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Phenotyping RIL Population to Identify Water Deficit Tolerant Lines in Groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Background: Genotype sensitivity to mid-season drought suggests the possibility of identifying genotypes with high yield potential and low sensitivity to mid-season droughts. The overall objective of the present research was to select genotypes with high tolerance to mid-season moisture stress. This effort includes assessing the variation of physiological and productive traits among RIL population lines, assessing the range of correlation for physiological and productive traits across two seasons and identifying a set of contrasting material under drought stress conditions.

Methods: To assess the recombinant inbred lines (RIL) under moisture stress, we screened RIL population of groundnut with 432 lines and parents under mid-season drought stress conditions in *rabi* for two years. During the drought stress period, soil moisture content (SMC) and temperatures were measured. SPAD chlorophyll meter reading (SCMR), specific leaf area (SLA), relative water content (RWC), visual wilting rate (VWR) and canopy temperature (CT) were recorded at the end of the stress period. Pod yield per plant (PYPP), kernel yield per plant (KYPP) and shelling percent (SP) were recorded at harvest.

Result: Physiological traits and yield attributes varied significantly among the RIL population. The Genotype by Trait biplot (GT biplot) revealed that the yield attributes showed a positive association with RWC, SCMR and a negative association with SLA, VWR and CT. The inbred lines were classified as visually healthy with high physiological and high or moderate yield traits; visually wilting with low physiological and low yield traits under a drought environment. The identified superior and inferior lines werefurther utilized to analyze genomic regions and genes for drought tolerance in groundnut.

Key words: Drought related traits, Groundnut RIL population, Mid-season drought stress, Yield traits.

INTRODUCTION

Groundnut, also known as the wonder nut or poor man's cashew nut, is an affordable food commodity, including a valuable source of all nutrients (Amarowicz and Pegg, 2020). Groundnut is mainly cultivated in Gujarat, Andhra Pradesh, Karnataka, Tamil Nadu and Maharashtra ofIndia. At the national level in India, this crop occupies an area of 47.5 lakh (l) hectares (ha) with the production of 62.2 l tonnes (t) and productivity of 1320 kilogram (kg) ha^{-1}, whereas it is cultivated in 6.61 l ha with 8.50 l t of production and productivity of 1285 kg^{-1} in Andhra Pradesh (Directorate of economics and statistics 2019-2020). In Andhra Pradesh, it is mainly grown under a rainfed situation in the Rayalaseema region.

Drought is undeniably one of the most significant abiotic stress that groundnut crop experience and it is undoubtedly the most important factor in limiting production and quality. Of India's 85 % rainfed groundnut region, 80% is a dry land with no irrigation facilities, leading to low productivity (Baig *et al*., 2013). Low rainfall and prolonged dry spells during the crop growth cycle are among the key factors limiting groundnut productivity in India. Since nearly 90% of the world's groundnut is grown in tropical and semi-arid tropical areas, efficient water use is a global concern in groundnut production (Hamidou *et al*., 2013). They also reported that losses due to water stress could be significant and exacerbated by increased temperature. They also observed pod yield decreases of up to 72 percent in drought conditions but no reductions in well-watered conditions at high temperatures. Yield losses are highly variable, depending on several factors such as the drought's timing, severity and duration (Nigam *et al*., 2005).

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Drought trends can be divided into three categories: early season, mid-season and end of season droughts, with the mid-season drought affecting the most vulnerable stages of groundnut plant growth such as pegging, pod and seed development etc. (Reddy *et al*., 2003). Researchers found a weak relationship between genotype sensitivity to mid-season drought and yield potential under sufficient water supply suggesting the possibility of identifying genotypes with high yield potential but low sensitivity to mid-season droughts (Fahad *et al*., 2017). Hubick *et al*. (1986) have demonstrated three potential solutions to the problem. Irrigating arid areas is one solution, but it is only feasible to a certain degree. A second option is to farm more intensively in humid regions, but water is typically scarce even in such humid areas. The third option is to select and breed plants that need less water to grow, thus increasing water-use efficiency (WUE).

The overall objective of the present study was to sort changes in physiological efficiency and yield attributes and select genotypes with high tolerance and high/moderate yields under water deficit conditions to boost groundnut production in rainfed/water-stressed soils. There are ample genomic resources available in groundnut and an array of advanced genomics approaches for trait discovery and marker development for target traits (Pandey *et al*., 2020). Precise phenotyping is expected to uncover genetic variations in drought and heat tolerance, which can be used to improve groundnut productivity through molecular breeding (Pandey *et al*., 2021). TMV2 is an elite variety known for its adaptation over the last 40 years and its EMS-derived mutant is TMV2NLM. The mapping population of 432 Recombinant Inbred Lines (RILs) derived from TMV2 and TMV2NLM would aid in the genetic dissection of drought, high temperature tolerance and other productivity traits. Thispaper aims to classify the RILs for drought tolerance with higher yield under mid-season drought stress conditions.

MATERIALS AND METHODS

Seeds of the parents (TMV2 and TMV2NLM), RIL population of 329 inbreds resulting from the cross of TMV2NLM (narrow leaf mutant) × TMV2 and 103 RILs resulting from the cross of TMV2 × TMV2NLM, developed at UAS, Dharwad, were obtained for drought tolerance analysis. All the RILs and their parents were planted using a randomized block design with two replications of two rows of two meters each during the *rabi*' 2018-19 and 2020-21at the research farm of Regional Agricultural Research Station,Acharya N G Ranga Agricultural University (ANGRAU), Tirupati, Andhra Pradesh, India (Plate1 and 2).

The total RIL population (432 lines) and parents were sown during the II fortnight of December 2018 and harvested during I fortnight of May 2019. The same lines were sown during the II fortnight of December 2020 and harvested during I fortnight of April 2021. The water deficit was naturally created in the field due to a complete lack of rain from pegging to pod formation, *i.e*., 40-90 days (mid-season drought), while adequate moisture was retained during the vegetative and maturity stages.

During the drought stress period, soil moisture and temperatures were measured using the field scout TDR 350 soil moisture meter. At the end of the mid-season stress period, the SCMR, SLA, RWC, VWR and CT were recorded. For the third completely expanded leaf from the top of the main stem, SCMR was measured using a Minolta SPAD 502 (Tokyo, Japan). SLA is the leaf area (cm^2) ratio to leaf dry weight (g). The leaf area was measured with an LI-3100 leaf area meter and the leaf samples were oven-dried for at least 48 hours at 80°C. To determine RWC, leaf samples were collected, recorded fresh weight, soaked for 6 hours to record turgid weight and the leaf samples were oven-dried for at least 48 hours at 80°C to record dry weight. RWC was calculated using the formula

$$\text{RWC (\%)} = \frac{\text{FW-DW}}{\text{TW-DW}} \times 100$$

Where

FW stands for fresh weight; DW= for dry weight; TW= for turgid weight.

As defined by Barrs and Weartherley (1962). A range of 1 to 5 score was assigned to describe the VWR during the moisture stress period in the morning hours (Kalariya *et al*., 2015).

The yield and its attributes were recorded by choosing ten plants at random after maturity and the average of their yields was used to calculate PYPP, KYPP and SP. Agro-meteorological data during the crop growth was collected from the Agromet Department, RARS, Tirupati, Andhra Pradesh.

The data collected from the two experiments (two years) was pooled and subjected to analysis of variance (ANOVA) using GENSTAT 6th Edition statistical tools (Payneet *et al*., 2012). The interrelationships between traits were computed through a GT biplot using the R software Program (Yan and Rajcan, 2002).

RESULTS AND DISCUSSION

Arid and semi-arid regions cover more than half of the production area and hence, groundnut is frequently subjected to abiotic stress (Pasupuleti *et al*., 2013). During critical growth stages such as emergence, flowering, pegging and pod filling, drought exposure will significantly reduce yield (Reddy *et al*., 2003). Furthermore, heat can intensify drought effects at all levels and increase the evaporation of soil moisture. Physiological traits such as RWC, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and CT have been evaluated on groundnut genotypes so far (Parkash and Singh, 2020). RILs of 432 and two parents, *viz*., TMV2 and TMV2NLM of groundnut, were sown and evaluated for drought tolerance during the *rabi* season of 2018-19 and 2019-20.

Variation in meteorological data

The data representing maximum and minimum temperatures, maximum and minimum humidity and total rainfall at the experimental site for two years are displayed

Plate 1: Field experimental view of the RIL population (432) and parents of groundnut during the mid season moisture stress period.

Plate 2: Field experimental view of the RIL population (432) and parents of groundnut before imposition of moisture stress.

in Fig 1. During the mid-season moisture stress period (40-90 DAS) of two growing seasons, there was no receipt of rain and recorded maximum temperatures. The meteorological data clearly shows no receipt of rain during the stress period and recorded high temperatures over the entire stress period (pod formation and pod filling stage), making the study suitable for evaluating RILs for drought tolerance.

The soil moisture content (SMC) in the field varied before and during moisture stress conditions. SMC was 26.6-33.6% and 18.2-20.6% at 0-15 and 15-30 cm soil depth, respectively at 40 days (the start of the moisture stress period) and 3.0-5.2% and 1.0- 2.5% at 0-15 and 15-30 cm soil depth respectively at 90 days (the end of the stress period) respectively at two growing seasons. Soil moisture content decreased during the stress period. In contrast, soil temperature increased during the stress period due to non-receipt of rainfall and high maximum and minimum temperatures recorded during the stress period (Kalariya *et al*., 2015).

Variability for the physiological traits

Recent research indicates that the drought-related traits SCMR, SLA and RWC are reliable for drought tolerance selection under drought stress (Banavath *et al.,* 2018; Hampannavar and Khan, 2019) and were significantly differentiated in the RIL population. Drought stress affected the physiological and yield parameters and the mean of the traits over two seasons is discussed below (Table 2). RILs of 201 lines showed higher SCMR than the mean SCMR, while 233 lines showed low SCMR. Several authors have reported that under drought stress, chlorophyll contents in drought tolerant cultivars were significantly higher than those in drought sensitive genotypes (Zhou *et al.,* 2017). As a result, SCMR may be a reliable predictor when screening germplasm for drought tolerance, as other studies demonstrated (Zaefyzadeh *et al.,* 2009). RIL population of 207 lines showed higher SLA than the mean and 227 lines had low values for SLA. SLA is commonly used as a fast, low-cost method for identifying and selecting groundnut

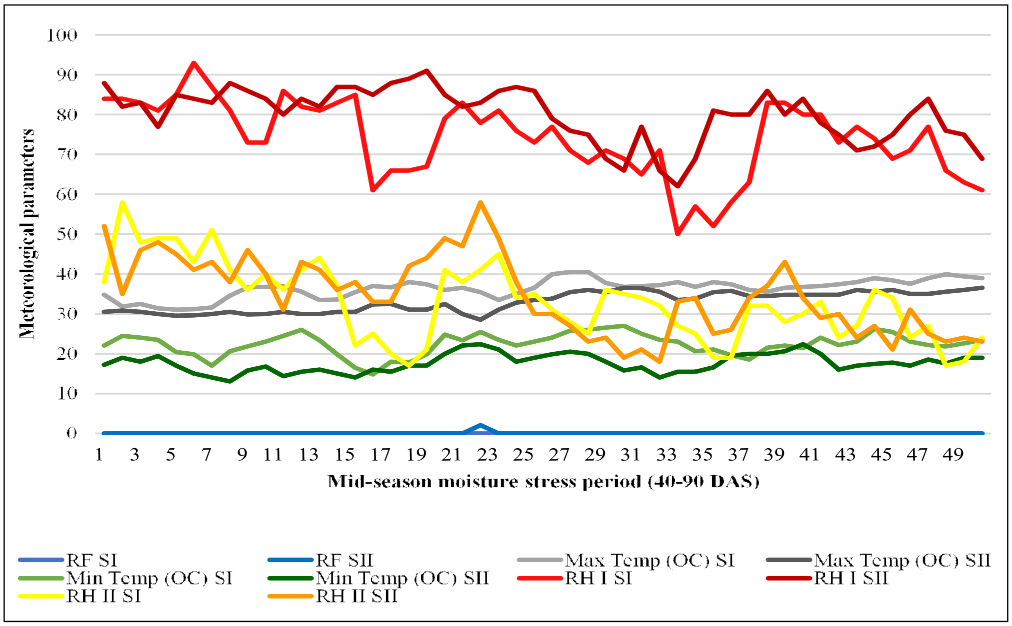

Fig 1: Maximum and minimum temperatures, maximum and minimum humidity and rainfall during the mid-season moisture stress period for two growing seasons.
Season I (2018-19) – SI, season II (2020-21) - SII.

genotypes with high WUE. Genotypes with thicker leaves have higher WUE, which has led to the conclusion that SLA is a viable surrogate trait.

RILs showed considerable variance in RWC, ranging from 52.42 to 82.88% and the mean was 72.62%. RILs of 245 had higher RWC than the mean RWC, while 189 lines showed lower RWC. The percent RWC of 65.6 and 68.8 were found in the parents TMV2 and TMV2NLM, respectively (Table 2). During the stress period, the decrease in RWC is due to decreased water uptake under deficit soil moisture conditions. The leaf relative water content representing the plant water status gives a biological baseline or reference. It is a significant determinant of metabolic activity and tissue or organ survival (Kalariya *et al.,* 2015; Shinde *et al.,* 2018). A maximum of 47.13°C, minimum of 30.68°C and mean of 40.08°C CT were recorded and it was lesser in TMV2 (41.4°C) than TMV2NLM (38.0°C) (Table 2). CT was higher in 231 lines than the mean, while 203 showed low CT. In many studies, CT has been suggested as a possible surrogate method for selecting genotypes with higher WUE in many legumes (Ainsworth and Rogers, 2007; Blum, 2009). Jongrungklang *et al.,* 2008 investigated the relationship between canopy temperature and WUE and found that CT measurements generally increased with drought conditions. Groundnut genotypes with lower canopy temperatures are preferable due to higher transpiration and thus higher CO2 exchange rate. The RIL population with the lowest VWR, *i.e.*, 1, was more resistant to stress conditions, while those with the highest value of 5 were more vulnerable to drought stress. Among 434 RILs, 101 lines showed 1, indicating the lines can withstand and combat stress conditions, while 93 showed 2, 121 with 3, 81 showed 4 and 38 lines recorded 5. Nonetheless, selecting drought-tolerant lines based on traits such as VWR, SCMR, SLA, RWC and CT would result in more stable lines (Kalariya *et al.,* 2015).

Variability for the yield traits

The RILs showed considerable variation in yield attributes and the drought stress lowered crop yield in most of the lines tested over two years. Table 2 shows the range of yield and its attributes under drought stress, including PYPP, KYPP and SP averaged across the two years and all of the yield traits observed in the RIL population showed considerable variation. The RIL population of 190 lines recorded higher PYPP than the mean PYPP, while 244 lines showed less than the mean, while KYPP was found to be higher in 187 lines than the mean and 247 lines showed lower yield than the mean KYPP. RIL population of 226 lines recorded higher values of SP, while 208 lines showed lower values than the mean.

A combined analysis of variance for the pooled data of mean squares is summarized in Table 1. The RILs differed significantly for drought related and productivity traits in response to mid-season moisture stress 40-90 DAS. The results of ANOVA demonstrated that the effects of yearonRIL population(treatments)were significant for all traits except for SCMR, CT and RWC. In the case of treatments, the mean of squares was significant for all the traits and even highly significant in the case of SCMR, SLA, CT, PYPP and KYPP. The mean squares for interaction between year and treatment (Y × T) were significant for all the traits.

Interrelationships of traits

The GT biplot of the mean performance of RILs explained 49.86% of the total variation of the standardized data (Fig 2),

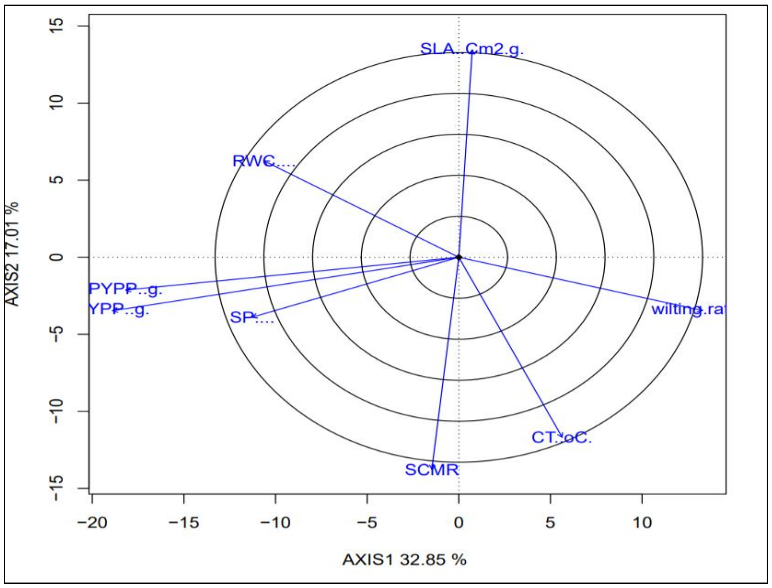

Fig 2: Vector view of the genotype-by-trait biplot showinginterrelationships among various physiological and yield traits measured in the RIL population under mid-season moisture stress conditions in 2018-19 and 2020-21 (combined over seasons). Horizontal and vertical axes are the first and second components, respectively.

Table 1: Combined Analysis of variance for pooled data of two years (Y) for WUE and productivity traits in 432 lines and two parents of RIL population tested under drought stress conditions in groundnut.

Source of	Mean squares							
Variation	DF	SCMR	SLA	CT	RWC	PYPP	KYPP	SP
Year	1	1,524.29	62,023.27*	39.552	1,653.12	270.235**	105.326**	15587.31**
RILs	433	56.893**	1,861.52**	29.046**	96.837*	7.534**	2.798**	277.18*
Year × RILs	433	34.367**	1,154.41**	22.129**	80.528**	6.012**	2.089**	231.029**
Pooled error	866	16.601	136.621	13.962	22.019	0.06	0.032	32.375
CD for year	2.218	6.945	4.814	2.738	0.03	0.07	1.137	
CD for RILs	10.702	62.025	8.588	16.382	4.476	2.638	27.747	
CD for Year × RILs	10.519	30.176	9.647	12.115	0.633	0.459	14.69	

*, **: Significant at 5% and 1% respectively; df: degrees of freedom; CD: Critical difference.

interpreting the low exploitation oftotal variation by the two principal components (PC 1 and PC 2) similar to the results of Samonte *et al.* (2013). The first PC contributed 32.85%, while the second PC explained 17.01% of the total variation in the traits tested and averaged across two growing seasons. This relatively moderate percentage reflects the complexity of the relationships among the measured traits (Yan and Rajcan, 2002). In the GT biplot, a vector drawn from the origin to each trait shows the visualization of the relationships among the traits. The line between the marked point of any trait and the origin of a biplot is termed as traits vector and cosine angle between trait vectors determines interrelationship among the traits (Yan and Rajcan, 2002).

The results of GT biplot analysis revealed the interrelationships among the physiological and yield traits under moisture stress conditions in the two growing seasons (Fig 2). This part of the analysis was performed to identify how far the field-measured traits related to better performance of genotypes under drought stress conditions. SLA indicates the thickness of the leaves and low SLA specifies thicker leaves and more chlorophyll content per unit area (Banavath *et al.,* 2018). In this study, SLA showed a negative association with SCMR, CT, VWR and yield traits. Thirumala Rao, 2016 reported a positive association between yield attributes and SCMR in groundnut. In the present study, all the yield components showed a positive relationship between themselves and SCMR and RWC while negatively correlated with VWR, CT and SLA. SCMR showed a weak association with RWC and a close association with CT and similar results were obtained by Krishnamurthy *et al*., 2007. This is contrary to several previous reports showing a close association between RWC and SCMR. However, these reports involved a minimal number of lines/genotypes (Sheshshayee *et al.,* 2006).

Table 2: Contrasting RIL entries (10 tolerant and 10 sensitive) along with trait values and mean of the WUE and productivity traits in 432 lines and two parents of RIL population tested across two growing seasons under mid-season moisture stress.

Line no.	SCMR	SLA (cm^2/g)	CT (°C)	RWC (%)	Visual Wilting rating	PYPP (g)	KYPP (g)	SP (%)
Tolerant lines								
414	56.5	127.6	42.5	74.3	2.0	8.5	5.6	65.7
208	44.4	158.9	39.9	74.3	1.0	9.7	6	63.2
196	51.2	166	37.8	76.1	1.0	6.3	4.8	74.9
238	45.9	173.3	41.5	78.7	1.0	8.6	5.4	61.3
102	48	171.4	34.4	75.6	1.5	6.6	4.4	62.9
343	48.7	151.4	39.6	79.4	1.5	4.8	3.3	66.5
351	48.2	143.9	40	74.2	1.0	6.0	3.5	56.1
203	47.1	153.1	38.6	74.3	1.0	4.2	2.7	63.6
354	54.2	130	39.4	76.5	2.0	3.6	2.1	62.9
288	46.1	163.1	37.4	76	2.5	5.3	3.4	64.5
Susceptible lines								
188	44.9	188.3	42.7	61.8	4.0	0.4	0.1	17.4
411	40	169.8	45.3	55.4	2.5	0.8	0.3	39.1
189	43.2	166.4	40.8	52.9	4.0	1.2	0.6	39.1
95	43.1	264.3	40.9	76.9	4.0	1.2	0.6	48.9
118	45.2	179.3	41.2	58.5	3.0	0.7	0.4	41.7
134	40.1	196.8	40.5	63.4	5.0	1.0	0.6	58.9
223	42.5	186.4	45.5	72.2	4.0	2.2	1.0	45.3
281	44.7	165.4	42.3	67	4.0	1.2	0.6	44.0
409	44.6	155.9	47.1	69.3	5.0	1.4	0.7	35.3
363	42.8	133.3	41.2	62.2	3.5	0.8	0.5	31.1
Parents								
TMV2	50.4	144	38	65.6	1.0	2.9	1.6	55.8
TMV2XNLM	45.7	164.2	41.4	68.8	1.0	2.7	1.6	56.1
RIL Mean	45.5	162.9	40.1	72.6	3.0	2.7	1.5	52.4
RIL Maximum	59.1	341.5	47.1	82.9	5.0	9.6	6.0	74.9
RIL Minimum	34.6	109.3	30.7	52.4	1.0	0.4	0.1	17.4

CONCLUSION

A significant variation in physiological traits of RILs indicates that the RILs were genetically diverse. The physiological and yield traits had correlations among themselves and also with each other across two years of study. Statistical analysis was performed to identify contrast entries among the RIL population under mid-season moisture stress. In the current study, phenotyping RIL population under moisture stress identified a few superior and inferior lines for WUE and yield attributes. The superior lines had high WUE and high yield attributes, recording higher values than the parents, while the inferior lines were sensitive to moisture stress with very low yields (Table 2). The five most superior RILs were 414, 208, 196, 238 and 102 and the five most inferior lines were 188, 411, 189, 95 and 118. Superior and inferior lines were chosen for further analysis to identify genomic regions and genes responsible for drought tolerance in groundnut. These RILs can also be used as genetic material in potential breeding programs and to find drought tolerance QTLs.

Conflict of interest: None.

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