46.00	4.00	3.00	23.40	4.80	35.20	33.62	33.32	28.62	52.88	52.24	89.04	98.54	41.50	72.97
339	32.00	47.00	2.00	4.00	22.00	19.56	17.70	24.85	11.44	20.36	51.32	53.64	213.10	210.42	64.63	65.89
165	31.00	46.00	5.00	3.00	15.20	20.40	31.12	12.56	27.50	12.56	51.46	46.86	171.50	186.45	67.45	70.17
412	34.00	48.00	4.00	3.00	10.00	10.80	14.70	15.32	22.32	10.32	50.30	50.30	117.68	110.47	49.36	62.30
175	32.00	46.00	4.00	3.00	29.60	16.80	32.34	20.56	31.76	10.56	48.76	48.76	210.92	222.54	64.12	72.33
139	31.00	47.00	2.00	4.00	19.60	31.00	32.92	23.00	29.40	13.00	47.50	49.00	207.67	217.78	61.47	54.62
S.Ed	0.14	0.14	0.13	0.12	1.15	1.53	1.81	1.08	1.76	0.83	1.04	2.76	10.68	13.01	2.19	1.87
CD @5%	0.28	0.28	0.27	0.25	2.33	3.09	3.65	2.19	3.57	1.68	2.10	5.58	21.62	26.29	4.43	3.79

Table 3: Mean performance of the genotypes of low SLA class related to growth, yield and surrogate traits related to WUE in F_7 and F_8 RILs of the cross NRCG 12568 × NRCG 12326 in groundnut

RIL No.	D to 50 *per cent* flowering		Primary branches per plant		Pod number per plant		Pod yield per plant (g)		Kernel yield per plant (g)		SCMR		SLA (cm^2/g)		Shelling percentage	
	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8
248	32.00	47.00	2.00	2.00	14.00	11.40	29.22	9.02	26.48	7.82	35.80	36.50	57.44	75.89	70.28	86.70
262	31.00	46.00	4.00	2.00	18.00	10.80	27.46	26.44	27.26	19.36	41.38	12.80	87.35	100.59	14.62	67.70
172	32.00	47.00	3.00	4.00	12.40	21.00	30.84	16.90	27.28	11.90	40.90	39.10	92.94	101.01	67.16	58.58
448	32.00	46.00	3.00	2.00	20.80	37.00	28.78	19.00	20.66	16.00	39.64	38.59	95.41	90.66	77.36	84.21
538	32.00	48.00	3.00	4.00	20.00	33.00	28.42	20.10	25.74	12.20	38.52	41.25	97.11	101.28	68.17	60.70
497	32.00	48.00	3.00	3.00	3.00	7.80	28.16	10.46	4.42	2.70	42.86	40.65	102.10	101.82	54.17	25.81
499	33.00	46.00	4.00	3.00	12.20	9.80	28.78	18.40	27.88	13.64	38.04	41.33	109.69	96.97	66.89	67.41
472	32.00	47.00	3.00	3.00	20.00	13.50	28.42	20.50	25.74	14.87	38.52	42.56	97.11	98.74	68.17	65.66
501	32.00	49.00	3.00	3.00	28.16	20.16	24.42	26.48	17.40	20.11	42.86	41.89	102.10	100.14	54.17	66.74
457	32.00	47.00	4.00	2.00	13.60	8.40	24.56	15.56	23.32	10.00	40.26	42.22	115.54	102.20	72.81	71.94
S.Ed	0.14	0.14	0.13	0.12	1.15	1.53	1.81	1.08	1.76	0.83	1.04	2.76	10.68	13.01	2.19	1.87
CD @5%	0.28	0.28	0.27	0.25	2.33	3.09	3.65	2.19	3.57	1.68	2.10	5.58	21.62	26.29	4.43	3.79

Table 4: Mean performance of the genotypes of high SLA class for growth, yield and surrogate traits related to WUE in F_7 and F_8 RILs of the cross NRCG 12568 × NRCG 12326 in groundnut

RIL No.	D to 50 *per cent* flowering		Primary branches per plant		Pod number per plant		Pod yield per plant (g)		Kernel yield per plant (g)		SCMR		SLA (cm^2/g)		Shelling percentage	
	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8	F_7	F_8
282	33.00	47.00	3.00	2.00	16.00	18.60	6.82	17.68	5.82	12.60	35.70	37.50	356.43	371.30	66.26	71.27
239	32.00	46.00	4.00	4.00	2.40	21.00	0.54	12.10	0.52	6.70	38.42	34.82	305.88	306.08	96.30	55.37
252	31.00	47.00	3.00	3.00	10.00	9.00	5.10	8.00	3.04	5.66	45.68	46.85	210.53	264.41	59.61	70.75
123	31.00	46.00	2.00	4.00	18.00	32.00	11.64	17.80	5.78	10.50	38.70	37.80	203.21	304.93	49.66	58.99
468	32.00	48.00	4.00	3.00	18.80	34.00	11.28	15.70	6.84	9.50	38.88	39.74	192.18	232.38	60.64	60.51
284	32.00	48.00	3.00	2.00	20.80	26.00	7.72	20.10	6.40	10.80	31.68	36.18	188.52	242.82	70.65	53.73
38	31.00	47.00	4.00	4.00	26.80	8.20	29.24	26.34	29.60	20.12	45.24	42.54	185.56	299.85	65.09	49.21
45	34.00	47.00	3.00	3.00	16.00	7.40	25.14	15.22	22.70	13.80	43.06	46.30	175.60	245.83	26.63	72.80
302	31.00	46.00	2.00	3.00	17.60	9.40	5.28	5.42	5.16	4.35	46.45	45.38	170.15	210.52	65.79	73.66
261	31.00	48.00	2.00	2.00	9.40	38.00	2.74	23.40	3.10	16.70	36.45	34.65	171.15	267.17	82.89	71.37
S.Ed	0.14	0.14	0.13	0.12	1.15	1.53	1.81	1.08	1.76	0.83	1.04	2.76	10.68	13.01	2.19	1.87
CD @5%	0.28	0.28	0.27	0.25	2.33	3.09	3.65	2.19	3.57	1.68	2.10	5.58	21.62	26.29	4.43	3.79

Table 5: t-test to compare the performance of extreme classes of SCMR and SLA for yield components and surrogate traits related to WUE in F_7 and F_8 RILs of the cross NRCG 12568 × NRCG 12326 in groundnut

Characters	For low SCMR genotypes		For High SCMR genotypes		For low SLA genotypes		For High SLA genotypes	
	Calculated t value	Table t value at 0.01	Calculated t value	Table t value at 0.01	Calculated t value	Table t value at 0.01	Calculated t value	Table t value at 0.01
Days to 50 per cent flowering	37.147**	2.552	28.107**	2.552	37.990**	2.552	34.000**	2.552
Primary branches/plant	0.512	2.552	1.574	2.552	1.212	2.552	0.275	2.552
SCMR	0.295	2.552	0.034	2.552	0.149	2.552	0.040	2.552
SLA (cm^2/g)	0.0013	2.552	0.010	2.552	0.020	2.552	0.056	2.552
Pods/plant	0.349	2.552	0.136	2.552	0.102	2.552	0.180	2.552
Kernel yield/plant (g)	0.080	2.552	2.471	2.552	0.578	2.552	0.021	2.552
Shelling percentage	0.041	2.552	0.228	2.552	0.031	2.552	0.015	2.552
SMK (%)	0.068	2.552	0.010	2.552	0.120	2.552	0.009	2.552
Pod yield/plant (g)	0.021	2.552	0.719	2.552	2.004	2.552	0.117	2.552

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