Mysore J. Agric. Sci., 54 (4) : 90-96 (2020)

Genetic Variability for Physiological Traits and Pod Yield in Groundnut (*Arachis hypogaea* L.) RILs under different Water Stress Conditions

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ABSTRACT

Groundnut is grown under non-irrigated conditions in most of the areas and is vulnerable to the effects of seasonal drought. Methods for efficiently evaluating drought tolerance and understanding the inherent principles of traits related to drought tolerance are critical for the success of groundnut breeding programme that aim to improve it. Therefore, the objective of the research is to study the extent of genetic variability for physiological traits under different water regimes. A mapping population of 147 recombinant inbred lines (RILs) resulting from the cross of NRCG 12568 × NRCG 12326 were evaluated in four different stress conditions *viz.,* well watered (WS), water stress-I (WS1) -with holding irrigation from 30-45 DAS (flowering period), water stress-II (WS2) -with holding irrigation from 45-65 DAS (flowering and peg initiation stage), water stress-III (WS3) -with holding irrigation from 65-85 DAS (peg penetration and pod development stage) for drought tolerance over two years at University of Agricultural Sciences, Bangalore during summer 2017 and 2018 in augmented design. Data was recorded on physiological traits namely specific leaf area (SLA) and SPAD chlorophyll meter reading (SCMR) before and after stress imposition at different water regimes along with well watered condition simultaneously. Low PCV and GCV were recorded by all the physiological traits measured at all stages across the seasons indicating low genetic variability in the population and difference between PCV and GCV for these traits indicated influence of environment on these traits during both the seasons. SCMR at 65-85 DAS and SLA at 30-45 DAS as well as at 45-65 DAS had moderate to high heritability and genetic advance as per cent of mean, indicating that selection for drought tolerance based on SCMR could be effective at later stages of intermittent stress. While based on SLA, selection could be effective only at the early stages of crop development.

Keywords: Groundnut, SCMR, SLA, Genetic variability, Water stress

GROUNDNUT (*Arachis hypogaea* L.) is an important oilseed crop for small holding farmer community of the semi-arid tropics of Asia and Sub-Saharan Africa. Drought is one of the main limiting factors for groundnut yield, especially during (1) germination and seedling stages, which determine plant survival and health and (2) pod development and maturity stages, which affect the final production. Further more, drought may easily aggravate *Aspergillus flavus* contamination, as well as infection with other diseases and insect pests. Therefore, it is vital to understand the mechanisms of drought tolerance in peanut and identifying drought tolerant lines.

Two-thirds of the global production occurs in rainfed regions of the semi-arid tropics where rainfall is generally erratic and insufficient, causing unpredictable drought stress, the most important constraint for groundnut production. Insufficient moisture at some point in the growing season is often the limiting factor in the production of a peanut crop. Additionally, the effects of drought can be economically devastating when it occurs at critical growth stages *i.e.,* during peg penetration and pod development stage. Water uptake is crucial during key stages like flowering and kernel filling at these stages can bring large yield benefits in groundnut.

Being mostly a rainy season crop, groundnut is exposed to intermittent water stress during gaps in rainfall, or at terminal water stress *i.e.,* at the end of the season when the rains are over. In these situations of water limitation, the strategy so far proposed to improve groundnut tolerance to drought is to identify lines with high water use efficiency.

In this context, WUE is defined as the ratio of dry matter production to water use, which provides a means to compare the variation among genotypes in their ability to produce dry matter in water-limiting conditions and thus to increase yield. Because groundnut production is habitually affected by drought, elevation of water use efficiency (WUE) is crucial to cope up with drought conditions. Soil plant analytical development (SPAD) chlorophyll meter reading (SCMR) and specific leaf area (SLA) are amongst easily assessable surrogates of WUE that can be used in breeding and selection schemes in crop plants. Thus, genetic variability is the pre-requisite for any successful breeding programme as the degree of response to selection depends on the quantum of variability. In the present study, genetic variability and heritability and genetic advance were studied for physiological traits and pod yield under different water regimes.

Material and Methods

The experimental material for the present study consisted of 147 recombinant inbred lines (RILs) derived from the cross NRCG 12568 × NRCG 12326. The two parents were contrasting for $\Delta^{13}C$ (delta C13), SCMR and SLA. NRCG 12568 has low $\Delta^{13}C$ (16.90) and NRCG 12326 has high $\Delta^{13}C$ (21.50) while SCMR and SLA were negatively correlated. The first season field evaluation of 147 (F_{11}) RILs was carried out during summer 2017 in augmented design along with parents and checks *viz*., GKVK 5 and TMV 2 at K-Block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru under three water regimes *i.e.,*well-watered (WW), water stress-II (WS2) -with holding irrigation from 45-65 DAS (flowering and peg initiation stage), water stress-III (WS3) -with holding irrigation from 65-85 DAS (peg penetration and pod development stage) to evaluate the RILs for physiological traits *viz.,* SCMR and SLA. The second season field evaluation of RILs was repeated with an addition of stress period at 30-45 DAS (flowering period) during summer 2018 under both well watered and water stress conditions.

All the lines were sown in one row of 2 m length with a spacing of 0.3 m between rows and 0.1 m between the plants. The recommended package of practices was followed for raising a good crop. All the four experiments including well watered plot and water stress plots under different water regimes were maintained separately. Imposition of stress was initiated only at specific periods in water stress plots *i.e.,* during WS1 (30-45 DAS), WS2 (45-65 DAS) and WS3 (65-85 DAS) while, irrigation was supplied to the WW plot at 7-10 days interval.

The SPAD chlorophyll meter (Minolta SPAD-502 m, Tokyo, Japan) reading was recorded on each leaflet of the tetra foliate leaf of five selected plants along the midrib. While recording the SCMR care was taken to ensure that the SPAD meter sensor fully covers the leaf lamina and that interference from veins and midribs was avoided. The unit value measured by the chlorophyll meter is termed as SCMR which provides information on the relative amount of leaf chlorophyll.

For recording SLA, fully expanded third leaf from the main axis was collected to record the leaf area by using leaf area meter. Leaves were dried in an oven at 80 °C for at least 48 hours to determine the leaf dry weight. Immediately after drying, the leaves were weighed and the SLA was derived as leaf area per unit leaf dry weight (cm^2 g^{-1}). Observations on SCMR and SLA were simultaneously recorded in the well watered and water stress plots before and after imposition of stress. The SLA was calculated using the following formula (Evans, 1972).

$$\text{Specific Leaf Area (SLA)} = \frac{\text{Leaf area (cm}^2\text{)}}{\text{Leaf dry weight (g)}}$$

Statistical Analysis

Statistical analyses of the data were carriedout according to augmented design. Analysis of variance was carried out as per the method suggested by Yates (1937). The genotypic and phenotypic coefficient of variation (Burton and Devane, 1953), heritability in broad sense (Hanson *et al.,* 1956) and genetic advance as per cent of mean (Johnson *et al.,* 1955) were computed. The data was analyzed by Windowstat 8.5 *ver.* Software.

Results and Discussion

Pooled analysis of variance carried out for two season data under different water stress conditions (Table 1) indicated significant differences among the RILs, season and also their interactions for most of the traits indicating the presence of genetic variability for all the traits studied. Mean and range of physiological traits and pod yield per plant for WW and WS conditions during the two seasons of summer 2017 and 2018 are presented in Table 2. In RILs the mean pod yield per plant over both the seasons under WW and WS were not on par, which might be because of the environmental interaction over both the seasons.

Table 1

Pooled analysis of variance for physiological traits and pod yield of RILs derived from NRCG 12568 × NRCG12326 in groundnut over two seasons of summer 2017 and 2018

Source	DF	DF	SCMR		SLA		Pod yield / plant (g)
			BIS	BRS	BIS	BRS	
Blocks (eliminating checks + RILs)	WW	6	1.66	7.07 *	1382.17 ***	854.96	26.45 **
	WS1		2.38 *	3.14 *	150.73	382.51 *	39.46
	WS2		0.90	8.23 **	255.86 *	323.13 **	33.05 *
	WS3		2.56	5.82	867.60 ***	281.37 *	31.16 **
RILs + Check	WW	150	35.72 **	7.22	361.77 *	515.05	17.21 *
	WS1		10.93 *	10.77	211.51	496.45	19.07 *
	WS2		5.66	6.43	556.68 **	906.95	20.04 *
	WS3		6.46 **	25.20 ***	354.68 **	426.13 **	13.97
Checks	WW	3	5.99	10.55	138.56	1661.21 *	17.23 ***
	WS1		5.40	16.78 **	102.34 **	1104.36 *	16.21
	WS2		1.80 **	18.76	194.91	262.95 *	42.96 *
	WS3		2.31	4.06	566.53 *	779.64 **	19.40 **
RILs	WW	146	6.35 **	7.11	367.65	489.09	16.83
	WS1		6.20	5.62	214.81 *	487.74	17.98
	WS2		5.74 **	6.17	566.53 ***	860.34	18.87 *
	WS3		6.56	25.67 ***	342.50 **	390.71 *	13.86
Checks *vs*. RILs	WW	1	33.09 *	13.28	173.05 *	867.14	73.00 **
	WS1		15.40	14.98 *	56.19	329.73 **	17.98
	WS2		5.81 **	7.08	605.80 **	255.25	21.44 **
	WS3		3.34 *	18.72 **	149.97 **	153.82 **	13.65 **
Error	WW	18	4.30	5.78	126.07	146.08	24.80
	WS1		5.94	10.92	125.06	73.29	39.01
	WS2		2.95	4.81	83.27	90.37	9.37
	WS3		4.76	3.79	73.38	60.31	15.74

Note: * Significant at 0.05 probability level, ** Significant at 0.01 probability level, *** Significant at 0.001 probability level
BIS - Before initiation of stress, BRS - Before release of stress, Results for WS1 is not pooled data

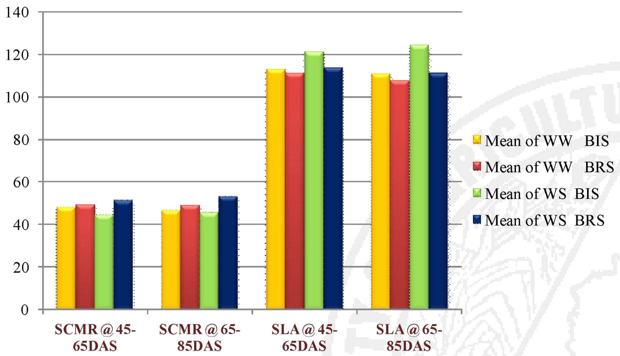
TABLE 2

Mean and range for physiological traits and pod yield per plant in RILs of NRCG 12568 × NRCG 12326 in groundnut under WW and WS conditions during summer 2017 and 2018

Character	Mean of WW				Mean of WS				Range of WW				Range of WS			
	S-2017		S-2018		S-2017		S-2018		S-2017		S-2018		S-2017		S-2018	
	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS	BIS	BRS
SCMR @ 30-45DAS	-	-	48.2	49.0	-	-	42.0	47.9	-	-	40.82-53.25	37.73-182.10	- -	- -	35.75 49.25	31.47-60.43
SCMR @ 45-65DAS	46.9	48.2	49.0	50.4	40.5	42.0	48.2	38.7	39.60-55.75	40.82-53.25	37.73-72.10	36.27-60.23	33.70-50.25	35.75-49.25	33.27-57.30	31.55-47.20
SCMR @ 65-85 DAS	45.1	46.6	48.2	51.3	38.7	40.3	52.1	53.7	37.15-58.50	31.55-47.20	40.82-53.25	33.10-62.70	31.55-47.20	34.23-46.98	37.60-62.40	43.63-166.83
SLA @ 30-45DAS	-	-	43.0	78.2	-	-	51.9	97.6	-	-	20.61-148.55	50.14-224.11	- -	- -	32.47 116.11	38.18-158.91
SLA @ 45-65 DAS	106.8	107.5	111.1	108.4	106.1	106.8	101.2	145.0	69.36-115.91	59.07-110.43	50.14-141.21	46.37-190.64	69.36-115.91	59.07-110.43	20.11-125.73	47.69-152.22
SLA @ 65-85 DAS	113.5	114.2	71.9	107.4	112.8	113.5	129.4	132.5	73.71-129.46	62.78-123.65	36.91-93.50	36.90-134.23	67.81-175.05	74.52-195.86	53-131.62	70.47-172.90
Pod yield per plant	14.1		10.6		WS2-12.97 WS3-10.13		WS1-9.09 WS2-10.09 WS3-6.94		5.58		1.30 36.20		WS2-2.78-36.18 WS3-2.30-33.00		SW1-1.20-26.20 WS2-2.00-30.80 WS3-1.5-20.30	

Note: " 66 " indicates no water stress experiment was taken up at 30-45 DAS during summer 2017

Pod yield per plant and SLA showed relatively high range. This indicating that these traits can be improved through individual plant selection. In the present study, the relative chlorophyll content of leaves was found lower in the initial stages of leaf development and increased as the dry matter accumulates and leaf matures. SCMR of the RILs under WS condition was higher compared to WW condition as indicated through mean at different stages of stress (Fig. 1). The chlorophyll density decides the photo synthetically active light-transmittance features of the leaf which is measured by the SCMR. Significant positive correlation between SCMR and chlorophyll density indicates that high SCMR value may have a higher photosynthetic activity per unit area. A strong negative relationship between WUE and SLA has been reported in several peanut studies, indicating that the genotypes with thicker leaves have greater WUE and leading to the conclusion that SLA can be used as a fast, relatively inexpensive technique for identifying and selecting genotypes with high WUE (Kalariya *et al.*, 2015 and Mashamba *et al.*, 2016). Water stress also decreased the SLA but increased SCMR.



Note : WW - Well watered, WS - Water stress, BIS - Before initiation of stress, BRS - Before releasing of stress

Fig. 1 : Mean of physiological traits in 147 RILs of NRCG 12568 x NRCG 12326 in groundnut under WW and WS conditions over two seasons

The estimates of mean performance, range, PCV (%) and GCV (%) heritability and genetic advance as per cent mean (GAM) of various characters are given in the Table 3. Lower difference between phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) indicates less influence of environment on expression of all the traits studied. Accordingly, Low PCV and GCV were recorded by the physiological traits measured while high PCV and GCV for pod yield per plant were recorded at all stages during the seasons (summer 2017 and 2018). The low genetic variation in the population and difference between PCV and GCV for SCMR and SLA indicated less prevalence of environment on these traits across the seasons. The similar results of significant genotypic

TABLE 3

Genetic parameters for physiological traits and pod yield in the RILs of NRCG 12568 × NRCG 12326 in groundnut during summer 2017 and 2018

Character	PCV (%)		GCV (%)		h^2 (broad sense)		GAM	
	S-2017	S-2018	S-2017	S-2018	S-2017	S-2018	S-2017	S-2018
SCMR @ 30-45 DAS	-	5.10	-	3.71	-	54.18	-	5.57
SCMR @ 45-65 DAS	4.29	5.10	3.67	3.71	74.81	54.42	6.46	5.57
SCMR @ 65-85 DAS	4.18	8.62	3.13	6.17	87.39	82.42	12.82	19.09
SLA @ 30-45 DAS	-	10.94	-	10.79	-	96.66	-	21.92
SLA @ 45-65 DAS	5.18	10.85	3.98	10.37	60.57	93.41	16.29	20.40
SLA @ 65-85 DAS	9.93	7.72	3.74	5.48	14.51	51.59	2.90	8.01
Pod yield^{-1} WW	34.12	44.94	24.19	44.23	44.70	28.20	36.87	45.76
Pod yield^{-1} WS1	-	48.55	-	45.57	-	88.14	-	35.87
Pod yield^{-1} WS2	48.23	47.76	25.55	27.71	58.80	53.68	29.65	31.67
Pod yield^{-1} WS3	52.42	56.04	50.02	41.25	93.82	54.18	48.65	36.88

Note: "-" indicates no water stress experiment was taken up at 30-45 DAS during summer 2017 variation for the traits related to WUE was reported in numerous reports depending upon the material used for their study by Mashamba *et al*. (2016), Srivalli *et al*. (2016) and Bhavya *et al*. (2017).

Higher estimates of heritability and low GAM for SCMR at 30-45 DAS was observed during summer 2018. High heritability and low GAM were recorded for SCMR at 45-65 DAS across the seasons. High heritability and moderate GAM were recorded for SCMR at 65-85 DAS indicating less influence of environment and wider scope for selection of this trait. Selection for drought tolerance based on SCMR could be effective only at later stages of stress imposition compared to the early stages. Similar kind of results of high heritability and moderate GAM was reported for SCMR by John *et al*. (2011). Srivalli *et al*. (2016) and Mashamba *et al*. (2016).

High heritability and moderate to high GAM was observed during summer 2018 for SLA at 30-45 DAS as well as at 45-65 DAS indicating the additive gene effects and selection for this trait could be effective in the early stages. The results were in accordance with the reports of high heritability and high GAM for SLA by Mahesh and Hasan (2019) and Savitha (2012). Low to moderate heritability along with low GAM were recorded for SLA at 65-85 DAS during summer 2017 and 2018 indicating the influence of environmental effects on the expression of SLA at this stage. Similar results of moderate $h^2_{(b)}$ and low GAM was reported by John *et al.* (2011). The heritability and GAM for pod yield per plant were moderate to high in well watered and water stress conditions indicating the prevalence for selection. Differential heritability and GAM estimates for physiological traits and pod yield in the present study are because of varied stages and duration of drought stress imposition. The mechanisms of physiological responses to early season drought might be different from those for long duration drought, which have been reported previously (Mashamba *et al.,* 2016). Kalariya *et al.* (2015) reported that broad sense heritability of drought tolerance traits varied among groundnut crosses, traits and stages depending on levels of genetic variation for the trait and stage of imposition of drought stress.

In the study, SLA with high heritability, moderate GAM under well-watered and water stress at 30-45 DAS indicated that SLA can be potentially used as selection tool as it is least influenced by the environment. SCMR at 65-85 DAS under well watered and water stress conditions showed high heritability, moderate GAM and hence, SCMR could be used as selection criteria along with other yield parameters for identifying superior genotypes for water stress. Thus, it is suggested to record SCMR at 65-85 DAS as a rapid technique for screening a large number of stable peanut breeding materials for water stress tolerance. This technique will help breeders to identify probable superior breeding materials based on SCMR before harvest which could be confirmed later based on pod yield under water stress conditions.

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(*Received :* August, 2020 *Accepted :* December, 2020)